University of Kentucky
UKnowledge

Theses and Dissertations--Animal and Food Sciences

Animal and Food Sciences

2021

Impact of Feeding Foods Containing Industrial Hemp-Derived Cannabidiol on Canine Health and Well-Being

Elizabeth M. Morris
University of Kentucky, efm5584@gmail.com
Author ORCID Identifier:
https://orcid.org/0000-0002-9650-3678
Digital Object Identifier: https://doi.org/10.13023/etd.2021.398

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation

Morris, Elizabeth M., "Impact of Feeding Foods Containing Industrial Hemp-Derived Cannabidiol on Canine Health and Well-Being" (2021). *Theses and Dissertations--Animal and Food Sciences*. 134.
https://uknowledge.uky.edu/animalsci_etds/134

This Doctoral Dissertation is brought to you for free and open access by the Animal and Food Sciences at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Animal and Food Sciences by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Elizabeth M. Morris, Student

Dr. David L. Harmon, Major Professor

Dr. David L. Harmon, Director of Graduate Studies

IMPACT OF FEEDING FOODS CONTAINING INDUSTRIAL HEMP-DERIVED CANNABIDIOL ON CANINE HEALTH AND WELL-BEING

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food, and Environment
at the University of Kentucky

By
Elizabeth M. Morris

Lexington, Kentucky

Director: Dr. David Harmon, Professor of Animal Science

Lexington, Kentucky

2021

Copyright © Elizabeth M. Morris 2021
https://orcid.org/0000-0002-9650-3678

ABSTRACT OF DISSERTATION

IMPACT OF FEEDING FOODS CONTAINING INDUSTRIAL HEMP-DERIVED CANNABIDIOL ON CANINE HEALTH AND WELL-BEING

Anecdotal evidence of beneficial behavioral and health effects of cannabidiol (CBD) use in companion animals has amplified the need to elucidate safety and potential impacts of CBD use. The purpose of this investigation was to determine the impact of industrial hemp-derived CBD administration on canine health and well-being. We hypothesized that CBD would produce beneficial effects on canine behavior without negatively impacting animal health. Dog treats were formulated to include CBD and shown to be palatable across a range of CBD inclusion levels. Dogs were supplemented with CBD treats and subjected to a noise-induced fear response test to assess potential anxiolytic effect of CBD. Behavioral response to the noise-induced fear response test was unaffected by CBD and did not support an anxiolytic effect of CBD when supplemented at 1.4 mg/kg BW/d. Next, triaxial accelerometers were fitted to dogs' collars to assess the impact of CBD treats on voluntary daily activity. While voluntary daily activity of healthy adult dogs was unaffected by 1.8 and 4.5 mg CBD/kg BW/d, a reduction in daily scratching suggested a potential antipruritic effect of CBD. Potential impacts on canine health were also assessed by evaluating changes in the canine metabolome, hematology, and serum chemistry, and immune response upon exposure to a novel antigen. The canine metabolome, including amine/phenol, carbonyl, carboxyl, and hydroxyl metabolites, was altered with 4.5 mg CBD/kg BW/d and suggested an impact on glucose, amino acid, vitamin, and nucleotide metabolism. Hematological and clinical indices of health and serum immunoglobulins (IgG and IgM) were largely unaffected by 5 mg CBD/kg BW/d; however, elevated liver enzyme alkaline phosphatase may suggest altered liver function. These findings may be used to formulate recommendations for CBD use in a clinical environment, spur investigation into other potential therapeutic effects of CBD, and may serve as a foundation for the development of regulations on CBD use, all of which are essential to supporting the health and well-being of companion animals.

KEYWORDS: canine, cannabidiol, noise phobia, voluntary activity, metabolomics, immunomodulation

Elizabeth M. Morris

10/3/2021
Date

IMPACT OF FEEDING FOODS CONTAINING INDUSTRIAL HEMP-DERIVED CANNABIDIOL ON CANINE HEALTH AND WELL-BEING

By

Elizabeth M. Morris

Dr. David L. Harmon
Director of Dissertation

Dr. David L. Harmon
Director of Graduate Studies

10/4/2021
Date

DEDICATION

To my husband, Brett, who moved halfway across the country for me to pursue my dream. Thank you for your relentless love and support.

ACKNOWLEDGMENTS

Firstly, I would like to thank my advisor, Dr. David Harmon, for providing me the opportunity to complete this graduate program, offering guidance when needed, and challenging me to become a better animal scientist. I would also like to thank Dr. Beth Kitts-Morgan for her support, direction, and hands-on assistance while completing experiments at Lincoln Memorial University. To my other dissertation committee members – Dr. Kyle McLeod, Dr. Amanda Adams, and Dr. Roberta Dwyer – I would like to express my gratitude for giving their time, input, and expertise over the course of this dissertation.

Secondly, I would like to thank the entire LMU DeBusk Veterinary Teaching Center faculty and staff for welcoming me and making this research a reality. Dr. Dawn Spangler, specifically, was essential for coordinating with shelters, providing animal care, and conducting necessary procedures during these experiments. Thank you for giving you time, input, and expertise to the care of our animals and the execution of this work. Thank you also to the animal caretakers, Vanessa Hellestad, Daniel McLain, and Lauren Beeler-Beistad, for their help ordering necessary supplies and providing excellent animal care over the course of this dissertation.

To all the veterinary students, graduate students, and undergraduate workers who helped with animal care, behavioral tests, and 24-hour blood collections, thank you for all the time and energy you poured into these projects. You were the backbone of these projects and the absolute best coworkers I could have ever asked for. I would like to specifically thank Jesse Gebert for stepping up to help manage projects as well as Ashlee

Hauss and Sarah Elefson, both of whom came down to LMU to help with late night collections and animal care while they were busy with their own projects back in Lexington.

Next, I would like to thank Winston Lin for all the time and assistance he gave ordering supplies and helping me with the HPLC (despite its multiple attempts to break). Additionally, I would like to express my gratitude to Susan Hayes and Cindi Roberts for their assistance in running cortisol RIA kits.

Importantly, I need to thank my family and friends for their support and encouragement throughout the completion of this program. To my husband, Brett Morris, thank you for your willingness to move across the country, your interminable support and encouragement, and your love and kindness (demonstrated even when I was the oppositie of loving or kind). Thank you to my parents, Lynne and Scott Miller, for supporting my dreams and providing timely advice – you can take anything for 3 years if it gets you what you want.

Thank you to all the dogs involved in these research projects. The work we completed together has drastically increased our knowledge of CBD use in dogs and will be instrumental to improving the lives of other animals all over the world. Additionally, thank you for being wonderful subjects – no matter how long the days or tiring the work, I am grateful that I got to spend my days with such sweet dogs. I am particularly grateful for Jazzy, who was so sweet and lovable she just had to come home with me.

Lastly, I need to thank my heavenly Father. You have done more in me and through me during my time in Lexington than I could have ever asked or imagined.

Thank You for Your Son's sacrifice that has allowed my relationship with you to be restored. Thank you for changing my life, for bringing me to the Center Point Church community, and for drawing me to Yourself through them. To You be the glory for ever and ever! Amen.

TABLE OF CONTENTS

ACKNOWLEDGMENTS iii
LIST OF TABLES ix
LIST OF FIGURES xi
LIST OF ADDITIONAL FILES xv
CHAPTER 1. INTRODUCTION 1
CHAPTER 2. LITERATURE REVIEW 3
Cannabidiol: Biosynthesis, History of Use, and Legal Status 3
The Endocannabinoid System 5
Phytocannabinoids and the Endocannabinoid System 6
Potential Physiological Effects of Cannabidiol 7
Analgesic 7
Anti-Arthritic 9
Anti-Cancer 10
Anti-Epileptic 12
Anti-Inflammatory and Immunomodulatory 14
Anti-Nausea and Anti-Emetic 18
Anti-Obesity 19
Antioxidant 21
Anti-Pruritic 23
Anxiolytic and Antipsychotic 24
Sedative 26
Bioavailability, Pharmacokinetics, and Metabolism of Cannabidiol 28
Cannabidiol Use in Dogs 29
Conclusions 32
CHAPTER 3. PALATABILITY OF CANNABIDIOL-CONTAINING DOG TREATS 33
Introduction 33
Materials and Methods 33
Subjects and Housing 34
Treatments and Diets 34
Data Collection 35
Statistical Analysis 36
Results 36
Discussion 37
Conclusions 38
Tables and Figures 40
CHAPTER 4. THE IMPACT OF FEEDING CANNABIDIOL CONTAINING TREATS ON CANINE RESPONSE TO A NOISE-INDUCED FEAR RESPONSE TEST 42
Introduction 42
Materials and Methods 44

Subjects and Housing 44
Diets and Treatments 45
Testing Room and Equipment 46
Acclimation 47
Open Field and Fireworks Tests 48
Experimental Design 49
Data Collection 50
Statistical Analysis 52
Results 53
Blood Cortisol 53
Heart Rate and Heart Rate Variability 54
Behavior 55
Discussion 56
Conclusions 67
Tables and Figures 69

CHAPTER 5. FEEDING CANNABIDIOL-CONTAINING TREATS DID NOT AFFECT CANINE DAILY VOLUNTARY ACTIVITY 75

Introduction 75
Materials and Methods 78
Subjects and Housing 78
Diets and Treatments 79
Experimental Design and Data Collection 80
Statistical Analysis 82
Results 84
Total Daily Activity 84
Quiet and Music Session Activity 84
Exercise Activity 86
Discussion 86
Conclusions 91
Tables and Figures 92

CHAPTER 6. ALTERATION OF THE CANINE METABOLOME AFTER A THREE-WEEK SUPPLEMENTATION OF CANNABIDIOL-CONTAINING TREATS 97

Introduction 97
Materials and Methods 98
Subjects and Housing 98
Experimental Design and Treatments 99
Blood Sample Collection 100
CIL/LC-MS-Based Untargeted Metabolomics Analysis 100
Metabolite Data Processing 101
Metabolite Identification 101
Statistical Analysis 101
Results 102
Amine/Phenol Metabolites 102
Carbonyl Metabolites 104
Carboxyl Metabolites 105

Hydroxyl Metabolites 106
Discussion 108
Amino Acid Metabolism 108
Carbohydrate Metabolism 114
Lipid Metabolism 117
Hydroxycinnamic Acid Derivatives 120
Vitamin and Nucleotide Metabolism 122
Additional Metabolites 124
Limitations, Strengths, and Weaknesses 126
Conclusions 126
Tables and Figures 128

CHAPTER 7. FEEDING TREATS CONTAINING CANNABIDIOL DID NOT ALTER CANINE IMMUNE RESPONSE TO IMMUNIZATION WITH KEYHOLE LIMPET HEMOCYANIN 145

Introduction 145
Materials and Methods 146
Subjects and Housing 146
Diets and Treatments 147
Experimental Design 148
Sample Collection and Analysis 148
Statistical Analysis 150
Results 151
Hematology and Serum Chemistry 151
Total Immunoglobulins 152
Anti-KLH Immunoglobulins 153
Discussion 153
Conclusions 156
Tables and Figures 157

CHAPTER 8. SUMMARY AND CONCLUSIONS 167

REFERENCES 171

VITA 225

LIST OF TABLES

Table 4.1. Schedule of events. 69

Table 4.2. Definition of heart rate (HR) and heart rate variability (HRV) variables..... 70

Table 4.3. Ethogram of behaviors tracked by a single trained observer blinded to treatments using The Observer XT (Noldus Information Technology Inc., Leesburg, VA). 71

Table 4.4. Effect of trazodone (T), CBD (C), CBD by trazodone (C*T) interaction, time (Pre-Noise and Noise), CBD by trazodone by time (C*T*Time) interaction, and period on mean heart rate (HR) and heart rate variability (HRV) parameters for 1-minute immediately prior to (Pre-Noise) and the first minute (Noise) of the noise-induced fear response tests administered after each 7-d treatment period. 73

Table 4.5. Effect of trazodone (T), CBD (C), CBD by trazodone interaction (C*T), time (Pre-Noise and Noise), CBD by trazodone by time interaction (C*T*Time), and period on the duration of behavioral parameters (s) for 1-minute immediately prior to (Pre-Noise) and the first minute (Noise) of the noise-induced fear response tests administered after each 7-d treatment period. 74

Table 5.1 Schedule of events for monitoring activity in dogs receiving cannabidiol containing treats. 92

Table 5.2. Activity variables measured by Vetrax® activity sensors (AgLogica Holdings, Inc., Norcross, GA). 93

Table 5.3. Effect of treatment (TRT), day, and TRT*day interaction on total daily activity variables collected via Vetrax® activity sensors (AgLogica Technology, Norcross, GA). Treatment effects are shown as the contrast between control (CON) and both CBD treatments (CBD) and the contrast between CBD treatments (LOW v. HIGH)..... 94

Table 5.4. Effect of treatment (TRT), day, session (Quiet or Music), time of day (AM or PM), and all relevant interactions on activity variables collected via Vetrax® activity sensors (AgLogica Technology, Norcross, GA) from 1000-1200 h (AM) and 1330-1530 h (PM) each day. Treatment effects are shown as the contrast between control (CON) and both CBD treatments (CBD) and the contrast between CBD treatments (LOW v. HIGH). 95

Table 5.5. Effect of treatment (TRT), day, and TRT*day interactions on activity parameters collected via Vetrax® activity sensors (AgLogica Technology, Norcross, GA) during the 2 periods of daily exercise, which included all data from 0700-0900 h and 1700-1900 h each day. Treatment effects are shown as the contrast between control (CON) and both CBD treatments (CBD) and the contrast between CBD treatments (LOW v. HIGH). 96

Table 6.1. Identified amine/phenol-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON. 129

Table 6.2. Identified carbonyl-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON. 134

Table 6.3. Identified carboxyl-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON. 137

Table 6.4. Identified hydroxyl-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON. 142

Table 7.1. Schedule of events for evaluating immune response to immunization with a novel antigen in dogs receiving cannabidiol containing treats compared to control. 157

Table 7.2. Complete blood count variables and their abbreviations. 158

Table 7.3. Effect of cannabidiol (CBD) treatment (Trt), day, and the treatment by day (Trt*Day) interaction on hematology variables in dogs compared to control (CON). ... 159

Table 7.4 Effect of cannabidiol (CBD) treatment (Trt), day, and the treatment by day (Trt*Day) interaction on serum chemistry variables indicative of liver function in dogs compared to control (CON). 161

LIST OF FIGURES

Figure 3.1. Mean intake (g/kg MBW/d) of all animals across all treatments during each 5-d adaptation and treatment period for both dry and wet food diets. Error bars represent the standard error of the treatment mean (SEM). 40

Figure 3.2. Mean treat consumption for animals across all treatments during each 5-d adaptation and treatment period for both dry and wet food diets. Scores of 2, 1, or 0 were assigned to each treat offering based on rapid, hesitant, or no consumption of the treat. Asterisks above bars represent a difference in treat consumption between periods ($P \leq 0.05$). Error bars represent the standard error of the treatment mean (SEM). 41

Figure 4.1. Cortisol concentration (ng/mL) for each treatment ($n = 16$), back transformed after analysis. Error bars represent the standard error of the treatment mean (SEM), which was calculated from the back-transformed confidence interval for each treatment: SEM = (upper limit – lower limit)/3.92. Due to lack of effect of time ($P = 0.189$) and any interactions with time ($P > 0.05$), all time points (Pre-Noise and Noise) have been combined. Trazodone treatment reduced overall cortisol concentration compared to control ($P < 0.001$), whereas there was no effect of CBD nor the CBD by trazodone interaction ($P = 0.104$, and 0.238, respectively). *Bars with asterisk differ from control at $P < 0.001$. 72

Figure 6.1. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential amine/phenol-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON). .. 128

Figure 6.2. Candidate amine/phenol-containing biomarkers altered by cannabidiol (CBD; in blue) compared to control (CON; in yellow) included **A.** aspartyl-glutamine (AUROC = 1.00; $P < 0.001$); **B.** gamma-aminobutyric acid (AUROC = 1.00; $P = 0.005$); **C.** gamma-glutamyl-gamma-aminobutyraldehyde (AUROC = 1.00; $P < 0.001$); **D.** L-glutamate-5-semialdehyde (AUROC = 1.00; $P < 0.001$); **E.** prolyl-glutamine (AUROC = 1.00; $P < 0.001$); **F.** pyrimidodiazepine (AUROC = 1.00; $P < 0.001$); **G.** 4-amino-4-deoxychorismate (AUROC = 0.98; $P < 0.001$); **H.** trans-2,3-dihydroxycinnamate (AUROC = 0.98; $P < 0.001$); **I.** alanyl-proline (AUROC = 0.97; $P = 0.002$); **J.** N-acetyl-L-asparagine (AUROC = 0.97; $P < 0.001$); **K.** (Z)-3-peroxyaminoacrylate (AUROC = 0.95; $P < 0.001$); **L.** 1,4-diaminobutane (AUROC = 0.95; $P < 0.001$); **M.** 2'-deamino-2'-hydroxy-6'-dehydroparomamine (AUROC = 0.95; $P = 0.004$); **N.** ascorbate (AUROC = 0.95; $P = 0.003$); **O.** D-lysopine (AUROC = 0.95; $P = 0.004$); **P.** *o*-tyrosine (AUROC = 0.95; $P = 0.001$); **Q.** phenylalanyl-glycine (AUROC = 0.95; $P = 0.002$); **R.** 2,4-dihydroxyhept-2-enedioate (AUROC = 0.94; $P < 0.001$); **S.** asparaginyl-aspartic acid (AUROC = 0.94; $P = 0.003$); **T.** isoferulic acid (AUROC = 0.94; $P < 0.001$); **U.** 7-carboxy-7-carbaguanine (AUROC = 0.92; $P = 0.001$); **V.** 3-(4-hydroxyphenyl)pyruvate (AUROC = 0.91; $P = 0.004$); **W.** aspartyl-threonine (AUROC = 0.91; $P = 0.005$); and **X.** isoleucyl-alanine (AUROC = 0.91; $P = 0.007$). 132

Figure 6.3. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential carbonyl-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON). 133

Figure 6.4. Candidate carbonyl-containing biomarker – Glucose (AUROC = 0.91; $P = 0.020$) – altered by cannabidiol (CBD; in blue) compared to control (CON; in yellow). 135

Figure 6.5. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential carboxyl-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON). 136

Figure 6.6. Biomarker analysis of carboxyl metabolites for cannabidiol (CBD) and control (CON) treatments identified **A.** jasmonic acid (AUROC = 1.00; $P < 0.001$); **B.** (S)-4-amino-5-oxopentanoate (AUROC = 1.00; $P < 0.001$); **C.** 3,4-dihydroxymandelic acid (AUROC = 1.00; $P < 0.001$); **D.** isomer of 1-aminocyclopropane-1-carboxylate (AUROC = 1.00; $P < 0.001$); **E.** isomer of hydroxypropionic acid (AUROC = 1.00; $P < 0.001$); **F.** L-1-pyrroline-3-hydroxy-5-carboxylate (AUROC = 1.00; $P < 0.001$); **G.** L-threonine/L-allothreonine (AUROC = 1.00; $P < 0.001$); **H.** 2-oxo-4-phenylbutyric acid (AUROC = 0.98; $P < 0.001$); **I.** Arginine (AUROC = 0.98; $P < 0.001$); **J.** citramalic acid (AUROC = 0.97; $P < 0.001$); **K.** 4-oxoproline (AUROC = 0.95; $P < 0.001$); **L.** 5-deoxy-D-glucuronate (AUROC = 0.95; $P < 0.001$); **M.** 9,10-12,13-diepoxyoctadecanoate (AUROC = 0.95; $P < 0.001$); **N.** isomer of 1-pyrroline-2-carboxylate (AUROC = 0.95; $P < 0.001$); **O.** isomer of aspartate (AUROC = 0.95; $P < 0.001$); **P.** 2-aminomuconate semialdehyde (AUROC = 0.94; $P < 0.001$); **Q.** isomer of 9-oxononanoic acid (AUROC = 0.94; $P < 0.001$); **R.** acetic acid (AUROC = 0.92; $P < 0.001$); **S.** ethyl malonate (AUROC = 0.92; $P < 0.001$); **T.** isomer of jasmonic acid (AUROC = 0.92; $P < 0.001$); **U.** 3-oxopropanoate (AUROC = 0.91; $P < 0.001$); **V.** D-glycerate (AUROC = 0.91; $P < 0.001$); and **W.** isomer of D-glycerate (AUROC = 0.91; $P < 0.001$) as candidate biomarkers..... 140

Figure 6.7. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential hydroxyl-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON). 141

Figure 6.8. Biomarker analysis of hydroxyl metabolites for cannabidiol (CBD) and control (CON) treatments identified **A.** L-fuculose/L-rhamulose (AUROC = 1.00; $P < 0.001$); **B.** glyceraldehyde (AUROC = 1.00; $P < 0.001$); **C.** L-rhamnofuranose (AUROC = 1.00; $P < 0.001$); **D.** L-rhamnono-1,4-lactone (AUROC = 1.00; $P < 0.001$); **E.** Xylitol/Arabitol/Ribitol (AUROC = 1.00; $P < 0.001$); **F.** D-tagatose/L-sorbose (AUROC = 0.98; $P < 0.001$); **G.** propane-1,3-diol (AUROC = 0.98; $P < 0.001$); **H.** 6-deoxy-L-galactose/L-rhamnose (AUROC = 0.97; $P = 0.002$); **I.** D-glucosamine (AUROC = 0.95; $P = 0.001$); **J.** phenethyl alcohol (AUROC = 0.94; $P = 0.002$); **K.** isomer of L-rhamnofuranose (AUROC = 0.92; $P = 0.001$); **L.** 3-phenoxybenzyl alcohol (AUROC = 0.91; $P = 0.003$); **M.** 3,4-dihydroxyphenylpropanoate (AUROC = 0.91; $P = 0.003$); **N.** cortolone (AUROC = 0.91; $P = 0.073$); and **O.** isomer of deoxyadenosine (AUROC = 0.91; $P = 0.008$) as candidate biomarkers..... 144

Figure 7.1. Serum alkaline phosphatase (U/L) of control (CON) and cannabidiol (CBD) treatments over time. Data were not normally distributed and were transformed by the square root for statistical analysis, then back-transformed for reporting purposes. Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92. Alkaline phosphatase levels remained within IDEXX normal reference range throughout the study and were similar between treatments at baseline (day 0, $P = 0.994$) and day 7 ($P = 0.183$), but were increased in CBD treatment on days 14, 21, and 28 compared to control ($P = 0.006$, 0.027, and 0.014, respectively). Asterisks (*) Indicate differences between treatments ($P < 0.05$) on that day. 162

Figure 7.2. Total serum **A)** IgG and **B)** IgM (mg/dL) of control (CON) and cannabidiol (CBD) treatments over the 28-d experiment. Dogs were challenged with keyhole limpet hemocyanin on days 0 and 14 after collection of blood samples. Both total IgG and total IgM concentrations were altered by day ($P < 0.001$). Day of experiment altered both total IgG and total IgM concentrations ($P < 0.001$). Total IgG was unaffected by both treatment and the treatment by day interaction ($P = 0.930$ and 0.897, respectively). Total IgM was not normally distributed and was log-transformed for statistical analysis, then back-transformed for reporting purposes. Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92. Total IgM was unaffected by treatment ($P = 0.817$) but a treatment by day interaction was observed ($P = 0.047$)..... 164

Figure 7.3. Anti-Keyhole Limpet Hemocyanin (KLH) **A)** IgG and **B)** IgM (kU/mL) of control (CON) and cannabidiol (CBD) treatments over the 28-d experiment. Dogs were challenged with KLH on days 0 and 14 after collection of blood samples. Anti-KLH IgG was not normally distributed and was transformed to the square root for statistical analysis, then back-transformed for reporting purposes. Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92. Day of experiment altered both anti-KLH IgG and IgM ($P < 0.001$). Both anti-KLH IgG and IgM were unaffected by treatment ($P = 0.422$ and 0.879, respectively) and the treatment by day interaction ($P = 0.510$ and 0.927, respectively)..... 166

LIST OF ADDITIONAL FILES

Supplemental Table 6.1 Tier_1_Metabolites ……..…….... [CSV 6 KB]
Supplemental Table 6.2. Tier_2_Metabolites …..……...……..…... [CSV 11 KB]
Supplemental Figure 6.1 Amine_Phenol_PLSDA_Permutation …..…...… [TIFF 4.3 MB]
Supplemental Figure 6.2 Carbonyl_PLSDA_Permutation ……....… [TIFF 4.3 MB]
Supplemental Figure 6.3 Carboxyl_PLSDA_Permutation ……....… [TIFF 70 KB]
Supplemental Figure 6.4 Hydroxyl_PLSDA_Permutation ……....… [TIFF 74 KB]

CHAPTER 1. INTRODUCTION

The 2018 amendment to the 2014 Farm Bill removed industrial hemp from the Controlled Substances Act (CSA) and cannabidiol (CBD) from the Schedule I drug list, opening up the possibility of expanding the market for and research on CBD products derived from industrial hemp (Johnson, 2019; Mead, 2017). Just one year later, the mean acreage devoted to hemp cultivation in the U.S. had increased by 54%, and the market for industrial hemp-derived CBD products was approximately $1.2 billion. This market is expected to grow to over $10 billion by 2024 (Hemp Industry Daily, 2019).

Much of this growth can be attributed to public perception of the supposed health benefits of CBD. In a set of surveys distributed by a research group at Colorado State University, 60-80% of over 1,500 pet owners said they were currently feeding or had previously fed hemp or marijuana products to their dogs (Kogan et al., 2016; 2018). These surveys provide insight on the overwhelmingly favorable perceptions of pet owners on the safety and efficacy of CBD, though they likely over-estimate CBD use in dogs and cats due to the surveys being shared primarily within social media groups dedicated to cannabis use in pets. On the other hand, a survey of over 2,100 veterinarians indicated that the majority of veterinarians are still hesitant to recommend or prescribe CBD products to patients, either because they did not feel knowledgeable enough or they felt that the field needed more research (Kogan et al., 2019a).

If CBD is shown to be safe and effective, it has the potential to improve the health and well-being of companion animals. Cannabidiol is already being supplemented to dogs for its potential therapeutic applications including, but not limited to, osteoarthritis, separation anxiety, noise phobias, glaucoma, and epilepsy (Blessing et al., 2015; Landa et

al., 2016). However, there is still limited *in vivo* work in canine models evaluating the safety of CBD supplementation and its efficacy in generating these beneficial effects. The literature available evaluating its therapeutic potential in osteoarthritic dogs has focused primarily on the subjective evaluation of CBD's effects by owners or veterinarians, with little quantitative data to support its use (Gamble et al., 2018). Thus, despite the overwhelming public opinion that CBD is a safe and effective treatment for the previously mentioned diseases, there remains a substantial gap in scientific literature for these anecdotal claims.

Therefore, the objective of this dissertation was to examine the potential impact of oral CBD supplementation on the health and well-being of dogs. The CBD was supplied in the form of treats, which were first assessed for palatability at increasing inclusion levels. These treats were then supplemented to assess the potential for CBD to influence canine behavior in both a normal, daily environment and while subject to fearful stimuli. Additionally, alteration of the canine metabolome, potential immunomodulatory effects, and impact on overall health indices by CBD were assessed.

CHAPTER 2. LITERATURE REVIEW

Cannabidiol: Biosynthesis, History of Use, and Legal Status

Hemp, a variety of *Cannabis sativa* L., is a dioecious, annual plant that is thought to be one of the earliest plants to be cultivated (Bonini et al., 2018; Russo et al., 2008). It is a multi-purpose plant, with its stem a source of both cellulosic and woody fibers that can be used to make animal bedding, textiles, paper, and more. Additionally, hemp seed is rich in protein and omega-3 fatty acids, which may make it a valuable feed additive in livestock and companion animal diets (Gibb et al., 2005; Skřivan et al., 2020; Zhang et al., 2018). The female flowers produce glandular trichomes, hair-like epidermal protrusions that cover the leaves, bracts, and stems. These trichomes are responsible for the production and storage of secondary metabolites, including terpenoids, flavonoids, and cannabinoids (Bonini et al., 2018; Grof, 2018; Huchelmann et al., 2017).

Cannabidiol is one of more than 100 cannabinoids produced by these glandular trichomes. It was first isolated in 1940 and its structure was elucidated in 1963 (Adams et al., 1940; Mechoulam and Shvo, 1963). Both CBD and Δ9-tetrahydrocannabinol (THC) – the other primary cannabinoid produced by *C. sativa* – are prenylated polyketides primarily derived from fatty acids and isoprenoids; however, their biosynthetic pathway has only recently been elucidated, and several steps in the pathway are still under investigation (Gagne et al, 2012; Stout et al., 2012; Taura et al., 2007). Within the polyketide pathway, hexanoyl-CoA is produced from the short-chain fatty acid hexanoate before being converted into olivetolic acid via polyketide synthase. Geranyl diphosphate (GPP), a product of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, is added to olivetolic acid to produce cannabigerolic acid, the precursor for the acid forms of both

THC and CBD (Stout et al., 2012). The concentration of cannabinoids and other secondary metabolites produced is dependent on a number of factors, including the different cultivars of *C. sativa*, whether or not the female plant has been pollinated, and environmental conditions like humidity, soil nutrients, and temperature (Bonini et al., 2018).

Due to both the psychoactive effects of THC and the rise of alternative sources for industrial fibers, the production, possession, and transfer of *C. sativa* was first restricted in the United States by the Marihuana Tax Act of 1937. This law required all hemp growers to be registered and licensed (Bonini et al., 2018, USDA, 2000). In 1970, hemp and all its products, including CBD, were classified as illegal, Schedule I drugs under the CSA, which further restricted access to CBD (Corron and Kight, 2018; Mead, 2017). These restrictions severely limited the potential for research on CBD and its effects on mammalian physiological systems.

Restrictions on hemp production first began to loosen at the state level in the early 2000's, with further federal relaxation of restrictions after the 2014 Farm Bill was enacted. This allowed research institutions to grow "industrial" hemp for research purposes, including the use of hemp-derived CBD (Mead, 2017). In order to be considered industrial hemp rather than marijuana, the plant and all its products must contain less than 0.3% THC on a dry weight basis (Johnson, 2019). Finally, in a 2018 amendment to the 2014 Farm Bill, industrial hemp was removed from the CSA entirely, and hemp-derived CBD was removed from the Schedule I drug list of the CSA. But THC, whether produced from hemp or marijuana, remains a Schedule I drug (Johnson, 2019; UDSA, 2000).

Despite the relaxation of restrictions on the production and research of industrial hemp-derived CBD, the Food and Drug Administration (FDA) still considers it illegal to market food or dietary supplements containing CBD, even if there are no therapeutic claims on the label (Johnson, 2019). The FDA has published several statements to the public emphasizing the lack of scientific evidence regarding the safety, proper dosage, and quality of products containing CBD, and they acknowledge the critical need to bridge public perception of the therapeutic effects of CBD and the scientific literature that is currently available (FDA, 2020; Hahn, 2020).

The Endocannabinoid System

The therapeutic potential of CBD is a major reason for the increased interest in its use, both in humans and companion animals. It has been proposed to induce a plethora of beneficial health effects through action on the endocannabinoid system (ECS). First described in the early 1990's, the ECS signaling system was discovered secondarily to the elucidation of the structure of THC and the search for its biological target. The discovery of the G-protein coupled receptor (GPCR) target for THC, dubbed cannabinoid receptor 1 (CB_1), during this investigation quickly led to the identification of a second cannabinoid receptor (CB_2) as well as the major endogenous ligands, or endocannabinoids, for these receptors: arachidonoyl ethanolamide (AEA; also known as anandamide) and 2-arachidonoyl glycerol (2-AG) (De Petrocellis and Di Marzo, 2009; Silver, 2019). These receptors and endocannabinoids, along with the enzymes involved in their synthesis, degradation, and regulation, make up the ECS. (Lu and Mackie, 2015; Pacher et al., 2006; Russo et al., 2016). Additional endocannabinoids and receptors like peroxisome proliferator activated receptors (PPARs) and transient receptor potential

(TRP) channels have since been shown to contribute to the widespread actions of the ECS (De Petrocellis and Di Marzo, 2009).

The ECS has been implicated in a number of physiological processes and is responsible for regulatory functions throughout the body, including the central nervous system (CNS), cardiovascular system, and immune system (Burstein, 2015; Russo, 2016; Silver, 2019). Receptor location and level of expression dictate their function. CB_1 receptors, for instance, are most abundant within the CNS, particularly in the cortex, basal ganglia, hippocampus, and cerebellum. Under normal conditions, CB_1 receptors exert a general protective or pro-homeostatic effect by helping to regulate synaptic strength of neurons involved in cognitive, affective, and motivational processes (De Petrocellis and Di Marzo, 2009). However, CB_1 receptors are also expressed to a lesser extent throughout the rest of the body, including, but not limited to, the enteric nervous system, liver, and thyroid (De Petrocellis and Di Marzo, 2009; Galiazzo et al., 2018; Pagotto et al., 2006). Conversely, CB_2 receptors are primarily expressed in immune cells like monocytes, macrophages, B-cells, and T-cells, and are in part responsible for the regulation of cytokine and chemokine release (Vučković et al., 2018). They are also expressed in the peripheral nervous system, the gastrointestinal tract, skin, and more (Galiègue et al., 1995; Silver, 2019; Wright et al., 2008).

Phytocannabinoids and the Endocannabinoid System

Plant-based cannabinoids, or phytocannabinoids, like CBD and THC are exogenous ligands for ECS receptors. For instance, THC is a high-affinity agonist for CB_1, and the psychotropic effects of THC are a result of the abundant expression of CB_1 within the CNS (Lu and Mackie, 2015; Pacher et al., 2006). This effect is magnified in

the canine nervous system as dogs have higher expression of CB_1 in the brain than humans, often leading to adverse reactions like static ataxia when a dog is exposed to THC (Silver, 2019). Conversely, CBD has low affinity for both CB_1 and CB_2 receptors, which explains its lack of psychotropic effects. It is, however, capable of acting as an antagonist of both cannabinoid receptors when in the presence of THC as well as a non-competitive negative allosteric modulator of CB_1 (Laprairie et al., 2015; Thomas et al., 2007). In addition to interactions with the primary cannabinoid receptors, CBD is a known agonist or antagonist for several ECS-associated receptors, including transient receptor potential (TRP) vanilloid receptors 1 and 2 (TRPV1 and 2), ankyrin 1 (TRPA1), melastatin 8 (TRPM8), G protein-coupled receptor 55 (GPR55) and more (Wang and Multhoff, 2021; Vučković et al., 2018). The potential physiological and therapeutic effects of CBD result from the action of CBD on the various ECS-associated receptors and interactions with endocannabinoids.

Potential Physiological Effects of Cannabidiol

Analgesic

Several ECS-associated receptors like TRPV1, serotonin (5-HT) 1A, and GPR55 are present throughout peripheral and central nervous system pain pathways (Burston and Woodhams, 2014; Jesus et al., 2019). Cannabidiol may exert an analgesic effect either via direct action on these receptors or indirectly by inhibiting the cellular uptake and degradation of AEA. In rats, repeated administration of CBD was shown to attenuate both thermal and mechanical hyperalgesia in a dose-dependent manner after either injection of complete Freund's adjuvant or injury to the sciatic nerve. The lower doses, ranging from 2.5 to 10 mg/kg, CBD reduced hyperalgesia whereas the 20 mg/kg dose

completely abolished hyperalgesia (Costa et al., 2007). As their findings saw no effect of CB_1 and CB_2 receptor antagonists, it was suggested that TRPV1 was the molecular target of this activity – either via direct action by CBD or indirect action through its effects on AEA (Costa et al., 2004; 2007).

Anandamide is an endocannabinoid produced in response to tissue injury or excessive pain signaling and acts on receptors to reduce pain and inflammation (Maccarrone et al., 2015; Vučković et al., 2018). However, AEA is rapidly taken up into cells via the anandamide membrane transporter (AMT) and degraded via hydrolysis by fatty acid amide hydrolase (FAAH) (Hillard and Jarrahian, 2000; Ueda et al., 2000). Cannabidiol is thought to indirectly produce analgesia by inhibiting both cellular uptake of AEA by AMT and AEA hydrolysis by FAAH, thus prolonging the analgesic effect of AEA (Bisogno et al., 2001).

Cannabidiol may also exert analgesic effects via activation of serotonin receptors. Jesus et al. (2019) investigated the potential analgesic effect of intraperitoneal CBD on pain in diabetic rats. The observed anti-allodynic effect of CBD treatment was not blocked by selective CB_1, CB_2, and glycine receptor antagonists; pretreatment with a selective 5-HT_{1A} antagonist completely prevented the antinociceptive effect. The role of 5-HT_{1A} receptors in the analgesic effect of CBD is supported by *in vitro* work demonstrating agonistic effects on 5-HT_{1A} receptors and inhibitory effects on serotonin reuptake (Rock et al., 2012; Russo et al., 2005).

The analgesic action of CBD may also be modulated via action on GPR55. An orphan receptor expressed in both central and peripheral nociceptive systems, GPR55 is believed to help modulate pain receptor excitability (Guerrero-Alba et al., 2019; Ray et

al., 2018). The activation of GPR55 increases intracellular Ca^{2+} in large dorsal root ganglia responsible for the transmission of both proprioceptive and nociceptive peripheral stimuli (Lauckner et al., 2008). Additionally, GPR55 activation has been shown to produce mechanical hypersensitivity in mice (Gangadharan et al., 2013). Cannabidiol may exert analgesic action by blocking the activation of GPR55, which has been demonstrated both *in vitro* and in mouse models (Kaplan et al., 2017; Ryberg et al., 2007).

Collectively, action on these receptors supports the analgesic potential of CBD, for which there are many possible applications. Its analgesic effect may inhibit mechanosensitivity of joint nociceptors, which would be useful in the management of arthritis or other inflammatory conditions (Philpott et al., 2017). Cannabidiol may also attenuate nociception involved with neuropathic or chronic pain, which could be used to mitigate the pain associated with conditions like nerve injury, diabetes, or cancer (Elikottil et al., 2013; Vučković et al., 2018). However, current *in vivo* work with canine models have focused primarily on the impact of CBD on symptoms rather than mechanisms of action (Brioschi et al., 2020; Gamble et al., 2018; Verrico et al., 2020). Thus, the mechanism by which CBD produces the reported analgesic effects in dogs has yet to be elucidated.

Anti-Arthritic

Arthritis is a general term encompassing more than 100 conditions in which one or more joints are inflamed. The most common types of arthritis – osteoarthritis and rheumatoid arthritis – are degenerative and characterized by chronic pain and inflammation (McDougall, 2006). The ECS is known to modulate joint homeostasis

through CB_1, CB_2, and TRPV1 (Richardson et al., 2008; Schuelert et al., 2008). These receptors are thought to be potential targets for the regulation of joint disease like arthritis (Philpott et al., 2017). To date, there is no evidence of CBD preventing or treating any form of arthritis. Instead, the so-called "anti-arthritic" effect of CBD is primarily attributable to its analgesic and anti-inflammatory effects discussed in pertinent sections of this work.

Anti-Cancer

Cancer is one of the leading causes of worldwide disease-related death; there is a great need for anti-cancer drugs that are both well-tolerated and highly effective against cancer cells (Siegel et al., 2020). Several ECS receptors, such as CB_2, TRPV1 and 2, TRPA1, TRPM8, and GPR55, are upregulated in cancer cells and have been shown to modulate cellular processes involved in tumor cell growth and proliferation (Lee et al., 2021; Wang and Multhoff, 2021). Therefore, CBD is thought to serve as an anti-cancer agent through several potential mechanisms, including induction of cell apoptosis, induction of autophagy, increasing sensitivity to cytotoxic therapies, and the inhibition of cell growth and proliferation.

Cannabidiol may induce tumor cell apoptosis through several mechanisms, including the inhibition of AKT/mammalian target of rapamycin (mTOR) signaling, activation of pro-caspases, and increasing the production of reactive oxygen species (ROS). Under normal conditions, the mTOR signaling pathway regulates cell growth and division, but can lead to tumor development when abnormally activated (Zou et al., 2020). In human breast cancer cells, CBD was shown to induce endoplasmic reticulum stress, thereby inhibiting AKT and mTOR signaling which led to cell death (Shrivastava

et al., 2011). In human gastric cancer and glioma cells, CBD has been shown to activate caspases-3/8/9, which directly trigger apoptosis (Massi et al., 2006; Zhang et al., 2019). Additionally, CBD has been shown to increase ROS production through both TRPV1 and TRPV 2 agonism (Kis et al., 2019; Massi et al., 2006; Wang and Multhoff, 2021). Increased ROS generation disrupts mitochondrial membrane potential, which leads to a release of cytochrome c, another pro-apoptotic factor (Olivas-Aguirre et al., 2019; Shrivastava et al., 2011). Remarkably, CBD did not impair normal primary glial cells under the same conditions (Massi et al., 2006).

In addition to inducing apoptosis, CBD is also thought to exert anti-cancer effects through the induction of autophagy. Autophagy is an intracellular degradation process that removes misfolded or aggregated proteins and damaged organelles from cells (Lee et al., 2021). Under normal conditions, autophagy helps maintain homeostasis, but can serve as a tumor suppressant in the early stages of cancer development or a tumor promoter in later stages (Amaravadi et al., 2019; Lee et al., 2021). Cannabidiol has been shown to induce autophagy in both human breast cancer and glioma cells through action on TRPA1 and TRPV2 (Nabissi et al., 2015; Shrivastava et al., 2011; Wang and Multhoff, 2021). Additionally, when combined with traditional cytotoxic cancer treatments, cannabinoids have been shown to enhance autophagy and increase the uptake of those chemotherapeutic compounds into cancer cells, suggesting a potential benefit of combining CBD with conventional treatments (Torres et al., 2011).

Lastly, CBD may exert anti-cancer effects by inhibiting tumor cell growth and proliferation. One potential mechanism for this antiproliferative effect is the antagonistic action of CBD on GPR55, which is known to activate downstream proliferation pathways

and is implicated in tumor migratory behavior (Kargl et al., 2016; Kis et al., 2019; Lee et al., 2021). Treatment of mice pancreatic cancer cells with GPR55 antagonists, including CBD, prevented MAPK signaling, cell growth, and cell cycle progression (Ferro et al., 2018; Lee et al., 2021). In male mice, treatment with 1 mg/kg CBD was shown to inhibit colon cancer cell growth (Aviello et al., 2012). In prostate cancer cell lines, CBD was also shown to inhibit cancer cell growth, potentially due to pro-apoptotic activity (Sharma et al., 2014; Sreevalsan et al., 2011). Another potential mechanism is CBD's anti-angiogenic properties. Angiogenesis, or the process of forming new blood vessels from pre-existing ones, is essential for tumor growth and metastasis (Kis et al., 2019). Cannabidiol has been shown to reduce growth, migration, and invasion of human umbilical vein endothelial cells (HUVEC), a cell line commonly used for proliferation assays as well as glioma cells *in vitro* (Solinas et al., 2012; 2013).

All this work points to the potential for CBD to be a potent anticancer agent. Currently, CBD is most commonly used to alleviate symptoms of cancer treatment. Tumor-related adverse effects were shown to be alleviated by 50-600 mg CBD/d in a human phase I clinical trial (Good et al., 2019), and a THC/CBD combination helped reduce pain intensity, nausea, and vomiting in patients undergoing chemotherapy (Johnson et al., 2010). However, there is currently little clinical evidence of anticancer effects of CBD outside of preclinical studies in cell lines and animal models, and more *in vivo* research with larger samples sizes is needed.

Anti-Epileptic

Epilepsy is a chronic neurological disease characterized by the occurrence of repeated and spontaneous seizures. In many cases, epilepsy can cause several

comorbidities that reduce a patient's quality of life, including cognitive deficits, psychiatric disorders, anxiety, and depression (Boleti et al., 2020). While many forms of epilepsy can be controlled with conventional antiepileptic medications, an estimated one-third of epileptic patients still suffer persistent seizures (Chen et al., 2018). Thus, investigation into alternative therapies such as CBD has increased substantially in recent years, culminating in the FDA approval of a CBD-based medication, Epidiolex®, for the treatment of seizures associated with Dravet and Lennox-Gastaut syndromes (Boleti et al., 2020).

There are several potential targets of CBD that may contribute to its anti-epileptic effect. The activation of TRPV1 has been implicated in the induction of epileptic seizures as it prompts a release of glutamate and an increase in Ca^{2+}, resulting in neuronal excitability (Nazıroğlu, 2015). While CBD is a known agonist of TRPV1, it acts more as a functional antagonist, desensitizing the receptor and preventing further release of glutamate and Ca^{2+} (Boleti et al., 2020; Silvestro et al., 2019). A similar mechanism of action has been postulated for the functional antagonism of GPR55 by CBD (Gray and Whalley, 2020; Rosenberg et al., 2015). Additionally, agonism of 5-HT_{1A} by CBD has been suggested to play a role through its inhibition of serotonergic neurons (Silvestro et al., 2019; Theodore, 2003). The antiseizure effect may also involve CBD's action on adenosine receptors A_1 and A_{2A}. Adenosine is an endogenous compound known to modulate neuronal excitability and is considered an endogenous anti-convulsant (Weltha et al., 2019). Activation of adenosine receptors by either adenosine or CBD inhibit the presynaptic influx of Ca^{2+}, thus preventing hyperpolarization (Boleti et al., 2020; Gray and Whalley, 2020).

Several human clinical trials have been completed evaluating the safety and efficacy of CBD for use in patients with epilepsy. Cannabidiol doses ranging from 5 to 50 mg CBD/kg/d have been shown to reduce seizure frequency and improve subjective quality of life in patients ranging from infants to adults suffering from a variety of seizure-related disorders (reviewed extensively in Silvestro et al., 2019). While CBD has been extensively studied in these clinical trials for safety and preliminary efficacy, work is ongoing to determine proper dosing and potential long-term psychiatric and cognitive effects. Adverse events are also of concern considering severe adverse events were reported in a number of these clinical trials as well as the possibility of interactions with other anti-seizure medication (Devinsky et al., 2018; Lattanzi et al., 2020). Altogether, CBD appears to be a promising prospect for the management of epileptic disorders.

Anti-Inflammatory and Immunomodulatory

One of the most common reasons for CBD use is its suspected anti-inflammatory effect (Kogan et al., 2016). Inflammation is a normal but complex physiological process in which the body responds to the presence of foreign bodies or damage by recruiting the immune system to eliminate the threat or repair the damage. In some chronic inflammatory disorders like osteoarthritis and rheumatoid arthritis, however, the immune system remains active, resulting in prolonged inflammation that can eventually be harmful to the body (Pahwa et al., 2020). Chronic inflammation is often marked by elevated production of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNFα as well as high-sensitivity C-reactive protein and NF-κB that are produced by immune cells as part of the immune response (Albensi, 2019; Zhang and An, 2007).

Cannabidiol is thought to exert anti-inflammatory effects through several mechanisms, including the reduction of pro-inflammatory cytokine production, alteration of T cell proliferation, activation of PPARγ, and more. Activation of ECS receptors like CB_1 have been shown to stimulate the pro-inflammatory response; as a negative allosteric modulator of CB_1, CBD may inhibit their activation and downstream inflammatory cascade (Han et al., 2009). Conversely, CBD can act as both a weak agonist and indirect agonist of CB_2 that, when activated, has been shown to decrease TNFα (Han et al., 2009; Muller et al., 2019; Pertwee, 2008). The indirect actions of CBD on both CB_1 and CB_2 may thus be a mechanism by which it produced anti-inflammatory effects. Decreased production of TNFα and other pro-inflammatory cytokines by CBD has also been shown to be mediated by its activation of A_{2A} receptors (Liou et al., 2008; Sunda and Arwolo, 2020), which may protect tissues from inflammation by down regulating over-reactive immune cells (Carrier et al., 2006; Ohta and Sitkovsky, 2001). Antagonism of GPR55 (Lanuti et al., 2015) and TRP channels (Atalay et al., 2020; Bujak et al., 2019; Minke, 2006) have also been suggested to facilitate the anti-inflammatory effect, though less studied. The anti-inflammatory effect of CBD may also be mediated by PPARγ activation as CBD has been shown to bind to and activate PPARγ (O'Sullivan, 2016). The inhibition of several cytokines by CBD has been shown to be PPARγ-mediated (Rockwell and Kaminski, 2004) along with the inhibition of myeloid-derived suppressor cell induction (Hegde et al., 2015) and reduced expression of inflammatory proteins in enteric glial cells (De Filippis et al., 2011), all of which may contribute to an anti-inflammatory effect. Cannabidiol has also been shown *in vitro* to modulate T cell proliferation through several mechanisms. Transcription of T-cell co-inhibitory

molecules – which are known to interfere with the interaction of T cells and antigen presenting cells required for differentiation – was enhanced by CBD treatment in encephalitogenic memory T cells (Kozela et al., 2011; 2016). Conversely, CBD has been shown to induce functional Tregs, which are a specialized population of T cells that suppress the immune response (Dhital et al., 2017).

The potential anti-inflammatory effect of CBD has been demonstrated in a limited number of studies in rats and humans. Rats with sodium monoiodoacetate-induced osteoarthritis demonstrated reduced joint inflammation when treated with localized CBD, and prophylactic CBD administration prevented the later development of pain and nerve damage from osteoarthritis induction (Philpott et al., 2017). Similarly, oral CBD administration reduced overexpression of prostaglandin E2 and nitric oxide in rats with induced unilateral neuropathy, though NF-κB and TNFα were unaffected (Costa et al., 2007). In a human pilot study, a single dose of oral CBD was administered, and blood drawn over the course of 6 hours. When human peripheral blood mononuclear cells (PBMCs) were isolated and stimulated by lipopolysaccharide, TNFα was decreased (Hobbs et al., 2020). However, this was a small study using only a single dose and administration of CBD. Other retrospective studies have been performed to evaluate current use of CBD and other cannabinoids for orthopedic issues (Gusho and Court, 2020) but no other controlled clinical trials have been published regarding the use of CBD for its anti-inflammatory effect.

While the anti-inflammatory effect of CBD has been pursued for potential treatments against chronic inflammatory conditions like multiple sclerosis and osteoarthritis, there is also evidence from *in vitro* work suggesting CBD may negatively

impact other functions of the immune system. Notably, CBD has been shown to inhibit IL-2, IL-10, and IFNγ production in mice (Condie et al., 1996; Jan et al., 2007). IL-2 is an essential cytokine for the activation and differentiation of T cells as well as the maintenance of peripheral tolerance, which is one mechanism for the regulation of self-reactive immune cells (Arenas-Ramirez et al., 2015). T cell activation is an essential component of the T cell-dependent B cell response to antigen encounter. Without proper levels of IL-2, T cells cannot be activated, and thus B cells are unable to undergo somatic hypermutation and isotype switching, the processes that generate high affinity antibodies to bind antigens (Jeurissen et al., 2004). This suppression of antigen-specific antibody production has been observed in mice after a single intraperitoneal dose of CBD (Jan et al., 2007).

There is also conflicting evidence regarding anti-inflammatory and immunomodulatory effects of CBD. Several studies have demonstrated an increase in IL-2 secretion through enhancement of gene transcription in activated T cells by CBD and other cannabinoids (Chen et al., 2012; Jan et al., 2002). Jenny et al. (2009) reported a biphasic response in human PBMCs, with CBD exhibiting a stimulatory effect at lower doses and inhibitory effects at higher doses. Cannabidiol has also been shown, under certain conditions, to enhance production of pro-inflammatory cytokine mRNA, thus exacerbating lipopolysaccharide-induced inflammation in mice (Karmaus et al., 2013). These discrepancies between responses may be dependent upon age, cell type, CBD dose, mode of administration, magnitude of cellular activation, or other unknown factors (Chen et al., 2012; Karamus et al., 2013). Although these immunomodulatory effects may be beneficial in some conditions, they may also be detrimental, particularly regarding

immune responses to antigen encounters. Continued investigation is essential to better understand these mechanisms and to elucidate potential risks to health especially because of the lack of *in vivo* studies and clinical trials.

Anti-Nausea and Anti-Emetic

Hemp has been used for centuries for its anti-nausea and anti-emetic effects (Russo, 2007), and has recently been suggested as a management strategy for chemotherapy-induced nausea and vomiting in cancer patients (Smith et al., 2015; Whitling et al., 2015). Cannabidiol is believed to exert a biphasic effect on nausea and vomiting. Low doses (2.5-20 mg/kg) of CBD have been shown to suppress toxin-induced vomiting while higher doses (40 mg/kg) exacerbated toxin-induced vomiting in musk shrews (Parker et al., 2004; Rock et al., 2020). One potential mechanism of action may be reduction of the firing rate of 5-HT afferents to the terminal forebrain regions via agonism of 5-HT_{1A} receptors, which has been demonstrated in musk shrews and rats (Rock et al., 2012). Alternatively, allosteric inhibition of 5-HT_{3A} receptors may be another mechanism of this effect. 5-HT_{3A} antagonists are known to be highly effective anti-nausea and anti-emetic drugs (Kaiser et al., 2004), and CBD has been shown to act as an allosteric inhibitor of 5-HT_{3A} receptors (Yang et al., 2010).

While there have been many clinical trials evaluating the use of cannabinoids or synthetic cannabinoids, either by themselves or in combination with other treatments, for nausea and vomiting, there have been no human clinical trials looking specifically at CBD. There have been a few phase I and II safety and efficacy studies using THC:CBD combinations (Duran et al., 2010; Mersiades et al., 2018) suggesting the combination is well-tolerated and may help reduce chemotherapy-induced nausea and vomiting. One

small phase II/III trial reported an association of oral THC:CBD with reduced nausea and vomiting, though approximately one-third of the participants experienced moderate to severe cannabinoid-related adverse events (Grimison et al., 2020). While this work is promising, additional investigation is necessary to elucidate what role CBD may play in this effect.

Anti-Obesity

The ECS plays a well-established role in glucose and energy metabolism. Activation of ECS receptors have been shown to increase food intake, to activate anabolic metabolic pathways in peripheral organs that favor energy storage, and to enhance triglyceride accumulation in adipocytes (Boon et al., 2014; Gruden et al., 2016; Kunos and Tam, 2011). The ECS is also known to be up regulated in metabolic disorders like insulin resistance and type 2 diabetes and its dysregulation is thought to contribute to the development of these disorders (Bielawiec et al., 2020; Silvestri and Di Marzo, 2013). Consequently, cannabinoids like CBD and THC are thought to possess therapeutic potential for these disorders.

The mechanisms by which CBD may exert an anti-obesity effect is unclear. It is possible that CBD may exert this effect through action on PPARγ or TRPV1. Cannabidiol is a known ligand for PPARγ, which plays an essential role in regulating glucose homeostasis and lipoprotein metabolism (Bielawiec et al., 2020; O'Sullivan, 2016). Stimulation of PPARγ has been shown to improve glucose tolerance, insulin sensitivity (Picard and Auwerx, 2002), and treatment with a PPARγ ligand reduced triglyceride levels by increasing the fractional clearance rate of triglycerides from very

low-density lipoproteins (Nagashima et al., 2005). Thus, the action of CBD on PPARγ may play a role in its suspected anti-obesity effect; however, this effect has not been directly investigated. TRPV1 is highly expressed on sensory nerve fibers in the pancreas and has been implicated in diabetes and obesity. Ablation of TRPV1-positive fibers by capsaicin has been shown to improve glucose tolerance in rat models of type 2 diabetes and prevented immune-mediated destruction of pancreatic β-cells in mice (Gram et al., 2007; Razavi et al., 2006). Silvestri et al. (2015), on the other hand, demonstrated a dose- and time-dependent reduction in hepatocyte lipid content *in vitro* that was shown to be independent of TRPV1, but their work was not able to identify a potential receptor responsible for the effect. They do suggest that the effect may have been a result of post-translational modifications as the expression of several proteins involved in lipid metabolism were upregulated (Silvestro et al., 2015). Thus, mechanisms by which CBD may exert a direct anti-obesity effect are currently unclear, and continued investigation is necessary.

Both CBD and THC have been shown to reduce hyperglycemia and increase insulin production in diabetic rats (Zorzenon et al., 2019). In a human pilot study, patients with type 2 diabetes receiving 100 mg CBD daily for 13 weeks had reduced resistin – a hormone linked to obesity and insulin resistance – compared to baseline, but there were no other changes in response to CBD treatment (Jadoon et al., 2016). However, it is possible these benefits of CBD may be attributable to its antioxidant and anti-inflammatory effects rather than a specific anti-obesity effect. In mice with induced type I diabetes, treatment with CBD reduced markers of inflammation in the microcirculation of the pancreas and reduced leukocyte activation, suggesting the influence of CBD on

diabetes and obesity may be attributed to its anti-inflammatory effect (Lehmann et al., 2016). Additionally, in a mouse model of type I diabetic cardiomyopathy, CBD attenuated the high glucose-induced increase in reactive oxygen species, cardiac fibrosis, and NF-κB activation (Rajesh et al., 2010). Collectively, current evidence points to CBD being beneficial for the management of complications associated with obesity and diabetes, though direct evidence of an anti-obesity effect remains elusive.

Antioxidant

Closely associated with the anti-inflammatory effect of CBD is its action as an antioxidant. Production of free radicals through aerobic metabolism is a normal physiological process tightly controlled by a complex network of antioxidant enzymes and molecules that remove ROS before they can damage a cell (Vertuani et al., 2004). During periods of oxidative stress, however, ROS production exceeds removal or damage repair by antioxidants, which can lead to permanent damage of cellular components like DNA, lipid peroxidation, and eventually cell death (Evans and Cooke, 2004). Cannabidiol has been shown to be a powerful antioxidant acting through direct and indirect mechanisms.

At the most basic level, CBD acts as an antioxidant by directly interrupting free radical chain reactions. Free radicals can be scavenged by the hydroxyl group on CBD's phenol ring, which then prevents further free radical propagation (Atalay et al., 2020; Borges et al., 2013). Cannabidiol can also directly reduce ROS generation by chelating transition metal ions involved in the production of free radicals by the Fenton reaction (Campos et al., 2016; Hamelink et al., 2005). Lastly, CBD prevents ROS production by

inhibiting pro-oxidant enzymes like xanthine oxidase, nitric oxide synthase, NADPH oxidases (Costa et al., 2007; Pan et al., 2009; Rajesh et al., 2007).

Indirect mechanisms by which CBD acts as an antioxidant include activation of PPARγ, reduction in lipid peroxidation, and enhanced expression and activity of antioxidant enzymes. Activation of PPARγ has been shown to inhibit cyclooxygenase 2, TNFα, IL-1, and IL-6 gene expression, and as CBD is a PPARγ agonist, it can indirectly exert antioxidant effects through this mechanism (Atalay et al., 2020; Hou et al., 2012). Administration of CBD also reduces lipid peroxidation, likely through its reduction of ROS production. Reduction of lipid peroxidation by CBD has been shown in both *in vitro* and rodent models by measuring malonodialdehyde (MDA) levels, which is a product of oxidative fragmentation of lipids (Ayala et al., 2014; Sun et al., 2017). Lastly, CBD has been shown to activate the redox-sensitive transcription factor nuclear erythroid 2-related factor (Nrf2), which regulates transcription of antioxidant genes (Atalay et al., 2020; Juknat et al., 2013). This activation leads to increased transcription of antioxidant enzymes like glutathione S-transferase and superoxide dismutase (SOD), whose activity is also increased by CBD (Jastrząb et al., 2019; Rajesh et al., 2010).

The antioxidant potential of CBD is one of the best studied. In combination with its potential effects on inflammation, the antioxidant effects of CBD make it a strong candidate for therapeutic use in diseases associated with oxidative stress and inflammation such as cancer, Alzheimer's, and diabetes mellitus. However, most of this work has been *in vitro* or in rodent models. Additional work in humans and companion animals is needed to determine if these effects translate to *in vivo* models and clinical trials.

Anti-Pruritic

Dermatologic conditions like allergic contact dermatitis, atopic dermatitis, and psoriasis are common medical problems for both humans and companion animals. In dogs, atopic dermatitis is one of the primary causes of pruritis and is associated with immune and skin barrier dysfunction (Brément et al., 2019). Emerging evidence suggests a protective role of the ECS in the skin. For instance, Campora et al. (2012) reported elevated ECS receptor immunoreactivity in dogs with atopic dermatitis compared to clinically normal dogs, suggesting an upregulation of these receptors due to inflammation. While the antipruritic effect of cannabinoids has been observed in humans and mice (Dvorak et al., 2003; Visse et al., 2017; Yuan et al., 2014), none of these studies have evaluated CBD *in vivo*. Palmitoylethanolamide (PEA), for example, has been shown to reduce itch and inflammation in mice (Vaia et al., 2016) and topical THC decreased contact allergic swelling and myeloid immune cell infiltration in a mouse model of allergic contact dermatitis (Gaffal et al., 2013).

While CBD has little affinity for CB_1 and CB_2 ECS receptors, it is a known agonist for the TRPV family of receptors (TRPV1-4), known ECS receptors widely expressed in the skin that are involved in itch sensation (Avila et al., 2020; Bisogno et al., 2001; Caterina and Pang, 2016; Tóth et al., 2019). As TRPV1 is rapidly desensitized after activation, it is thought that CBD may exert antipruritic effects by keeping TRPV1 desensitized, thus preventing neuronal activation by irritants (Imamachi et al., 2009; Muller et al., 2019; Xie and Hu, 2018). This potential mechanism is supported by an *in vitro* model of allergic contact dermatitis in which CBD elevated AEA and dose-

dependently inhibited the release of pro-inflammatory IL-6, IL-8, and TNFα, an effect that was reversed by both CB_2 and TRPV1 antagonists (Petrosino et al., 2018).

Other potential mechanisms include action on other receptors or through genetic regulation. CBD has been shown to be an antagonist for transient receptor potential melastatin 8 (TRPM8) receptors (De Petrocellis et al., 2008; Muller et al., 2019). In the skin, TRPM8 is responsible for environmental cold detection and has been suggested to contribute to the perception of pain and itch, which may indicate it is another target for the potential antipruritic effect of CBD (Caterina and Pang, 2016; Jankowski et al., 2017). In an *in vitro* model using canine keratinocytes, CBD downregulated several genes associated with inflammatory pathways though increased DNA methylation of key sites (Massimini et al., 2021), which may indicate that CBD is affecting gene transcription in addition to action on receptors. However, as this effect has not been evaluated *in vivo*, more research is needed to determine if any of these mechanisms produce a physiologically relevant response.

Anxiolytic and Antipsychotic

Cannabis has been used for centuries to treat anxiety and to improve mood (Graczyk et al., 2021), and the ECS is known to play an essential role in the regulation of cognitive abilities, stress, sleep, and mood (Hill et al., 2013; Morena et al., 2016). While THC induces psychotic symptoms through its action on CB_1 (D'Souza et al., 2004; Morrison et al., 2011), CBD is a non-psychotropic that can inhibit anxiety and psychotic-like symptoms produced by THC use (Englund et al., 2013; Klein et al., 2011). Because of this, CBD has been investigated for its therapeutic potential for a wide range of

medical conditions, including psychiatric disorders like anxiety, schizophrenia, and depression.

One possible mechanism by which CBD exerts antipsychotic effects is through the inhibition of FAAH and subsequent increase in AEA. Elevated AEA is associated with psychiatric symptom improvement (Giuffrida et al., 2004; Leweke et al., 2012), so CBD may indirectly produce an antipsychotic effect by inhibiting AEA breakdown by FAAH (Bisogno et al., 2001). Additionally, both CBD and AEA are agonists of TRPV1, which plays a role in emotional and cognitive responses in the brain in addition to pain detection (Campos et al., 2012; Nazıroğlu and Demirdaş, 2015). Anxiety was reduced in TRPV1 knockout mice, and the desensitization of TRPV1 by either CBD or AEA may produce a similar response (Marsch et al., 2007). The antipsychotic effect of CBD may alternatively be a result of its action on 5-HT_{1A} receptors (Bonaccorso et al., 2019). A 5-HT_{1A} antagonist was shown to attenuate the reduction in anxiety by CBD in rats using contextual fear conditioning, elevated plus maze, and Vogel conflict test models (Campos et al., 2008; Gomes et al., 2012).

In addition to the work examining CBD use as an anxiolytic and antipsychotic in rodent models, there is also considerable evidence of this effect in humans. In human studies evaluating the impact of CBD/THC ratios in cannabis on psychosis measures, CBD content was inversely related to psychosis symptoms like memory impairment, depression, and anxiety (Morgan et al., 2010; 2012) as well as age of onset of psychiatric disorders (Di Forti et al., 2014; Iseger and Bossong, 2015). In a study of patients with Parkinson's disease, CBD improved psychosis scores and did not alter motor function (Zuardi et al., 2008). In a clinical trial of CBD versus a conventional antipsychotic, CBD

was as effective as the conventional medication in improving symptoms of schizophrenia and displayed less side effects (Leweke et al., 2012). Additionally, CBD supplementation was reported to attenuate the cortisol decrease associated with the circadian rhythm of the hormone in humans (Appiah-Kusi et al., 2020; Zuardi et al., 1993), which may also contribute to anxiolytic effects outside of its effects on cognition. Despite these promising clinical trials, larger scale, long-term, placebo-controlled studies still need to be conducted in the future, especially considering CBD has been proposed for use in psychiatric issues that have not been investigated, like depression or post-traumatic stress disorder

Sedative

The ECS has been implicated in the regulation of the circadian sleep–wake cycle (Sanford et al., 2008; Vaughn et al., 2010) and has been suggested to be the link between the superchiasmatic nucleus – which regulates circadian regulation systems – and associated behavioral and physiological processes like sleep (Babson et al., 2017). Considering approximately 10% of adults suffer from chronic insomnia and the resulting decrease in productivity (Kuhathasan et al., 2019), the potential sedative effect of CBD may be a therapeutic benefit if shown to aid in treatment of sleep disorders through its actions on the ECS.

The primary mechanism by which CBD is thought to exert a sedative effect is through inhibition of AEA transport and breakdown. Cannabidiol is thought to indirectly enhance AEA signaling through both the inhibition of AEA uptake via anandamide membrane transporters and prevention of AEA degradation via FAAH inhibition (Bisogno et al., 2001; Rakhshan et al., 2000; Leweke et al, 2012). Anandamide is thought

to promote sleep through both action on the CB_1 receptor and an increase in adenosine, which is a sleep-inducing molecule (Murillo-Rodriguez, 2008; Murillo-Rodriguez et al., 2003; 2016). Thus, the delayed transport and degradation of AEA may result in a sedative effect of CBD indirectly by elevating AEA levels in the brain.

Cannabidiol has been shown to have differential effects on sedation and sleep – larger doses exerting sedative effects and low doses increasing wakefulness. In humans and rats, CBD doses ranging from 2 to 40 mg/kg BW/d have been reported to induce sedative effects, improve sleep quality, and increase total sleep time (Chagas et al., 2013; Hsiao et al., 2012; Zuardi et al., 1993). In 15 patients with insomnia, 160 mg CBD/d increased total sleep time and decreased the frequency of arousals during the night, (Carlini and Cunha, 1981), and a long-term study assessing the effect of CBD on sleep quality in adults found a modest improvement in sleep (Shannon et al., 2019). However, some work has demonstrated no influence of CBD on the sleep cycle in humans (Linares et al., 2018), and others report that CBD promotes alertness and wakefulness rather than sedation. Nicholson et al. (2004) reported that 15 mg CBD increased wakefulness of young adults during sleeping time, and increased alertness has been observed in rats following CBD administration (Murillo-Rodríguez et al., 2006; 2008; 2014). These discrepancies may be due to differences in length of CBD supplementation, small sample sizes, route of administration, vehicle used, CBD dose, combination of CBD with other cannabinoids, or subject species. Based on the numerous conflicting reports on the potential sedative or wakefulness effects of CBD, it is clear that additional research is needed in the area of CBD use for sleep disorders.

Bioavailability, Pharmacokinetics, and Metabolism of Cannabidiol

Compared to intravenous or inhalation administration, the bioavailability of oral CBD by itself is low, ranging from 5% to 19% due to factors such as low absorption rate and extensive first-pass metabolism in the liver (Lim et al., 2020; Samara et al., 1988). Variable increases in bioavailability of oral CBD have been demonstrated when administered in lipid formulations, such as infused in oil, encapsulated, or formulated into a chew or treat (Atsmon et al., 2018; Bartner et al., 2018; Deabold et al., 2019). Administering CBD with a fat meal may also contribute to increased bioavailability by increasing bile release while slowing gastric emptying and gastrointestinal motility, which increases the time available for CBD dissolution and absorption (Lim et al., 2020; Welling, 1996). The increase in oral bioavailability when combined with lipids may also be a result of intestinal lymphatic absorption and transport. A study in rats demonstrated an almost 3-fold increase in the bioavailability of oral CBD when administered in a lipid-based formulation compared to a lipid-free vehicle (Zgair et al., 2016).

Pharmacokinetic data from oral CBD administration suggests, in both human and canine models, that the half-life of elimination ($t_{1/2}$) ranges from 1 to 4 hours while the maximal concentration (C_{max}) and area under the curve (AUC) vary considerably depending on the dose and whether the CBD was administered with food (Bartner et al., 2018; Deabold et al., 2019; Gamble et al., 2018; Hobbs et al., 2020; Millar et al., 2018). Major elimination pathways of CBD may include both urinary excretion and fecal excretion via biliary secretion. In dogs, unmetabolized CBD is the predominant compound excreted within the first several hours after administration (Samara et al., 1990a). Once absorbed, CBD undergoes extensive metabolism in the liver by various

cytochrome P450 enzymes, involving hydroxylation, oxidation, side-chain degradation, and conjugation (Jiang et al., 2011; Samara et al., 1990a). Metabolites produced during these processes differ considerably between species. In humans, major routes of transformation include 7-hydroxylation, oxidation of C9 to the alcohol and carboxylic acid, and side-chain oxidation (Harvey et al., 1991a, b; Huestis, 2007). In mice and rats, hydroxylation at C2 is a major route of biotransformation alongside acid products of beta-oxidation (Harvey et al., 1991b; Samara et al., 1991). In dogs, 6β-hydroxylation and oxidation of resulting alcohol is the dominant oxidative pathway compared to the 7-hydroxylation in humans. Additionally, side-chain hydroxylation is less extensive in dogs than in humans (Samara et al., 1990b). Canine and human CBD metabolite production is similar in the production of glucoside conjugates, like 6-oxo CBD, while similar conjugates are not found in rat urine (Harvey et al., 1991a; Samara et al., 1990a). Rats and dogs are similar in the production of acids via beta-oxidation whereas acids formed in humans are primarily derived from side-chain degradation and 3" hydroxylation (Harvey et al., 1991a).

Cannabidiol Use in Dogs

Cannabidiol is currently supplemented to dogs for its potential therapeutic applications including osteoarthritis, separation anxiety, noise phobias, and epilepsy (Kogan et al., 2018; Landa et al., 2016). Several studies have evaluated its effectiveness in dogs with osteoarthritis (Brioschi et al., 2020; Gamble et al, 2018; Verrico et al., 2020), behavior (Corsetti et al., 2021) and epilepsy (McGrath et al., 2019) with mixed results. Considerable work has also been done investigating pharmacokinetics (Bartner et al., 2018; Deabold et al., 2019; Gamble et al., 2018; Wakshlag et al., 2020) of oral CBD

administration. However, there is still limited *in vivo* work in canine models evaluating long-term safety of CBD supplementation and its efficacy in generating these beneficial effects.

A preliminary investigation of the safety of escalating CBD doses in 20 healthy dogs reported mild constitutional adverse events recorded for dogs receiving 1.7-64.7 mg/kg CBD oil, which included both lethargy and hyperesthesia (Vaughn et al., 2020). A similar investigation into the safety of a 1:20 THC:CBD herbal extract reported mild neurological adverse events, like ataxia and delayed hopping, after single and multiple oral doses of 2 and 5 mg/kg CBD extract (Chicoine et al., 2020). While adverse events in both studies were mild and rare, they do highlight the potential for CBD to cause undesirable side effects as well as the need for continuing research evaluating the safety and efficacy of CBD use in dogs.

The literature available evaluating the therapeutic potential of CBD in dogs is sparce. Work in osteoarthritic dogs has focused primarily on the subjective evaluation of CBD's effects by owners or veterinarians, with little quantitative data to support its use. In those studies, administration of either oral or transmucosal CBD oil at 2 mg/kg increased canine brief pain inventory (CBPI) and Hudson scores in dogs with osteoarthritis (Brioschi et al., 2020; Gamble et al., 2018), suggesting an increase in activity and comfort with CBD use. Before this work, only one other study evaluated behavioral effects of CBD, in which the effect of a CBD-predominant hemp extract on aggressive behaviors was assessed in shelter dogs. Authors reported a reduction in aggressive behaviors in dogs towards humans after treatment but no differences between CBD and placebo, suggesting acclimation of animals to humans and the environment

rather than a result of CBD (Corsetti et al., 2021). McGrath et al. (2019) reports the first randomized, blinded clinical trial assessing the addition of CBD to conventional antiepileptic treatment in dogs with epilepsy. Treatment with 2.5 mg CBD/kg reduced seizure frequency compared to placebo, but the proportion of responders to treatment was similar between groups. Lastly, an *in vitro* model of canine atopic dermatitis, a primary cause of pruritus in dogs, demonstrated the potential for a combination of CBD and polyphenols to modulate transcription of inflammatory genes related to atopic dermatitis (Massimini et al., 2021).

Despite this lack of scientific evidence, use of CBD by dog owners is gaining in popularity due to the possibility of therapeutic benefits. Some owners have turned to hemp products because it is from a natural source versus conventional medications while others believe hemp products to be better at managing specific symptoms than conventional medicine (Wallace et al., 2020). Kogan et al. (2016; 2018) reported that of dog owners who gave hemp products to their pets, most said they perceived the products to be helpful in the reduction of symptoms associated with pain, anxiety, nausea and vomiting, noise phobias, and more. A survey of Canadian dog owners reported that owners generally perceived the cannabis products to be equally or more effective than conventional medications for similar issues (Kogan et al., 2019b). Veterinarians, on the other hand, are more hesitant to use or recommend hemp products. While the majority of veterinarians and veterinary students responding to surveys report their hesitancy is due to awareness of the lack of research, they still expressed positive views on the potential benefit of the use of hemp products in animals and overwhelmingly supported continued research in animals (Kogan et al., 2019a; Vogt et al., 2019).

Conclusions

Despite centuries of cannabis use and favorable public opinion of CBD use for a variety of ailments, there is limited evidence of safety or efficacy of CBD in both humans and companion animals. While investigations into CBD effects have increased since its removal from the CSA in 2018, evidence supporting its use is largely limited to *in vitro* work, rodent models, or under-powered clinical trials. Regardless of desired effect, more research is necessary to clarify safety of use, proper dosing, and which, if any, actions of CBD have physiological relevance in dogs.

CHAPTER 3. PALATABILITY OF CANNABIDIOL-CONTAINING DOG TREATS

Introduction

Palatability is the capacity of a food or ingredient to stimulate the appetite to encourage eating and satiety. It is dependent on several factors, including formulation of ingredients, processing method, texture, and the freshness and stability of raw materials (Aldrich and Koppel, 2015). Additionally, palatability can be difficult to quantify as it is individually perceived and therefore highly subjective (Tobie et al., 2015). Despite the difficulty, assessment of food palatability is essential in the development of any new product. It does not matter how well a product is formulated if animals will not consume it.

In the case of CBD, there are reports of human oromucosal sprays like Sativex® having an unpleasant taste (Lus et al., 2018). However, there is no literature available regarding the palatability of CBD-containing canine products like treats nor their potential effect on daily diet consumption. Therefore, the objective of this study was to determine the palatability of dog treats formulated with increasing amounts of industrial hemp-derived CBD and to examine the potential for CBD inclusion in treats to alter daily food consumption. The underlying hypothesis was that inclusion of CBD in treats would not alter food consumption nor treat consumption compared to control.

Materials and Methods

This study was approved by the Lincoln Memorial University (LMU) Institutional Animal Care and Use Committee (IACUC) (protocol 1801-RES) before the start of the study. All housing and husbandry received were in accordance with the Animal Welfare

Act, the Guide for the Care and Use of Laboratory Animals (8th ed.), and all applicable LMU standard operating procedures (SOPs).

Subjects and Housing

Twenty-four dogs (12 male, 12 female, 16.0 ± 3.6 kg BW) were received from a local shelter for inclusion in this study. The shelter was asked to provide dogs weighing 16 ± 4 kg. Additionally, the shelter was informed and gave consent for the use of the dogs for research purposes prior to their arrival. Prior to beginning the experiment, each dog had a complete blood count (CBC) and serum chemistry analysis (IDEXX Laboratories, Inc., Westbrook, ME) performed, along with physical evaluation by a veterinarian and a fecal examination to rule out any underlying disease that might preclude enrollment. Dogs were excluded if they demonstrated serious behavioral issues, such as aggression that would endanger research personnel, were severely emaciated, classified as a body condition score < 3.5 or > 7.5 on a 9-point scale (where 1 is emaciated and 9 is obese; Laflamme, 1997), or if their initial evaluations revealed an underlying disease that required more than routine treatments (such as heartworm positive dogs). Three dogs were excluded for being spayed and one dog was excluded for behavioral issues. Dogs were individually housed in 1.2 x 1.8 m kennels within one of two dog wards at the LMU DeBusk Veterinary Teaching Center (DVTC, Ewing, VA). Dogs were stratified by treatment and sex and evenly distributed between the two wards.

Treatments and Diets

Twenty dogs were included in this study (10 male, 10 female, 16.1 ± 3.8 kg BW) and were stratified by weight and sex before being randomized into four treatment groups

(*n* = 5 per treatment). The treatments consisted of 0, 5, 10, or 25 mg CBD/d. The CBD was the primary constituent of a proprietary industrial hemp isolate (AgTech Scientific, Paris, KY) that was incorporated into treats and administered in the form of 1 treat twice daily with each treat containing half the daily dose. Treats were offered as a reward within 30 min of a meal upon kennel re-entry following twice daily exercise, with consumption scored for five days. Based on an average body weight, dogs received approximately 0, 0.313, 0.625, or 1.563 mg CBD/kg BW/d.

Dogs were fed Purina Pro Plan EN Gastroenteric Dry Dog Food (Nestle Purina, Inc., St. Louis, MO) during the initial measure and Purina Pro Plan EN Gastroenteric Wet Dog Food during the final as these foods provide a large difference in palatability of the basal diet. Both foods were fed to meet the daily metabolizable energy requirements of intact adult dogs at maintenance, calculated as $(70 * BW^{0.75}) * 1.8$ and split into two meals per day.

Data Collection

Prior to scoring treat consumption for 5 days (Treatment Period), dogs were adapted to each test food for 5 days (Adaptation Period). Daily intake of the basal food was monitored to determine if treat consumption affected food intake. The dogs were then adapted to the second commercial food (the canned food) for 5 days before offering the treatment treats for 5 days and again scoring consumption.

Palatability was scored based on their consumption of each treat: Rapid, Hesitant or No consumption. Consumption was considered “rapid” if the treat was consumed within 5 seconds of being offered and was given a numerical score of 2 for statistical analysis.

Consumption was considered “hesitant” if the treat was consumed between 5-10 seconds of being offered, where it was then given a numerical score of 1. Lastly, “no consumption” was recorded if the treat was not consumed within 10 seconds of being offered, in which case the numerical score was 0. If the treat was not consumed within 10 seconds, it was left in the dog’s food bowl until the next feeding. If the treat was still present before the next feeding, its presence was noted, and the treat was removed prior to receiving the next meal. Persons administering the treats were blinded as to the actual IHE content.

Statistical Analysis

Food intake and treat consumption were analyzed using the MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC) including the fixed effects of treatment (CBD), period (Adaptation and Treatment), and treatment by period interaction. Food intake was assessed as mean intake per kg of metabolic body weight per day (g/kg $BW^{0.75}$/d) over each 5-d period. Treat consumption was analyzed as the mean numerical treat consumption score (range of 0-2) over each 5-d period. Results are presented as the mean ± SE and effects were considered significant when $P \leq 0.05$.

Results

Food intake was unaffected by treatment when dogs were fed either dry (31.4 ± 0.66 g/kg $BW^{0.75}$/d) or wet dog food (102.9 ± 3.94 g/kg $BW^{0.75}$/d; Figure 3.1; $P = 0.280$ and 0.669, respectively). Similarly, food intake did not differ between adaptation and treatment periods for either the dry or wet foods ($P = 0.968$ and 0.500, respectively). Cannabidiol inclusion had no effect on the consumption of treats when fed either a dry or wet dog food ($P = 0.308$ and 0.571, respectively). Similarly, treat consumption did not

differ between adaptation and treatment periods when dogs were fed wet food (P = 0.171). However, when fed dry dog food, treat consumption was increased during the Treatment period compared to the Adaptation period (Figure 3.2; P = 0.005).

Discussion

During the first adaptation phase of this study, when the dogs were being adapted to the dry food diet, the control treats offered were a commercially available treat (Purina Pro Plan Gentle Snackers). During the remainder of the study, the control treats were the test treats prepared for the study (0 mg CBD) and were given during the Adaptation period. The test treats were quite different from the commercial treats in smell, texture, and taste and were extremely palatable. The consumption of the commercial treats during the dry food adaptation phase was lower than the consumption of the test treats during the treatment period because of the differences in the treats. There were several dogs who refused to eat a single commercial treat but consumed every single test treat without hesitation. During the second adaptation phase, when dogs were being adapted to the wet food diet, the test treats were offered and no difference was found between those control treats and the treatment treats. Additionally, as there were no differences between treatments, there was no apparent effect of increasing levels of CBD on the palatability of the treats when included at up to 25 mg CBD per day.

The presence of CBD in these treats also did not alter daily intake. There were no differences in intake on a metabolic body weight basis between the different treatments, or between the adaptation and treatment periods for both dry and wet food diets. Intake was greater when dogs were fed wet food; however, this was likely due to the increased intake required to meet daily dietary maintenance requirements. Wet dog foods have a

much lower caloric density than dry dog food because of the high-water content, and as such, larger amounts needed to be offered each day to meet requirements. Since intake did not differ between the adaptation and treatment periods while dogs were on wet foods, individual dog preference was potentially a more important factor affecting daily food intake. The majority of dogs displayed no hesitation in the consumption of their twice daily meals. A few of the dogs were picky eaters, and those few dogs were the primary source of refusals for their treatment groups during both adaptation and treatment periods.

It is worth noting, however, that all dogs exhibited loose stools when switched to the strictly wet food diet, regardless of treatment. The wet dog food fed was much lower in fiber than the dry dog food, despite being the same brand and type of food, indicating a much more gradual adaptation to the wet food would be needed for all dogs. There were no recorded reports of diarrhea while the dogs were receiving test treats during the dry food treatment period, and diarrhea presented during the adaptation to the wet food before the test treats were being administered. As such, it is unlikely that this gastrointestinal upset was a result of the CBD supplemented in the treatment treats. It does, however, reinforce that any change in diet should take place over a longer period of time and should involve a slow transition from one food to another.

Conclusions

These results suggest the palatability of CBD-containing treats is comparable to treats without CBD, and that the presence of CBD does not affect daily feed intake when supplemented up to 25 mg CBD per day. Future studies can utilize these treats to

examine any potential effects of CBD on various physiological processes in a canine model.

Tables and Figures

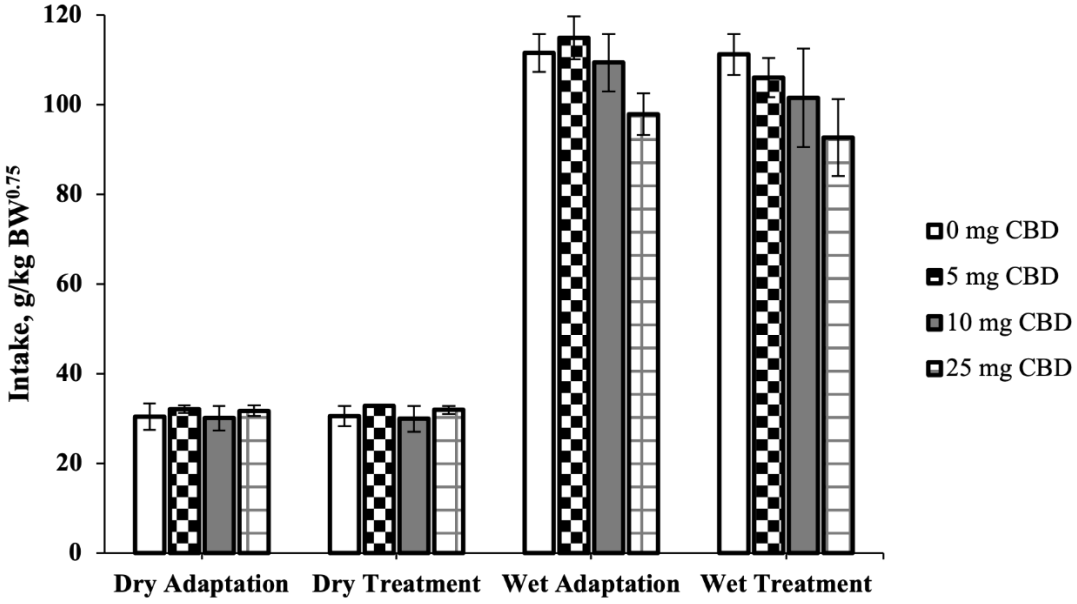


Figure 3.1. Mean intake (g/kg $BW^{0.75}$/d) of all animals across all treatments during each 5-d adaptation and treatment period for both dry and wet food diets. Error bars represent the standard error of the treatment mean (SEM).

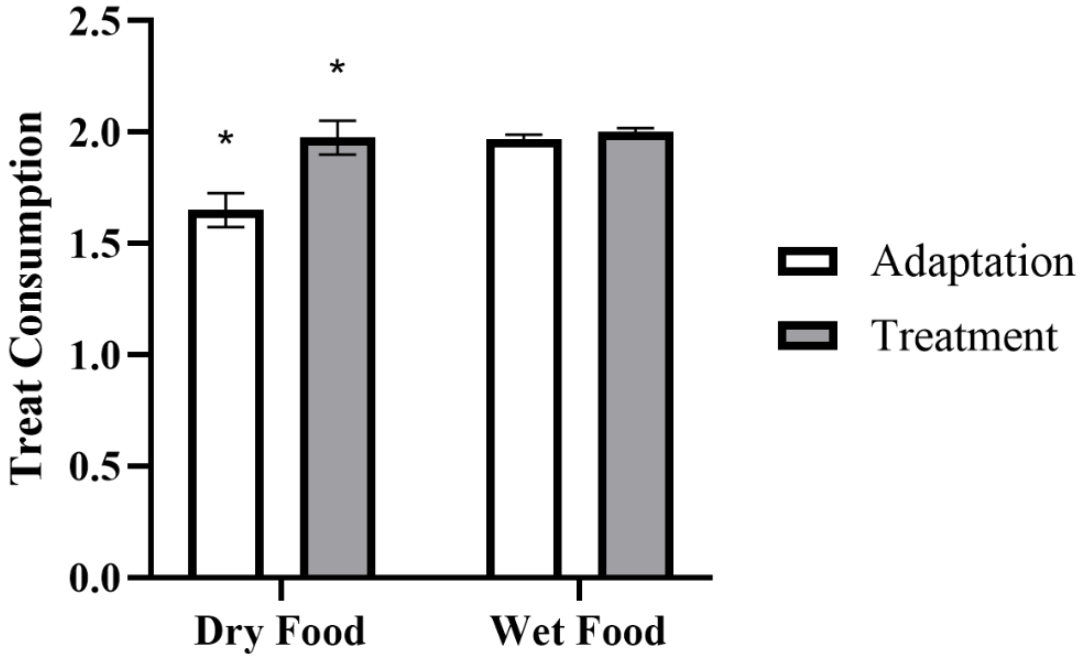


Figure 3.2. Mean treat consumption for animals across all treatments during each 5-d adaptation and treatment period for both dry and wet food diets. Scores of 2, 1, or 0 were assigned to each treat offering based on rapid, hesitant, or no consumption of the treat. Asterisks above bars represent a difference in treat consumption between periods ($P \leq 0.05$). Error bars represent the standard error of the treatment mean (SEM).

CHAPTER 4. THE IMPACT OF FEEDING CANNABIDIOL CONTAINING TREATS ON CANINE RESPONSE TO A NOISE-INDUCED FEAR RESPONSE TEST

Frontiers in Veterinary Science, 2020, 7:569565

Introduction

Noise aversion or reactivity is one of the most common fearful behaviors in dogs, with 40 to 50% of dogs demonstrating at least one fearful behavior in response to noise exposure (Blackwell et al., 2013; Tiira et al., 2016). There is, however, considerable variation in the behavioral responses to noise. Some dogs will reduce activity while others become hyperactive. Some behavioral changes, such as panting and hiding, are mild, while others, like destructiveness and self-trauma, are more extreme and potentially hazardous to the health and well-being of both dog and owner (Sherman and Mills, 2008). Such extreme and detrimental stress associated with fear reduces overall health and lifespan (Dreschel, 2010; Dreschel and Granger, 2005).

Despite the prevalence of noise aversion behaviors in dogs, they frequently go untreated with less than one-third of dog owners reporting that they would seek advice for the treatment of noise aversion (Blackwell et al., 2013). Potential treatment regimens for various noise aversion behaviors include systematic desensitization with a compact disk-based training system and administration of medications or natural products (Sherman and Mills, 2008). There are several commonly prescribed drugs for the treatment of canine behavior disorders associated with fear and anxiety, including but not limited to benzodiazepines, selective serotonin reuptake inhibitors, and tricyclic anti-depressants (Overall, 2013; Gruen et al., 2014). However, some owners may be hesitant to administer such medications, whether due to the possibility of undesirable side effects,

personal bias against drug use, or cost. This has led to increased interest in the use of natural extract products to alter fearful behaviors, like dog-appeasing pheromones or oral supplementations such as L-theanine, a tryptic hydrolysate of milk protein and fish hydrolysate (Araujo et al., 2010; Landsberg et al., 2015; Mills et al., 2006; Palestrini et al., 2010; Sheppard and Mills, 2003). Additionally, there has been renewed interest in the use of cannabinoids, CBD in particular, to regulate anxiety disorders in both humans and companion animals (Tambaro and Bortolato, 2012).

The potential anxiolytic effects of CBD have been attributed to several mechanisms, including its activation of 5-HT_{1A} receptors and its ability to indirectly activate cannabinoid receptors by inhibiting the metabolism of the endocannabinoid anandamide (Blessing et al., 2015; Lee et al., 2011). This has produced great interest in using CBD as a potential alternative to conventional therapies to reduce anxiety. While there is considerable work examining its use as an anxiolytic in human and rodent models (reviewed extensively in Lee et al., 2017), this effect has yet to be examined in a canine model. But despite the lack of evidence, canine anxiety and noise aversion are some of the most common reasons that pet owners seek information on and administer CBD to their pets (Kogan et al., 2019a).

The objective of this study was to evaluate the influence of CBD on behavioral responses to fear-inducing stimuli in dogs, with the underlying hypothesis was that CBD would reduce fearful and anxious responses. This hypothesis was tested using a fireworks model of noise-induced fear and anxiety in which the effectiveness of CBD was assessed by comparing CBD to both a positive and negative control and to the combination of

CBD with the positive control. All treatments were expected to reduce fearful and anxious responses compared to the negative control.

Materials and Methods

This study was reviewed and approved by the LMU IACUC (protocol 1811-RES) prior to the start of the study. All housing and husbandry received were in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals (8th ed.), and all applicable LMU SOPs.

Subjects and Housing

Twenty-four intact, adult dogs (12 male, 12 female; 1 to 5 years old; 17.7 ± 3.9 kg) of various mixed breeds, including cur, Labrador, hound, Boxer, shepherd, dane, Schipperkee, Springer Spaniel, and terrier mixes were received from a local shelter for inclusion in this study. The shelter was asked to provide dogs weighing 16 ± 4 kg. Additionally, the shelter was informed and gave consent for the use of the dogs for research purposes prior to their arrival. Prior to beginning the experiment, each dog had a CBC and serum chemistry analysis (IDEXX Laboratories, Inc., Westbrook, ME) performed, along with physical evaluation by a veterinarian and a fecal examination to rule out any underlying disease that might preclude enrollment. Dogs were excluded if they demonstrated serious behavioral issues, such as aggression that would endanger research personnel, were severely emaciated, classified as a body condition score < 3.5 or > 7.5 on a 9-point scale (where 1 is emaciated and 9 is obese; Laflamme, 1997), or if their initial evaluations revealed an underlying disease that required more than routine treatments (such as heartworm positive dogs). Two dogs were excluded from the experiment due to positive heartworm tests and 4 additional dogs were excluded due to

other health or behavioral concerns. Dogs were individually housed in 1.2 x 1.8 m kennels within one of two dog wards at the LMU DeBusk Veterinary Teaching Center. Dogs were stratified by sex and evenly distributed between the two wards.

Diets and Treatments

Dogs were fed Purina Pro Plan EN Gastroenteric Dry Dog Food (Nestle Purina Inc., St. Louis, MO) to meet the daily metabolizable energy requirements of intact adult dogs at maintenance, calculated as $(70 * BW^{0.75}) * 1.8$ and split into two meals fed at approximately 0730 and 1830 h each day. Dogs were weighed and body condition scored (5-point scale) weekly and diets adjusted accordingly. Treatments were arranged in a 2 x 2 factorial and consisted of 1) control (placebo treats), 2) 25.2 mg CBD/d, 3) Trazodone + 0 mg CBD, and 4) 25.2 mg CBD/d + Trazodone. Trazodone, a serotonin antagonist and reuptake inhibitor, was dosed at 100 mg for dogs weighing 10.0-20.0 kg and at 200 mg for dogs weighing 20.1-40 kg as recommended by the veterinarian and based on previous work (Jay et al., 2013). Because trazodone does not require an extensive adaptation period, trazodone tablets were dosed via a Pill Pocket (Mars Petcare US, Franklin, TN) the evening prior and morning of the behavioral assessment.

The CBD was the primary constituent of a proprietary industrial hemp isolate (AgTech Scientific, Paris, KY) that was incorporated into treats and administered in the form of 2 treats daily, with each treat containing half the daily dose. Groups not receiving CBD treatment received control treats (0 mg CBD). Both control and CBD treats were composed of the following ingredients: chicken, chicken liver, Asian carp, catfish, and in the case of the CBD treats, industrial hemp extract. Dosage of CBD was selected based on previous work utilizing oral CBD in dogs (Gamble et al., 2018). CBD was the primary

constituent of the industrial hemp extract (12.6 ± 1.2 mg/treat); no other cannabinoids were detected in the treats. Treats were formulated to target CBD at 2 mg/kg BW/d based on an estimation that dogs would weigh an average of 16 kg. However, based on the mean weight of dogs included on the study, actual dosage of CBD was 1.4 mg/kg BW/d.

Treats were offered solely as a reward upon kennel re-entry following twice daily exercise at approximately 0700 and 1800 h each day. Trazodone tablets hidden in Pill Pockets were administered at approximately 1830 h the evening before and 1000 h the morning of each noise-induced fear response test. Empty Pill Pockets were administered to the control and CBD treatment groups on those days to ensure that research personnel administering the treats were blinded as to the treatments administered.

Testing Room and Equipment

The testing room was an approximately 2.72 x 3.38 m isolation room located on the opposite side of the building relative to where dogs were housed. The room contained a wall-mounted table and cabinets, a set of closed metal kennels, and a cloth dog bed. The dogs could interact with these objects, but none obstructed the dogs from view of the cameras. Two recording cameras (Model BRC-Z700, Sony Co., New York, NY and Model B07DQPS3KY, QallExpress International, China) were secured on opposite sides of the room near the ceiling – approximately 2 m from the floor – to ensure the dogs would be within sight at all times. Dogs were isolated in the testing room; handlers monitored the dogs from the adjacent room via the cameras and could not be seen by dogs. Two Bluetooth speakers (Bose Co., Framingham, MA) were placed on opposite sides of the room near the cameras to create a surround-sound effect during the noise tests. Between each dog's test, the room was cleaned with Rescue™ Concentrate (Virox

Animal Health, Oakville, ON, Canada), an accelerated hydrogen peroxide-based disinfectant.

Acclimation

After intake and entrance into the study, all dogs were adapted to their environment, diet, daily routine, and the testing room for 3 d (Table 4.1), in which the dogs spent 6 minutes in the testing room where behavior was monitored, but not scored. A baseline open field test followed the 3-d adaptation, where dogs were placed in the testing room, behavior was scored, but no noise track was played (described below). The next day, a 6-min baseline fireworks test was conducted (described below). Both the open field test and baseline fireworks test were used solely to select dogs for inclusion in the study. Dogs not exhibiting at least one behavioral change between the open field test and the fireworks test, behaviors such as cowering, shaking, vocalization, destructiveness, or tail tucking, were excluded from the study. Dogs included in the study spent 6 min in the test room every day throughout the experiment to eliminate the possibility of behavioral changes due to the novel environment of the test room. In order to acclimate dogs to the testing procedure, heart rate monitors bands were placed on the dogs for each adaptation to the test room. Additionally, blood draws were simulated on non-testing days by restraining dogs and holding off cephalic and jugular veins prior to placing them in the testing room. The fireworks test was conducted on the last day of each 7-d period (Table 4.1).

Open Field and Fireworks Tests

A fireworks model of noise-induced fear and anxiety was utilized to assess the effectiveness of the treatments. All dogs received from the shelter (n = 24) received 1 open field test and 1 baseline fireworks test. All dogs included on the study (n = 16) also received 1 fireworks test per 7-d period (5 total fireworks tests), each lasting 6 min. During the open field test, the dogs were placed in the testing room and their behavior was recorded in two 3-min blocks where no fireworks track was played in order to assess baseline behavior of dogs in the testing room. During the fireworks tests, the first 3-min block was the same as the open field test where no noise was played (**Pre-Noise**), and the fireworks track was played over a stereo speaker system (mean) during the second 3-min block (**Noise**).

In previous work using this model, a thunderstorm track was utilized to test the noise-induced fear response in dogs (Araujo et al., 2013; Landsberg et al., 2015). However, a fireworks video (https://www.youtube.com/watch?v=5eLcHJLDlI8) was used according to Blackwell et al. (2013) that reported a larger percentage of dogs respond to fireworks than to thunderstorms. This noise-induced fear response test used in this study was a modified version of the one developed and validated by Araujo et al. (2013). They utilized a 9-min test that included a "before," "during," and "after" thunderstorm time points. Because they saw no behavioral differences (i.e. near door duration, inactivity duration) between the "during" and "after" thunder time points, the test for this study was shortened to 6 min, ending immediately after the fireworks track (Noise time point) ended. This allowed for the immediate post-test blood sample collection to be obtained more quickly after the fireworks test. The mean of 90 dB was

selected based on previous work (Araujo et al., 2013; Landsberg et al., 2015) that were both successful in generating a response using equal or lesser decibel thunderstorm track. Behaviors in each 3-min time block were recorded and analyzed as separate time points (Pre-Noise and Noise).

Experimental Design

Sixteen dogs were included in this study (7 male, 9 female; 1 to 4 years old, mean BW 18.1 ± 0.2 kg). Dogs were selected based on their behavioral response to the baseline noise-induced fear test (described above), in which behaviors such as cowering, shaking, vocalization, destructiveness, and tucking tail upon the start of the fireworks track indicated the dog was reactive to noise. These behaviors were selected as they have been previously used to assess noise reactivity (Franzini de Souza et al., 2017; Landsberg et al., 2015; Sheppard and Mills, 2003). Included dogs were then arranged in a multiple square, replicated 4 x 4 Latin Square design in which dogs within each square (4 dogs per square) were randomly assigned to receive one of the four treatments each week (Periods 1 – 4). Each square was tested on successive days for scheduling purposes. Dogs received each treatment for a 7-d period prior to each of the noise-induced fear response tests (Table 4.1).

On testing days, all experimental procedures started at 1200 h. CBD treats were administered approximately 4 to 6 hours prior to the test, and the morning dose of trazodone was administered approximately 2 to 4 hours prior to the test. At the time of the completion of this study (July 2018), there was little literature available on the pharmacokinetics of oral CBD administration. Samara et al. (1988) reported that the half-life of IV CBD administration was 6 to 9 hours but had no estimate for an oral dose. For a

similar dose of trazodone, Jay et al. (2013) reported a mean half-life of elimination of 166 min. No washout period was included between treatment periods. From the reported half-lives of both CBD and trazodone, it was decided that the 7-d treatment period would be sufficient to allow for elimination of previous treatments prior to the next test while also allowing for acclimation to the next treatment. Additionally, time constraints on the availability of the kennels in which the dogs were housed prevented the inclusion of washout periods. Dogs received the test at the same time each week.

Because of scheduling constraints, the test started as soon as the dogs entered the testing room on testing days. This did not allow for either HRV or behavior to return to normal after movement from kennel to testing room. To account for this, only data from the last minute of the Pre-Noise time point was utilized to represent the behavior and HRV of dogs during that time point, which served as a reference of their normal behavior prior to the fireworks track starting. Additionally, only the first minute of the Noise time point was utilized to represent the dogs' behavior and HRV during that time point in order to assess the dogs' initial reaction to the fireworks track.

Data Collection

Consumption of food and treats, consistency of stool, frequency of elimination, activity during exercise, mucus membrane color, and other indicators of general health status were monitored twice daily by research personnel. Evidence of any adverse event – defined as any symptom occurrence that would not be expected in normal dogs – was also monitored. However, no adverse events were observed in any dogs following the administration of CBD treats during this study.

On the day of each fireworks test, blood samples (5 mL) were collected via jugular or cephalic venipuncture 1 h prior to testing, immediately after testing (5-10 min after cessation of noise exposure), and again 1 h post testing for cortisol analysis. Blood samples were collected into EDTA plasma tubes, centrifuged at 1645 x *g*, and stored at -80°C for later analysis. Plasma samples were analyzed in duplicate for cortisol using a commercial radioimmunoassay kit (MP Biomedicals, LLC, Solon, OH). The sensitivity reported for the radioimmunoassay was 1.7 ng/mL, and the intra- and inter-assay coefficients of variation were 5.3-8.9% and 7.5-9.3%, respectively.

Polar H10 (Polar Electro Inc., Bethpage, NY) heart rate sensors were used for the collection of heart rate (HR) and heart rate variability (HRV) parameters via Bluetooth connection to an iPhone app (Heart Rate Variability Logger, Marco Altini). Parameters measured are defined in Table 4.2. In general, HR will increase and HRV will decrease in response to stressful stimuli as a result of an increase in sympathetic nervous system activity (Kim et al., 2018). Thus, an effective treatment would be expected to decrease HR and increase HRV, indicating higher parasympathetic activity. Just prior to the open field and fireworks tests, the heart rate monitor bands were placed around the chest of the dogs immediately behind the front legs, with the rubberized surface placed ventrally immediately behind the left front leg. Electrode gel was applied liberally to the rubberized surface of the transmitter band to promote conductivity. Due to all dogs having short hair and the use of electrode gel, dogs did not have to be shaved to promote conductivity.

Two cameras mounted approximately 2 m from the floor on opposite corners of the testing room continuously recorded all video and audio data for each test. The

duration of behaviors given in Table 4.3 were logged by a single trained observer who was blinded to treatments using The Observer XT software (Noldus Information Technology Inc., Leesburg, VA). Three of the dogs included on the study had docked tails, and as such had no data on tail posture. The behaviors assessed were selected based on behavioral measures used in previous work evaluating canine anxiety and fear (Dreschel and Granger, 2005; Franzini de Souza et al., 2017; Korpivaara et al., 2017). Based on these previous studies, duration of fearful behaviors such as panting, cowering, and tail tuck were expected to increase during the fireworks test. Thus, an effective treatment was expected to decrease the duration of such fearful behaviors. Behaviors in different behavioral categories (i.e. Movement vs. Tail Posture) were not mutually exclusive, whereas behaviors within a behavioral category were mutually exclusive.

Statistical Analysis

The normality of data distribution was tested using the UNIVARIATE procedure in SAS 9.4 (SAS Institute, Cary, NC) on the residual of the data. In instances where data did not meet normality assumptions, statistical analysis was performed on the natural logarithm transformation of the data. However, data were then back transformed for reporting purposes. The standard error of the back transformed data was calculated from the confidence limits of the transformed data as follows: SEM = (back-transformed upper limit – back-transformed lower limit)/3.92. The denominator relates to the Z-value of a 95% confidence interval (± 1.96). Cowering, pacing, destruction, tail wagging, tail tucked, and all muzzle behaviors could not be analyzed due to insufficient occurrences that prevented data from meeting normality assumptions. With the exception of HR,

pNN50, and HF, parameters were not normally distributed and were analyzed using the natural logarithm of the data.

Blood cortisol was then analyzed using the MIXED procedure in SAS including the fixed effects of CBD, trazodone, period (Weeks 1 – 4), time (-60, 0, and 60 min), the interaction of CBD and trazodone, and the interaction of CBD by trazodone by time. Random effects included square and dog nested within square and repeated effect of time. All behavioral and HRV data from the 1-min immediately prior to (Pre-Noise) and the first min of the noise-induced fear response test (Noise) were also analyzed using the MIXED procedure in SAS including the fixed effects of CBD, trazodone, period (Weeks 1 – 4), time (Pre-Noise and Noise), and all accompanying interactions. Random effects included square and dog nested within square and repeated effect of time. Effects were considered significant when $P \leq 0.05$ and considered a tendency when $P \leq 0.10$.

Results

Blood Cortisol

There was an overall effect of period on blood cortisol ($P = 0.024$). Blood cortisol was reduced in period 1 compared to both periods 3 and 4 ($P = 0.003$ and 0.003, respectively), but was similar across all other periods ($P > 0.05$). Blood cortisol was unaffected by time of collection and CBD ($P = 0.189$, 0.104, respectively). Similarly, neither the CBD x trazodone, time x CBD, time x trazodone, nor the time x CBD x trazodone interactions affected blood cortisol ($P = 0.238$ 0.772, 0.667, and 0.812, respectively). However, trazodone lowered overall blood cortisol concentrations (Figure 4.1; $P < 0.0001$).

Heart Rate and Heart Rate Variability

There was a period effect on both HR and AVNN (Table 4.4; $P = 0.005$ and 0.046, respectively). Heart rate in period 4 tended to be lower than in period 1 ($P = 0.075$) and was lower than in periods 2 and 3 ($P = 0.004$ and 0.001, respectively). Heart rate was similar between periods 1, 2, and 3 ($P > 0.05$). The mean beat-to-beat intervals (AVNN) was increased in period 4 compared to all other periods ($P = 0.021$, 0.018, and 0.030, respectively), but was similar between all other periods ($P > 0.05$). All other HR and HRV variables were unaffected by period ($P > 0.05$).

With the exception of SDNN and RMSSD, HR and all other HRV variables were affected by the time point (Pre-Noise vs. Noise) (Table 4.4; $P < 0.05$). HR was lower during the Pre-Noise time point compared to the Noise time point ($P < 0.001$), while HRV parameters affected by time – AVNN, pNN50, LF, and HF – were all higher during the Pre-Noise time point than the Noise time point ($P < 0.05$). The LF/HF ratio tended ($P = 0.053$) to be higher in the Pre-Noise time point compared to the Noise time point.

CBD tended to increase overall HR (Table 4.4; $P = 0.093$) and decreased LF regardless of time point ($P = 0.011$). All treatments reduced HF compared to control ($P < 0.05$). AVNN, SDNN, RMSSD, and pNN50 were unaltered by CBD and trazodone ($P < 0.05$). No HRV variables were affected by the CBD by time nor the trazodone by time interaction ($P > 0.05$). The CBD by trazodone by time interaction influenced the LF/HF ratio ($P = 0.039$). During the Pre-Noise time point, trazodone tended ($P = 0.061$) to increase the LF/HF ratio compared to control and increased the LF/HF ratio compared to the combination of CBD and trazodone ($P = 0.038$). During the Noise time point, the

combination of CBD and trazodone tended ($P = 0.083$) to reduce the LF/HF ratio compared to control.

Behavior

There were no period effects on any behavioral variables (Table 4.5; $P > 0.05$). With the exception of Facing Door and Tail Relaxed, all other behaviors were affected by time point (Pre-Noise vs. Noise; $P < 0.05$). During the Noise time point, duration of inactivity ($P = 0.011$), Glancing Around ($P < 0.001$), and Ears Moving ($P < 0.001$) were increased compared to their duration during the Pre-Noise time point. Conversely, the duration of Other Eyes, Ears Relaxed, Ears Erect, and Tail Stiff were reduced during the Noise time point compared to the Pre-Noise time point ($P < 0.05$). Across both time points, dogs fed CBD tended ($P = 0.072$) to spend less time focused on something in the room (Other Eyes). Conversely, trazodone increased overall duration of Other Eyes ($P = 0.044$) and time spent with Tail Relaxed (P = 0.001), but CBD did not alter tail posture ($P = 0.753$). No behavioral variables were affected by the CBD by time nor the trazodone by time interaction ($P > 0.05$).

These changes between the Pre-Noise and Noise time points may indicate that the fireworks test generated the desired fearful behavioral response. However, the behaviors Glancing Around and Ears Moving could be considered a normal response to hearing a loud noise and may not necessarily indicate a fearful response to the noise. However, since the common fearful behaviors measured – cowering, pacing, vocalizations, etc. – could not be analyzed due to insufficient occurrences, it is difficult to determine if the fireworks test was severe enough to generate a fearful response.

Discussion

Since the passage of the Agriculture Improvement Act of 2018, which removed industrial hemp from the Controlled Substances Act and removed CBD from the Schedule I drug list, the market for industrial hemp-derived CBD has be able to expand considerably (Johnson, 2019). Just one year after the act passed, the market was estimated to be $1.2 billion and is expected to grow to over $10 billion by 2024 (Hemp Industry Daily, 2019). Much of this growth can be attributed to public perception of the supposed health benefits of CBD, including analgesic, antioxidant, anti-inflammatory, and anti-anxiety effects. However, despite general public opinion that CBD is a safe and effective treatment for these conditions, the lack of scientific clarity on the safety, dosage, and efficacy of CBD makes it critical for continued research in both humans and companion animals.

The present study is one of the first to describe the effect of CBD on the fear and anxiety response of dogs. The fear-response test was developed and validated by Araujo et al. (2013), in which dogs were placed in the test room for 9 min and a thunderstorm track was played from 3-6 min. A modified version of this test was used in the current study, with a fireworks track being used instead of a thunderstorm track as previous literature has shown a greater percentage of dogs to be fearful of fireworks than of thunderstorms (Blackwell et al., 2013). Additionally, because Araujo et al. (2013) saw no behavioral differences during the “after thunder” time period, the test for this study was shortened to 6 min, ending immediately after the fireworks track ended. This allowed for the immediate post-test blood sample collection to be obtained within 10 min of the end of the fireworks test.

If cortisol concentrations had decreased with each subsequent period of the experiment, it would have been an indication that the dogs were adapting to the sound stimulus. While there was a period effect on cortisol, it was due to cortisol in periods 3 and 4 being increased compared to period 1. This may indicate a heightened response to the sound stimulus upon repeated exposure, which suggests that the dogs were being conditioned to be stressed in the testing room despite being placed in the room on non-testing days to avoid such conditioning. The potential for conditioned place aversion is a limitation of the crossover design used in this study. It may be beneficial in future work to either include washout periods or utilize a different design to reduce the number of tests administered to each dog to prevent this conditioning; however, the latter would require a larger sample size than is needed when using a Latin Square design.

The lack of a time effect on blood cortisol concentration was also unexpected. It is possible that cortisol concentrations did not change because the fireworks test may not have produced a sufficient change in fear or stress in these dogs. However, Landsberg et al. (2015) demonstrated that the use of a thunderstorm noise-induced fear response test – also averaging 90 dB – resulted in a time-dependent change in blood cortisol, with higher concentrations 5 min post-test compared to 1 hour pre- and post-test samples. This time effect was not replicated in this study. Instead, cortisol concentrations decreased at each subsequent timepoint, though not enough to produce an overall effect of time. Other studies have also demonstrated that blood and saliva cortisol concentrations peak between 5 and 20 minutes after noise exposure and begin to decline as early as 30 minutes post-exposure (Franzini de Souza et al., 2017; 2018; Hydbring-Sandberg et al., 2004). For this study, while the blood sample taken immediately after the test was taken

within this window, it is possible that cortisol levels had not yet peaked after noise exposure. It would be beneficial in future work to take additional blood samples throughout the first hour after noise exposure to better show cortisol changes after noise exposure. Alternatively, it is also possible that the lack of time effect on cortisol may have been due to elevated initial stress due to the use of shelter animals. Franzini de Souza et al. (2017) demonstrated differences in endocrine and behavioral responses between laboratory and companion dogs in response to sound stimuli. While shelter animals were not represented in that study, it is possible that increased stress from the shelter environment, transport, and new environment could impact cortisol concentrations and warrants further investigation.

It is also possible that the time of testing influenced cortisol concentrations. Kolevská et al. (2003) showed that dogs not undergoing an exercise regimen had the highest blood cortisol concentrations between 1000 h and 1300 h and the lowest concentrations between 1600 h and 1900 h. A similar pattern was seen in this experiment, with the highest cortisol concentrations at the 60-min pre-test sample, which would have been taken between 1200 h and 1400 h, and the lowest concentrations at the 60-min post-test sample period, which would have been taken between 1400 h and 1600 h. While blood cortisol concentrations in samples taken from 1300 h to 1600 h were lower than those taken between 1000 h and 1300 h (Kolevská et al., 2003), that effect was not seen in this study. This could indicate that the noise-induced fear response test did in fact affect blood cortisol concentrations, maintaining the elevated levels through the afternoon rather than the normal drop expected from the circadian rhythm of the hormone. These results warrant further investigation, and future work should consider administering the

noise test earlier in the day to account for possible influence of the circadian rhythm of cortisol.

In humans, trazodone has been shown to decrease plasma cortisol concentrations compared with placebo and is commonly prescribed for the treatment of anxiety, depression, and to facilitate sleep (Gruen et al., 2014; Monteleone, 1991). While trazodone is not currently labeled for use in dogs, off-label use of trazodone is common for the treatment of anxiety disorders as well as to reduce the agitation and distress associated with post-surgery confinement and reduced exercise (Chea and Giorgi, 2017; Gruen and Sherman, 2008). In this experiment, treatment with trazodone lowered blood cortisol concentrations compared to all other treatments. On the other hand, CBD did not alter plasma cortisol concentrations compared to control in this experiment. In humans, CBD administration has been shown to attenuate the cortisol decrease associated with the circadian rhythm of the hormone (Appiah-Kusi et al., 2020; Zuardi et al., 1993). While other anxiolytic supplements seem to reduce anxiety in dogs at least in part by reducing the cortisol response to stressors (Landsberg et al., 2015), the results of this study may suggest that CBD does not exert an anti-anxiety effect by lowering blood cortisol concentrations. However, Hurd et al. (2019) demonstrated a decrease in salivary cortisol when CBD was dosed to humans at ~5 and 10 mg/kg BW, which may indicate that the CBD dosage selected for this study (1.4 mg/kg BW) was too low to exert an effect on cortisol.

Another possibility is that CBD was administered too early the day of the fireworks test. Recent work with other oral CBD products with similar dosages to this study demonstrated the time of maximum CBD concentration to be around 1.5 hours after

administration and the half-life of elimination to be between 1 and 4 hours (Bartner et al., 2018; Deabold et al., 2019; Gamble et al., 2018). However, at the time this study was completed (July 2018), these works on CBD pharmacokinetics had not yet been published, and earlier literature (Samara et al., 1988) reported much longer half-life for IV administration of CBD. This resulted in CBD treats being administered between 4 and 6 hours prior to the test in this study. In the future, it may be necessary to administer treatments within 2 hours of the noise test in order for CBD to have the greatest effect. This was accounted for in the administration of trazodone, as Jay et al. (2013) reported that the same dose of oral trazodone had a mean half-life of 166 min in dogs.

Even if CBD was administered too early to exert an anxiolytic effect, CBD did appear to inhibit the ability of trazodone to lower blood cortisol in the combination treatment compared with trazodone alone. This observation may support previous work that shows CBD to be a potent inhibitor of the cytochrome P450 family of enzymes, which is responsible for the metabolism of trazodone to its active metabolite, m-chlorophenylpiperazine, in the liver (Rotzinger et al., 1998; Yamaori et al., 2011). Several studies have highlighted these potential CBD-drug interactions as well as the lack of information regarding CBD doses that can be deemed safe for use – whether administered alone or in combination with other medications (Ewing et al., 2019; Foster et al., 2019; Iffland and Grotenhermen, 2017; Zendulka et al., 2016). The potential interaction between CBD and trazodone demonstrated in this study lends support to these concerns. While there has been some work investigating specific CBD-drug interactions (Manini et al., 2015), it may be inadvisable to administer CBD concomitantly with other products or medications until these interactions are more fully elucidated.

In agreement with previous work in both dogs and other species, oral CBD administration in this experiment was well-tolerated. No gastrointestinal or constitutional adverse events were observed in dogs receiving CBD during this study. Additionally, food consumption and body weight remained consistent throughout the experiment. However, other studies evaluating the safety of oral CBD administration in dogs have reported the potential for adverse events, including lethargy, gastrointestinal issues such as vomiting or diarrhea, and hematological changes such as increases in liver enzymes (Deabold et al., 2019; Gamble et al., 2018; McGrath et al., 2018; Vaughn et al., 2020). However, aside from initial bloodwork evaluated upon animal intake from the shelter, hematological changes were not evaluated during this experiment. As increases in liver enzymes may be indicative of altered liver function, the potential effects of oral CBD administration on clinical chemistry parameters should be monitored in future work.

Heart rate variability has been used as a measure of stress and anxiety in a number of animal species, including dogs. In particular, considerable work has been done using HRV as an indicator of canine fear and anxiety in response to stressful stimuli, in which HRV generally decreases and HR increases when animals are under stress, indicating impaired parasympathetic function and autonomic nervous system dysregulation (Craig et al., 2017; Gácsi et al., 2013; Wormald et al., 2017). The results of this study concur, showing increased HR and decreased HRV – AVNN, pNN50, LF, and HF – during the fireworks stimulus compared to the Pre-Noise time point when no sound was played. As AVNN represents the interval between heart beats, the decrease in AVNN was expected alongside the increase in HR during the fireworks stimulus. The pNN50 is thought to relate to parasympathetic activity and was also expected to decrease with increased stress

from the fireworks stimulus (Kim et al., 2018; Shaffer and Ginsberg, 2017). The low frequency band (LF) mainly reflects baroreceptor activity in the heart while at rest, but can be generated by parasympathetic, sympathetic, or baroreceptor activity depending on the situation. Unlike other HRV parameters, the LF band is expected to increase with stress as an increase in baroreceptor activity would be expected to accompany a rise in blood pressure (Kim et al., 2018; McCraty and Shaffer; 2015). This was not replicated in this study, where LF actually decreased during the fireworks stimuli. The high frequency band (HF), or respiratory band, corresponds to heart rate variations related to the respiratory cycle. Unlike LF, HF only reflects parasympathetic activity, and lower HF is correlated with stress and anxiety (Grossman and Taylor, 2007; Thayer et al., 2010). Because LF can be influenced by both sympathetic and parasympathetic activity while HF is only produced by parasympathetic activity, the LF/HF ratio has been used as a way to estimate sympathetic versus parasympathetic activity (Shaffer et al., 2014). An increased LF/HF ratio is thought to indicate higher sympathetic drive, which would be expected when exposed to stressful stimuli and has been demonstrated in dogs exposed to sound stimuli (Franzini de Souza et al., 2017; 2018; Maccariello et al., 2018). In this study, however, the LF/HF ratio tended to be reduced during the fireworks track compared to the Pre-Noise time point. This, combined with the reduction in LF, may indicate that the fireworks track was not sufficient to cause a fearful or stress response.

Additionally, the fireworks tract did not alter SDNN nor RMSSD in this study. The standard deviation of interbeat-intervals (SDNN) measures how interbeat-intervals change over time and has been shown to be reduced by stress (Kim et al., 2018; Schaffer and Ginsberg, 2017). As such, SDNN is generally measured over a 24 h collection

period, though short-term periods have also been used to evaluate short-term variability (Baek et al., 2015; Katayama et al., 2016). The RMSSD reflects beat-to-beat variance and is used to estimate vagally mediated changes in HRV, which reflects self-regulatory capacity (Shaffer et al., 2014). Reduced RMSSD has been associated with smoking, high LDL cholesterol, and work stress in humans and has been shown to be reduced in sound-sensitive dogs in response to sound exposure (Franzini de Souza et al., 2018; Thayer et al., 2010). As some of the findings of this study concur with previous work and other results conflict with what was expected upon exposure to fireworks, it is possible that the fireworks test was not successful in generating the desired fearful response. However, some of this conflicting evidence may be a result of the ultra-short time frame used for recording HRV, particularly for some variables that are more commonly measured over longer time periods. Future work should consider recording HRV over longer time frames in order to better assess changes. Only HR and AVNN were affected by the period of the experiment, where HR was reduced in period 4 and AVNN was increased in period 4 compared to all other periods. This may suggest that the dogs were acclimating to the fireworks stimulus, a limitation to this study design. Future work should consider either washout periods or a study design that does not require multiple noise-induced fear response tests in order to avoid this problem.

To our knowledge, no work has been done to evaluate the effect of CBD or trazodone administration on HRV in dogs, though there is some evidence that CBD may improve HRV in healthy humans (Schmid et al., 2010). Since an increase in stress and anxiety due to sound stimuli has been shown to increase HR, LF, and the LF/HF ratio while decreasing RMSSD, and HF (Franzini et al., 2017; 2018; Maccariello et al., 2018),

it was expected that both CBD and trazodone would attenuate these changes. In contrast to these expectations, both LF and HF were decreased by CBD in this study compared to control. Conversely, CBD tended to increase HR, while SDNN, RMSSD, and pNN50 were unaffected by treatment. While the reduction in LF would indicate that CBD attenuated the increase in cardiac sympathetic modulation, the increase in HR and decrease in HF suggest the opposite. Trazodone, again in contradiction to expectations, reduced overall HF in this study, tended to increase the LF/HF ratio during the Pre-Noise time point, and did not affect any other HRV parameters. The combination of CBD and trazodone also reduced HF compared to control and tended to reduce the LF/HF ratio compared to all other treatments during the Noise time point when the fireworks track was playing. The lack of effect on other HRV parameters such as SDNN and RMSSD may be due to the fireworks track not producing a change in these variables rather than a lack of treatment effect. These conflicting results warrant further investigation, particularly considering the lack of information available regarding the effects of both CBD and trazodone on HRV in dogs.

When the fireworks track started, there was a visible change in the demeanor of the dogs compared to both the open field test and the first 3-min block of the noise-induced fear response tests (Pre-Noise). While this may indicate that the fireworks track was able to generate the desired behavioral response, it is also possible that the change in behavior was a result of the dogs' interest in the noise rather than a fearful response. However, the considerable variability in the type of observed responses makes it difficult to elucidate whether the change was due to fear or if it was just a reflexive response. The predominant response was a decrease in activity, which may or may not have been

accompanied by a variety of other behaviors, such as a tucked tail, shaking, or nervous vocalizations like whining. These fearful behaviors would have been a better representation of the behavior changes due to the fireworks test as they have been used to evaluate such changes in other work (Franzini de Souza, 2017; 2018; Landsberg et al., 2015; Maccariello et al., 2018). However, these behaviors occurred too infrequently in this study to allow for statistical analysis. This may be indicative of a lack of behavioral response to the fireworks test. However, as all dogs were selected for this experiment based on the presentation of one or more fearful behaviors during baseline testing, this may simply highlight the variation in behavioral responses to sound exposure. Other anxiolytic supplements and medications have been shown to increase activity or distance travelled using this model (Araujo et al., 2013; Landsberg et al., 2015); however, neither CBD nor trazodone treatment changed activity compared to control. This is particularly surprising for the treatment groups receiving trazodone, which has previously been shown to visibly reduce behaviors associated with a number of stressful situations (Gilbert-Gregory et al., 2016; Gruen and Sherman, 2008; Herron and Shreyer, 2014). However, several of these studies relied on owner-completed surveys rather than objective data to assess effectiveness.

In contrast, CBD has been shown to reduce anxious behaviors in mouse, rat, and human models, but at this time there is little to no literature regarding its effect on canine behavior. In mouse and rat models, responses to threatening or unpleasant stimuli were assessed by several methods, including the elevated plus-maze, Vogel-conflict test, contextual fear conditioning, and elevated T maze (Campos et al., 2013; Marinho et al., 2015; Moreira et al., 2006). The use of these models has shown that intraperitoneal

administration of CBD in doses ranging from 1 to 20 mg/kg produced anxiolytic effects with some responses being dose-dependent (Blessing et al., 2015; Lee et al., 2017). Though different models of anxiety were used in rodents, this may indicate that a higher dose is necessary to produce the desired behavioral changes associated with reduced stress and anxiety, particularly if dosed orally due to the considerable first-pass effect on CBD in the liver (Samara et al., 1988; Trevaskis et al., 2009). Future research should investigate the effect of higher dosage of CBD for dogs above the dose tested in this study. Another important consideration is the time of CBD administration prior to noise exposure. As previously mentioned, oral CBD has been shown to have a half-life of less than 4 hours (Bartner et al., 2018; Deabold et al., 2019; Gamble et al., 2018), but CBD treats in this experiment were dosed between 4 and 6 hours of testing. Thus, it is possible that the dose used in this study would be sufficient to generate an anxiolytic effect if dosed closer to the fireworks test. Alternatively, CBD may need to be dosed for longer than 7 days in order to produce anxiolytic effects. Future investigation into these possibilities is warranted.

While there was no period effect on any behavioral variables, the lack of behavioral response to treatment could also have been due to acclimation of some of the animals to the firework track. While dogs were selected for inclusion into the study based on their reaction to the baseline noise-induced fear response test, it is possible that the weekly exposure to the stimulus diminished the reaction of some of the dogs during the later tests. This hypothesis is supported by the effect of period on other variables measured in this study, including plasma cortisol, HR, and AVNN. This highlights an important limitation of this study design, where time constraints prevented washout periods. To

avoid this issue in future work, dogs could be blocked by their reaction to the baseline test and assigned to just one treatment for the duration of the study. This would eliminate the need for multiple firework tests and would allow baseline and treatment tests to be spaced out over time but would also require a much larger sample size. However, considering the high level of variability in behavioral responses to the fireworks test, it would be difficult to ensure even distribution of dogs even with blocking. If feasible, it would be ideal to utilize the crossover design with longer washout periods to minimize the potential for acclimation to the stressful stimulus. The variability in behavioral responses also makes it difficult to quantify different fear responses. Several of the most common fearful behaviors (shaking, cowering, panting, etc.) were measured, but could not be analyzed due to insufficient occurrences, which may be accounted for in future work by aggregating such behaviors together into one behavioral category. The inclusion of a non-fearful control group should also be considered for future work as it would allow for better evaluation of changes in fearful behaviors in reactive dogs.

Conclusions

The results of the current study do not provide strong support of an anxiolytic effect of CBD in dogs when supplemented at 1.4 mg CBD/kg BW/d. Trazodone, but not CBD, decreased plasma cortisol concentration. When combined with trazodone, CBD appeared to attenuate the effects of trazodone on plasma cortisol. Cannabidiol decreased LF and HF, tended to increase HR, and tended to decrease duration of Other Eyes. Conversely, trazodone increased duration of Other Eyes, increased time spent with tail relaxed, reduced HF, increased the LF/HF ratio.

It would be beneficial in future studies to use increasing doses of CBD to clarify any potential anxiolytic effect, if present, and the dose necessary to elicit that effect. This study demonstrates the considerable variation in canine anxiety behaviors, which makes it difficult to accurately measure the response to treatments. It may be inadvisable to administer CBD concomitantly with other products or medications as the results from this study highlight potential drug interactions associated with CBD use. Considering the increased interest of CBD use in companion animals, continued research is essential to understanding the mechanisms by which CBD may exert anxiolytic effects as well as possible risks, like drug interactions, associated with CBD administration.

Tables and Figures

Table 4.1. Schedule of events.

Study Day	Key Event
-7 to -6	Animal intake, physical exam, and bloodwork (CBC/serum chemistry)
-5 to -3	Acclimation to diet, daily routine, and testing room
-2	Open Field Test
-1	Baseline Fireworks Test
1 to 4	Start of treatment 1 (Squares 1 – 4 started on consecutive days)
7 to 10	Period 1 Fireworks Test, start of treatment 2 evening after test
14 to 17	Period 2 Fireworks Test, start of treatment 3 evening after test
21 to 24	Period 3 Fireworks Test, start of treatment 4 evening after test
28 to 31	Period 4 Fireworks Test

Table 4.2. Definition of heart rate (HR) and heart rate variability (HRV) variables.

Variable	Definition
HR	Heart rate, bpm
AVNN	Mean beat-to-beat intervals, ms
SDNN	Standard deviation of beat-to-beat intervals, ms
RMSSD	Square root of the mean squared difference of successive RRs or inter-beat intervals, ms
pNN50	Percentage of successive RR intervals that differ by more than 50 ms, %
LF	Peak frequency of the low-frequency band (0.04 - 0.15 Hz)
HF	Peak frequency of the high-frequency band (0.15 - 0.40 Hz)
LF/HF	Ratio of LF-to-HF

Table 4.3. Ethogram of behaviors tracked by a single trained observer blinded to treatments using The Observer XT (Noldus Information Technology Inc., Leesburg, VA).

Behavioral Category	Behavior	Definition Used
Movement	Inactive	Standing still, sitting, or laying down
	Cowering	Sudden cessation of movement in response to a stimulus
	Pacing	Frantically moving back and forth, restlessness
	Destruction	Scratching or chewing at room furnishings
Eyes	Facing Door	Eyes are focused on the door of the room
	Glancing Around	Eyes are shifting back and forth, possibly looking for the source of a sound
	Other	Eyes are focused on something else in the room
Ears	Ears Relaxed	Ears are held in natural position
	Ears Erect	Ears raised in response to stimulus
	Ears Moving	Ears moving back and forth
Tail Posture	Tail Relaxed	Tail is not rigid and is lower than the top of the body
	Tail Stiff	Tail is rigid and horizontal
	Tail Wagging	Tail is wagging back and forth
	Tail Tucked	Tail is tucked between hind legs
Muzzle	Barking	Emitting a short, loud sound
	Whining	Emitting a long, high pitch sound, often repeated
	Panting	Mouth open wide with tongue protruding while breathing heavily
	Licking	Using the tongue on own body or another object
	Yawning	Opening the mouth wide and inhaling
	Biting	Using teeth on the door or object

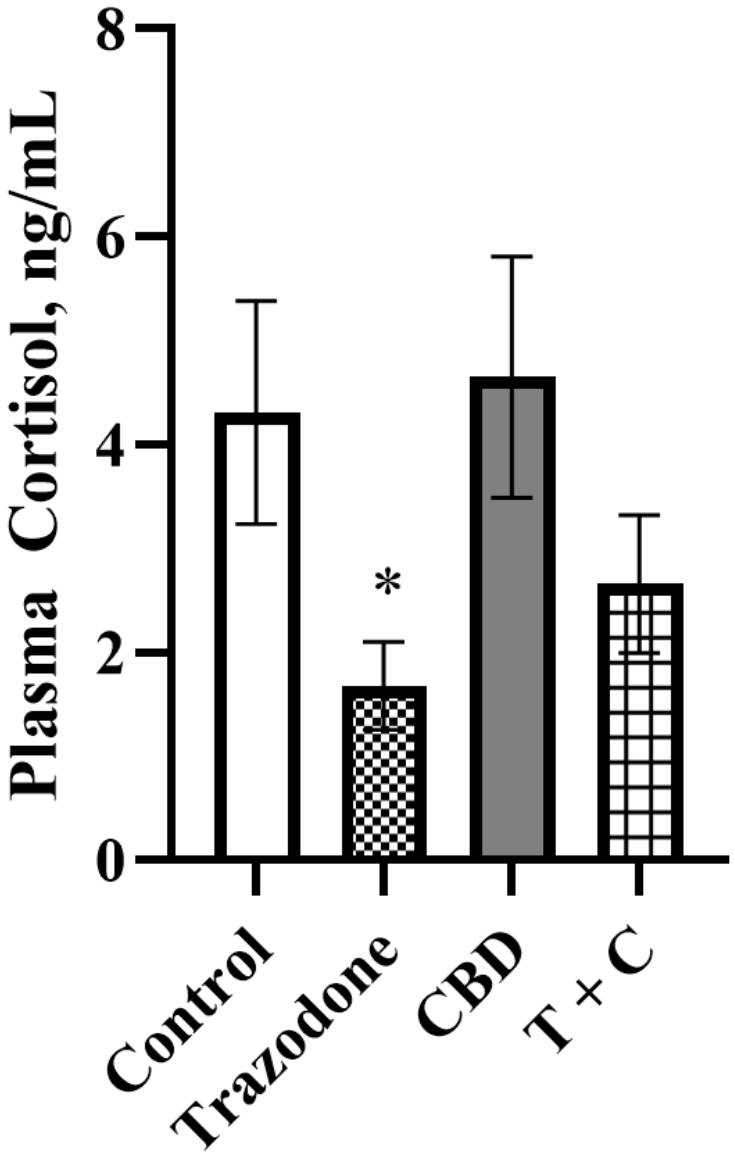


Figure 4.1. Cortisol concentration (ng/mL) for each treatment (n = 16), back transformed after analysis. Error bars represent the standard error of the treatment mean (SEM), which was calculated from the back-transformed confidence interval for each treatment: SEM = (upper limit – lower limit)/3.92. Due to lack of effect of time (P = 0.189) and any interactions with time (P > 0.05), all time points (Pre-Noise and Noise) have been combined. Trazodone treatment reduced overall cortisol concentration compared to control (P < 0.001), whereas there was no effect of CBD nor the CBD by trazodone interaction (P = 0.104, and 0.238, respectively). *Bars with asterisk differ from control at P < 0.001.

Table 4.4. Effect of trazodone (T), CBD (C), CBD by trazodone (C*T) interaction, time (Pre-Noise and Noise), CBD by trazodone by time (C*T*Time) interaction, and period on mean heart rate (HR) and heart rate variability (HRV) parameters for 1-minute immediately prior to (Pre-Noise) and the first minute (Noise) of the noise-induced fear response tests administered after each 7-d treatment period.

	Treatment					*P*-value					
Variable[1]	Control	Trazodone (T)	CBD (C)	T+C[2]	SE[3]	Trazodone	CBD	C*T	Time	C*T*Time	Period
HR, bpm	118.03	118.02	124.20	124.07	10.968	0.985	0.093	0.987	<.001	0.637	0.005
AVNN, ms	555.96	539.85	539.42	517.50	25.988	0.276	0.266	0.850	0.040	0.807	0.046
SDNN, ms	108.16	102.96	106.54	88.18	7.129	0.200	0.359	0.450	0.977	0.419	0.695
RMSSD, ms	100.35	89.28	91.70	69.71	12.649	0.130	0.189	0.538	0.366	0.654	0.538
pNN50, %	41.19	36.99	36.88	33.73	5.774	0.180	0.168	0.847	0.032	0.773	0.306
LF, Hz	0.090	0.062	0.050	0.048	0.0071	0.188	0.011	0.315	0.010	0.273	0.533
HF, Hz	0.142[a]	0.076[b]	0.059[b]	0.068[b]	0.0221	0.205	0.022	0.071	0.036	0.853	0.481
LF/HF Ratio	0.729	0.803	0.711	0.540	0.1052	0.595	0.126	0.183	0.053	0.039	0.908
Pre-Noise	0.651[a,b]	0.992[a*]	0.907[a,b]	0.607[b]	0.1402						
Noise	0.808[a]	0.615[a]	0.515[a]	0.474[a*]	0.1461						

[1] With the exception of HR, pNN50, and HF, variables were not normally distributed and were analyzed using the natural logarithm. Data were back transformed for reporting purposes. In the event of a treatment by time interaction, parameters are given as their treatment mean within each time point (Pre-Noise and Noise).

[2]Treatment T+C indicates the combination treatment of CBD and trazodone

[3]The standard error (SE) of the back transformed data was calculated from the confidence limits of the transformed data as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92.

[ab*]Within rows, values with different letters differ at $P \leq 0.05$ and asterisks indicate a trend at $P < 0.10$.

Table 4.5. Effect of trazodone (T), CBD (C), CBD by trazodone interaction (C*T), time (Pre-Noise and Noise), CBD by trazodone by time interaction (C*T*Time), and period on the duration of behavioral parameters (s) for 1-minute immediately prior to (Pre-Noise) and the first minute (Noise) of the noise-induced fear response tests administered after each 7-d treatment period.

	Treatment					*P*-value					
Variable[1], s	Control	Trazodone (T)	CBD (C)	T+C[2]	SE[3]	Trazodone	CBD	C*T	Time	C*T*Time	Period
Inactive	55.35	56.33	55.21	56.26	1.214	0.329	0.918	0.971	0.011	0.092	0.993
Facing Door	37.45	33.90	34.96	37.70	4.198	0.872	0.796	0.217	0.561	0.556	0.786
Glancing Around	16.90	15.65	17.93	15.91	3.460	0.396	0.736	0.841	<.001	0.142	0.819
Other Eyes	5.10	13.33	4.10	5.48	1.885	0.044	0.072	0.182	<.001	0.469	0.792
Ears Relaxed	11.37	7.76	12.35	11.43	4.913	0.179	0.168	0.422	<.001	0.868	0.567
Ears Erect	29.33	34.29	29.93	29.80	5.614	0.304	0.408	0.279	<.001	0.747	0.982
Ears Moving	19.25	17.79	17.20	18.78	2.076	0.970	0.742	0.351	<.001	0.457	0.493
Tail Relaxed	37.90	49.86	38.93	50.96	4.857	0.001	0.753	0.992	0.611	0.898	0.990
Tail Stiff	18.45	5.55	16.39	6.65	4.582	0.002	0.887	0.644	0.010	0.757	0.896

[1]Variables were not normally distributed and were analyzed using the natural logarithm. Data were back transformed for reporting purposes.

[2]Treatment T+C indicates the combination treatment of CBD and trazodone

[3]The standard error (SE) of the back transformed data was calculated from the confidence limits of the transformed data as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92.

CHAPTER 5. FEEDING CANNABIDIOL-CONTAINING TREATS DID NOT AFFECT CANINE DAILY VOLUNTARY ACTIVITY

Frontiers in Veterinary Science, 2021, 8:645667

Introduction

Pet owners and caretakers are increasingly interested in monitoring their animal's behavior and activity as indicators of health and well-being. While several subjective measures like the canine brief pain inventory and the Hudson visual analog scale are available for use (Brown et al., 2008; Hudson et al., 2004), the ability to measure activity through objective, non-invasive means such as accelerometers is a potentially preferable tool that can provide an impartial measure of animal activity (Dow et al., 2009; Eskander et al., 2020; Hansen et al., 2007; Michel and Brown, 2011). The use of accelerometers, kinesiology, and gait analysis are becoming popular methods by which to evaluate the health status of an animal as well as response to treatment. Several triaxial accelerometers have been validated for the measurement of canine activity and can be easily attached to a collar or harness for home use (den Uijl et al., 2017; Griffies et al., 2018; Preston et al., 2012; Yam et al., 2011). They have been used to evaluate the effectiveness of treatments for osteoarthritis and pruritic behaviors (Brown et al., 2010; Meijia et al., 2019; Muller et al., 2018; Wernimont et al., 2018), the effects of exercise and rest on the voluntary activity of active sled dogs (Robinson et al., 2020; 2021), and to predict rest in dogs and sleep in humans (Clarke and Fraser, 2015; Ladha and Hoffman, 2018; Nam et al., 2016).

Normal activity of healthy dogs is influenced by many factors, including breed, age, degree of socialization, and amount of exercise (Pickup et al., 2017; Rosado et al., 2012; Siwak et al., 2001). Additionally, canine activity may be negatively influenced by

factors such as disease, chronic illnesses like osteoarthritis, or behavioral issues such as anxiety (Brown et al., 2010; Overall et al., 2001; Tiira et al., 2016). There are also certain circumstances where canine activity needs to be reduced as a result of normal activity in high energy dogs, pruritic behaviors like scratching, or anxious behaviors like pacing and destruction. Activity may also need to be prevented or reduced following an illness, medical treatment or surgical procedure (Tiira et al., 2016; Wernimont et al., 2018). In such instances, many turn to medications like sedatives or antidepressants that have been shown to reduce canine activity (Gruen et al., 2014). However, some pet owners may be hesitant to use such medications due to potential side effects, cost, or personal bias against their use. Instead, they often investigate alternatives to conventional medications such as CBD (Kogan et al., 2016; 2018).

There has been considerable interest in the use of CBD for both humans and companion animals due to its reported benefits, such as analgesia, anti-inflammatory, anxiolytic, and sedative effects (Andre et al., 2016; Bonini et al., 2018; Huchelmann et al., 2017). The analgesic effect of CBD has been documented in rodent and human models (Costa et al., 2007; van de Donk et al., 2018; Yassin and Robinson, 2017), and the use of oral and transmucosal CBD oil formulations increased canine brief pain inventory (CBPI) and Hudson scores in dogs with osteoarthritis, suggesting an increase in activity and comfort with CBD use (Brioschi et al., 2020; Gamble et al., 2018). However, despite evidence of an anxiolytic effect of CBD in both rodents and humans with doses ranging from 2.5 to 10 mg/kg (Appian-Kusi et al., 2020; Campos et al., 2013; Crippa et al., 2011), a recent report failed to demonstrate an anxiolytic effect of treats containing 1.4 mg CBD/kg BW in dogs exposed to a noise-induced fear response test (Morris et al.,

2020). Other effects attributed to CBD, such as sedative effects, are thought to be biphasic. Larger doses have been shown to exert sedative effects in both rats and humans, whereas low doses of CBD may increase wakefulness (Babson et al., 2017; Chagas et al., 2013; Nicholson et al., 2004). While the effect of CBD on sedation has not been specifically investigated in a canine model, a preliminary investigation of the safety of escalating CBD doses in 20 healthy dogs reported mild constitutional adverse events recorded for dogs receiving 1.7-64.7 mg/kg CBD oil, which included both lethargy and hyperesthesia (Vaughn et al., 2020). A similar investigation into the safety of a 1:20 THC:CBD herbal extract reported mild neurological adverse events, like ataxia and delayed hopping, after single and multiple oral doses of 2 and 5 mg/kg CBD extract (Chicoine et al., 2020). While adverse events in both studies were mild and rare, they do highlight the potential for CBD to cause undesirable side effects as well as the need for continuing research evaluating the safety and efficacy of CBD use in dogs.

Despite the lack of scientific evidence demonstrating the safety and efficacy of CBD use in dogs, a recent survey of over 1000 dog owners recruited on social media showed that almost 80% of owners surveyed had purchased hemp or marijuana products for their dogs to provide pain relief, relieve anxiety, aid with sleep, and treating other health conditions. Many also indicated that they believed hemp products were more effective than conventional medications (Kogan et al., 2016; 2018). The study population included owners of both healthy and diseased animals as well as owners that either had or had not ever purchased hemp products for their dogs; however, this is likely an over-estimation of overall hemp use in companion animals due to the surveys being shared primarily within social media groups dedicated to cannabis use in pets. Nevertheless,

these surveys provide insight on the overwhelmingly favorable perceptions of pet owners on the safety and efficacy of CBD use in companion animals. Due to this interest in the use of CBD in companion animals, there is a critical need for further evaluation of CBD use in dogs and its potential effects on canine activity. Thus, the objective of the current study was to determine the impact of CBD on the daily activity of healthy adult dogs with the underlying hypothesis that CBD would reduce the overall daily activity of dogs compared with control. This hypothesis was tested using triaxial accelerometers to measure the activity of dogs receiving two levels of CBD administration compared to a control.

Materials and Methods

This study was approved by the LMU IACUC (protocol 1911-RES) prior to the start of the study. All housing and husbandry received were in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals (8th ed.), and all applicable LMU SOPs.

Subjects and Housing

Thirty neutered, adult dogs (15 male, 15 female, 9 months to 4 years old, 17.6 ± 3.4 kg) of various mixed breeds, including terrier, hound, Bassett, shepherd, Border Collie, Husky, cur, Labrador, Boxer, and Pug mixes were received at the LMU DVTC from a local shelter for inclusion in this study. The shelter was asked to provide dogs weighing 16 ± 4 kg. Additionally, the shelter was informed and gave consent for the use of the dogs for research purposes before their arrival. Before beginning the experiment, each dog had a complete blood count (CBC) and serum chemistry analysis (IDEXX Laboratories, Inc., Westbrook, ME) performed, along with a physical evaluation by a

veterinarian and a fecal examination to rule out any underlying disease that might preclude enrollment. Dogs were excluded if they demonstrated serious behavioral issues, such as extreme fear or aggression that would endanger research personnel, were severely emaciated or obese, classified as a body condition score < 3.5 or > 7.5 on a 9-point scale (where 1 is emaciated and 9 is obese; Laflamme, 1997), or if initial evaluations revealed an underlying disease that required more than routine treatments, such as heartworm infection, metabolic or infectious disease, and mobility issues. Three dogs were excluded due to positive heartworm tests and another three dogs were excluded for behavioral concerns. The remaining 24 dogs (12 male, 12 female, 9 months to 4 years old, 18.0 ± 3.4 kg) were selected for inclusion in the study. Dogs were individually housed in 1.2 x 1.8 m kennels within one of two dog wards at the LMU DVTC for the duration of the study.

Diets and Treatments

Dogs were fed Purina Pro Plan EN Gastroenteric Fiber Balance Dry Dog Food (Nestle Purina Inc., St. Louis, MO) to meet the daily metabolizable energy requirements of neutered adult dogs at maintenance, calculated as (70 * $BW^{0.75}$) * 1.6 and split into two meals per day fed between 0700 and 0900 h in the morning and between 1700 and 1900 h in the evening each day. Dogs were weighed and body condition scored (5-point scale) weekly and diets adjusted accordingly. Treatments were arranged in a randomized complete block design and consisted of 0 (placebo treats; **CON**), 34.0 ± 1.16 (**LOW**), or 75.6 ± 5.86 (**HIGH**) mg CBD/d. The CBD was the primary constituent of a proprietary industrial hemp isolate (AgTech Scientific, Paris, KY) that was incorporated into treats and administered in the form of 2 treats daily, each containing half the daily dose. Both control and CBD treats were composed of the following ingredients: chicken, chicken

liver, Asian carp, catfish, and – in the case of CBD treats – industrial hemp extract. While CBD was the primary constituent of the industrial hemp extract, trace Δ9-tetrahydrocannabinol (THC) was present in both LOW and HIGH treatments (1.1 ± 0.37 and 2.9 ± 0.22 mg THC/d, respectively). Treats were formulated to target CBD at doses of 2.5 and 5.0 mg/kg BW/d for LOW and HIGH treatments, respectively, based on an estimation that dogs would weigh an average of 16 kg. The LOW dose was selected based on previous literature that utilized a similar dose in dogs to assess single-dose pharmacokinetics of CBD and to evaluate its potential to alleviate pain in dogs with osteoarthritis (Gamble et al., 2018). That dose was then doubled to achieve the HIGH dosage. However, based on the mean BW of dogs included in the study and analysis of the treats, mean doses of CBD were 1.8 and 4.5 mg CBD/kg BW/d for LOW and HIGH treatments, respectively. Treats were offered solely as a reward upon kennel re-entry following twice-daily exercise, which occurred within 30 min of meals.

Experimental Design and Data Collection

Upon completion of intake exams, dogs underwent a 7-d acclimation period for adjustment to environment, diet, collars, and daily routine (Table 5.1). Kennels were maintained on a 12-h light schedule. Dogs received two 15-min exercise periods each day, with morning exercise occurring between 0700 and 0900 h and evening exercise occurring between 1700 and 1900 h. During exercise periods, dogs that were aggressive towards other dogs were individually hand-walked by research personnel; all other dogs were allowed to exercise freely in playgroups of 2 – 4 dogs in one of two grassy enclosures. The number of dogs being hand-walked and in playgroups was balanced across all treatments. Four hours each day – 1000 to 1200 h (**AM**) and 1330 to 1530 h

(**PM**) – were designated as the time when no persons were allowed to enter the kennels. Two of those hours were designated as Quiet time, and the other two as Music time where calming classical music was played over speakers in each kennel. Quiet and Music sessions were randomly allotted to either AM or PM times each day. All dogs started receiving control treats (0 mg CBD) twice daily as a reward for kennel re-entry after the twice-daily exercise.

After the acclimation period, Vetrax® activity sensors (AgLogica Holdings, Norcross, GA) were fitted to dogs' collars using the attachment provided by the manufacturer and placed ventral to the mandible. These triaxial accelerometers were used for the continuous collection of activity variables – activity points, activity duration (min), duration of no activity (h), duration of resting (h), running duration (min), walking duration (min), scratching duration (s), head shaking duration (s), and sleep quality (Table 5.2). Data collected by the sensors was automatically uploaded to the Vetrax® server via Wi-Fi once an hour for behavior algorithm processing, which has been previously validated (Griffies et al., 2018). Except for a weekly consistent 2-3 h charging period, sensors remained on the dogs at all times. Before the start of the experiment, data were collected over a 14-d baseline period to block dogs by mean daily activity – high (mean 118.6 min; range 88.6-157.5 min) or low (mean 59.3 min, range 30.2-85.1 min) – before stratifying dogs by age, weight and sex and randomly assigning dogs within each block to treatments. Dogs were stratified by treatment and sex, evenly distributed between the two wards, and adapted to treatments for 7 d before another 14-d collection of activity via Vetrax® sensors (Table 5.1).

Consumption of food and treats, consistency of stool, frequency of elimination, subjective assessment of activity during exercise, mucus membrane color, and other indicators of general health status were monitored twice daily by research personnel. Evidence of any adverse event – defined as any symptom occurrence that would not be expected in normal dogs – was also monitored. However, no adverse events were observed in any dogs following the administration of CBD treats during this study.

Statistical Analysis

Based on variation in activity and behaviors reported in previous work using these sensors (Griffies et al., 2018; Wernimont et al., 2018), it was calculated that $n = 8$ dogs/treatment was sufficient to detect a 25% change with a 16% CV (Berndtson, 1991). Activity monitors for two of the dogs in the control group (one in the high activity block and one in the low activity block) spontaneously stopped transmitting halfway through the treatment period, and activity data from the last 7 d of the experiment for those two dogs were lost.

The normality of the residuals was tested using the UNIVARIATE procedure in SAS 9.4 (SAS Institute, Cary, NC). In instances where data did not meet normality assumptions, statistical analysis was performed on transformed data. However, data were then back-transformed for reporting purposes. The standard error of the back-transformed data was calculated from the confidence limits of the transformed data as follows: SEM = (back-transformed upper limit – back-transformed lower limit)/3.92. The denominator relates to the Z-value of a 95% confidence interval (± 1.96). For the baseline period, activity duration, running, scratching, and head shaking were not normally distributed

and were log-transformed for statistical analysis. Activity points and walking were not normally distributed and were transformed into the square root for statistical analysis.

During the baseline period, dogs allotted to CBD treatments tended ($P = 0.061$) to run more than control; thus, the mean duration of running from the baseline period was utilized as a covariate for the duration of running in the treatment period. All other variables were similar across treatments in the baseline period. For overall daily activity during the treatment period, running, scratching, and head shaking were not normally distributed and were log-transformed for statistical analysis. For Quiet and Music session activity periods, all variables – except for No Activity and Resting – were not normally distributed and were log-transformed for statistical analysis. For exercise activity periods, activity points, activity duration, and scratching were not normally distributed and were log-transformed for statistical analysis, whereas running and head shaking were not normally distributed and were transformed to the cube root for statistical analysis.

From the treatment period, overall daily activity and activity during exercise periods (0700-0900 h and 1700-1900 h) were analyzed using the MIXED procedure in SAS including the fixed effects of treatment, day, and the treatment by day interaction. Dog nested within activity block (high or low) was included as a random effect and day was included as a repeated measure with dog nested within treatment as the subject. Activity during the Quiet and Music sessions were analyzed using the MIXED procedure in SAS including the fixed effects of treatment, day, session (Quiet or Music), time of day (AM or PM), and all accompanying interactions. Dog nested within the activity block was again included as a random effect. Time (AM or PM) was included as a repeated measure with dog nested within treatment as the subject. Treatment effects are described

as the contrast between CON and both CBD treatments and the contrast between LOW and HIGH CBD treatments. Results are presented as the mean ± SE. Effects were considered significant when $P \leq 0.05$ and considered a tendency when $P < 0.10$.

Results

Total Daily Activity

CBD did not alter total activity points, activity duration, no activity, resting, running, walking, head shaking, or sleep quality compared to CON (Table 5.3; $P > 0.05$). However, CBD tended to reduce scratching compared with CON ($P = 0.071$) but was not different between LOW and HIGH treatments ($P = 0.209$). Level of CBD inclusion (LOW vs. HIGH) did not affect any variables measured ($P > 0.05$). With the exceptions of activity duration, running, walking, and scratching, all variables were affected by day of treatment ($P < 0.05$), but no treatment by day interactions were observed ($P > 0.05$).

Quiet and Music Session Activity

Overall, dogs were more active in the PM sessions than the AM, with all variables affected by the time of day (Table 5.4; $P < 0.001$). Activity points, activity duration, running, walking, and resting increased in the PM compared to the AM ($P < 0.001$) while the duration of No Activity decreased in the PM compared to AM ($P < 0.001$). During these sessions, the Music session tended to reduce activity points ($P = 0.055$) and running ($P = 0.098$) compared to the Quiet session. The Music session reduced activity duration ($P = 0.002$), walking ($P < 0.001$), and resting ($P = 0.045$) while increasing duration of no activity ($P = 0.014$) compared to the Quiet session.

Session by time interactions were observed for activity points, no activity, and resting (Table 5.4; $P < 0.05$), and a trend for session by time interactions was observed for activity duration, running, and walking ($P = 0.076$, 0.078, and 0.084, respectively). Type of session (Quiet or Music) did not alter activity points or duration of activity during the AM session ($P = 0.502$ and 0.522, respectively). When the Quiet session was allotted to the PM, however, activity points ($P = 0.002$), duration of activity ($P < 0.001$), resting ($P < 0.001$), running ($P < 0.001$), and walking ($P < 0.001$) were increased compared to when the Music session was allotted to the PM. Duration of No Activity was similar between Quiet and Music sessions when allotted to the AM ($P = 0.230$) but was increased in the Music session compared to the Quiet session when allotted to the PM ($P < 0.001$).

Activity points, running, and head shaking were unaffected by treatment, and all treatment interactions during the Quiet and Music sessions (Table 5.4; $P > 0.05$). Scratching was reduced by CBD during Quiet and Music sessions compared to CON ($P = 0.030$), but the level of CBD inclusion did not affect time spent scratching ($P = 0.612$).

A treatment by time interaction was observed for No Activity and resting (Table 5.4; $P = 0.013$ and 0.006, respectively) and a trend for a treatment by time interaction was observed for activity and walking duration ($P = 0.079$ and 0.060, respectively). Regardless of Quiet or Music session, activity duration was similar across treatments in the AM ($P > 0.05$), but dogs receiving HIGH CBD tended (1.44 ± 0.172 min; $P = 0.091$) to be less active than CON (2.64 ± 0.324 min) in the PM and tended (1.37 ± 0.162 min; $P = 0.059$) to walk less than CON (2.60 ± 0.326 min) in the PM. Similarly, duration of No Activity was unaffected by treatment in the AM ($P > 0.05$) but in the PM tended to

increase in the HIGH CBD treatment (1.13 ± 0.060 h) compared to both CON (0.95 ± 0.063 h) and LOW (0.97 ± 0.060 h) treatments ($P = 0.054$ and 0.068, respectively). Conversely, resting duration increased in the LOW treatment (0.96 ± 0.051 h) compared to the HIGH treatment (0.80 ± 0.051 h) in the PM ($P = 0.038$), but was similar across all other time points and treatments ($P > 0.05$). No treatment by session nor treatment by session by time interactions were observed ($P > 0.05$) for any variables measured. All activity variables were affected by day of treatment period ($P < 0.05$), but no treatment by day interactions were observed ($P > 0.05$).

Exercise Activity

Neither CBD treatment nor inclusion level affected any variables measured during the exercise periods (Table 5.5; $P > 0.05$). Day of treatment period tended ($P = 0.066$) to affect scratching and affected head shaking ($P = 0.003$), but no other variables were impacted by day of treatment ($P > 0.05$). Additionally, no treatment by day interactions were observed ($P > 0.05$).

Discussion

Triaxial accelerometer sensors were used in this study to determine the effect of daily CBD dosing on activity in healthy adult dogs by measuring daily activity, pruritic behaviors, and an assessment of rest and sleep quality. The objective of this study was to evaluate the impact of CBD on the daily activity of healthy adult dogs with the hypothesis that CBD would reduce overall daily activity compared to control. However, results showed that oral CBD administration did not alter the overall daily activity of healthy adult dogs. The lack of effect on overall daily activity and sleep quality was unexpected based on previous reports of sedative and hypnogenic effects of CBD in

rodent, human, and canine models. In humans and rats, CBD doses ranging from approximately 2 to 40 mg/kg BW/d have been reported to induce sedative effects, improve sleep quality, and increase total sleep time (Chagas et al., 2013; Carlini and Cunha, 1981; Zuardi et al., 1993). However, more recent work has reported CBD to have no influence on the sleep cycle in humans, (Linares et al., 2018) and others argue that CBD by itself does not produce sedative effects but rather modulates the sedative effect of Δ9-tetrahydrocannabinol (THC), even if THC is only present in minute amounts (Kesner and Lovinger, 2020; Nicholson et al., 2004; Spindle et al., 2020).

The potential for the sedative effect of CBD to be caused by the presence of THC may be supported by an escalating dose study in dogs where placebo, CBD-predominant, THC-predominant, and CBD/THC combination oils were administered to dogs to evaluate the occurrence and severity of adverse events after administration (Vaughn et al., 2020). Doses for the CBD-predominant oil started at 1.7 mg CBD/kg BW/d and was incrementally increased to a maximum of 64.7 mg CBD/kg BW/d over 30 days. Lethargy was reported with the CBD-predominant oil formulation, however, that oil was not THC-free; it was reported to contain 0.7 mg/mL THC (Vaughn et al., 2020). The industrial hemp extract included in the CBD treats used in this experiment contained similar THC content as the oil reported in Vaughn et al. (2020) but did not produce a similar effect. The reason for these conflicting results remains unclear. These differences could be due to the difference in animals utilized for the study – shelter vs. research-bred dogs – or the different modes of delivery – eating a treat versus oral gavage of an oil. There have been reports of variation in the pharmacokinetics of CBD depending on the mode of delivery. In one experiment, CBD-infused oil demonstrated increased maximum plasma CBD

concentration than the same dose administered as microencapsulated oil beads and a CBD-infused transdermal cream (Bartner et al., 2018). Other reports using similar doses of oral CBD oil and chews report an increased maximum plasma CBD concentration when administered as a chew compared to an oil; however, this has yet to be investigated in a single, controlled experiment (Deabold et al., 2019; Gamble et al., 2018) Additionally, dogs used in Vaughn et al. (2020) fasted before administration of the CBD oil whereas dogs in the current experiment consumed CBD treats within 30 min of a meal. It has been suggested that administering cannabinoids with a fat-meal increased bioavailability (Zgair et al., 2017). Since the CBD used in Vaughn et al. (2020) was mixed in a lipid-based formulation, it is unclear if these differences in methodology would lead to the difference in sedative effects observed between their report and the current study. Additional investigation using THC-free CBD is needed to evaluate the potential for CBD to exert a sedative effect in dogs.

While there was no observed effect on overall daily activity with CBD treatment, it tended to influence activity during different times of the day. Dogs in the current study were more active in the PM than the AM regardless of treatment and type of session. Playing calming music in the kennels (Music session) did reduce activity compared to when no music was played (Quiet session), which supports previous work showing that playing music can reduce stress and increase relaxed behaviors in kenneled dogs (Amaya et al., 2020; Bowman et al., 2017; Engler and Bain, 2017). This effect, however, appears to be independent of the effect of CBD as there was no interaction between treatment and session nor a treatment by session by time interaction. The tendency for dogs in the HIGH CBD treatment to be less active than CON dogs in the PM may indicate that CBD

exerted some sedative or calming effect on the dogs. However, this potential sedative effect was expected to be observed in the AM as previous pharmacokinetic reports showed a half-life for CBD of 1-4 h (Deabold et al., 2019; Bartner et al., 2018; Gamble et al., 2018; Wakshlag et al., 2020). As this effect was not observed during the AM sessions, exercise periods, nor overall daily activity, these collective results do not support a sedative or calming effect of CBD in dogs. Thus, the claim that CBD exerts a sedative or calming effect in dogs remains unsubstantiated, but further investigation may provide clarification to these results.

In the present study, dogs were necessarily regimented into a strict schedule of daily activities. It is possible that in a setting where dogs were entirely free to choose their activities, such as a home, the outcome could have been different. The strict, consistent schedule of the kennel environment did not allow for much activity outside of scheduled exercise periods, which may have prevented normally high-energy dogs from being as active as they might be with consistent free access to more space. Conversely, shelter environments have been shown to increase activity in dogs compared to a home environment and may prevent dogs from resting due to increased stress (Hoffman et al., 2019; Part et al., 2014). This may have artificially increased activity in dogs that would have otherwise been less active. As a result, it may be preferable to evaluate the effect of these treatments in familiar environments that have more space for dogs to exhibit normal activity and rest behaviors. Additionally, the small sample size and use of healthy adult dogs were limitations of this study. The dogs included in this study exhibited high variability in voluntary activity despite being blocked by baseline activity and having no known mobility or behavioral issues. These limitations may preclude extrapolation of

these results to other canine populations. Since CBD is often used to increase comfort and activity in dogs with mobility issues like osteoarthritis or to decrease the activity of anxious or hyperactive dogs (Kogan et al., 2018), future work should evaluate voluntary activity in animals with mobility or behavioral issues like osteoarthritis or anxiety.

Results from this study suggest a potential antipruritic effect of CBD. Phytocannabinoids like CBD act on the body through the endocannabinoid system (ECS), which is a signaling system including endocannabinoids like anandamide and 2-arachidonylglycerol, their receptors, and regulatory enzymes (Pacher et al., 2006). The ECS helps regulate metabolic homeostasis, thermoregulation, epidermal homeostasis, and more (Avila et al., 2020; Bellocchio et al., 2008). While CBD has little to no affinity for CB1 and CB2 ECS receptors, it is a known agonist for the transient receptor potential vanilloid family of receptors (TRPV1-4), which are known ECS receptors widely expressed in the skin and plays a role in itch sensation (Avila et al., 2020; Bisogno et al., 2001; Caterina and Pang, 2016; Tóth et al., 2019). As TRPV1 is rapidly desensitized after activation, it is thought that CBD may exert antipruritic effects by keeping TRPV1 desensitized, thus preventing neuronal activation by irritants (Imamachi et al., 2009; Muller et al., 2019; Xie and Hu, 2018).

Additionally, CBD has been shown to be an antagonist for transient receptor potential melastatin 8 (TRPM8) receptors (De Petrocellis et al., 2008; Muller et al., 2019). In the skin, TRPM8 is responsible for environmental cold detection and has been suggested to contribute to the perception of pain and itch, which may indicate it is another target for the potential antipruritic effect of CBD (Caterina and Pang, 2016; Jankowski et al., 2017). The antipruritic effect of cannabinoids has been observed in

humans (Dvorak et al., 2003; Visse et al., 2017; Yuan et al., 2014), but this is the first report of a potential antipruritic effect of CBD in dogs as a reduction in scratching duration was observed in dogs. While this experiment was not designed to assess the antipruritic effect of CBD, these results may suggest a potential for CBD to be beneficial in the treatment of skin conditions and pruritic behaviors in dogs. To investigate this potential effect, it would be beneficial for future work to specifically examine the effect of CBD in dogs with skin issues such as allergies, atopic dermatitis, or unexplained pruritus.

Conclusions

The results of the current study indicate that when supplemented with up to 4.5 mg/kg BW/d, CBD does not impact the overall daily activity of adult dogs. Total daily activity including duration of the activity, sleep quality, and resting were unaffected by CBD. Similarly, activity during exercise periods was also unaffected by CBD. During Quiet and Music session periods, 4.5 mg CBD/kg BW/d tended to reduce activity in dogs compared to both 1.8 mg CBD/kg BW/d and CON, but this did not translate to an overall daily effect. Playing classical music in the kennels reduced activity compared to having no music played but did not alter the response to CBD. Cannabidiol reduced total daily scratching as well as scratching during Quiet and Music sessions, which may indicate a possible antipruritic effect. Future work examining the effect of CBD on activity is warranted, particularly in dogs with mobility and behavioral issues like osteoarthritis and anxiety. Additionally, the potential antipruritic effect of CBD should be investigated using dogs with dermatological issues like skin allergies or atopic dermatitis.

Tables and Figures

Table 5.1 Schedule of events for monitoring activity in dogs receiving cannabidiol containing treats.

Day of Study	Event	Data Collection	Treats
-2 and -1	Intake and initial health exams	None	Control
1 to 7	Acclimation	None	Control
8 to 21	Baseline Period Activity Collection	Vetrax activated	Control
22 to 28	Treatment Adaptation	None	Treatment
29 to 43	Treatment Period Activity Collection	Vetrax activated	Treatment

Table 5.2. Activity variables measured by Vetrax® activity sensors (AgLogica Holdings, Inc., Norcross, GA).

Variable	Definition
Activity Points	Total activity of dogs weighted by each individual activity (ex. running worth more points than walking), calculated using a proprietary algorithm.
Activity, min	Duration of total activity including running and walking.
No Activity, h	Duration of complete inactivity.
Resting, h	Duration of time not actively walking or running, but not completely inactive.
Running, min	Duration of running.
Walking, min	Duration of walking.
Scratching, s	Time spent scratching.
Head Shaking, s	Time spent shaking head.
Sleep	Scale of sleep quality measured using a proprietary algorithm based on absence of night-time disturbance; scaled 0-100 with 100 being undisturbed sleep.

Table 5.3. Effect of treatment (TRT), day, and TRT*day interaction on total daily activity variables collected via Vetrax® activity sensors (AgLogica Technology, Norcross, GA). Treatment effects are shown as the contrast between control (CON) and both CBD treatments (CBD) and the contrast between CBD treatments (LOW v. HIGH).

	Treatment				*P*-value			
Variable[1]	Control (CON)	1.8 mg CBD/kg BW/d (LOW)	4.5 mg CBD/kg BW/d (HIGH)	SE[2]	CON v. CBD	LOW v. HIGH	Day	TRT*Day
Activity Points	56834	57079	56634	4952.5	0.985	0.950	0.017	0.857
Activity, min	80.1	82.7	82.0	11.90	0.882	0.966	0.421	0.773
No Activity, h	13.6	13.9	14.3	0.58	0.496	0.652	0.002	0.394
Resting, h	8.8	8.5	8.1	0.43	0.364	0.513	<.001	0.389
Running, min	5.8	5.6	6.9	0.36	0.612	0.172	0.172	0.447
Walking, min	76.2	76.5	71.8	10.94	0.878	0.760	0.531	0.749
Scratching, s	69.6	35.8	51.9	7.33	0.071	0.209	0.162	0.485
Head Shaking, s	32.4	27.3	42.4	5.49	0.878	0.229	0.006	0.194
Sleep Quality	77.2	76.1	75.1	2.21	0.500	0.722	0.029	0.679

[1]Running, Scratching, and Shaking were not normally distributed and were transformed for statistical analysis. Data were back-transformed for reporting purposes.
[2]Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92.

Table 5.4. Effect of treatment (TRT), day, session (Quiet or Music), time of day (AM or PM), and all relevant interactions on activity variables collected via Vetrax® activity sensors (AgLogica Technology, Norcross, GA) from 1000-1200 h (AM) and 1330-1530 h (PM) each day. Treatment effects are shown as the contrast between control (CON) and both CBD treatments (CBD) and the contrast between CBD treatments (LOW v. HIGH).

	Treatment				*P*-value							
Variable[1]	Control (CON)	1.8 mg CBD/kg BW/d (LOW)	4.5 mg CBD/kg BW/d (HIGH)	SE[2]	CON vs. CBD	LOW vs. HIGH	Time of Day	Session	Session *Time	TRT* Time	TRT* Session	Day
Activity Points	2884	2509	2308	148.6	0.152	0.550	<.001	0.055	0.014	0.287	0.465	<.001
Activity, min	1.23	0.87	0.85	0.144	0.204	0.937	<.001	0.002	0.076	0.079	0.257	<.001
No Activity, h	1.21	1.25	1.34	0.059	0.273	0.327	<.001	0.014	<.001	0.013	0.443	0.002
Resting, h	0.71	0.70	0.62	0.050	0.384	0.234	<.001	0.045	<.001	0.006	0.203	0.002
Running, min	0.04	0.03	0.04	0.006	0.602	0.544	<.001	0.098	0.078	0.369	0.834	0.223
Walking, min	1.29	0.87	0.82	0.148	0.138	0.857	<.001	<.001	0.084	0.060	0.217	<.001
Scratching, s	4.18	2.29	2.62	0.413	0.030	0.612	<.001	0.930	0.274	0.326	0.886	0.730
Head Shaking, s	1.78	1.65	1.82	0.128	0.882	0.600	<.001	0.926	0.305	0.319	0.212	0.375

[1]Except for No Activity and Resting, variables were not normally distributed and were transformed for statistical analysis. Data were back-transformed for reporting purposes. There was no effect of the treatment by day nor the Session by time by treatment interactions on any variable ($P > 0.05$) and thus are not shown.

[2]Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92.

Table 5.5. Effect of treatment (TRT), day, and TRT*day interactions on activity parameters collected via Vetrax® activity sensors (AgLogica Technology, Norcross, GA) during the 2 periods of daily exercise, which included all data from 0700-0900 h and 1700-1900 h each day. Treatment effects are shown as the contrast between control (CON) and both CBD treatments (CBD) and the contrast between CBD treatments (LOW v. HIGH).

	Treatment				*P*-value			
Variable[1]	Control (CON)	1.8 mg/kg BW/d CBD (LOW)	4.5 mg/kg BW/d CBD (HIGH)	SE[2]	CON v. CBD	LOW v. HIGH	Day	TRT*Day
Activity Points	21736	25735	26122	2096.8	0.143	0.910	0.117	0.283
Activity, min	37.66	48.09	48.07	3.213	0.143	0.998	0.528	0.305
No Activity, h	0.82	0.75	0.77	0.132	0.708	0.899	0.359	0.842
Resting, h	2.47	2.24	2.31	0.116	0.312	0.838	0.338	0.847
Running, min	4.9	4.5	5.3	0.31	0.940	0.298	0.258	0.531
Walking, min	35.0	43.8	40.7	0.12	0.207	0.446	0.958	0.576
Scratching, s	21.1	14.1	20.3	2.53	0.442	0.267	0.066	0.875
Head Shaking, s	17.8	14.7	25.8	0.27	0.682	0.167	0.003	0.273

[1]Except for No Activity and Resting, variables were not normally distributed and were transformed for statistical analysis. Data were back-transformed for reporting purposes.
[2]Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92.

CHAPTER 6. ALTERATION OF THE CANINE METABOLOME AFTER A THREE-WEEK SUPPLEMENTATION OF CANNABIDIOL-CONTAINING TREATS

Frontiers in Veterinary Science, 2021, 8:685606;
Journal of Animal Science and Research, 2021, 5(2)

Introduction

Mass spectrometry-based metabolomics has been increasingly used to assess the health and status of animals and to analyze metabolic alterations caused by diet, disease, or other factors (Sethi and Brietzke, 2015; Ogunade et al., 2018; Yang et al., 2018). Targeted metabolomics can be used to quantify defined groups of metabolites, whereas untargeted metabolomics provides a comprehensive analysis of all measurable analytes in a sample, including any unknowns (Adeyemi et al., 2019; Roberts et al., 2012). In instances where the specific metabolites of interest are unknown, untargeted metabolomics can also be used to discover specific biomarkers for later use in targeted metabolomics studies as well as pathway analysis (Xia et al., 2013; Goldansaz et al., 2020). In recent years, Chemical isotope labeling (CIL) liquid chromatography mass spectrometry (LC–MS)-based untargeted metabolomics has provided an opportunity to analyze metabolites based on chemical groups, including metabolites containing amine/phenol chemical groups, which are common intermediate products of amino acid metabolism; metabolites containing carbonyl groups, which are common intermediate products of energy metabolism; metabolites containing carboxyl groups, including fatty acids and their derivatives; and metabolites containing hydroxyl groups, which include important biological compounds like homones (Ogunade et al., 2021; Zhao et al., 2016; 2019).

To date, there has been no evaluation of the effect of CBD on the canine metabolome. Therefore, the objective of this explorative study was to evaluate the impact of CBD supplementation on the canine metabolome with the underlying hypothesis that after 3 weeks of supplementation, CBD would alter the canine metabolome compared with control. This was accomplished through the use of untargeted metabolomics and biomarker analysis of amine/phenol-, carbonyl-, carboxyl-, and hydroxyl-containing metabolites.

Materials and Methods

This study was approved by the LMU IACUC (protocol 1911-RES) before the start of the study. All housing and husbandry received were in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals (8th ed.), and all applicable LMU SOPs.

Subjects and Housing

Sixteen dogs (8 male, 8 female, 9 months to 4 years of age, 18.2 ± 3.4 kg BW) of various mixed breeds, including terrier, hound, Bassett, shepherd, Border Collie, Husky, cur, Labrador, Boxer, and Pug mixes were received from a local shelter for inclusion in this study. The shelter was asked to provide dogs weighing 16 ± 4 kg. Additionally, the shelter informed of and gave consent for the use of the dogs for research purposes before their arrival. Prior to beginning the experiment, each dog had a CBC and serum chemistry analysis (IDEXX Laboratories, Inc., Westbrook, Maine) performed, along with physical evaluation by the attending veterinarian and a fecal examination to rule out any underlying disease that might preclude enrollment. Dogs were excluded if they demonstrated serious behavioral issues, such as aggression that would endanger research

personnel, were severely emaciated, classified as a body condition score < 3.5 or > 7.5 on a 9-point scale (where 1 is emaciated and 9 is obese; Laflamme, 1997), or if their initial evaluations revealed an underlying disease that required more than routine treatments (such as heartworm positive dogs). Dogs were individually housed in 1.2 x 1.8 m kennels within one of two dog wards at the LMU DVTC. Dogs were stratified by treatment and sex and evenly distributed between the two wards. Dogs were fed Purina Pro Plan EN Gastroenteric Fiber Balance Dry Dog Food to meet the daily metabolizable energy requirements of neutered adult dogs at maintenance, calculated as $(70 * BW^{0.75}) * 1.6$ and split into two meals per day. Dogs were weighed and body condition scored (5-point scale) weekly for the adjustment of diets. Dogs arrived from the shelter and were started on the study diet more than 37 days prior to starting treatments and 58 days before collecting samples for this study.

Experimental Design and Treatments

These dogs were participating in a concurrent study evaluating the impact of CBD on canine voluntary activity (Morris et al., 2021) with treatments consisting of 0 (placebo treats; **CON**) or 75.6 ± 5.86 mg CBD/d (**CBD**). Dogs were blocked by baseline activity before being stratified by age, weight, and sex and randomly assigned to treatments within each block. The CBD was the primary constituent of a proprietary industrial hemp isolate (AgTech Scientific, Paris, KY) that was incorporated into treats and administered in the form of 2 treats daily, each containing half the daily dose. Both CON and CBD treats were composed of the following ingredients: chicken, chicken liver, Asian carp, catfish, and in the case of the CBD treats, industrial hemp extract. Cannabidiol was the primary constituent of the industrial hemp extract; however, trace THC was present in the

CBD treatment (2.9 ± 0.22 mg THC/d). Based on the mean BW of dogs included in the study and analysis of the treats, mean dose of CBD was 4.5 ± 0.77 mg CBD/kg BW/d. Treats were offered solely as a reward upon kennel re-entry following twice-daily exercise, which was within 30 min of meals.

Blood Sample Collection

After 21 d of treatment administration, approximately 6 mL of blood was collected via cephalic catheter or jugular venipuncture approximately 2 hours after the final treat administration. The selection of this time point was based on previous work demonstrating the half-life of elimination of CBD to be between 1 and 4 hours after oral administration (Bartner et al., 2018; Deabold et al., 2019; Gamble et al., 2018). Blood samples were collected into tubes containing sodium heparin and were immediately centrifuged at 1645 x *g* for 10 min. Plasma was collected after centrifugation then stored at -20°C (<12 hours) before long-term storage at -80°C.

CIL/LC-MS-Based Untargeted Metabolomics Analysis

Untargeted metabolomic profiling was done using a CIL/LC-MS-based technique with an Agilent 1100 LC system (Palo Alto, CA) connected to a Bruker Impact HD quadrupole time-of-flight (QTOF) MS (Billerica, MA). This technique uses a differential isotope labelling (^{12}C and ^{13}C-labelling) to separate metabolites based on chemical groups followed by LC-MS analysis (Zhao et al., 2019). Detailed information regarding sample preparation, labeling, normalization, LC-UV and LC-MS setup, and metabolite quantification have been reported elsewhere (Wu and Li, 2012; Mung and Li, 2017). Typical coefficient of variation for this high-performance chemical isotope labeling LC-

MS method for metabolome analysis is in the range of 5–10% for individual metabolites (Guo and Li, 2009; Zhao et al., 2016; Zhao et al., 2017). In this study, the amine/phenol-, carbonyl-, carboxyl-, and hydroxyl-containing metabolites were analyzed. A total of 19 LC-MS data files were generated (3 quality control samples, 8 CBD samples, and 8 CON samples).

Metabolite Data Processing

Raw data processing on the 19 LC-MS data files was performed using ISOMS Pro 1.0 according to procedures described by Mung and Li (2017). Peak pairs whose mean (sample) / mean (blank) was ≤ 4.0 were filtered out. Peak pairs with no data present in at least 80% of the samples were filtered out. The final metabolite-intensity table was generated using IsoMS-Quant (Huan and Li, 2015).

Metabolite Identification

A two-tier identification approach was used to perform metabolite identification. In tier 1, peak pairs were searched against a chemical isotope labeled metabolite library (CIL Library) based on accurate mass and retention time (Huan and Li, 2015). In tier 2, a linked identity library (LI Library) was used for identification of the remaining peak pairs. The LI Library includes over 2000 human endogenous metabolites from 68 metabolic pathways, providing high-confidence putative identification results based on accurate mass and predicted retention time matches (Li et al., 2013).

Statistical Analysis

The final metabolite intensity tables for the amine/phenol-, carbonyl-, carboxyl-, and hydroxyl-containing metabolome were imported separately into MetaboAnalyst 5.0

software package (www.metaboanalyst.ca; Chong et al., 2019) for statistical analysis. Prior to statistical analysis, the data were log-transformed, normalized by median, and auto-scaled. Median scaling was performed to eliminate unwanted inter-sample variations to make the individual samples more comparable to each other. Auto-scaling was used to make the metabolites more comparable in magnitude to each other.

Univariate (volcano plot) and multivariate analysis (Partial least squares discriminant analysis [PLS-DA] scores plot) were then generated to identify overall treatment differences across the multivariate dataset. The volcano plot was constructed by plotting the fold change (FC; CBD/CON) of each metabolite against its *P*-value. The volcano plot was constructed by plotting the FC (CBD/CON) of each metabolite against its adjusted *P*-value. Metabolites with FC ≥ 1.2 or ≤ 0.83 having a false discovery ratio (FDR) ≤ 0.05 were considered to be differentially increased or decreased relative to CON, respectively.

The utility of the metabolites with FC ≥ 1.2 or ≤ 0.83 and FDR ≤ 0.05 to serve as potential biomarkers of the effects of CBD was tested using receiver operating characteristic (ROC) curves as calculated by the ROCCET web server using MetaboAnalyst 5.0 software package. Metabolites with an area under ROC (AUROC) ≥ 0.90 and a $P \leq 0.05$ were considered excellent biomarkers as defined in Xia et al. (2013).

Results

Amine/Phenol Metabolites

Within the amine/phenol analysis, a total of 2681 unique peak pairs (representing different compounds) were detected. Of those peak pairs, 134 metabolites were positively

identified in tier 1 (CIL Library; Supplementary Table 6.1) and 103 metabolites were putatively identified with high confidence in tier 2 (LI Library; Supplementary Table 6.2). The PLS-DA scores plot (Figure 6.1A) shows clear separation between CON and CBD samples, and the permutation test ($P < 0.01$) confirms the validity of the PLS-DA model (Supplementary Figure 6.1).

Volcano plot analysis showed that 32 metabolites were differentially altered (FC ≥ 1.2 or ≤ 0.83, FDR ≤ 0.05) by CBD (Table 6.1; Figure 6.1B). Eighteen of those metabolites – pyrimidodiazepine, 4-amino-4-deoxychorismate, isoferulic acid, an isomer of D-glucosamine, 7-carboxy-7-carbaguanine, 2,4-dihydroxyhept-2-enedioate, ascorbate, 2'-deamino-2'-hydroxy-6'-dehydroparomamine, trans-2,3-dihydroxycinnamate, gamma-glutamyl-gamma-aminobutyraldehyde, 1,4-diaminobutane, tyramine, an isomer of 2-deoxy-scyllo-inosamine, isoleucyl-alanine, 3,4-hydroxyphenylpyruvate, aspartyl-threonine, vanillic acid, and D-lysopine – were differentially increased (FC ≥ 1.2, FDR ≤ 0.05) by CBD. The other 14 metabolites – N-acetyl-L-asparagine, alanyl-proline, asparaginyl-aspartic acid, seryl-aspartic acid, phenylalanyl-glycine, prolyl-glutamine, *o*-tyrosine, N-acetyl-L-adrenaline, L-threo-3-methylaspartate, Z-3-peroxyaminoacrylate, L-glutamate-5-semialdehyde, 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylate, gamma-aminobutyric acid, and aspartyl-glutamine – were differentially reduced (FC ≤ 0.83, FDR ≤ 0.05) by CBD compared to CON.

Univariate ROC analysis of the 32 identified amine/phenol-containing metabolites that were differentially increased or decreased by CBD revealed 24 metabolites – aspartyl-glutamine, gamma-aminobutyric acid, gamma-glutamyl-gamma-aminobutyraldehyde, L-glutamate-5-semialdehyde, prolyl-glutamine, pyrimidodiazepine,

4-amino-4-deoxychorismate, trans-2,3-dihydroxycinnamate, alanyl-proline, N-acetyl-L-asparagine, (Z)-3-peroxyaminoacrylate, 1,4-diaminobutane, 2'-deamino-2'-hydroxy-6'-dehydroparomamine, ascorbate, D-lysopine, *o*-tyrosine, phenylalanyl-glycine, 2,4-dihydroxyhept-2-enedioate, asparaginyl-aspartic acid, isoferulic acid, 7-carboxy-7-carbaguanine, 3-(4-hydroxyphenyl)pyruvate, aspartyl-threonine, and isoleucyl-alanine – that appear to be highly predictive of the metabolomic changes between CBD and CON (AUROC ≥ 0.90; $P < 0.001$; Figure 6.2).

Carbonyl Metabolites

Within the carbonyl analysis, a total of 612 unique peak pairs were detected. Of those peak pairs, 6 peak pairs were positively identified in tier 1 (CIL Library; Supplementary Table 6.1) and 15 peak pairs were putatively identified with high confidence in tier 2 (LI Library; Supplementary Table 6.2). The PLS-DA scores plot (Figure 6.3A) shows clear separation between CON and CBD samples, and the permutation test ($P < 0.01$) confirms the validity of the PLS-DA model (Supplementary Figure 6.2).

Volcano plot analysis showed that 5 metabolites were differentially altered (FC ≥ 1.2 or ≤ 0.83, FDR ≤ 0.05) by CBD (Figure 6.3B; Table 6.2). Glucose and 2-formylglutarate were differentially increased (FC ≥ 1.2, FDR ≤ 0.05) by CBD, while glyceraldehyde, isomer of glyceraldehyde, and 4-oxoglutaramate were differentially reduced (FC ≤ 0.83, FDR ≤ 0.05) by CBD compared to control.

Univariate ROC analysis of the 5 carbonyl metabolites positively and putatively identified that were differentially altered by CBD revealed that plasma glucose appears to

be highly predictive of the metabolomic changes between CBD and CON (AUROC = 0.91; $P = 0.020$; Figure 6.4).

Carboxyl Metabolites

Within the carboxyl analysis, a total of 2943 unique peak pairs were detected. Of those peak pairs, 29 peak pairs were positively identified in tier 1 (CIL Library; Supplementary Table 6.1) and 144 peak pairs were putatively identified with high confidence in tier 2 (LI Library; Supplementary Table 6.2). The PLS-DA scores plot (Figure 6.5A) shows clear separation between CON and CBD samples, and the permutation test ($P = 0.02$) confirms the validity of the PLS-DA model (Supplementary Figure 6.3).

Volcano plot analysis showed that 42 metabolites were differentially altered (FC ≥ 1.2 or ≤ 0.83, FDR ≤ 0.05) by CBD (Figure 6.5B; Table 6.3) Twenty-eight metabolites – 2,5-dioxopentanoate, isomer of hydroxypropionic acid, 4-cumarate, isomer of D-glycerate, acetic acid, D-glycerate, isomer of glycolate, isomer of threonate, 5-deoxy-D-glucuronate, citramalic acid, glycolate, 3-oxopropanoate, ethyl malonate, isomer of 4-oxopropanoate, isomer of 3-hydroxybutyric acid, 2-hydroxy-3-oxopropanoate, hydroxyisobutyric acid, isovaleric acid, butyric acid, glyoxylate, nicotinate, 3-hydroxybutyric acid, (S)-5-amino-3-oxohexanoic acid, hydroxypropionic acid, lactic acid, isomer of lactic acid, and isovanillic acid – were differentially increased (FC ≥ 1.2, FDR ≤ 0.05) by CBD. Fourteen metabolites – 4-oxoproline, 2-aminomuconate semialdehyde, isomer of 9-oxononanoic acid, L-1-pyrroline-3-hydroxy-5-carboxylate, (S)-4-amino-5-oxopentanoate, isomer of 1-aminocyclopropane-1-carboxylate, L-allothreonine, arginine, isomer of aspartate, isomer of jasmonic acid, isojasmonic acid, 2-

oxo-4-phenylbutyric acid, 9,10-12,13-diepoxyoctadecanoate, jasmonic acid, and isomer of 1-pyrroline-2-carboxylate – were differentially reduced (FC ≤ 0.83, FDR ≤ 0.05) by CBD compared to control.

Univariate analysis of the 42 carboxyl metabolites positively and putatively identified that were differentially increased or decreased by CBD revealed that 23 metabolites – jasmonic acid, (S)-4-amino-5-oxopentanoate, 3,4-dihydroxymandelic acid, isomer of 1-aminocyclopropane-1-carboxylate, isomer of hydroxypropionic acid, L-1-pyrroline-3-hydroxy-5-carboxylate, L-allothreonine, 2-oxo-4-phenylbutyric acid, arginine, citramalic acid, 4-oxoproline, 5-deoxy-D-glucuronate, 9,10-12,13-diepoxyoctadecanoate, isomer of 1-pyrroline-2-carboxylate, isomer of aspartate, 2-aminomuconate semialdehyde, isomer of 9-oxononanoic acid, acetic acid, ethyl malonate, isomer of jasmonic acid, 3-oxopropanoate, D-glycerate, and isomer of D-glycerate – appear to be highly predictive of the metabolomic changes between CBD and CON (AUROC ≥ 0.90; $P < 0.001$; Figure 6.6).

Hydroxyl Metabolites

Within the hydroxyl analysis, a total of 3759 unique peak pairs were detected. Of those peak pairs, 141 peak pairs were positively identified in tier 1 (CIL Library; Supplementary Table 6.1) and 65 peak pairs were putatively identified with high confidence in tier 2 (LI Library; Supplementary Table 6.2). The PLS-DA scores plot (Figure 6.7A) shows clear separation between CON and CBD samples, and the permutation test ($P < 0.01$) confirms the validity of the PLS-DA model (Supplementary Figure 6.4).

Volcano plot analysis showed that 32 metabolites were differentially altered (FC ≥ 1.2 or ≤ 0.83, FDR ≤ 0.05) by CBD (Figure 6.7B; Table 6.4). Fifteen metabolites – 17α,20α-dihydroxypregn-4-en-3-one, D-tagatose, 3,4-dihydroxyphenylpropanoate, L-rhamnono-1,4-lactone, L-rhamnofuranose, isomer of L-rhamnofuranose, 6-deoxy-L-galactose, D-galactosamine, sepiapterin, isomer of sepiapterin, L-fuculose, isomer of deoxyadenosine, deoxyadenosine, and 3-hydroxy-L-proline – were differentially increased (FC ≥ 1.5, FDR ≤ 0.05) by CBD. Eighteen metabolites – N-acetyl-trans-3-hydroxy-L-proline, ethanolamine, 2,3-dihydroxyindole, N2'-acetyl-3'-hydroxykynurenamine, cyanate, isomer of cyanate, glycerol, alpha-ribazole, isomer of 3-phenoxybenzyl alcohol, dihydroshikonofuran, glycoaldehyde, N-acetyl-2-carboxy-2,3-dihydro-5,6-dihydroxyindole, allotetrahydrodeoxycorticosterone 3-O-glucuronide, phenethyl alcohol, propane-1,3-diol, cortolone, arabitol, and cortisol 21-O-sulfate – were differentially reduced (FC ≤ 0.83, FDR ≤ 0.05) by CBD compared to control.

Univariate analysis of the 35 hydroxyl metabolites positively and putatively identified that were differentially increased or decreased by CBD revealed that 15 metabolites – L-fuculose, glyceraldehyde, L-rhamnofuranose, L-rhamnono-1,4-lactone, arabitol, D-tagatose, propane-1,3-diol, 6-deoxy-L-galactose, D-galactosamine, phenethyl alcohol, isomer of L-rhamnofuranose, isomer of 3-phenoxybenzyl alcohol, 3,4-dihydroxyphenylpropanoate, cortolone, and isomer of deoxyadenosine – appear to be highly predictive of the metabolomic changes between CBD and CON (AUROC ≥ 0.90; $P < 0.001$; Figure 6.8).

Discussion

Amino Acid Metabolism

Increased concentrations of tyramine, 3-(4-hydroxyphenyl)pyruvate, 2,4-dihydroxyhept-2-enedioate, gamma-glutamyl-gamma-aminobutyraldehyde, 1,4-diaminobutane, 2,5-dioxopentanoate, 3-hydroxy-L-proline, D-lysopine, D-glycerate, isomer of D-glycerate, glycolate, isomer of glycolate, glyoxylate, 2-hydroxy-3-oxopropanoate, glycolaldehyde 3,4-dihydroxymandelic acid, hydroxyisobutyric acid, isovaleric acid, butyric acid, S-5-amino-3-oxohexanoic acid, 3-hydroxypropionic acid, and isomer of hydroxypropionic acid indicate that CBD altered amino acid metabolism.

Tyramine, 3-(4-hydroxyphenyl)pyruvate, and 2,4-dihydroxyhept-2-enedioate are intermediates in tyrosine and phenylalanine metabolism (Lehmann and Pollmann, 2009; Miller and Litwack, 1971; Wang et al., 2010). Tyramine, in particular, is also involved in the biosynthesis of many secondary metabolites in plants, such as isoquinoline alkaloids, flavonoids, and hydroxycinnamic acid amines (Leonard et al., 2020; Sato et al., 2007).

Gamma-glutamyl-gamma-aminobutyraldehyde, 2,5-dioxopentanoate, 3-hydroxy-L-proline, and 1,4-diaminobutane (i.e. putrescene) are intermediates in arginine, proline, hydroxyproline, and ornithine degradation pathways (Kurihara et al., 2005; Jo et al., 2008; Visser et al., 2012; Watanabe et al., 2012). Putrescene is also known to play a role in the regulation of cell growth, protein synthesis, apoptosis, and other cellular processes (Igarashi and Kashiwagi, 2010; Larqué et al., 2007). 2,5-dioxopentanoate can be converted into 2-oxoglutarate, by which it can enter the citric acid cycle through conversion into succinyl-CoA in several prokaryotic organisms, including several species

of *Pseudomonas*, *Escherichia coli*, and *Haloferax volcanii* (Brouns et al., 2006; Johnsen et al., 2009; Watanabe et al., 2006).

D-lysopine is an amino opine derivative of L-lysine found in crown gall tumors produced by pathogenic bacteria that infect plants, including *C. sativa*. While not known to be produced in mammalian systems, other opines like saccharopine are known intermediates in the metabolism of lysine in mammals (Darling and Larsen, 1961; Lippincott et al., 1972; Moore et al., 1997). 3,4-Dihydroxymandelic acid is a metabolite of norepinephrine with potent antioxidant activity (Ley et al., 2002). If the increase in 3,4-dihydroxymandelic acid, it is possible that its increase may contribute to the antioxidant effect of CBD, which warrants further investigation.

Hydroxyisobutyric acid is an intermediate in valine degradation that in humans is used as a biomarker of 3-hydroxyisobutyric aciduria and methylmalonic semialdehyde dehydrogenase deficiency, rare metabolic diseases (Podebrad et al., 2000). In dogs, alpha-hydroxyisobutyric acid was shown to be upregulated in a small group of dogs with diabetes compared to healthy controls (O'Kell et al., 2017), though it was not identified as a potential CBD biomarker in this study. Isovaleric acid is a branched-chain fatty acid intermediate in leucine catabolism (Parimoo and Tanaka, 1993), and S-5-Amino-3-oxohexanoic acid is an intermediate in lysine degradation (Bellinzoni et al., 2011; Kreimeyer et al., 2007).

D-glycerate is an essential intermediate in the catabolism of glycine, serine, and threonine in plants. Once formed, D-glycerate can then feed into either glyoxylate metabolism via hydroxypyruvate or be converted into 3-phospho-D-glycerate and fed into glycolysis (Bartsch et al., 2008; Randall and Tolbert, 1971). In humans with a

glycerate kinase mutation, D-glycerate levels are elevated to the point of acidemia which, if left untreated, can lead to progressive neurological impairment, hypotonia, seizures, failure to thrive, and metabolic acidosis (Guo et al., 2006; Sass et al., 2010). In plants, glycolate is an intermediate in glyoxylate metabolism, converting hydroxypyruvate into glyoxylate for further metabolism in the glyoxylate cycle (Kisaki and Tolbert, 1969). In mammalian systems, glyoxylate is produced either through oxidation of glycolate in peroxisomes or the catabolism of hydroxyproline before being converted into glycine via alanine-glyoxylate aminotransferases present in peroxisomes (Belostotsky et al., 2012; Salido et al., 2012). The glyoxylate cycle, a pathway that converts fatty acids into glucose once believed to be absent in mammalian systems, may be present in the liver (Davis and Goodman, 1992; Song, 2000). Genetic defects in glyoxylate metabolic enzymes have been attributed to metabolic diseases like primary hyperoxalurias and insulin resistance, though this has not been investigated in canine models (Salido et al., 2012; Song, 2000). Additionally, plasma glyoxylate has been indicated as an early marker for type II diabetes development in humans (Nikiforova et al., 2014; Padberg et al., 2014). 2-Hydroxy-3-oxopropanoate and glycolaldehyde are additional intermediates in glyoxylate metabolism (Barkulis and Krakow, 1956; Gupta and Vennesland, 1964). Glycolaldehyde is also precursor to pyridoxine synthesis and is an intermediate in folate synthesis in bacteria (Vella et al., 1980). The increase in these metabolites may suggest that CBD enhanced amino acid degradation.

Decreased concentrations of N-acetyl-L-asparagine, o-tyrosine, N-acetyl-L-adrenaline, L-threo-3-methylaspartate, L-glutamate-5-semialdehyde, 4-oxoglutaramate, 4-oxoproline, L-1-pyrroline-3-hydroxy-5-carboxylate, isomer of 1-pyrroline-2-

carboxylate, N-acetyl-trans-3-Hydroxy-L-proline, arginine, 2-aminomuconate semialdehyde, isomer of 1-aminocyclopropane-1-carboxylate, L-allothreonine, isomer of aspartate, 2-oxo-4-phenylbutyric acid, 2,3-dihydroxyindole, N2'-acetyl-3'-hydroxykynurenamine, cyanate, isomer of cyanate, and N-acetyl-2-Carboxy-2,3-dihydro-5,6-dihydroxyindole may also suggest that CBD altered amino acid metabolism. N-acetyl-L-asparagine is a derivative of asparagine that is N-acetylated by N-acetyltransferase 1 (NAT1), one of several acetyltransferases known to play a role in drug metabolism (Carlisle et al., 2018). This enzyme has been suggested to play a role in the regulation of mTOR complex I activation, cancer cell proliferation, and mitochondrial function (Butcher and Minchin, 2012; Camporez et al., 2017; Carlisle et al., 2018). As CBD is suspected to exert an anti-cancer effect, it may be prudent in future work to investigate if CBD supplementation alters NAT1 activity.

o-Tyrosine is a structural isomer of tyrosine and a phenylalanine derivative. It is considered a marker for oxidative stress as it is produced through free-radical hydroxylation of phenylalanine (Molnar et al., 2005; 2016). If the decrease in *o*-tyrosine was due to CBD supplementation, this may contribute to the suspected antioxidative effects of CBD. N-acetyl-L-adrenaline is a methylated form of epinephrine, an adrenal hormone involved in the regulation of visceral functions (Malenka et al., 2015; Smith et al., 1992). 2-Aminomuconate semialdehyde, 2,3-dihydroxyindole, and N2'-acetyl-3'-hydroxykynurenamide are intermediates in tryptophan metabolism. 2-Aminomuconate is part of the kynurenine pathway, a metabolic pathway used for NAD biosynthesis (Colabroy et al., 2005; Nishizuka et al., 1965). Kynurenine pathway metabolites like 2-aminomuconate are thought to help regulate processes like immune cell response,

neuronal excitability, and host-microbiome signaling (Cervenka et al., 2017). N2'-Acetyl-3'-Hydroxykynurenamine is an acetylated intermediate in tryptophan metabolism (Thiele et al., 2013). 2-3-Dihydroxyindole is an intermediate in an indole degradation pathway present in several bacterial species but is not known as a mammalian metabolite (Ma et al., 2018). 2-Oxo-4-phenylbutyric acid is an intermediate in phenylalanine, tyrosine, and tryptophan metabolism that, in microbial species, is a precursor to homophenylalanine (Koketsu et al., 2013); however, this pathway is not known to be present in mammalian species. N-Acetyl-2-Carboxy-2,3-dihydro-5,6-dihydroxyindole (i.e. leucodopachrome) is an intermediate in tyrosine metabolism and in betalain melanogenesis pathway (Land et al., 2003; Olivares et al., 2001). While these results would suggest an alteration of phenolic-containing amino acid metabolism by CBD, the implications remain unclear as the relative concentrations of phenylalanine, tyrosine, and tryptophan remained unchanged by treatment.

L-Glutamate-5-semialdehyde is a non-proteinogenic amino acid that is an intermediate in both proline and arginine biosynthesis from glutamate (Fons et al., 1991; Ginguay et al., 2017). L-1-pyrroline-3-hydroxy-5-carboxylate, and 1-pyrroline-2-carboxylate are intermediates in arginine and proline metabolism (Abaskharon et al., 2019; Hu et al., 1996; Watanabe et al., 2016). N-Acetyl-trans-3-hydroxy-L-proline (i.e. oxaceprol) is a derivative of L-proline that is an established anti-inflammatory drug used in the treatment of osteoarthritis (Durg et al., 2019). Combined with the decrease in arginine, these results suggest an impact of CBD on arginine and proline metabolism; however, since the relative concentration of proline was unaffected by treatment, the biological significance is unclear. 4-oxoglutaramate is an intermediate in one of the

histidine catabolism pathways that leads to the production of 2-oxoglutarate (i.e. α-ketoglutarate), which then feeds into the citric acid cycle (Brown and Kies, 1959; Hassall and Greenberg, 1963).

L-threo-3-methylaspartate is an amino acid formed by glutamate mutase and can be metabolized by methylaspartate ammonia-lyase. It is found in the structures of the antibiotics friulimicin and vicenistatin and in carbon metabolism of haloarchaea (Khomyakova et al., 2011; Raj and Poelarends, 2013). Cyanate is an intermediate in nitrogen metabolism that can be produced spontaneously from urea. It has been suggested to improve insulin sensitivity and potentially exert antioxidative effects (Kang et al., 2018). Aspartyl-glutamine, aspartyl-threonine, alanyl-proline, asparaginyl-aspartic acid, isoleucyl-alanine, phenylalanyl-glycine, prolyl-glutamine, and seryl-glycine are products of the incomplete breakdown of protein digestion or catabolism. While some dipeptides are known to have physiological or cell-signaling effects, none of the affected dipeptides have been identified as one of these bioactive molecules (Naka et al., 2015; Nakato et al., 2019).

The altered concentrations of these metabolites suggest an effect of CBD on amino acid metabolism. However, since the relative concentrations of the individual amino acids like glutamate and proline were unaffected by treatment, the biological significance of the changes in these metabolites is unclear. Additional research is needed to assess the potential for CBD to alter amino acid metabolism.

Carbohydrate Metabolism

The increase in glucose, lactic acid, acetic acid, an isomer of glucosamine, 2'-deamino-2'-hydroxy-6'-dehydroparomamine, an isomer of 2-deoxy-schyllo-inosamine, D-glycerate, D-tagatose, D-galactosamine, L-rhamnono-1,4-lactone, L-rhamnofuranose, L-fuculose, 6-deoxy-L-galactose, and 5-deoxy-D-glucuronate may suggest that carbohydrate metabolism was altered by CBD. The endocannabinoid system (ECS) – by which CBD and other cannabinoids exert physiological effects – plays a well-established role in glucose and energy metabolism, marking it as a target for the treatment of metabolic diseases like type 2 diabetes (Bielawiec et al., 2020). Cannabinoids like CBD and THC have been suggested to reduce hyperglycemia and increase insulin production in rodents (Jadoon et al., 2016; Zorzenon et al., 2019), but this has yet to be investigated in a canine model. Lactic acid is generated from pyruvate during anaerobic conditions in the muscle and red blood cells. The Cori cycle, or lactic acid cycle, removes lactate from tissues and transports lactate via blood to the liver where it can be converted back into glucose (Katz and Tayek, 1998; Nuttall et al., 2008). As both this cycle and lactic acid play vital roles in glucose homeostasis, the increase in plasma lactic acid indicates alteration of glucose metabolism by CBD. Similarly, acetic acid plays a role as an intermediate in several essential energetic pathways, including glycolysis, pyruvate metabolism, and glyoxylate metabolism via acetyl-CoA.

Glucosamine is an amino sugar that is readily synthesized in the body from glucose and glutamine. It is an essential component of mucopolysaccharides that are incorporated into connective tissue, mucous secretions, skin, tendons, ligaments, and cartilage. Additionally, it helps regulate the synthesis of collagen in cartilage (Anderson

et al., 2005; Beale, 2004; Bhathal et al., 2017). Because of its high concentration in joint tissues, glucosamine is commonly used as a dietary supplement in humans, horses, and dogs as a support for joint health and function and to relieve symptoms of osteoarthritis, though there is little scientific evidence supporting these effects (Henroitin et al., 2012). Both 2-deoxy-scyllo-inosamine and 2'-deamino-2'-hydroxy-6'-dehydroparomamine are intermediates in the biosynthesis of aminoglycoside antibiotics, like kanamycin, from glucose in *Streptomyces* bacterial species (Kudo et al., 2005; Park et al., 2011). However, since these metabolites are not known to be generated in mammalian systems, the biological significance of their changes is unclear.

D-glycerate is an intermediate in several metabolic pathways. Regarding carbohydrate metabolism, D-glycerate is an intermediate in the conversion of other D-sugars and glyoxylate to 2-phosphoglycerate, which can then feed into glycolysis (Dawkins and Dickens, 1965; Heinz et al., 1968). D-tagatose is a monosaccharide often found in fruit or dairy products that is commonly used as an artificial sweetener due to its low glycemic index (Mu et al., 2018). It can also be produced as an intermediate in the catabolism of D-sugars like D-galactose prior to entry into the pentose phosphate pathway (Bohren et al., 1989). D-galactosamine is an amino sugar derived from galactose that is a constituent of glycoprotein hormones like luteinizing hormone and follicle-stimulating hormone (Saracyn et al., 2015; Wu et al., 2014). L-rhamnono-1,4-lactone, L-rhamnofuranose, L-fuculose, and 6-deoxy-L-galactose (i.e. fucose) are intermediates in fructose and mannose metabolism (Becker and Lowe, 2003; Rigo et al., 1985; Wen et al., 2016). Fucose, in particular, is a common *N*-linked glycan that has been shown to play an important role in mammalian health, with disruption of fucosylated glycan expression

implicated in a number of disease mechanisms (Becker and Lowe, 2003; Vanhooren and Vandamme et al., 1999).

5-deoxy-D-glucuronate is an intermediate in the catabolism of myo-inositol in bacterial species like *Bacillus subtilis*, a common species found in the gastrointestinal tract of humans and ruminants (Hong et al., 2009; Yoshida et al., 2008). While not known to be produced in canine metabolic pathways, production of 5-deoxy-D-glucuronate in the gastrointestinal tract by microflora may have been absorbed into the body. The increase in this metabolite may suggest CBD supplementation affected gastrointestinal microflora populations. Decreased concentrations of arabitol may also support an impact of CBD on the microbiome. Arabitiol is a sugar alcohol commonly produced by several yeast species (Kordowska-Wiater, 2015). Elevated levels of arabitol in urine have been suggested as a biomarker of fungal infections (Salonen et al., 2001), and elevated plasma arabitol has been linked to other diseases like congenital liver cirrhosis and acute mountain sickness in humans (Verhoeven et al., 2001; Zhu et al., 2015). While CBD is believed to be beneficial in an assortment of gastrointestinal conditions (McCabe and Cital, 2021), little work has been done regarding its influence on microbial populations in the canine gastrointestinal tract, highlighting a potential avenue for future investigation of CBD supplementation.

The decrease in gamma-aminobutyric acid (GABA), glyceraldehyde, and an isomer of glyceraldehyde may also indicate that CBD altered carbohydrate metabolism. Best known as the primary inhibitory neurotransmitter in the central nervous system, GABA is also produced by insulin-producing β cells of the pancreas, endothelial cells, gut microbiota, and immune cells (Bansal et al., 2011; Franklin and Wollheim, 2004). In

the pancreas, GABA inhibits glucagon secretion from neighboring α cells and modulates glucose homeostasis (Bansal et al., 2011; Purwana et al., 2014; Rorsman et al., 1989). This action of GABA in the pancreas has highlighted its potential as a target for diabetes treatment (Wan et al., 2015). It has also been shown to regulate cytokine secretion from PBMCs and $CD4^+$ T cells and is thought to exert anti-inflammatory effects (Bhandage et al., 2018; Jin et al., 2013; Prud'homme et al., 2013). Glyceraldehyde, a triose monosaccharide, is an intermediate in glycolysis in its phosphorylated form (GAP). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the enzyme that catalyzes the conversion of GAP into 1,3-bisphosphoglycerate, is a major regulator of carbon flux in the body (Hildebrandt et al., 2015). It is also known to play a role in other cellular functions such as redox sensing, membrane fusion, iron homeostasis, and cell death (White and Garcin, 2017). The decrease in glyceraldehyde might lend support to the suspected anti-obesity and anti-diabetic effects of CBD; however, the relative increase in glucose and decrease in GABA would appear to be incongruous with this potential effect. These results highlight a relatively unexplored avenue of CBD research that warrants further investigation.

Lipid Metabolism

Increased concentrations of 17alpha,20alpha-dihydroxypregn-4-en-3-one, ethyl malonate, 3-hydroxybutyric acid, an isomer of 3-hydroxybutyric acid, 3-hydroxypropionic acid, an isomer of hydroxypropionic acid, and butyric acid may indicate an alteration of lipid metabolism with CBD supplementation. Primarily known as a steroid hormone elevated in late pregnancy in mammals, 17alpha,20alpha-dihydroxypregn-4-en-3-one is also thought to play a role in progesterone homeostasis in

non-pregnant animals (Mahajan et al., 1983; Shikita et al., 1967). Ethyl malonate is a branched fatty acid associated with several fatty acid metabolism disorders in humans, including short-chain acyl-CoA dehydrogenase deficiency and ethylmalonic encephalopathy (Tiranti et al., 2004; Wolfe et al., 2011), and has also been identified as a potential biomarker for the detection of human breast cancer (Wang et al., 2018). 3-hydroxybutyric acid is a ketone body and a typical intermediate in the partial breakdown of branched-chain amino acids like valine in muscles (Miyazaki et al., 2015). 3-Hydroxypropionic acid is an intermediate in beta-alanine, propanoate, and uracil metabolism (Den et al., 1959; Hawes et al., 1996). Butyric acid is an intermediate in butanoate metabolism, which is often formed by bacterial fermentation in the gut (Louis and Flint, 2009; Macfarlane and Macfarlane, 2003).

Additionally, decreased concentrations of propane-1,3-diol, glycerol, ethanolamine, isomer of 9-oxononanoic acid, 9,10-12,13-diepoxyoctadecanoate, allotetrahydrodeoxycorticosterone 3-O-glucuronide, cortolone, and cortisol 21-O-sulfate may also suggest that CBD altered lipid metabolism. Propane-1,3-diol is a product of glycerol fermentation in bacterial species but is not known as a mammalian metabolite (Marçal et al., 2020). Glycerol is the backbone of glycerides and plays an essential role in lipid metabolism, carbohydrate metabolism, and other metabolic processes (Blötz and Stülke, 2017). Blood glycerol is also a biomarker for liver disease, hyperglycemia, and type II diabetes (Jin et al., 2018; Johnston et al., 1982; Mahendran et al., 2013). Additionally, glycerol is involved in endocannabinoid signaling as a product of endocannabinoid 2-arachidonylglycerol (2-AG) hydrolysis (Baggelaar et al., 2018). Ethanolamine, an amino alcohol, is an intermediate in glycerophospholipid metabolism.

It is also involved in retrograde endocannabinoid signaling as a product of arachidonoyl ethanolamide (AEA) degradation by fatty acid amide hydrolase (FAAH) (Giang and Cravatt, 1997). These results may suggest CBD inhibited AEA degradation, which supports previous work showing inhibition of cellular uptake and breakdown of AEA by CBD via inhibition of fatty acid binding proteins (Elmes et al., 2015; Leweke et al., 2012; Schroeder et al., 2016). However, as the relative concentrations of plasma AEA and 2-AG were unaffected by treatment, the physiological significance of the changes in these metabolites is unclear. Future work using targeted metabolomics and pathway analysis may further elucidate this potential effect.

9-Oxononanoic acid is a medium chain fatty acid product of alpha-linoleic acid oxidation in plants (Otte et al., 2013). Early studies showed elevated lipid peroxides and reduced lipogenesis in rat livers after oral administration of 9-oxononanoic acid (Kanazawa et al., 1986; Minamoto et al., 1988); more recent work indicates this is a result of arachidonic cascade induction by 9-oxononanoic acid through its activation of phospholipase A_2 (Ren et al., 2013). If the decrease in 9-oxononanoic acid is a result of CBD supplementation, this may be one potential mechanism contributing to the suspected anti-inflammatory effects of CBD. Jasmonic acid is a plant stress hormone synthesized from linolenic acid that serves as a natural pesticide and promotes plant growth (Behr et al., 2019). Additionally, jasmonic acid has been suggested to exhibit anti-cancer and anti-inflammatory properties *in vitro* (Henriet et al., 2017). 9,10-12,13-Diepoxyoctadecanoate is an intermediate in the conversion of linoleic acid into tetrahydrofurandiols (THF-diols) (Falck et al., 2007).

Allotetrahydrodeoxycorticosterone (THDOC) 3-O-glucuronide is an endogenous neurosteroid that acts as a potent positive allosteric modulator of the $GABA_A$ receptor and exhibits sedative, anxiolytic, and anticonvulsant effects (Reddy, 2006; Reddy and Rogawski, 2002). Cortolone and cortisol 21-O-sulfate are metabolites in steroid hormone catabolism (Kornel et al., 1995). Cortolone is often linked to a glucuronide to help in its excretion (Hosoda et al., 1984; Yang et al., 2017), and cortisol 21-O-sulfate may be used as a biomarker for Cushing's syndrome (Kornel et al., 1995).

Altogether, these results support an effect of CBD on lipid metabolism and may indicate potential mechanisms for its suspected anti-inflammatory effect. However, as the animals on this study were healthy, it is unclear how this would translate to a diseased population in which these metabolomic changes might be more influential, such as animals with Cushing's disease. Further studies are needed in unhealthy or diseased populations of dogs to determine the potential physiological applications of these potential effects of CBD.

Hydroxycinnamic Acid Derivatives

The increase in plasma isoferulic acid (IFA), trans-2,3-dihydroxycinnamate, and vanillic acid may suggest that CBD altered the metabolism of hydroxycinnamic acid derivatives. Isoferulic acid is a naturally occurring hydroxycinnamic acid derivative commonly found in *Lobelia* and *Cimicifuga* species. It is an isomer of ferulic acid, a phenolic compound that is a component of lignin, which is commonly found in cell walls of plants, including *C. sativa* (Zimniewska et al., 2018). Ferulic acid has also been isolated from hemp seed meal, a byproduct of hemp oil processing (Pojić et al., 2014). Both ferulic acid and IFA have been reported to have anti-inflammatory, anti-viral, anti-

oxidative, and anti-diabetic properties and are commonly used as ingredients in herbal medicines in Japan and China (Meeprom et al., 2013; Zhao et al., 2008). Isoferulic acid has been shown to reduce plasma glucose in diabetic rats and to inhibit IL-8 production in mice (Liu et al., 2000; Hirabayashi et al., 1994). Additionally, IFA has been suggested to act as an anti-glycation compound. Protein glycation is a non-enzymatic reaction associated with oxidative stress and reactive oxygen species (ROS) production; it is thought to be a contributor to age-related diseases (Meeprom et al., 2015). In several studies, IFA protected against fructose- and glucose-mediated glycation and inhibited ROS production *in vitro* (Meeprom et al., 2013; Arfin et al., 2018). In humans, IFA has been shown to be an intermediate in the metabolism of plant-derived phenolic compounds like caffeic acid (Clifford et al., 2019), with humans obtaining the majority from dietary consumption.

Trans-2,3-dihydroxycinnamate is a derivative of cinnamic acid, which is an intermediate in the biosynthesis of lignin, flavonoids, and other secondary metabolites produced by plants like *C. sativa* (Vogt, 2010). Cinnamic acid and its derivatives, like trans-2,3-dihydroxycinnamate, possess antioxidizing activity (Santos and Vieira, 2013). Vanillic acid is a dihydroxybenzoic acid derivative commonly used as a flavoring agent. It is also an intermediate in the synthesis of vanillin from ferulic acid and a phenolic compound that, like IFA, is a component of lignin present in the secondary cell wall of plants, including *C. sativa* (Civolani et al., 2000; Khan et al., 2014). Like other lignin-associated aromatic acids, vanillic acid has been reported to exert antimicrobial properties (Yemiş et al., 2011). If the increase in these metabolites is a result of CBD supplementation, it is possible that these compounds may contribute to the suspected

anti-microbial, anti-inflammatory, and antioxidative effects of hemp; however, additional work is warranted to further investigate these potential effects.

Vitamin and Nucleotide Metabolism

Increased concentrations of pyrimidodiazepine, 4-amino-4-deoxychorismate, 7-carboxy-7-carbaguanine, 2-formylglutarate, ascorbate, an isomer of threonate, 2-hydroxy-3-oxopropanoate, nicotinate, 3-oxopropanoate, an isomer of 3-oxopropanoate, deoxyadenosine, and an isomer of deoxyadenosine may indicate an alteration of vitamin and nucleotide metabolism. Pyrimidodiazepine is a derivative of uracil and a substrate for pyrimidodiazepine synthase, an enzyme that can contribute to glutathione synthesis. Uracil derivatives like pyrimidodiazepine are also thought to possess antimicrobial and antioxidant properties (El-Kalyoubi et al., 2017). 3-Oxopropanoate is an intermediate in uracil and beta-alanine metabolism that can then be converted into malonate and enter fatty acid biosynthetic pathways (Scholem and Brown, 1983). Deoxyadenosine is a derivative of adenosine and an intermediate in purine metabolism. In the absence of adenosine deaminase in humans, deoxyadenosine will accumulate in and kill T lymphocytes, leading to the development of adenosine deaminase severe combined immunodeficiency disease (Bradford et al., 2017). Nicotinate, or vitamin B3, is required for the formation of coenzymes NAD and NADP. While it can be synthesized from tryptophan in humans and dogs, the process is inefficient and is thus considered an essential nutrient (Kirkland, 2009; Krehl and Torbet, 1946). 4-amino-4-deoxychorismate is a precursor for *para*-aminobenzoic acid (pABA) biosynthesis, which is a precursor for folic acid (vitamin B9) synthesis in plants and microorganisms (Sahr et al., 2006; Wegkamp et al., 2007). While vitamin B9 is an essential cofactor that facilitates methyl

transfers, mammals do not possess the enzymes to produce folic acid and instead rely on dietary consumption of the vitamin (Sahr et al., 2006). 7-carboxy-7-carbaguanine is pyrrolopyrimidine that, like 4-amino-4-deoxychorismate, is involved in the biosynthesis of vitamin B9 (McCarty et al., 2009). 2-formylglutarate is an intermediate in nicotinamide metabolism in several bacterial species (Alhapel et al., 2006; Kress et al., 2008). Threonate is an intermediate in ascorbate metabolism that is thought to play a role in bone mineralization, prevent androgen-driven balding, and improve learning and memory when combined with magnesium (Kwack et al., 2010; Sun et al., 2016; Wang and Jiang, 2011). Ascorbate, or vitamin C, can be synthesized in dogs from glucose or ingested in the diet (Linster and Schaftingen, 2007; Meister, 1994). Vitamin C serves as a cofactor in several essential reactions, including collagen synthesis and wound healing, as well as an antioxidant. If the increase in ascorbate was due to CBD supplementation, this may contribute to the suspected antioxidative effects of CBD.

Decreased concentrations of 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylate (Z)-3-peroxyaminoacrylate, and alpha-ribazole may also support an effect of CBD on vitamin and nucleotide metabolism. 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylate is an intermediate in the pyridoxine (vitamin B6) degradation pathway (Yokochi et al., 2009). Vitamin B6 is an essential cofactor in several enzymatic reactions, including the synthesis of glutathione, an important antioxidant (Dalto and Matte, 2017). alpha-Ribazole is an intermediate in vitamin B12 synthesis in plants and bacterial species that can be used as a marker for vitamin B12 content in foodstuffs (Gray and Escalante-Semerena, 2010; Pakin et al., 2005). (Z)-3-peroxyaminoacrylate is an intermediate in bacterial pyrimidine degradation pathway known as the Rut pathway. However, this

pathway and intermediate are not known to play a role in mammalian pyrimidine metabolism (Knapik et al., 2012).

Since the relative concentrations of pyridoxine, uracil, and folate were not affected by treatment, and since several of these metabolites are not known to be generated in mammalian systems, the biological significance of these changes is unclear. However, increased pyrimidodiazepine and ascorbate, along with decreased 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylate, may indicate an influence of CBD on antioxidant status. Further studies are needed to determine the roles of these metabolites and the potential effects of CBD on these pathways.

Additional Metabolites

Increased relative concentrations of 4-coumarate, 3,4-dihydroxyhpenylpropanoate, isovanillic acid, citramalic acid, sepiapterin, and an isomer of sepiapterin may further suggest an alteration of metabolism by CBD supplementation. 4-Coumarate, or 4-hydroxycinnamate, is a hydroxycinnamic acid derivative and an intermediate in several plant metabolic pathways, including phenylpropanoid biosynthesis, isoquinoline alkaloid biosynthesis, tyrosine metabolism, and more (Li et al., 2005; Schneider et al., 2005). 3,4-Dihydroxyphenylpropanoate, or dihydrocaffeic acid, is an intermediate in tyrosine metabolism, specifically the conversion of L-DOPA to rosmarinate, and is suspected to possess antioxidant and anti-inflammatory properties (Ranjith et al., 2007; Wang et al., 2018). Isovanillic acid is a metabolite of isovanillin with suspected antibacterial properties (Panoutsopoulos and Beedham, 2005; Strand and Scheline, 1975). While not known as a mammalian metabolite, it can be found in plasma after consumption of flavonoid consumption (Loke et al., 2009). Citramalic acid is most

commonly produced by yeast or anaerobic bacteria as an intermediate in C5-branched dibasic acid metabolism (Zarzycki et al., 2009). As it is not a mammalian metabolite, citramalic acid is most commonly found as a urine metabolite in cases of yeast or bacterial overgrowth (Shaw et al., 1995). Sepiapterin is an intermediate in folate biosynthesis that in mammals can be metabolized into tetrahydrobiopterin, which is an essential cofactor in mammals for the metabolism of aromatic amino acids and biosynthesis of neurotransmitters (Smith et al., 2019; Wang et al., 2011). It has been suggested as a treatment for atherosclerosis and tetrahydropterin deficiencies in humans (Smith et al., 2019; Tarpey, 2002; Vásquez-Vivar et al., 2002).

Decreased concentrations of L-glutamate-1-semialdehyde, 1-aminocyclopropane-1-carboxylate, L-allothreonine, an isomer of 3-phenoxybenzyl alcohol, dihydroshikonofuran, and phenethyl alcohol also indicate an effect of CBD on metabolism. L-Glutamate-1-semialdehyde, 1-aminocyclopropane-1-carboxylate, and L-allothreonine are plant metabolites. L-Glutamate-1-semialdehyde is produced as an intermediate in porphyrin and chlorophyll synthesis (Campanini et al., 2013). 1-Aminocyclopropane-1-carboxylate is the direct precursor of the plant hormone ethylene and plays a role in the regulation of plant development (Polko and Kieber, 2019). L-Allothreonine is a stereoisomer of threonine and an intermediate in plant glycine, serine, and threonine metabolism (Fiehn et al., 2000). 3-Phenoxybenzyl alcohol is a mammalian metabolite of the insecticide permethrin produced by carboxylesterases (Crow et al., 2007; Stok et al., 2004). Dihydroshikonofuran is a monoterpenoid product of ubiquinone and other terpenoid-quinone biosynthesis from the same metabolic pathway that produces shikonin, a plant pigment with anti-inflammatory, antibacterial, and wound-healing

properties (Boehm et al., 2000; Yazaki et al., 1987). Phenethyl alcohol is a natural fragrance produced by rose, carnation, and other plants (Guterman et al., 2006; Politano et al., 2013).

Limitations, Strengths, and Weaknesses

This study is the first to evaluate the impact of CBD supplementation on the canine metabolome. This analysis was not intended to be all-encompassing but rather a first look into the potential for CBD supplementation to alter the canine metabolome. Thus, this study was limited by the relatively short duration of CBD supplementation, lack of baseline sampling, small sample size, and the use of only a single CBD dosage. Even so, identifying potential changes in the metabolome is essential for directing future investigation into both the physiological relevance of these changes as well as elucidating potential mechanisms leading to these observed effects.

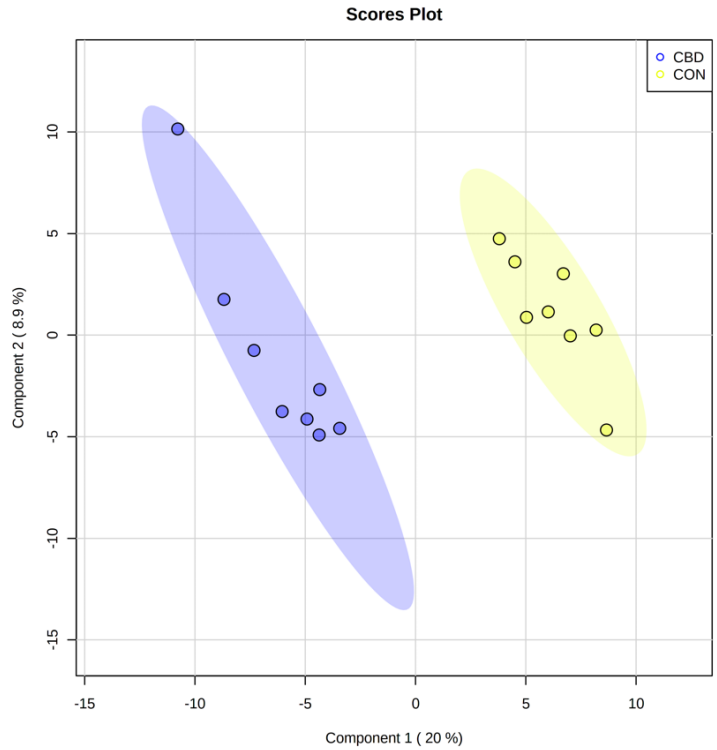
Conclusions

This study demonstrated the canine metabolome was altered with 4.5 mg CBD/kg BW/d supplementation for 3 weeks. Altered metabolites may suggest a potential for CBD to influence glucose, carbohydrate, lipid, amino acid, vitamin, and nucleotide metabolism. Additionally, the difference in relative concentrations of metabolites like 9-oxononanoic acid, *o*-tyrosine, IFA, glucosamine, pyrimidodiazepine, 9-oxononanoic acid, and 3,4-dihydroxymandelic acid may indicate potential pathways by which CBD may exert suspected anti-inflammatory, antioxidant, anti-obesity, and antimicrobial effects. Several metabolites were identified as potential biomarkers for changes in the canine metabolome by CBD. Further studies with larger sample sizes, longer supplementation

periods and baseline comparisons to refine metabolites are necessary to elucidate the physiological relevance of these changes.

Tables and Figures

A.



B.

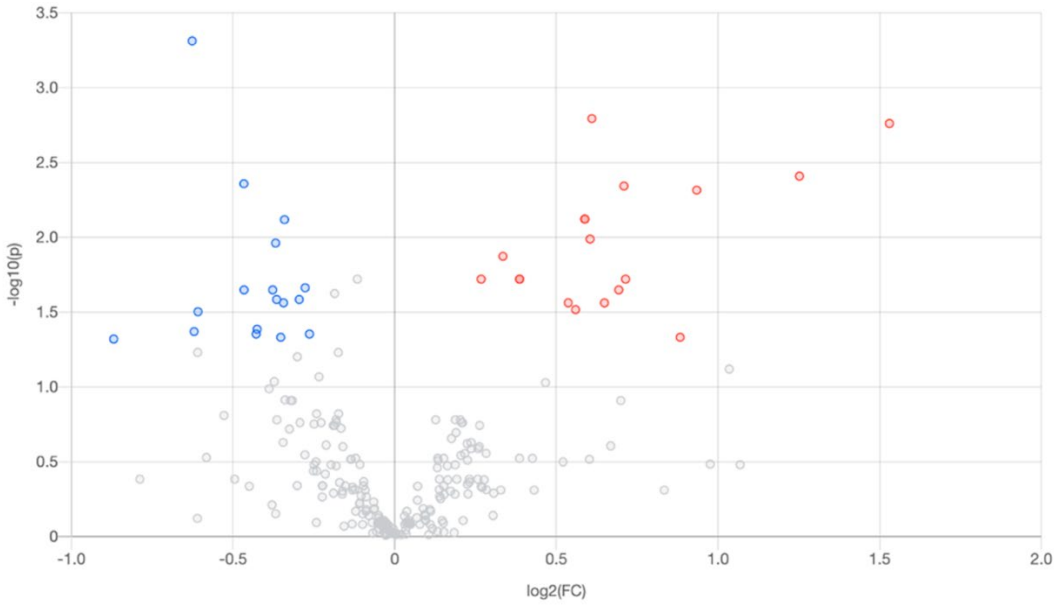


Figure 6.1. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential amine/phenol-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON).

Table 6.1. Identified amine/phenol-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON.

Metabolite	Normalized RT[1]	FC	FDR	Identification Level[2]
Pyrimidodiazepine	1029.2	2.89	0.002	Tier 2
4-Amino-4-deoxychorismate	504.2	2.38	0.004	Tier 2
Isoferulic acid	1075.1	1.91	0.005	Tier 1
Isomer of D-Glucosamine	152.6	1.85	0.045	Tier 2
7-Carboxy-7-carbaguanine	369.6	1.64	0.019	Tier 2
2,4-Dihydroxyhept-2-enedioate	680.1	1.64	0.004	Tier 2
Ascorbate	530.4	1.62	0.023	Tier 2
2'-Deamino-2'-hydroxy-6'-dehydroparomamine	761.9	1.57	0.026	Tier 2
trans-2,3-Dihydroxycinnamate	856.8	1.53	0.001	Tier 2
gamma-Glutamyl-gamma-aminobutyraldehyde	337.7	1.52	0.010	Tier 2
1,4-Diaminobutane	1281.7	1.50	0.007	Tier 1
Tyramine	1538.7	1.50	0.007	Tier 1
Isomer of 2-Deoxy-scyllo-inosamine	208.1	1.47	0.031	Tier 2
Isoleucyl-Alanine	586.8	1.45	0.026	Tier 1
3-(4-Hydroxyphenyl)pyruvate	1068.7	1.31	0.019	Tier 2
Aspartyl-Threonine	236.0	1.31	0.019	Tier 1
Vanillic acid	1026.1	1.26	0.012	Tier 1
D-Lysopine	999.8	1.20	0.019	Tier 2
N-Acetyl-L-Asparagine	492.6	0.83	0.023	Tier 2
Alanyl-Proline	477.2	0.82	0.026	Tier 1
Asparaginyl-Aspartic acid	149.6	0.79	0.026	Tier 1

Seryl-Aspartic acid	162.3	0.78	0.045	Tier 1
Phenylalanyl-Glycine	569.9	0.78	0.026	Tier 1
Prolyl-Glutamine	335.6	0.78	0.012	Tier 1
o-Tyrosine	1313.2	0.77	0.023	Tier 1
N-Acetyl-L-Adrenaline	1650.1	0.75	0.041	Tier 2
L-threo-3-Methylaspartate	491.9	0.74	0.045	Tier 2
Z-3-Peroxyaminoacrylate	666.3	0.72	0.024	Tier 2
L-Glutamate 5-semialdehyde	367.9	0.72	0.004	Tier 2
2-Methyl-3-hydroxy-5-formylpyridine-4-carboxylate	652.3	0.66	0.032	Tier 2
Aspartyl-Glutamine	194.8	0.65	0.001	Tier 1
Gamma-Aminobutyric acid	466.3	0.55	0.049	Tier 1

[1]Normalized RT (retention time) shows the corrected retention time of the peak pair with Universal RT Calibrant data.
[2]Tier 1 indicates positive metabolite identification within the chemical isotope labeling (CIL) metabolite library whereas Tier 2 indicates high confidence putative identification within the linked identity (LI) library.

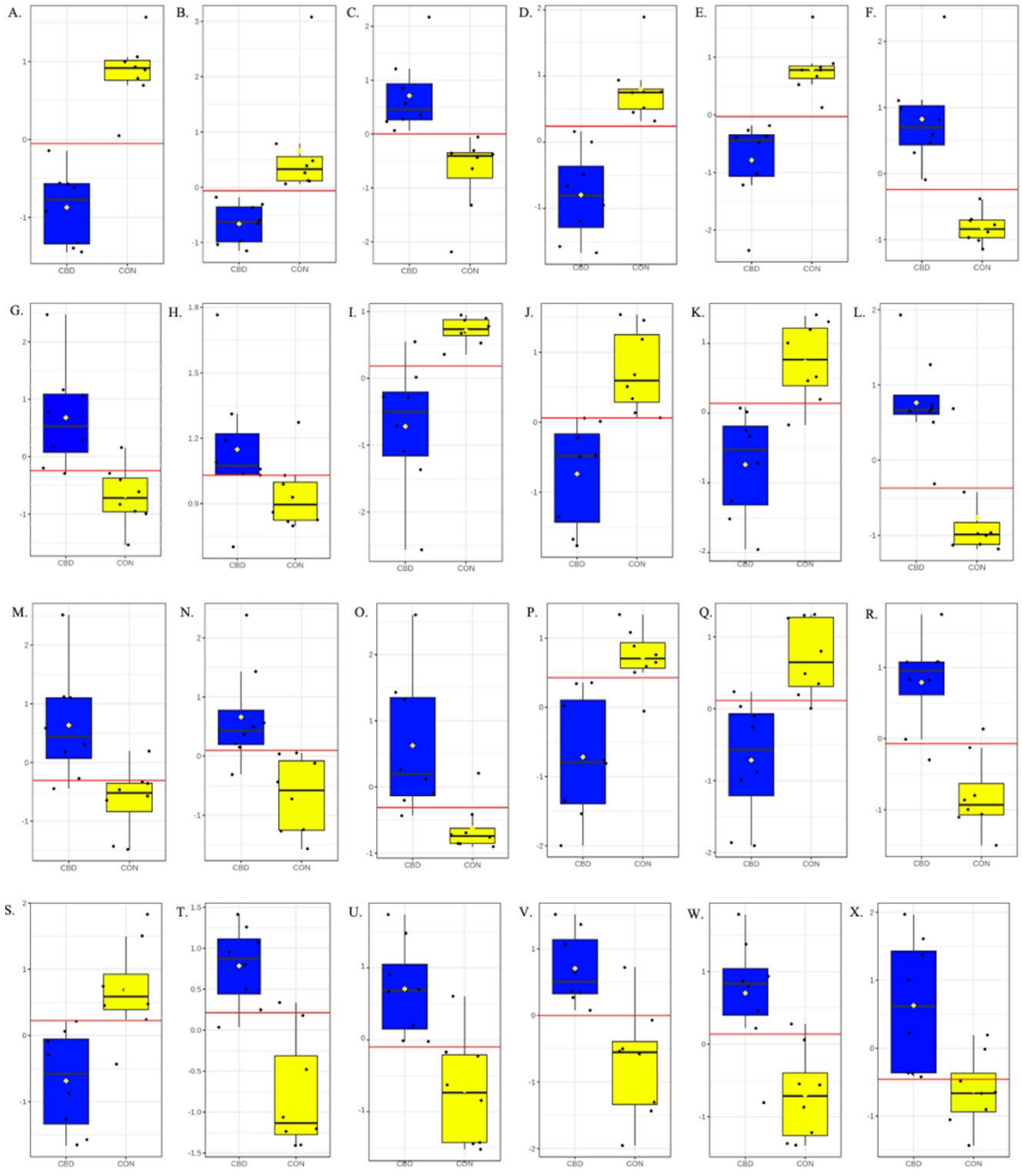
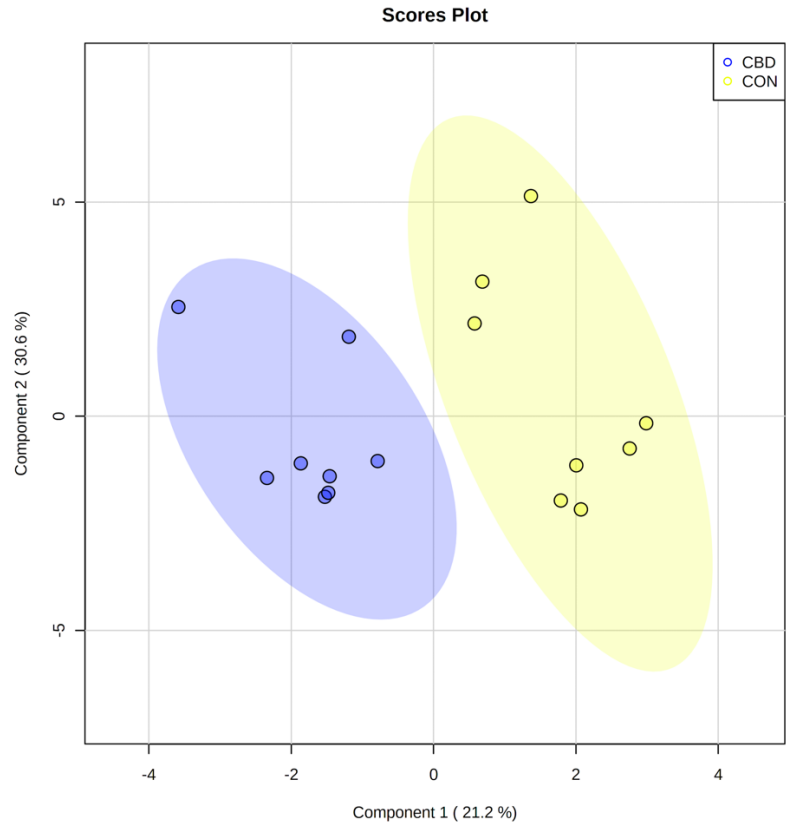

A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.
L.
M.
N.
O.
P.
Q.
R.
S.
T.
U.
V.
W.
X.
CBD
CON

Figure 6.2. Candidate amine/phenol-containing biomarkers altered by cannabidiol (CBD; in blue) compared to control (CON; in yellow) included **A.** aspartyl-glutamine (AUROC = 1.00; $P < 0.001$); **B.** gamma-aminobutyric acid (AUROC = 1.00; $P = 0.005$); **C.** gamma-glutamyl-gamma-aminobutyraldehyde (AUROC = 1.00; $P < 0.001$); **D.** L-glutamate-5-semialdehyde (AUROC = 1.00; $P < 0.001$); **E.** prolyl-glutamine (AUROC = 1.00; $P < 0.001$); **F.** pyrimidodiazepine (AUROC = 1.00; $P < 0.001$); **G.** 4-amino-4-deoxychorismate (AUROC = 0.98; $P < 0.001$); **H.** trans-2,3-dihydroxycinnamate (AUROC = 0.98; $P < 0.001$); **I.** alanyl-proline (AUROC = 0.97; $P = 0.002$); **J.** N-acetyl-L-asparagine (AUROC = 0.97; $P < 0.001$); **K.** (Z)-3-peroxyaminoacrylate (AUROC = 0.95; $P < 0.001$); **L.** 1,4-diaminobutane (AUROC = 0.95; $P < 0.001$); **M.** 2'-deamino-2'-hydroxy-6'-dehydroparomamine (AUROC = 0.95; $P = 0.004$); **N.** ascorbate (AUROC = 0.95; $P = 0.003$); **O.** D-lysopine (AUROC = 0.95; $P = 0.004$); **P.** *o*-tyrosine (AUROC = 0.95; $P = 0.001$); **Q.** phenylalanyl-glycine (AUROC = 0.95; $P = 0.002$); **R.** 2,4-dihydroxyhept-2-enedioate (AUROC = 0.94; $P < 0.001$); **S.** asparaginyl-aspartic acid (AUROC = 0.94; $P = 0.003$); **T.** isoferulic acid (AUROC = 0.94; $P < 0.001$); **U.** 7-carboxy-7-carbaguanine (AUROC = 0.92; $P = 0.001$); **V.** 3-(4-hydroxyphenyl)pyruvate (AUROC = 0.91; $P = 0.004$); **W.** aspartyl-threonine (AUROC = 0.91; $P = 0.005$); and **X.** isoleucyl-alanine (AUROC = 0.91; $P = 0.007$).

A.



B.

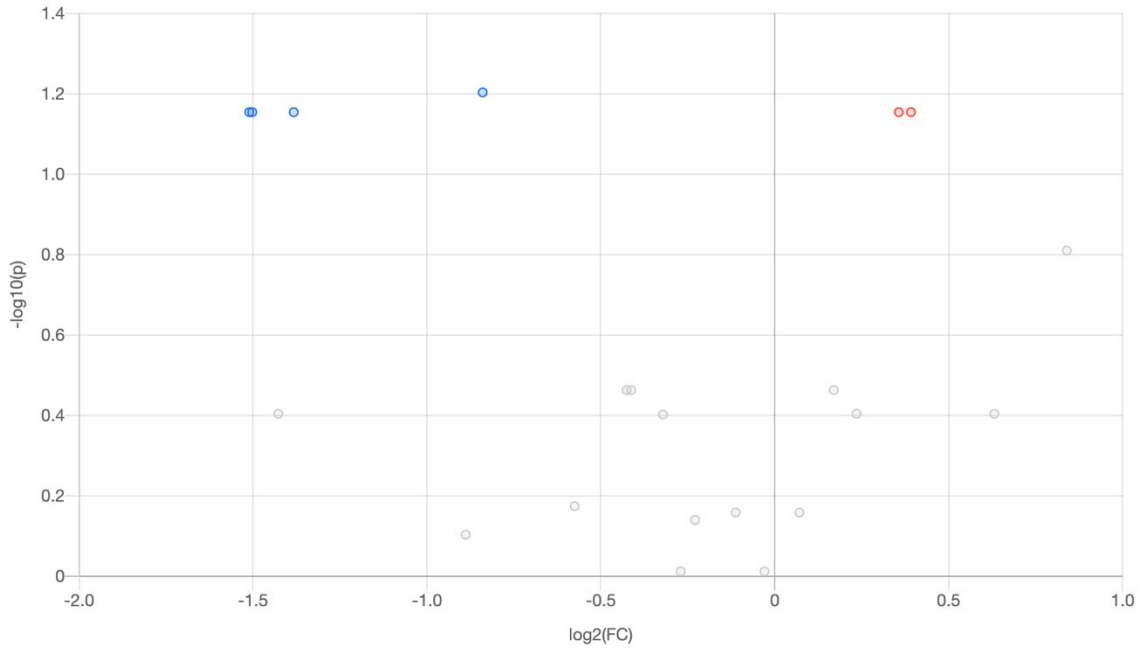


Figure 6.3. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential carbonyl-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON).

Table 6.2. Identified carbonyl-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON.

Metabolite	Normalized RT[1]	FC	FDR	Identification Level[2]
2-Formylglutarate	569.3	1.99	0.021	Tier 2
Glucose	371.2	1.54	0.018	Tier 1
4-Oxoglutaramate	394.4	0.62	0.050	Tier 2
Isomer of Glyceraldehyde	471.1	0.42	0.035	Tier 1
Glyceraldehyde	453.2	0.38	0.040	Tier 1

[1]Normalized RT (retention time) shows the corrected retention time of the peak pair with Universal RT Calibrant data.
[2]Tier 1 indicates positive metabolite identification within the chemical isotope labeling (CIL) metabolite library whereas Tier 2 indicates high confidence putative identification within the linked identity (LI) library.

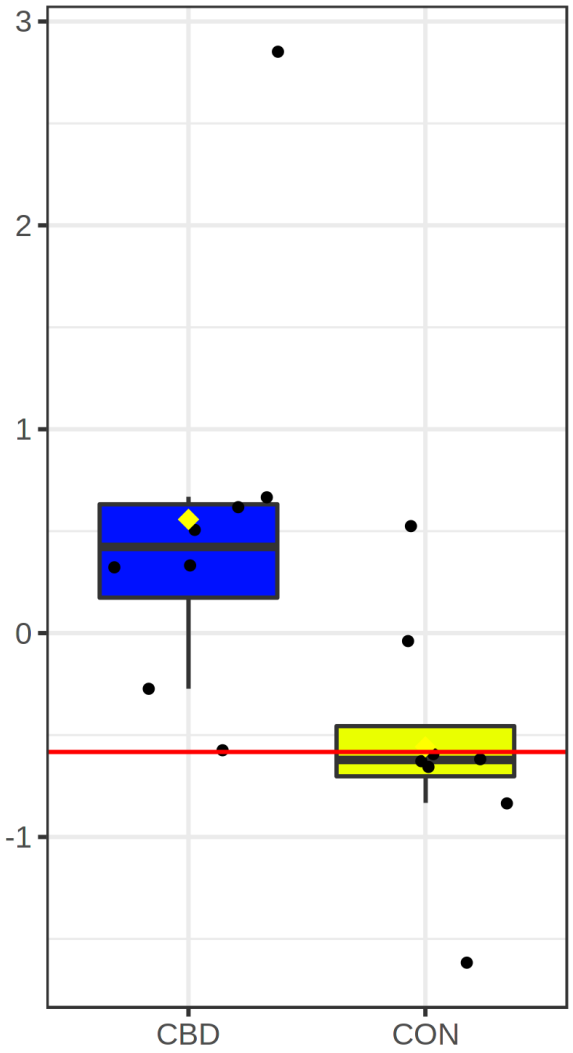
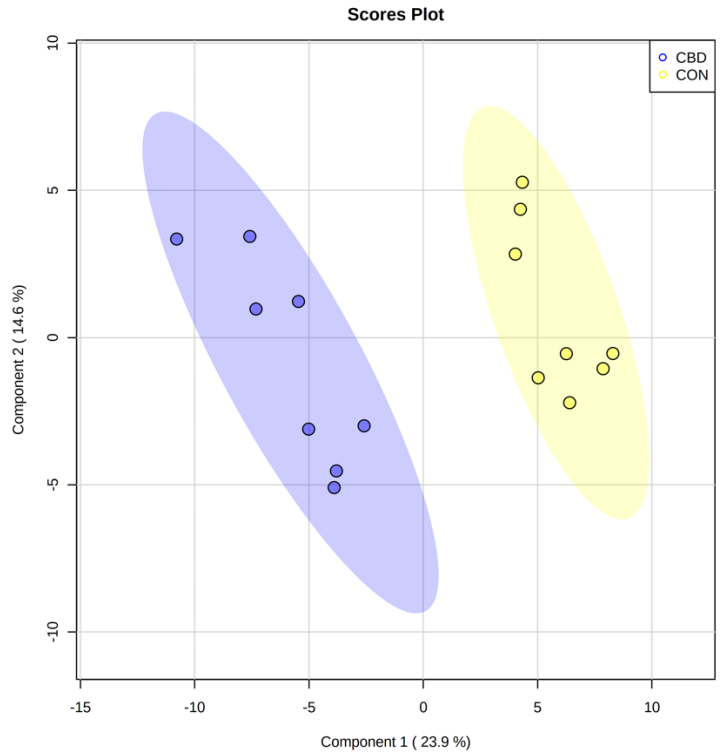


Figure 6.4. Candidate carbonyl-containing biomarker – Glucose (AUROC = 0.91; *P* = 0.020) – altered by cannabidiol (CBD; in blue) compared to control (CON; in yellow).

A.



B.

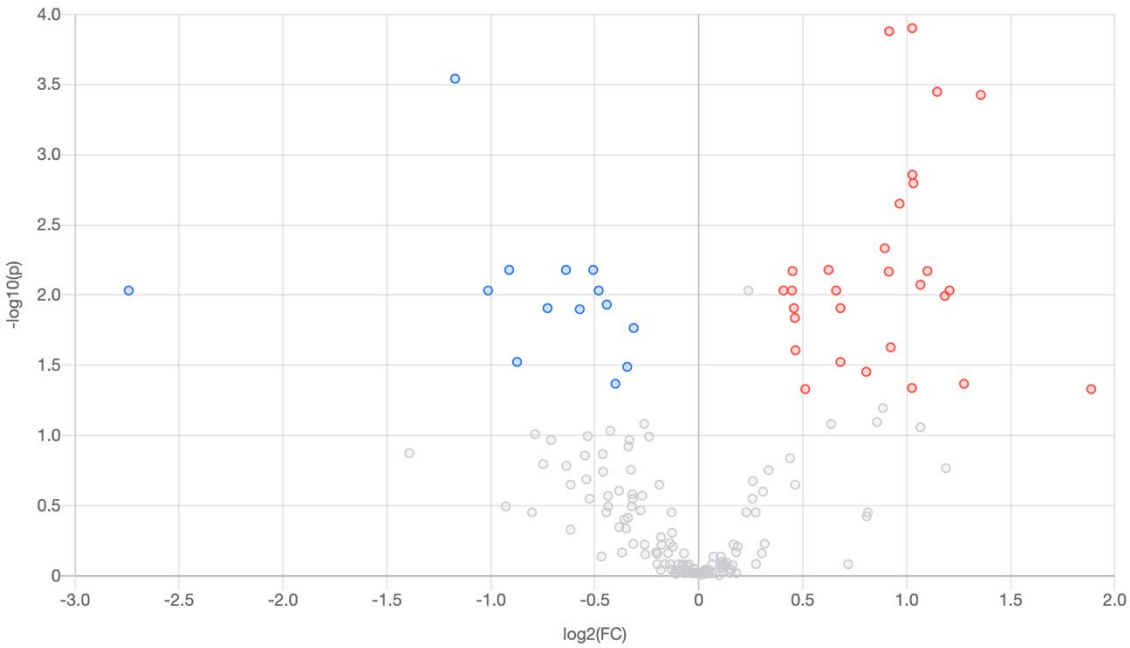


Figure 6.5. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential carboxyl-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON).

Table 6.3. Identified carboxyl-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON.

Metabolite	Normalized RT[1]	FC	FDR	Identification Level[2]
2,5-Dioxopentanoate	741.4	3.70	0.047	Tier 2
Isomer of Hydroxypropionic acid	380.9	2.56	<.001	Tier 1
4-Coumarate	802.6	2.42	0.043	Tier 2
Isomer of D-Glycerate	255.0	2.31	0.009	Tier 2
Acetic acid	560.5	2.21	<.001	Tier 1
D-Glycerate	299.4	2.14	0.007	Tier 2
Isomer of Glycolate	282.4	2.09	0.008	Tier 2
Isomer of Threonate	272.2	2.09	0.088	Tier 2
5-Deoxy-D-glucuronate	361.4	2.04	0.002	Tier 2
Citramalic acid	449.1	2.04	0.001	Tier 1
3,4-Dihydroxymandelic acid	493.9	2.04	<.001	Tier 1
Glycolate	299.4	2.03	0.046	Tier 2
3-Oxopropanoate	361.4	1.95	0.002	Tier 2
Ethyl Malonate	739.7	1.89	<.001	Tier 1
Isomer of 3-Oxopropanoate	332.4	1.88	0.007	Tier 2
Isomer of 3-Hydroxybutyric acid	474.9	1.86	0.005	Tier 1
2-Hydroxy-3-oxopropanoate	389.2	1.81	0.080	Tier 2
Hydroxyisobutyric acid	548.8	1.60	0.012	Tier 1
Isovaleric acid	1039.8	1.60	0.030	Tier 1
Butyric acid	870.3	1.58	0.009	Tier 1
Glyoxylate	528.3	1.55	0.083	Tier 2
Nicotinate	574.9	1.54	0.007	Tier 2

3-Hydroxybutyric acid	501.4	1.43	0.047	Tier 1
S-5-Amino-3-oxohexanoic acid	596.0	1.38	0.025	Tier 2
Hydroxypropionic acid	424.8	1.38	0.015	Tier 1
Lactic acid	452.2	1.37	0.012	Tier 1
Isomer of Lactic acid	471.2	1.36	0.009	Tier 1
Isovanillic acid	739.5	1.33	0.009	Tier 1
4-Oxoproline	798.3	0.80	0.017	Tier 2
2-Aminomuconate semialdehyde	799.6	0.79	0.032	Tier 2
Isomer of 9-Oxononanoic acid	1048.9	0.74	0.093	Tier 2
L-1-Pyrroline-3-hydroxy-5-carboxylate	372.5	0.74	0.012	Tier 2
S-4-Amino-5-oxopentanoate	439.3	0.70	0.007	Tier 2
Isomer of 1-Aminocyclopropane-1-carboxylate	272.2	0.67	0.013	Tier 2
L-Allothreonine	439.3	0.64	0.007	Tier 2
Arginine	279.8	0.60	0.012	Tier 2
Isomer of Aspartate	574.6	0.58	0.098	Tier 2
Isomer of Jasmonic acid	1525.6	0.55	0.030	Tier 2
2-Oxo-4-phenylbutyric acid	1150.3	0.53	0.007	Tier 2
9,10-12,13-Diepoxyoctadecanoate	1333.8	0.50	0.009	Tier 2
Jasmonic acid	1437.0	0.44	<.001	Tier 2
Isomer of 1-Pyrroline-2-carboxylate	1160.7	0.15	0.009	Tier 2

[1]Normalized RT (retention time) shows the corrected retention time of the peak pair with Universal RT Calibrant data.
[2]Tier 1 indicates positive metabolite identification within the chemical isotope labeling (CIL) metabolite library whereas Tier 2 indicates high confidence putative identification within the linked identity (LI) library.

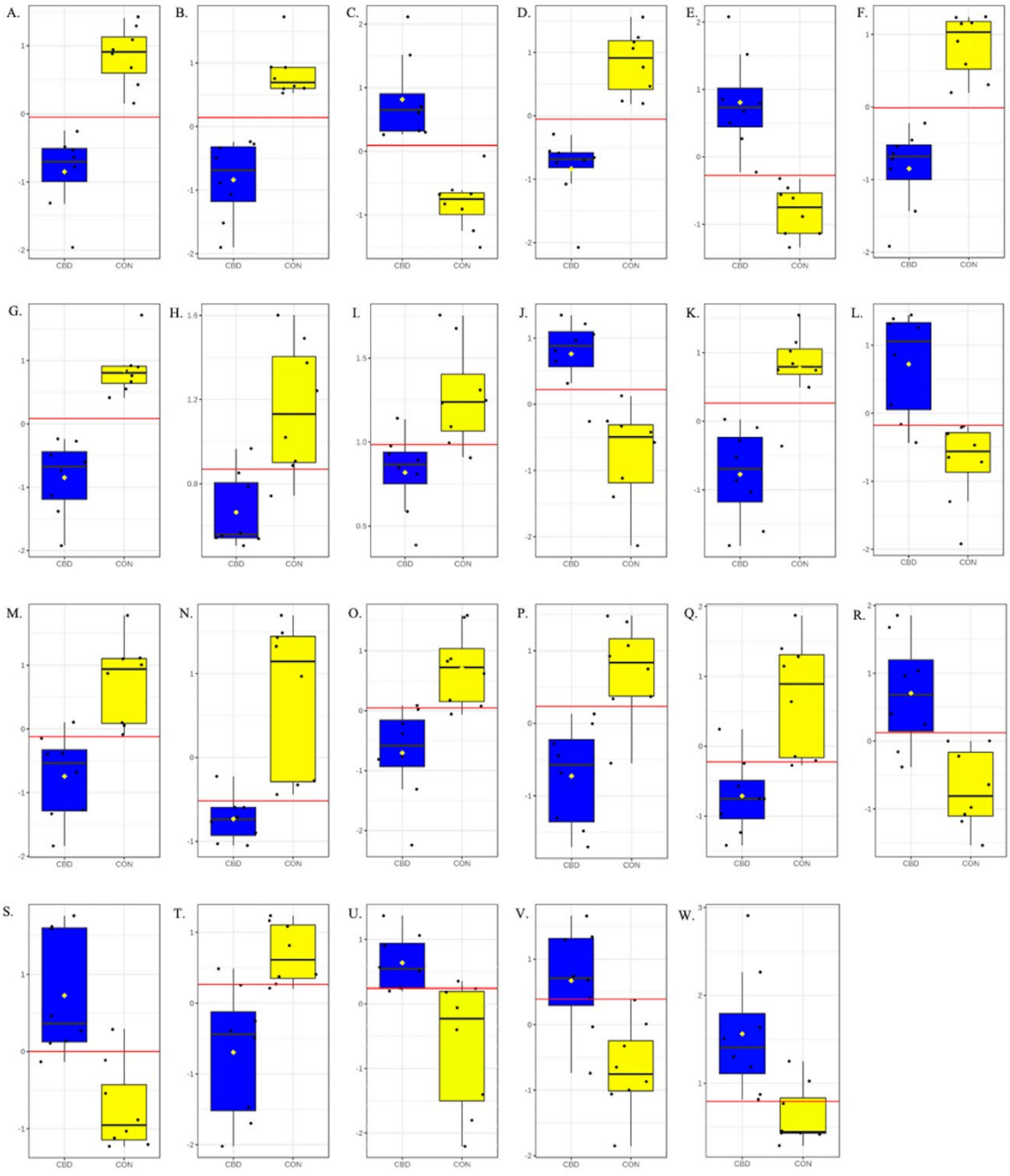
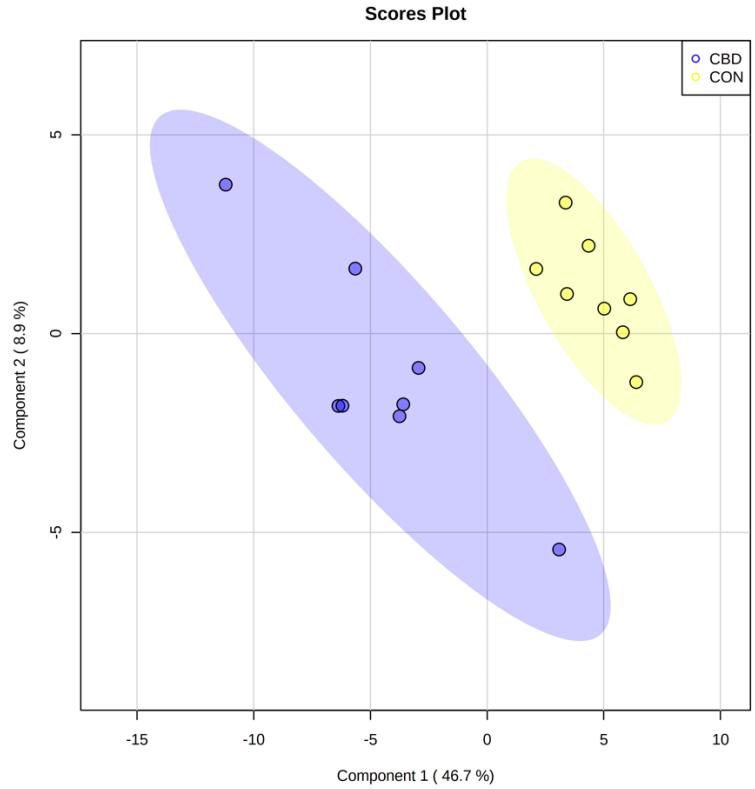

A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.
L.
M.
N.
O.
P.
Q.
R.
S.
T.
U.
V.
W.
CBD
CON

Figure 6.6. Biomarker analysis of carboxyl metabolites for cannabidiol (CBD) and control (CON) treatments identified **A.** jasmonic acid (AUROC = 1.00; $P < 0.001$); **B.** (S)-4-amino-5-oxopentanoate (AUROC = 1.00; $P < 0.001$); **C.** 3,4-dihydroxymandelic acid (AUROC = 1.00; $P < 0.001$); **D.** isomer of 1-aminocyclopropane-1-carboxylate (AUROC = 1.00; $P < 0.001$); **E.** isomer of hydroxypropionic acid (AUROC = 1.00; $P < 0.001$); **F.** L-1-pyrroline-3-hydroxy-5-carboxylate (AUROC = 1.00; $P < 0.001$); **G.** L-threonine/L-allothreonine (AUROC = 1.00; $P < 0.001$); **H.** 2-oxo-4-phenylbutyric acid (AUROC = 0.98; $P < 0.001$); **I.** Arginine (AUROC = 0.98; $P < 0.001$); **J.** citramalic acid (AUROC = 0.97; $P < 0.001$); **K.** 4-oxoproline (AUROC = 0.95; $P < 0.001$); **L.** 5-deoxy-D-glucuronate (AUROC = 0.95; $P < 0.001$); **M.** 9,10-12,13-diepoxyoctadecanoate (AUROC = 0.95; $P < 0.001$); **N.** isomer of 1-pyrroline-2-carboxylate (AUROC = 0.95; $P < 0.001$); **O.** isomer of aspartate (AUROC = 0.95; $P < 0.001$); **P.** 2-aminomuconate semialdehyde (AUROC = 0.94; $P < 0.001$); **Q.** isomer of 9-oxononanoic acid (AUROC = 0.94; $P < 0.001$); **R.** acetic acid (AUROC = 0.92; $P < 0.001$); **S.** ethyl malonate (AUROC = 0.92; $P < 0.001$); **T.** isomer of jasmonic acid (AUROC = 0.92; $P < 0.001$); **U.** 3-oxopropanoate (AUROC = 0.91; $P < 0.001$); **V.** D-glycerate (AUROC = 0.91; $P < 0.001$); and **W.** isomer of D-glycerate (AUROC = 0.91; $P < 0.001$) as candidate biomarkers.

A.



B.

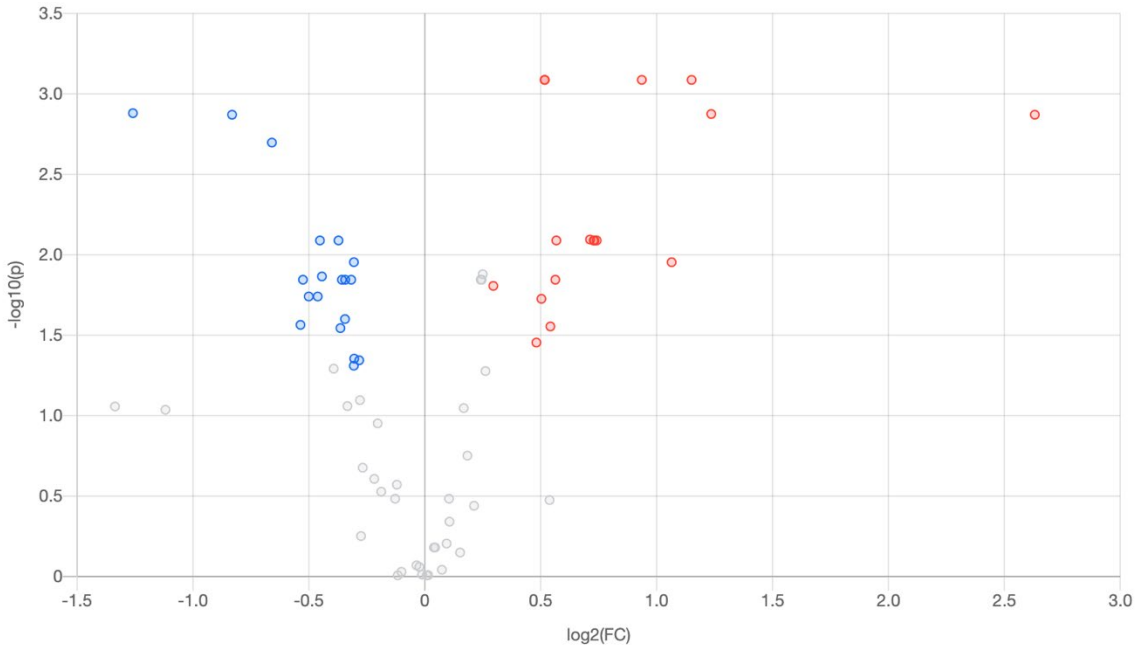


Figure 6.7. **A.** Partial least squares discriminant analysis (PLS-DA) scores plot and **B.** volcano plot showing the differential hydroxyl-containing metabolites. Fold change (FC) ≥ 1.2 (in red) or ≤ 0.83 (in blue) with false discovery ratio (FDR) ≤ 0.05 are differentially increased or reduced by cannabidiol (CBD) relative to control (CON).

Table 6.4. Identified hydroxyl-containing metabolites affected by cannabidiol (CBD) compared to control (CON). Metabolites with a fold change (FC) ≥ 1.2 relative to CON and a false discovery ratio (FDR) ≤ 0.05 considered increased in the CBD compared to CON. Metabolites with a FC ≤ 0.83 and an FDR ≤ 0.05 considered reduced in CBD compared to CON.

Metabolite	Normalized RT[1]	FC	FDR	Identification Level[2]
17alpha,20alpha-Dihydroxypregn-4-en-3-one	1618.8	6.19	0.001	Tier 2
D-Tagatose	235.4	2.35	0.001	Tier 2
3,4-Dihydroxyphenylpropanoate	678.7	2.09	0.011	Tier 2
L-Rhamnono-1,4-lactone	280.5	1.91	0.001	Tier 2
L-Rhamnofuranose	198.0	1.67	0.008	Tier 2
Isomer of L-Rhamnofuranose	735.3	1.66	0.008	Tier 2
6-Deoxy-L-galactose	649.6	1.66	0.008	Tier 2
D-Galactosamine	162.8	1.48	0.008	Tier 2
Sepiapterin	240.1	1.48	0.014	Tier 2
Isomer of Sepiapterin	182.5	1.46	0.028	Tier 2
L-Fuculose	128.6	1.43	0.001	Tier 2
Isomer of Deoxyadenosine	261.6	1.42	0.019	Tier 2
Deoxyadenosine	243.3	1.40	0.035	Tier 2
3-Hydroxy-L-proline	211.6	1.23	0.016	Tier 2
N-Acetyl-trans-3-Hydroxy-L-proline	602.8	0.82	0.080	Tier 2
Ethanolamine	398.0	0.82	0.045	Tier 2
2,3-Dihydroxyindole	578.9	0.81	0.044	Tier 2

N2'-Acetyl-3'-Hydroxykynurenamine	425.7	0.79	0.087	Tier 2
Cyanate	469.9	0.79	0.014	Tier 2
Isomer of Cyanate	505.6	0.77	0.008	Tier 2
Glycerol	447.9	0.76	0.051	Tier 2
alpha-Ribazole	894.1	0.74	0.014	Tier 2
Isomer of 3-Phenoxybenzyl alcohol	538.1	0.73	0.008	Tier 1
Dihydroshikonofuran	1341.0	0.73	0.018	Tier 2
Glycolaldehyde	613.0	0.71	0.018	Tier 2
N-Acetyl-2-Carboxy-2,3-dihydro-5,6-dihydroxyindole	404.7	0.69	0.014	Tier 2
Allotetrahydrodeoxycorticosterone 3-O-glucuronide	1167.4	0.69	0.027	Tier 2
Phenethyl alcohol	653.4	0.63	0.002	Tier 1
Propane-1,3-diol	945.6	0.56	0.001	Tier 2
Cortolone	1011.9	0.46	0.092	Tier 2
Arabitol	238.7	0.42	0.001	Tier 2
Cortisol 21-O-sulfate	875.1	0.40	0.088	Tier 2

[1]Normalized RT (retention time) shows the corrected retention time of the peak pair with Universal RT Calibrant data.
[2]Tier 1 indicates positive metabolite identification within the chemical isotope labeling (CIL) metabolite library whereas Tier 2 indicates high confidence putative identification within the linked identity (LI) library.

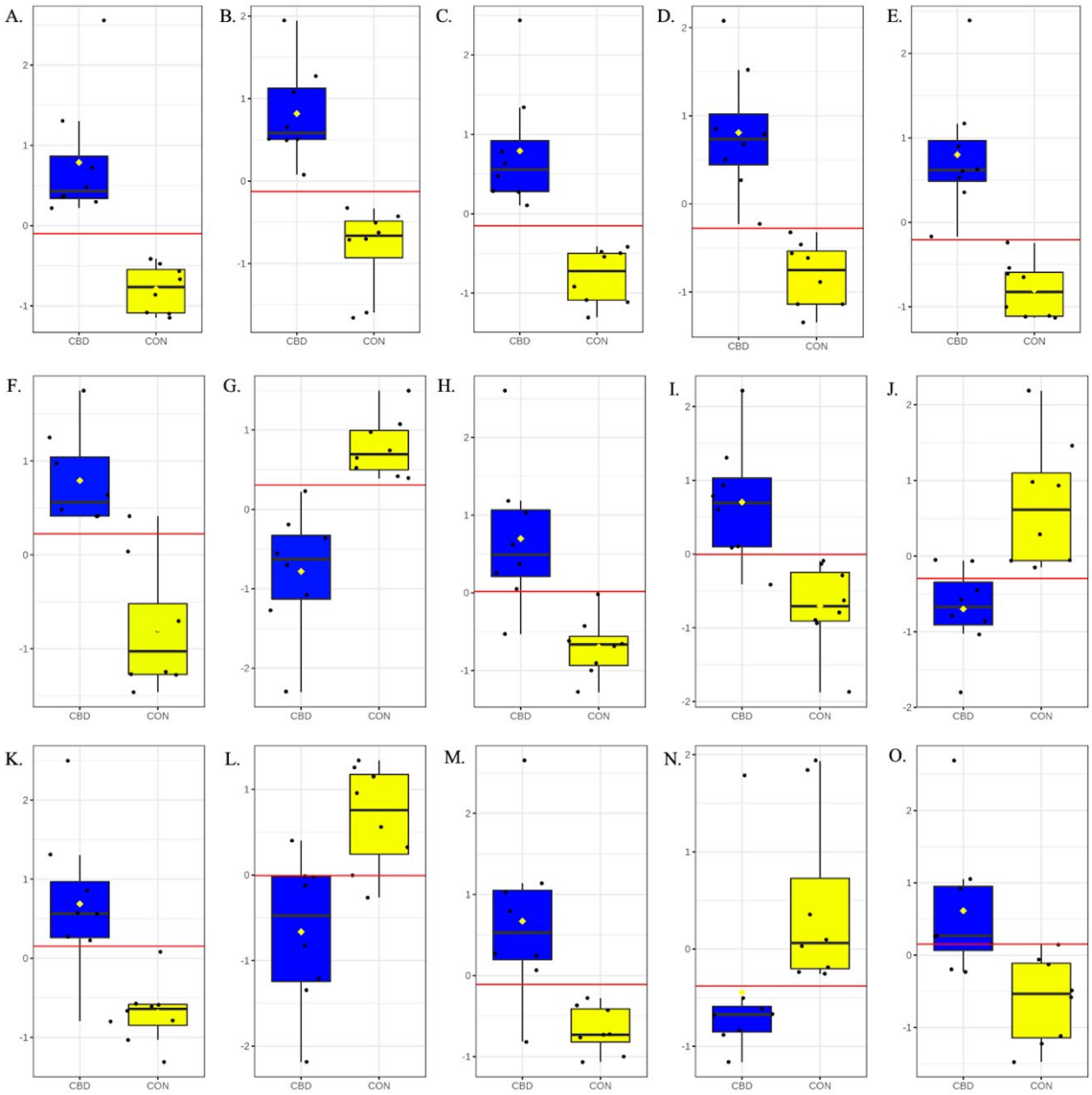


Figure 6.8. Biomarker analysis of hydroxyl metabolites for cannabidiol (CBD) and control (CON) treatments identified **A.** L-fuculose/L-rhamulose (AUROC = 1.00; $P < 0.001$); **B.** glyceraldehyde (AUROC = 1.00; $P < 0.001$); **C.** L-rhamnofuranose (AUROC = 1.00; $P < 0.001$); **D.** L-rhamnono-1,4-lactone (AUROC = 1.00; $P < 0.001$); **E.** Xylitol/Arabitol/Ribitol (AUROC = 1.00; $P < 0.001$); **F.** D-tagatose/L-sorbose (AUROC = 0.98; $P < 0.001$); **G.** propane-1,3-diol (AUROC = 0.98; $P < 0.001$); **H.** 6-deoxy-L-galactose/L-rhamnose (AUROC = 0.97; $P = 0.002$); **I.** D-glucosamine (AUROC = 0.95; $P = 0.001$); **J.** phenethyl alcohol (AUROC = 0.94; $P = 0.002$); **K.** isomer of L-rhamnofuranose (AUROC = 0.92; $P = 0.001$); **L.** 3-phenoxybenzyl alcohol (AUROC = 0.91; $P = 0.003$); **M.** 3,4-dihydroxyphenylpropanoate (AUROC = 0.91; $P = 0.003$); **N.** cortolone (AUROC = 0.91; $P = 0.073$); and **O.** isomer of deoxyadenosine (AUROC = 0.91; $P = 0.008$) as candidate biomarkers.

CHAPTER 7. FEEDING TREATS CONTAINING CANNABIDIOL DID NOT ALTER CANINE IMMUNE RESPONSE TO IMMUNIZATION WITH KEYHOLE LIMPET HEMOCYANIN

Introduction

While there have been preliminary investigations into the safety of its use in dogs (Deabold et al., 2019; Gamble et al., 2018; Vaughn et al., 2020), concerns remain regarding the safety of CBD use as it poses potential risks of hepatoxicity and drug interactions (Ewing et al., 2019; Morris et al., 2020; Yamaori et al., 2011). An additional concern regarding the safety of CBD is its effects on the immune system. It has been proposed to exert anti-inflammatory effects by enhancing adenosine signaling via the A_{2A} receptor, which may protect tissues from inflammation by down-regulating over-reactive immune cells (Carrier et al., 2006; Ohta and Sitkovsky, 2001). Although these actions of CBD have been pursued as potential treatments against chronic inflammatory conditions, CBD may also negatively impact other functions of the immune system, such as responses to vaccination, through the same mechanisms. In several human case studies, the use of marijuana was associated with a decrease in serum IgG and IgM, B and T lymphocytes, and natural killer cells compared to non-smokers (El-Gohary and Eid, 2004; Huemer et al., 2007). The production of these cells and immunoglobulins is essential to the generation of a proper immune response to infection or immunization. Thus, the potential for CBD to inhibit these processes poses a potential risk to the health of animals supplemented with CBD. However, this effect has yet to be tested in a canine model.

Keyhole limpet hemocyanin (KLH) is considered an ideal antigen to assess primary and secondary immune responses to vaccination because it is harmless, has a low

risk of cross-reactivity, and produces an adequate immune response that allows for measureable differences between treatments (Swaminathan et al., 2014). The use of KLH as an antigen in immunotoxicology studies has been validated for use in dogs to evaluate both primary and secondary responses to KLH immunization (Finco-Kent and Kawabata, 2005; Kawai et al., 2013) and has also been used to detect immunosuppressive effects of cyclophosphamide and cyclosporine in dogs (Legrand et al., 2012).

The primary objective of this study was to determine the effect of industrial hemp-derived CBD on the immune response to immunization with a novel antigen in dogs. A secondary objective was to determine if CBD altered the overall health indices of dogs receiving immunization. The underlying hypothesis was that CBD would not negatively impact health indices, nor would it alter or negatively affect and KLH-specific antibody production of dogs receiving CBD.

Materials and Methods

This study was approved by the LMU IACUC (protocol 2005-RES) prior to the start of the study. All housing and husbandry received were in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals (8th ed.), and all applicable LMU SOPs.

Subjects and Housing

Forty adult dogs (20 intact male, 17 female, 3 spayed females; 2.0 ± 1.3 years old; 21.9 ± 6.7 kg) of various mixed breeds were received from several local shelters for inclusion in this study. The shelters were informed and gave consent for the use of the dogs for research purposes before their arrival. Before beginning the experiment, each dog had a hematology and serum chemistry analysis (IDEXX Laboratories, Inc.,

Westbrook, ME) performed, along with a physical evaluation by a veterinarian and a fecal examination to rule out any underlying disease that might preclude enrollment. Dogs were excluded if they demonstrated serious behavioral issues such as aggression that would endanger research personnel; were severely emaciated, classified as a body condition score < 3.5 or > 7.5 on a 9-point scale (where 1 is emaciated and 9 is obese; Laflamme, 1997); or if initial evaluations revealed an underlying disease that required more than routine treatments (i.e. heartworm infection, metabolic or infectious disease, and mobility issues). Thirty-two dogs (16 intact males, 13 intact females, 3 spayed females; 2.1 ± 1.3 years old; 22.4 ± 6.3 kg) were selected for inclusion in the study. Dogs were individually housed in 1.2 x 1.8 m kennels within one of two dog wards at the LMU DeBusk Veterinary Teaching Center. Dogs were stratified by treatment and sex and evenly distributed between the two wards.

Diets and Treatments

Dogs were fed Purina Pro Plan EN Gastroenteric Dry Dog Food (Nestle Purina Inc., St. Louis, MO) to meet the daily metabolizable energy requirements of intact adult dogs at maintenance, calculated as (70 * $BW^{0.75}$) * 1.8 and split into two meals at approximately 0800 h and 1800 h each day. Dogs were weighed and body condition scored (5-point scale) weekly and diets adjusted accordingly. Dogs were stratified by BW and sex before being randomly assigned to treatments arranged in a completely randomized design consisting of 0 (placebo treats; **CON**) or 114.5 ± 6.6 mg CBD/d (**CBD**). Based on the mean BW of dogs included in the study and analysis of the treats, mean dose of CBD was 5.0 ± 3.7 mg CBD/kg BW/d. No other cannabinoids were detected in the treats. The CBD was the primary constituent of a proprietary industrial

hemp isolate (AgTech Scientific, Paris, KY) that was incorporated into treats administered twice daily with each treat containing half the daily dose. Both control and CBD treats were composed of the following ingredients: chicken, chicken liver, Asian carp, catfish, and – in the case of CBD treats – industrial hemp extract. Treats were offered within 30 min of meals solely as a reward upon kennel re-entry following twice-daily exercise. Research personnel administering the treats were blinded as to the treatments administered.

Experimental Design

Upon intake, all dogs underwent a 7-d acclimation period for adjustment to treatments, environment, diet, and daily routine (Table 7.1). Following the initial 7-d acclimation to treatments, dogs were immunized with 10 mg KLH/dog (product #: H7017-5X20MG; Sigma-Aldrich, St. Louis, MO) via intramuscular injection into the semimembranosus muscle region. The KLH was dissolved in physiological saline at a concentration of 10 mg/mL with a total injection volume of 1 mL/dog. This dosage of KLH was selected based on previous work in dogs (Kawai et al., 2013). The solution was filtered using a sterile 0.02 μm membrane filter before injection. The second immunization of KLH was administered in the same manner on d 14 in the contralateral leg immediately after collection of blood samples.

Sample Collection and Analysis

Blood samples (~6 mL) were collected via jugular or cephalic venipuncture upon intake (d -9 and -8) and on d 7, 14, 21, and 28 (Table 7.1). Blood samples were split between serum (~5 mL) and EDTA plasma (~1 mL) tubes. Whole blood samples in

EDTA plasma tubes from each time point were used for hematology analysis using a ProCyte Dx Hematology Analyzer (Table 7.2; IDEXX Laboratories, Inc., Westbrook, ME). Serum samples were allowed to clot for 1 h before centrifugation at 1645 x *g* for 10 min. Approximately 0.5 mL of serum was then used for serum chemistry analysis using a Catalyst Dx Chemistry Analyzer (IDEXX Laboratories, Inc., Westbrook, ME) while the remaining serum was temporarily (24-48 hr) stored at -20°C before being stored at -80°C for later immunoglobulin analysis.

Serum IgG and IgM (mg/dL) were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Dog IgG and IgM; Immunology Consultants Laboratory, Inc., Portland, OR) with inter- and intra-assay coefficients of variation of 7.2% and 6.7%, respectively for IgG and 6.4% and 4.1%, for IgM. Anti-KLH IgG and IgM immunoglobulins (kU/L) was analyzed using commercially available ELISA kits (Canine Anti-KLH IgG and IgM; Alpha Diagnostic Intl. Inc., San Antonio, TX) with inter- and intra-assay coefficients of variation of 8.4% and 3.6%, respectively for IgG and 6.86% and 5.43%, for IgM assays.

Consumption of food and treats, stool consistency, frequency of elimination, mucus membrane color, subjective assessment of activity during exercise, and other indicators of general health status were monitored twice daily by research personnel. Body temperature and injection sites were also monitored immediately and 12 h after KLH immunization for evidence of any reaction to the immunization. Evidence of any adverse event – defined as any symptom occurrence that would not be expected in normal dogs – was also monitored. However, no adverse events were observed in any dogs following KLH immunization nor the administration of CBD treats during this study.

Statistical Analysis

The normality of the residuals was first tested using the UNIVARIATE procedure in SAS 9.4 (SAS Institute, Cary, NC). In instances where data did not meet normality assumptions, statistical analysis was performed on transformed data. Data were then back-transformed for reporting purposes. The standard error of the back-transformed data was calculated from the confidence limits of the transformed data as follows: SEM = (back-transformed upper limit – back-transformed lower limit)/3.92 where the denominator relates to the Z-value of a 95% confidence interval (± 1.96). HGB, RETIC%, RETIC, MONO%, LYM, MONO, MPV, PDW, total protein, alanine aminotransferase, and total IgM were not normally distributed and were log-transformed for statistical analysis. BASO%, the blood urea nitrogen-to-creatinine ratio, alkaline phosphatase, and anti-KLH IgG were not normally distributed and were transformed to the square root for statistical analysis. Glucose and creatinine were not normally distributed and were transformed to the cube root for statistical analysis. Lastly, EOS%, EOS, and BASO were not normally distributed and were transformed to the fourth root for statistical analysis. At baseline, blood urea nitrogen and globulin were increased while albumin and the albumin-to-globulin ratio were decreased in CON compared with CBD; thus, baseline values were utilized as a covariate for the remaining measurements.

Data were then analyzed using the MIXED procedure in SAS including the fixed effects of treatment, day, and the treatment by day interaction. Day was included as a repeated measure with dog nested within treatment as the subject. Results are presented as the mean ± SE. Effects were considered significant when $P \leq 0.05$ and considered a tendency when $P \leq 0.10$.

Results

Hematology and Serum Chemistry

Hematological variables were generally normal for dogs across both treatments throughout the study (Table 7.3). While there were differences or trends in RBC (P = 0.065), hematocrit (P = 0.013), hemoglobin (P = 0.027), mean corpuscular hemoglobin concentration (P = 0.068), and platelets (P = 0.071) between treatments, all were within IDEXX normal reference ranges. Effects of experiment day were observed for almost all variables apart from red cell distribution width, reticulocyte %, and reticulocytes (P = 0.260, 0.285, and 0.309, respectively). Treatment by day interactions were observed for platelet distribution width (P = 0.027) and a trend observed for monocytes (P = 0.065). No other treatment by day interactions were observed (P > 0.05). Monocytes were similar across treatments on each day of the experiment (P > 0.05) except for d 28, where CON tended to be lower than CBD (P = 0.085); platelet distribution width was similar across treatments on each individual day of the experiment (P > 0.05). Again, despite these minor differences between treatments, all remained within the laboratory's normal reference ranges.

With respect to the serum chemistry, again the variables were generally normal for dogs across both treatments (Table 7.4). Day of experiment affected concentrations of all serum chemistry variables (P < 0.05). Blood urea nitrogen and the ratio of blood urea nitrogen to creatinine were increased in CBD compared to CON (P = 0.027 and 0.016, respectively), but both treatments were within normal reference range and no treatment by day interactions were observed (P = 0.105 and 0.689, respectively). A treatment effect and treatment by day interaction were observed for serum albumin (P = 0.017 and 0.044,

respectively), where CON albumin tended to increase compared with CBD on d 14 (P = 0.057) and increased on d 21 and 28 ($P < 0.05$). Again, despite the differences, serum albumin remained within normal reference range in all dogs for the duration of the study. Treatment by day interactions were observed for both globulin ($P = 0.047$) and the albumin to globulin ratio ($P < 0.001$). However, both were similar across treatments on each individual day of the experiment ($P > 0.05$). Both a treatment effect and a treatment by day interaction were observed for alkaline phosphatase ($P = 0.045$ and 0.014, respectively). While similar between treatments at baseline and on d 7 (Figure 7.1; P = 0.994 and 0.183, respectively), alkaline phosphatase was elevated in CBD compared with CON on d 14, 21, and 28 ($P = 0.006$, 0.027, and 0.014, respectively). Again, despite the treatment differences, all stayed within laboratory reference ranges for the duration of the study; however, alkaline phosphatase did increase 2-fold in the CBD group from baseline to d 21.

Total Immunoglobulins

Total IgG was unaffected by both treatment and treatment by day interaction (P = 0.930 and 0.897, respectively) but increased over time (Figure 7.2A; $P < 0.001$). Total IgM was unaffected by treatment ($P = 0.817$) but was affected by day ($P < 0.001$) and a treatment by day interaction (Figure 7.2B; $P = 0.047$). The CON IgM peaked 7 days after initial KLH immunization and returned to baseline levels by day 21, whereas CBD IgM did not peak until day 21 and returned to baseline levels on day 28.

Anti-KLH Immunoglobulins

Anti-KLH immunoglobulins were undetectable in all samples on day 0. Anti-KLH IgG was affected by day (Fig. 7.3A; $P < 0.001$), peaking on day 21 (218.45 ± 8.743 kU/L), but was unaffected by both treatment and treatment by day interaction ($P = 0.422$ and 0.510, respectively). Similarly, anti-KLH IgM was unaffected by treatment and treatment by day interaction (Fig. 7.3B; $P = 0.879$ and 0.927, respectively) but an effect of day was observed ($P < 0.001$). Anti-KLH IgM peaked in both treatments 7 d after the initial KLH immunization (354.27 ± 15.130 kU/L) before declining each subsequent week.

Discussion

Due to the potential impact of CBD on the immune system, the purpose of this investigation was to determine if CBD would alter the humoral immune response of dogs to immunization with KLH and to assess its potential to alter overall health indices. Acute toxicity studies in mice and monkeys have demonstrated the potential for CBD to cause liver injury at extremely high doses (mouse equivalent dose of 200-300 mg/kg) (Ewing et al., 2019; Rosenkrantz et al., 1981). While those doses are not applicable to most real-life scenarios, they do demonstrate the potential risk of CBD use if proper dosing is not established. For example, a dog may get into a box of CBD treats and consume all treats at once. In sub-acute studies in mice, mouse equivalent doses of 15 mg/kg produced only hepatocyte cytoplasmic swelling, but repeated doses of 50 mg/kg elevated several liver enzymes and caused hepatocyte cytoplasmic swelling (Ewing et al., 2019). This demonstrates the potential risk of liver injury at both lower doses and

repeated dosing regimens. The need for continued investigation is evident, particularly since neither toxicity nor long-term dosing studies have been performed in dogs.

Several studies have evaluated changes in hematology and serum chemistry in response to short-term CBD supplementation in dogs. At doses ranging from 2 to 65 mg/kg, CBD has been shown to increase serum alkaline phosphatase in dogs as early as 14 d after treatment initiation (Gamble et al., 2018; McGrath et al., 2018; Vaughn et al., 2020). Results from this study concur with these previous findings as serum alkaline phosphatase, though remaining within the IDEXX normal reference range, was increased in the CBD treatment compared with control on 14 d and remained elevated throughout the study. Whether this increase presents any potential detriment remains uncertain, particularly considering that other serum chemistry variables were similar between treatments. It would be beneficial for future work to investigate the potential for hepatoxic effects in dogs over long-term CBD supplementation as well as the use of multiple CBD doses.

The anti-inflammatory actions of CBD have most often been suggested as potential treatments against chronic inflammatory conditions like multiple sclerosis and osteoarthritis; however, there is also evidence from *in vitro* work suggesting CBD may negatively impact other functions of the immune system. Notably, CBD has been shown to inhibit IL-2, IL-10, and IFNγ production in mice (Condie et al., 1996; Jan et al., 2007), which are essential for both the activation and differentiation of T and B cells in response to an immune challenge (Arenas-Ramirez et al., 2015; Jeurissen et al., 2004). The suppression of antibody production has been demonstrated in mice after a single intraperitoneal dose of CBD (Jan et al., 2007) and is associated with marijuana use in

humans (El-Gohary and Eid, 2004; Huemer et al., 2007). There is, however, conflicting evidence on the immunomodulatory effects of CBD. Jenny et al. (2009) reported a biphasic response in human peripheral blood mononuclear cells (PBMCs), with CBD exhibiting a stimulatory effect at lower doses and inhibitory effects at higher doses. Collectively, these studies highlight the possibility of CBD acting negatively on the immune system and the need for work evaluating this potential risk.

The present study is one of the first to describe the potential effect of CBD on the immune response of dogs after immunization. Despite a minor shift in the timing of total IgM peak between control and CBD, the IgG and IgM responses of both groups were similar throughout the study. Anti-KLH immunoglobulin responses were similar to previous studies using this model in dogs (Finco-Kent and Kawabata, 2005; Kawai et al., 2013), and CBD did not appear to exhibit immunosuppressive effects such as those seen with known immunosuppressants cyclophosphamide and cyclosporine (Legrand et al., 2012). While these results suggest CBD does not negatively impact the immune response of dogs to immunization, the work is preliminary. This work utilized healthy, adult dogs receiving no concommittant medication that might futher impact the immune response; CBD may affect aged or diseased populations differently. Future work should investigate the possibility of different responses to CBD supplementation in diseased, young, or aged populations compared to healthy adults using common canine vaccines rather than KLH. Additionally, our study utilized a single dose (mg/kg) of CBD supplemented over 5 weeks; longer-term CBD dosing using multiple CBD doses is necessary as well as futher investigation into potential drug interactions. It would be beneficial for future work to

evaluate B and T cell population changes with CBD supplementation as this will better describe any potential impact on the immune system.

Conclusions

Results of the current study provide initial evidence that CBD does not negatively impact the normal canine immune response to immunization when supplemented at 5 mg/kg BW/d. Neither total nor KLH-specific immunoglobulin production was reduced by CBD supplementation. However, this work does highlight, alongside several other studies in dogs, the potential for CBD to alter liver function and need for further safety evaluations of CBD use in dogs. Considering these concerns and the increased use of CBD in companion animals, continued investigation into the safety of CBD use in companion animals is critical.

Tables and Figures

Table 7.1. Schedule of events for evaluating immune response to immunization with a novel antigen in dogs receiving cannabidiol containing treats compared to control.

Day of Study	Event	Data Collection	Treats
-9 & -8	Intake Exams	Baseline blood draw	Control
-7 – -1	Acclimation	None	Treatment
0	Primary immunization	None	Treatment
7	Blood Draw	Day 7 post-primary immunization blood draw	Treatment
14	Blood Draw; Secondary immunization	Day 14 post-primary immunization blood draw prior to secondary immunization	Treatment
21	Blood Draw	Day 7 post-secondary immunization blood draw	Treatment
28	Blood Draw	Day 14 post-secondary immunization blood draw	Treatment

Table 7.2. Complete blood count variables and their abbreviations.

Abbreviation	Variable
RBC, M/μL	Red blood cells
HCT, %	Hematocrit
HGB, g/dL	Hemoglobin
MCV, fL	Mean corpuscular volume
MCH, pg	Mean corpuscular hemoglobin
MCHC, g/dL	Mean corpuscular hemoglobin concentration
RDW, %	Red cell distribution width
RETIC %	Reticulocyte percentage
RETIC, K/μL	Reticulocytes
RETIC-HGB, pg	Reticulocyte/Hemoglobin ratio
WBC, K/μL	White blood cells
NEU, %	Percentage neutrophils
LYM, %	Percentage lymphocytes
MONO, %	Percentage monocytes
EOS, %	Percentage eosinophils
BASO, %	Percentage basophils
NEU, K/μL	Neutrophils
LYM, K/μL	Lymphocytes
MONO, K/μL	Monocytes
EOS, K/μL	Eosinophils
BASO, K/μL	Basophils
PLT, K/μL	Platelets
MPV, fL	Mean platelet volume
PDW, fL	Platelet distribution width
PCT, %	Plateletcrit

Table 7.3. Effect of cannabidiol (CBD) treatment (Trt), day, and the treatment by day (Trt*Day) interaction on hematology variables in dogs compared to control (CON).

Variable	IDEXX Reference Interval	CON	CBD	SEM	*P*-value		
					Trt	Day	Trt*Day
RBC, M/μL	5.65-8.87	6.98	7.34	0.135	0.065	<.001	0.657
HCT, %	37.3-61.7	44.35	47.75	0.943	0.013	<.001	0.710
HGB, g/dL	13.1-20.5	16.12	17.12	0.183	0.027	<.001	0.684
MCV, fL	61.6-73.5	63.56	64.92	0.655	0.141	<.001	0.717
MCH, pg	21.2-25.9	23.16	23.44	0.229	0.384	0.070	0.600
MCHC, g/dL	32.0-37.9	36.46	35.97	0.189	0.068	0.004	0.323
RDW, %	13.6-21.7	18.39	18.06	0.308	0.437	0.260	0.929
RETIC %		0.43	0.39	0.027	0.654	0.285	0.610
RETIC, K/μL	10.0-110.0	28.79	28.79	2.014	0.972	0.309	0.637
RETIC-HGB, pg	22.3-29.6	25.83	26.57	0.375	0.164	<.001	0.781
WBC, K/μL	5.05-16.76	12.05	12.75	0.809	0.528	0.001	0.316
NEU, %		55.89	60.13	1.881	0.111	<.001	0.629
LYM, %		27.25	25.73	1.650	0.506	<.001	0.451
MONO, %		6.23	6.17	0.161	0.884	<.001	0.187
EOS, %		7.78	5.62	0.684	0.205	<.001	0.795
BASO, %		0.60	0.57	0.028	0.754	0.037	0.140
NEU, K/μL	2.95-11.64	6.39	7.41	0.292	0.231	<.001	0.493
LYM, K/μL	1.05-5.10	2.97	2.92	0.132	0.883	<.001	0.100
MONO, K/μL	0.16-1.12	0.72	0.76	0.023	0.582	<.001	0.065
EOS, K/μL	0.06-1.23	0.89	0.72	0.087	0.403	<.001	0.551
BASO, K/μL	0.00-0.10	0.07	0.07	0.004	0.754	0.008	0.152
PLT, K/μL	148-484	259.5	225.1	13.346	0.071	0.040	0.755
MPV, fL	8.7-13.2	12.06	12.18	0.133	0.895	0.012	0.972
PDW, fL	9.1-19.4	12.06	12.18	0.202	0.800	0.006	0.027
PCT, %	0.14-0.46	0.31	0.27	0.015	0.044	0.104	0.730

RBC, red blood cells; HCT, hematocrit; HGB, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; RETIC, reticulocyte; WBC, white blood

cells; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; PLT, platelets; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit

Table 7.4 Effect of cannabidiol (CBD) treatment (Trt), day, and the treatment by day (Trt*Day) interaction on serum chemistry variables indicative of liver function in dogs compared to control (CON).

Variable	IDEXX Reference Interval	CON	CBD	SEM	*P*-value		
					Trt	Day	Trt*Day
Glucose, mg/dL	74-143	88.72	88.12	1.116	0.941	<.001	0.280
Creatinine, mg/dL	0.5-1.8	1.12	1.09	0.018	0.495	<.001	0.643
BUN, mg/dL	7-27	16.29	18.73	0.777	0.027	<.001	0.103
BUN/CREA		14.71	17.53	0.808	0.016	0.082	0.689
Total Protein, g/dL	5.2-8.2	6.75	6.69	0.066	0.667	0.003	0.996
Albumin, g/dL	2.3-4.0	3.44	3.24	0.066	0.017	<.001	0.044
Globulin, g/dL	2.5-4.5	3.46	3.29	0.072	0.455	<.001	0.047
ALB/GLOB		1.00	0.99	0.045	0.818	<.001	<.001
ALT, U/L	10-125	56.83	55.70	2.729	0.874	<.001	0.387
ALP, U/L	23-212	57.61	93.32	7.099	0.045	<.001	0.014

BUN, blood urea nitrogen; BUN/CREA, blood urea nitrogen/creatinine ratio; ALB/GLOB, albumin/globulin ratio; ALT, alanine aminotransferase; ALP, alkaline phosphatase

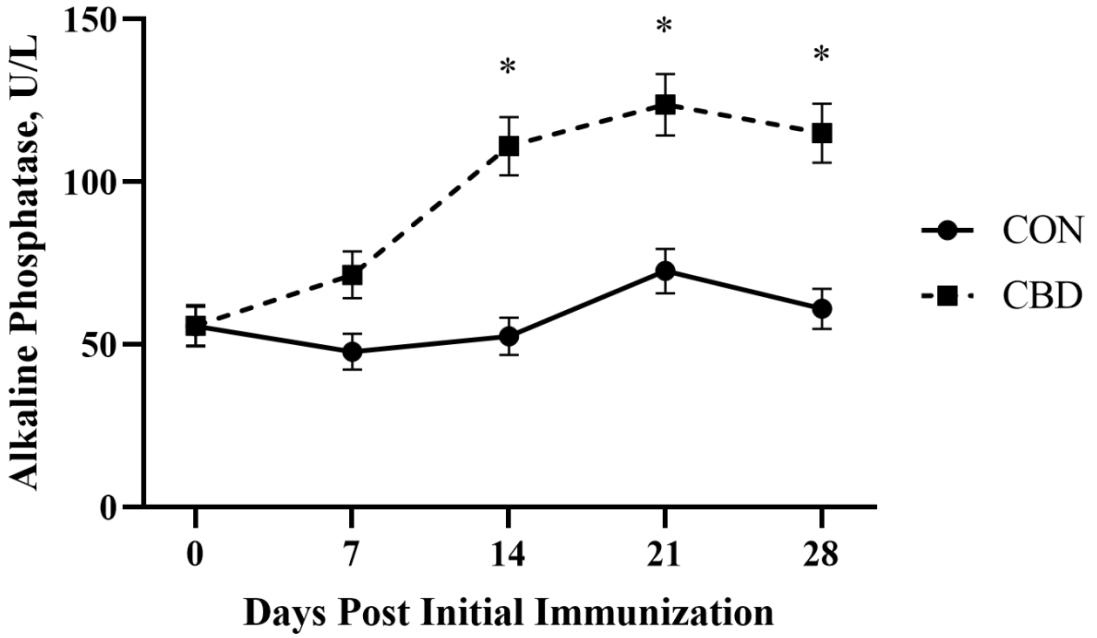
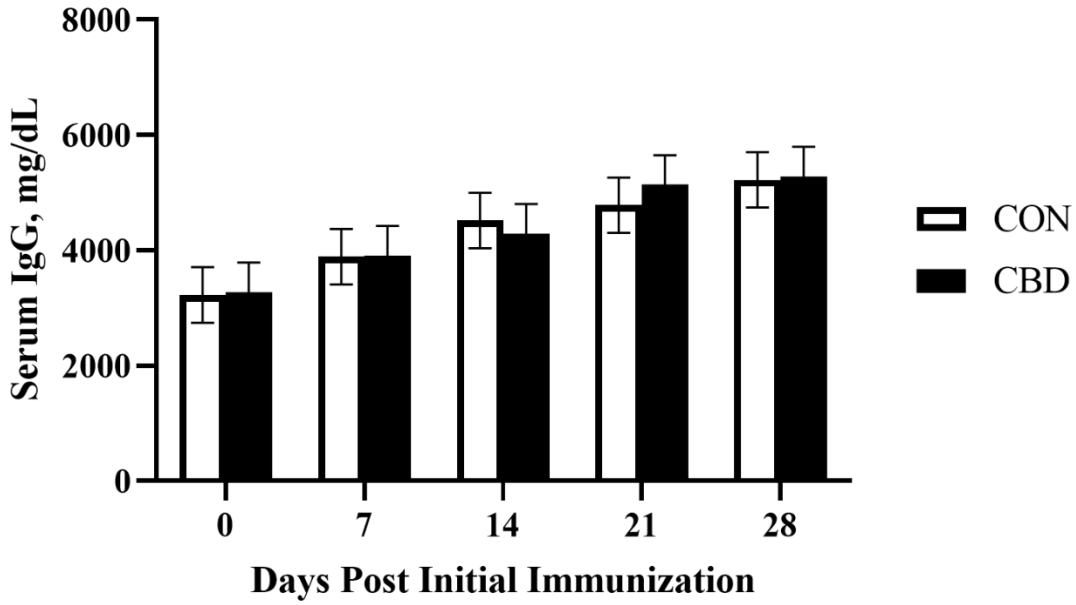


Figure 7.1. Serum alkaline phosphatase (U/L) of control (CON) and cannabidiol (CBD) treatments over time. Data were not normally distributed and were transformed by the square root for statistical analysis, then back-transformed for reporting purposes. Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92. Alkaline phosphatase levels remained within IDEXX normal reference range throughout the study and were similar between treatments at baseline (day 0, $P = 0.994$) and day 7 ($P = 0.183$), but were increased in CBD treatment on days 14, 21, and 28 compared to control ($P = 0.006$, 0.027, and 0.014, respectively). Asterisks (*) Indicate differences between treatments ($P < 0.05$) on that day.

A.


8000
6000
4000
2000
0
Serum IgG, mg/dL
0
7
14
21
28
Days Post Initial Immunization
CON
CBD

B.

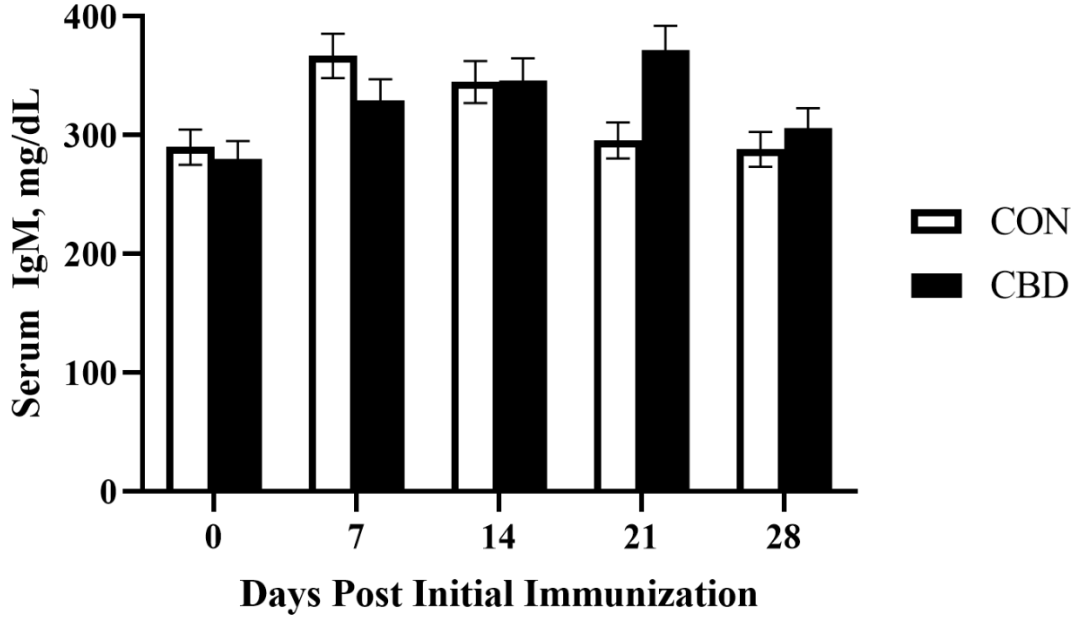
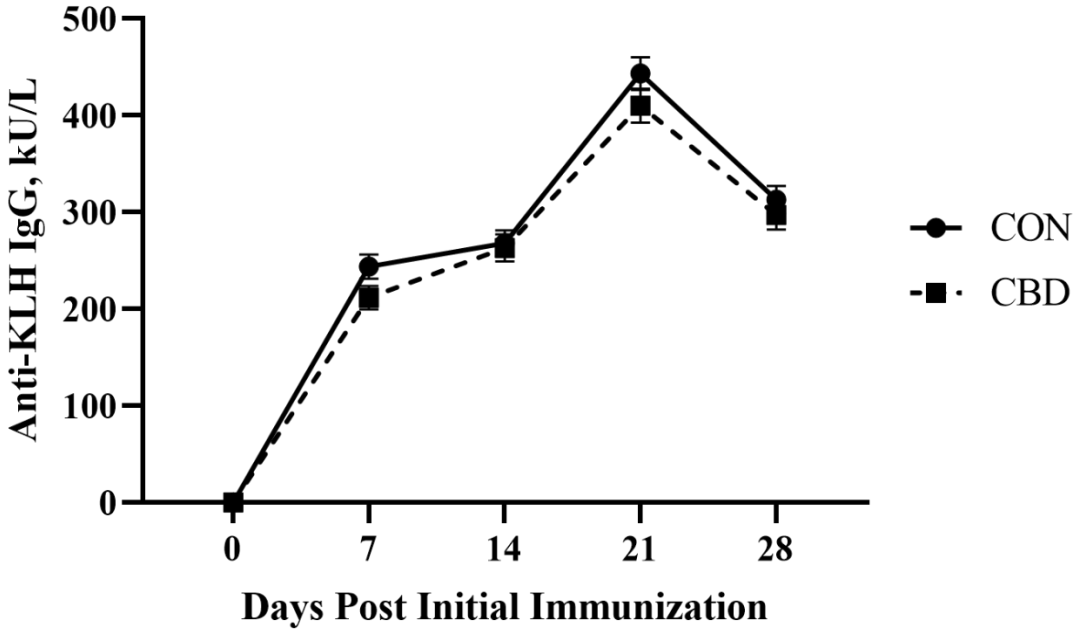

400
300
200
100
0
Serum IgM, mg/dL
0
7
14
21
28
Days Post Initial Immunization
CON
CBD

Figure 7.2. Total serum **A)** IgG and **B)** IgM (mg/dL) of control (CON) and cannabidiol (CBD) treatments over the 28-d experiment. Dogs were challenged with keyhole limpet hemocyanin on days 0 and 14 after collection of blood samples. Both total IgG and total IgM concentrations were altered by day ($P < 0.001$). Day of experiment altered both total IgG and total IgM concentrations ($P < 0.001$). Total IgG was unaffected by both treatment and the treatment by day interaction ($P = 0.930$ and 0.897, respectively). Total IgM was not normally distributed and was log-transformed for statistical analysis, then back-transformed for reporting purposes. Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92. Total IgM was unaffected by treatment ($P = 0.817$) but a treatment by day interaction was observed ($P = 0.047$).

A.


500
400
300
200
100
0
Anti-KLH IgG, kU/L
0
7
14
21
28
Days Post Initial Immunization
CON
CBD

B.

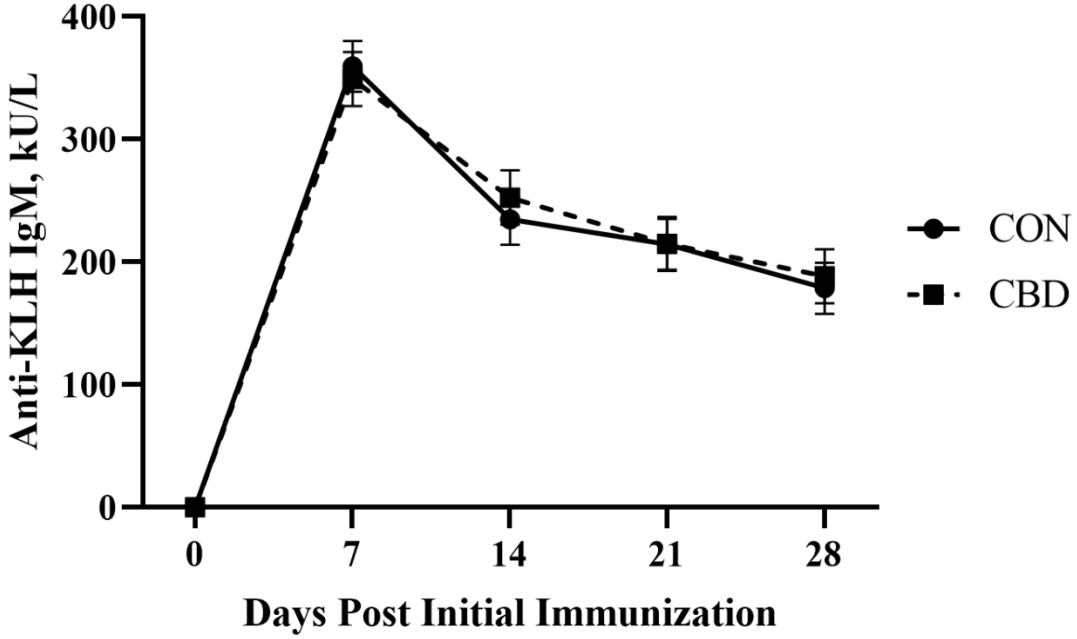

400
300
200
100
0
Anti-KLH IgM, kU/L
0
7
14
21
28
Days Post Initial Immunization
CON
CBD

Figure 7.3. Anti-Keyhole Limpet Hemocyanin (KLH) **A)** IgG and **B)** IgM (kU/mL) of control (CON) and cannabidiol (CBD) treatments over the 28-d experiment. Dogs were challenged with KLH on days 0 and 14 after collection of blood samples. Anti-KLH IgG was not normally distributed and was transformed to the square root for statistical analysis, then back-transformed for reporting purposes. Standard error (SE) of the back-transformed data was calculated as follows: SE = (back-transformed upper limit – back-transformed lower limit)/3.92. Day of experiment altered both anti-KLH IgG and IgM ($P < 0.001$). Both anti-KLH IgG and IgM were unaffected by treatment ($P = 0.422$ and 0.879, respectively) and the treatment by day interaction ($P = 0.510$ and 0.927, respectively).

CHAPTER 8. SUMMARY AND CONCLUSIONS

Interest and use of CBD in companion animals is increasing due to suspected benefits like antioxidant, anti-inflammatory, and anxiolytic effects. Yet despite increased use and favorable public opinion, scientific literature detailing mechanisms of action are inconsistent, and evidence of safety and efficacy of CBD use in dogs is shockingly limited. Therefore, the primary goal of this dissertation was to investigate the potential impact of industrial hemp-derived CBD treat supplementation on the health and well-being of dogs. This objective was accomplished through assessments of canine anxiety, voluntary activity, metabolome, overall health indices, and immune response during CBD supplementation.

To study the effect of oral CBD on canine health and well-being, industrial hemp-derived CBD extract was included in a dog treat formulation for supplementation. Palatability of treats was first assessed when dogs were fed a variety of diet types. Neither food consumption nor treat acceptance were altered by increasing CBD inclusion in treats. These treats provide a simple, palatable method of administering oral CBD at precise dosages for research, which is an improvement from use of oils that must either be top-dressed or dosed via syringe.

Despite the lack of evidence regarding efficacy of CBD as an anxiolytic, canine anxiety and noise aversion are some of the most common reasons that pet owners administer CBD to their dogs (Kogan et al., 2019a). The second part of this series of experiments was the first to investigate the potential anxiolytic effect of CBD in dogs with noise aversion through the use of a noise-induced fear response test. Cannabidiol

supplemented at 1.4 mg CBD/kg BW/d did not demonstrate an anxiolytic effect in this model. The positive control, trazodone, decreased plasma cortisol, increased heart rate variability variables, and increased duration of the relaxed behaviors, Other Eyes and tail relaxed. Cannabidiol by itself decreased duration of relaxed behavior Other Eyes and did not alter plasma cortisol. When supplemented in combination with trazodone, however, CBD appeared to attenuate the effects of trazodone on plasma cortisol, which may be indicative of cytochrome P450 inhibition by CBD that could lead to drug interactions. Instead of decreasing heart rate and increasing heart rate variability as expected from an anxiolytic, CBD increased heart rate and decreased some heart rate variability variables. The lack of anxiolytic effect by CBD may have been a result of the low dose, short duration of supplementation, or use of Latin square design.

The next study in this series of experiments did not show a change in voluntary daily activity in dogs supplemented with 1.8 or 4.5 mg CBD/kg BW/d. The higher dose tended to reduce voluntary activity during Quiet and Music periods, but this did not translate to an overall daily effect. As this experiment utilized healthy animals to examine voluntary daily activity, this effect may be more evident in dogs with mobility and behavioral issues like osteoarthritis or anxiety. Unexpectedly, CBD reduced scratching, which may indicate a possible antipruritic effect warranting investigation in dogs with dermatological issues like skin allergies or atopic dermatitis.

Using the same cohort of dogs from the previous study, alteration of the plasma metabolome was observed after 21 d of 4.5 mg CBD/kg BW/d supplementation. Metabolites altered by CBD may suggest a potential influence on glucose, carbohydrate, lipid, amino acid, vitamin, and nucleotide metabolism. Potential biomarkers identified

may indicate potential pathways by which CBD may exert suspected anti-inflammatory, antioxidant, anti-obesity, and antimicrobial effects. The identification of these metabolic changes will help direct future research into the elucidation of potential mechanisms and evaluation of the physiological relevance of these changes.

The last experiment in this series was one of the first to assess the potential risk for CBD to diminish the normal immune response to immunization. When supplemented to healthy dogs at 5 mg CBD/kg BW/d, both total and KLH-specific immunoglobulin production were unaffected by CBD. While CBD demonstrated no immunosuppressive effects after 5 weeks of supplementation, alkaline phosphatase was elevated in dogs receiving CBD, highlighting the potential for CBD to alter liver function and need for further safety and hepatoxicity evaluations of CBD use in dogs, particularly in aged or diseased populations that may have compromised immune function.

While concerns of long-term safety remain as potential drug interactions and altered liver function were observed, the potential therapeutic applications of CBD make further investigation into these effects critical. Future work utilizing larger sample sizes and several doses is needed to assess long-term safety and proper dosing of CBD in dogs. Additionally, as our work utilized only healthy dogs, additional investigation is needed to evaluate these potential therapeutic benefits in aged populations, dogs with diabetes, dogs with atopic dermatitis, or other specific disorders.

In conclusion, this work provides initial evidence that industrial hemp-derived CBD may impact the health and well-being of dogs. Formulating dog treats to include CBD extract was shown to be a simple and palatable means of administering CBD that can be used by both researchers and pet owners. Although CBD did not appear to

influence behavioral measures in this work, it may have the potential to improve the well-being of dogs. As the use of CBD in companion animals continues to increase, increasing our understanding of physiological mechanisms by which CBD exerts these effects is crucial to further elucidate how CBD may improve animal health and well-being.

REFERENCES

Abaskharon, R.M., Mukherjee, D., and Gai, F. (2019). 4-Oxoproline as a Site-Specific Infrared Probe: Application To Assess Proline Isomerization and Dimer Formation. J. Phys. Chem. B. 123(24):5079-5085. doi: 10.1021/acs.jpcb.9b03766

Adams, A.A., Breathnach, C.C., Katepalli, M.P., Kohler, K., and Horohov, D.W. (2008). Advanced age in horses affects divisional history of T cells and inflammatory cytokine production. Mech Ageing Dev. 129(11):656-64. doi: 10.1016/j.mad.2008.09.004

Adams, R., Hunt, M., and Clark, J.H. (1940). Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp. J Am Chem Soc. 62(1):196-200. doi: 10.1021/ja01858a058

Adeyemi, J.A., Harmon, D.L., Paulus Compart, D.M., and Ogunade, I.M. (2019). Effects of a blend of Saccharomyces cerevisiae-based direct-fed microbial and fermentation products in the diet of newly weaned beef steers: growth performance, whole-blood immune gene expression, serum biochemistry, and plasma metabolome. J. Anim. Sci. 97(11):4657-4667. doi: 10.1093/jas/skz308

Albensi, B.C. (2019). What is nuclear factor kappa B (NF-κB) doing in and to the mitochondrion? Front. Cell Dev. Biol. 7:154. doi: 10.3389/fcell.2019.00154

Aldrich, G.C. and Koppel, K. (2015). Pet food palatability evaluation: A review of standard assay techniques and interpretations of results with a primary focus on limitations. Animals. 5(1):43-55. doi: 10.3390/ani5010043

Alhapel, A., Darley, D.J., Wagener, N., Eckel, E., Elsner, E., and Pierik, A.J. (2006). Molecular and functional analysis of nicotinate catabolism in Eubacterium barkeri. Proc. Natl. Acad. Sci. USA. 103(33):12341-12346. doi: 10.1073/pnas.0601635103

Amaravadi, R.K., Kimmelman, A.C., and Debnath, J. (2019). Targeting autophagy in cancer: Recent advances and future directions. Cancer Discov. 9(9):1167-1181. doi: 10.1158/2159-8290.CD-19-0292

Amaya, V., Paterson, M.B.A., and Phillips, C.J.C. (2020). Effects of olfactory and auditory enrichment on the behavior of shelter dogs. Animals, 10(4):581. doi: 10.3390/ani10040581

Anderson, J.W., Nicolosi, R.J., and Borzelleca, J.F. (2005). Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food Chem. Toxicol. 43(2):187-201. doi: 10.1016/j.fct.2004.11.006

Andre, C.M., Hausman, J.-F., and Guerriero, G. (2016). Cannabis sativa: The plant of the thousand and one molecules. Front. Plant Sci. 7:19. doi: 10.3389/fpls.2016.00019

Appiah-Kusi, E., Petros, N., Wilson, R., Colizzi, M., Bossong, M.G., Valmaggia, L., et al. (2020). Effects of short-term cannabidiol treatment on response to social stress in subjects at clinical high risk of developing psychosis. Psychopharmacology. 237:1121-1130. doi: 10.1007/s00213-019-05442-6

Araujo, J.A., de Rivera, C., Ethier, J.L., Landsberg, G.M., Denenberg, S., Arnold, S., et al. (2010). ANXITANE® tablets reduce fear of human beings in a laboratory model of anxiety-related behavior. J. Vet. Behav. 5(5):268-275. doi: 10.1016/j.jveb.2010.02.003

Araujo, J.A., C. de Rivera, C., Landsberg, G.M., Adams, P.E., and Milgram, N.W. (2013). Development and validation of a novel laboratory model of sound-induced fear and anxiety in Beagle dogs. J. Vet. Behav. 8:204-212. doi: 10.1016/j.jveb.2012.10.008

Arenas-Ramirez, N., Woytschak, J., and Boyman, O. (2015). Interleukin-2: Biology, design, and application. Trends Immunol. 36(12):763-777. doi: 10.1016/j.it.2015.10.003

Arfin, S., Siddiqui, G.A., Naeem, A., and Moin, S. (2018). Inhibition of advanced glycation end products by isoferulic acid and its free radical scavenging capacity: An in vitro andmolecular docking study. Int. J. Biol. Macromol. 118:1479-1487. doi: 10.1016/j.ijbiomac.2018.06.182

Ashraf, A., Darley, D.J., Wagener, N., Eckel, E., Elsner, N., and Pierik, A. (2006). Molecular and functional analysis of nicotinate catabolism in Eubacterium barkeri. Proc. Natl. Acad. Sci. USA. 103(33):12341-12346. doi: 10.1073/pnas.0601635103

Atalay, S., Jarocka-Karpowicz, I., and Skrzydlewska, E. (2019). Antioxidative and anti-inflammatory properties of cannabidiol. Antioxidants (Basel). 9(1):21. doi: 10.3390/antiox9010021

Atsmon, J., Heffetz, D., Deutsch, L., Deutsch, F., and Sacks, H. (2018). Single-dose pharmacokinetics of oral cannabidiol following administration of PTL101: A new formulation based on gelatin matrix pellets technology. Clin. Pharmacol. Drug Dev. 7(7):751-758. doi: 10.1002/cpdd.408

Aviello, G., Romano, B., Borrelli, F., Capasso, R., Gallo, L., Piscitelli, F., Di Marzo, V., and Izzo, A.A. (2012). Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. J. Mol. Med. (Berl). 90(8):925-34. doi: 10.1007/s00109-011-0856-x

Avila, C., Massick, S., Kaffenberger, B.H., Kwatra, S.G., and Bechtel, M. (2020). Cannabinoids for the treatment of chronic pruritus: A review. J. Am. Acad. Dermatol. 82:1205-1212. doi: 10.1016/j.jaad.2020.01.036

Ayala, A., Muñoz, M.F., and Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell Longev. 2014:360438. doi: 10.1155/2014/360438

Babson, K.A., Sottile, J., and Morabito, D. (2017). Cannabis, cannabinoids, and sleep: A review of the literature. Curr. Psychiatry Rep. 19:23. doi: 10.1007/s11920-017-0775-9

Baek, H.J., Cho, C.H., Cho, J., and Woo, J.M. (2015). Reliability of ultra-short-term analysis as a surrogate of standard 5-min analysis of heart rate variability. Telemed. J. E. Health. 21(5):404-414. doi: 10.1089/tmj.2014.0104

Bansal, P., Wang, S., Liu, S., Xiang, Y-Y., Lu, W-Y., et al. (2011). GABA Coordinates with Insulin in Regulating Secretory Function in Pancreatic INS-1 β-Cells. PLoS One. 6(10):e26225. doi: 10.1371/journal.pone.0026225

Barkulis, S.S. and Krakow, G. (1956). Conversion of glyoxylate to hydroxypyruvate by extracts of Escherichia coli. Biochim. Biophys. Acta. 21(3):593-4. doi: 10.1016/0006-3002(56)90208-6

Bartner, L.R., McGrath, S., Rao, S., Hyatt, L.K., and Wittenburg, L.A. (2018). Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. Can. J. Vet. Res. 82:178-183. doi: 10.3389/fvets.2018.00165

Bartsch, O., Hagemann, M., and Bauwe, H. (2008). Only plant-type (GLYK) glycerate kinases produce d-glycerate 3-phosphate. FEBS Lett. 582(20):3025-8. doi: 10.1016/j.febslet.2008.07.038

Beale, B.S. (2004). Use of nutraceuticals and chondroprotectants in osteoarthritic dogs and cats. Vet. Clin. North Am. Small Anim. Pract. 34(1):271-289. doi: 10.1016/j.cvsm.2003.09.008

Becker, D.J. and Lowe, J.B. (2003). Fucose: Biosynthesis and biological function in mammals. Glycobiology. 13(7):41R-53R. doi: 10.1093/glycob/cwg054

Behr, M., Pokorna, E., Dobrev, P.I., Motyka, V., Guignard, C., Lutts, S., et al. (2019). Impact of jasmonic acid on lignification in the hemp hypocotyl. Plant Signal Behav. 14(6):1592641. doi: 10.1080/15592324.2019.1592641

Bellinzoni, M., Bastard, K., Perret, A., Zaparucha, A., Perchat, N., Vergne, C., et al. (2011). 3-Keto-5-aminohexanoate cleavage enzyme: a common fold for an

uncommon Claisen-type condensation. J. Biol. Chem. 286(31):27399-405. doi: 10.1074/jbc.M111.253260

Bellocchio, L., Cervino, C., Pasquali, R., and Pagotto, U. (2008). The endocannabinoid system and energy metabolism. J. Neuroendocrinol. 20(6):850-857. doi: 10.1111/j.1365-2826.2008.01728.x

Belostotsky, R., Pitt, J.J. and Frishberg, Y. (2012). Primary hyperoxaluria type III—a model for studying perturbations in glyoxylate metabolism. J. Mol. Med. 90:1497–1504. doi: 10.1007/s00109-012-0930-z

Bergamaschi, M.M., Queiroz, R.H.C., Crippa, J.A.S., and Zuardi, A.W. (2011). Safety and side effects of cannabidiol, a *Cannabis sativa* constituent. Curr Drug Saf. 6(4):237-249. doi: 10.2174/157488611798280924

Berndtson, W. E. (1991). A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. J. Anim. Sci. 69(1):67-76. doi: 10.2527/1991.69167x

Bhandage, A.K., Jin, Z., Korol, S.V., Shen, Q., Pei, Y., Deng, et al. (2018). GABA Regulates Release of Inflammatory Cytokines From Peripheral BloodMononuclear Cells and CD4+ T Cells and Is Immunosuppressive in Type 1 Diabetes. EBioMedicine. 30:283-294. doi: 10.1016/j.ebiom.2018.03.019

Bhathal, A., Spryszak, M., Louizos, C., and Frankel, G. (2017). Glucosamine and chondroitin use in canines for osteoarthritis: A review. Open Vet. J. 7(1):36-49. doi: 10.4314/ovj.v7i1.6

Bielawiec, P., Harasim-Symbor, E., and Chabowski, A. (2020). Phytocannabinoids: Useful Drugs for the Treatment of Obesity? Special Focus on Cannabidiol. Front. Endocrinol. 11:114. doi: 10.3389/fendo.2020.00114

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D.E., Brandi, I., Moriello, A.S., Davis, J.B., Mechoulam, R., and Di Marzo, V. (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br. J. Pharmacol. 134(4):845-852. doi: 10.1038/sj.bjp.0704327

Blackwell, E.J., Bradshaw, J.W.S., and Casey, R.A. (2013). Fear responses to noises in domestic dogs: Prevalence, risk factors and co-occurrence with other fear related behaviour. Appl. Anim. Behav. Sci. 145:15-25. doi: 10.1016/j.applanim.2012.12.004

Blessing, E.M., Steenkamp, M.M., Manzanares, J., and Marmar, C.R. (2015). Cannabidiol as a potential treatment for anxiety disorders. Neurotherapeutics. 12:825-836. doi: 10.1007/s13311-015-0387-1

Blötz, C. and Stülke, J. (2017). Glycerol metabolism and its implication in virulence in Mycoplasma. FEMS Microbiol. Rev. 41(5):640-652. doi: 10.1093/femsre/fux033

Boehm, R., Sommer, S., Li, S.M., and Heide, L. (2000). Genetic engineering on shikonin biosynthesis: expression of the bacterial ubiA gene in Lithospermum erythrorhizon. Plant Cell Physiol. 41(8):911-9. doi: 10.1093/pcp/pcd013

Bogucki, Sz. and Noszczyk-Nowak, A. (2015). Short-term heart rate variability (HRV) in healthy dogs. Pol. J. Vet. Sci. 18(2):307-312. doi:10.1515/pjvs-2015-0040

Bohren, K.M., Bullock, B., Wermuth, B., and Gabbay, K.H. (1989). The aldo-keto reductase superfamily. cDNAs and deduced amino acid sequences of human aldehyde and aldose reductases. J. Biol. Chem. 264(16):9547-51

Boleti, A.P.A., Frihling, B.E.F., Silva, P.S.E., Cardoso, P.H.O., de Moraes, L.F.R.N., Rodrigues, T.A.A., Biembengute, M.E.F., Koolen, H.H.F., and Migliolo, L. (2020). Biochemical aspects and therapeutic mechanisms of cannabidiol in epilepsy. Neurosci. Biobehav. Rev. S0149-7634(20)30583-2. doi: 10.1016/j.neubiorev.2020.09.027

Bonaccorso, S., Ricciardi, A., Zangani, C., Chiappini, S., and Schifano, F. (2019). Cannabidiol (CBD) use in psychiatric disorders: A systematic review. Neurotoxicology. 74:282-298. doi: 10.1016/j.neuro.2019.08.002

Bonini, S.A., Premoli, M., Tambaro, S., Kumar, A., Maccarinelli, G., Memo, Maurizio, and Mastinu, A. (2018). Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. J. Ethnopharmacol. 227:300-315. doi: 10.1016/j.physbeh.2017.01.024

Boon, M.R., Kooijman, S., van Dam, A.D., Pelogrom, L.R., Berbee, J.F.P., Visseren, C.A.R., et al. (2014). Peripheral cannabinoid 1 receptor blockade activates brown adipose tissue and diminishes dyslipidemia and obesity. FASEB J. 28(12):5361-75. doi: 10.1096/fj.13-247643

Borges, R.S., Batista, J. Jr., Viana, R.B., Baetas, A.C., Orestes, E., Andrade, M.A., et al. (2013). Understanding the molecular aspects of tetrahydrocannabinol and cannabidiol as antioxidants. Molecules. 18(10):12663-74. doi: 10.3390/molecules181012663

Bowman, A., Scottish SPCA, Dowell, F.J., and Evans, N.P. (2017). The effect of different generes of music on the stress levels of kennelled dogs. Phys. Behav. 171:207-215. doi: 10.1016/j.physbeh.2017.01.024

Bradford, K.L., Moretti, F.A., Carbonaro-Sarracino, D.A., Gaspar, H.B., and Kohn, D.B. (2017). Adenosine deaminase (ADA)-deficient severe combined immune

deficiency (SCID): Molecular pathogenesis and clinical manifestations. J. Clin. Immunol. 37(7):626-637. doi: 10.1007/s10875-017-0433-3

Braggelaar, M.P., Maccarrone, M., and van der Stelt, M. (2018). 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. Prog. Lipid Res. 71:1-17. doi: 10.1016/j.plipres.2018.05.002

Brément, T., Laly, M.J., Combarros, D., Guillemaille, D., Bourdeau, P.J., and Bruet, V. (2019). Reliability of different sets of criteria in diagnosing canine atopic dermatitis applied to a population of 250 dogs seen in a veterinary teaching hospital. Vet. Dermatol. 30(3):188-e59. doi: 10.1111/vde.12729

Brioschi, F.A., Di Cesare, F., Gioeni, D., Rabbogliatti, V., Ferrari, F., D'Urso, E.S., Amari, M., and Ravasio, G. (2020). Oral transmucosal cannabidiol oil formulation as part of a multimodal analgesic regimen: Effects on pain relief and quality of life improvement in dogs affected by spontaneous osteoarthritis. Animals. 10(9):1505. doi: 10.3390/ani10091505

Brouns, S.J.J., Walther, J., Snijders, A.P.L., van de Werken, H.J.G., Willemen, H.L.D.M., Worm, P., et al. (2006). Identification of the missing links in prokaryotic pentose oxidation pathways: Evidence for enzyme recruitment. J. Biol. Chem. 281(37):27378-27388. doi: 10.1074/jbc.M605549200

Brown, D.C., Boston, R.C., Coyne, J.C., and Farrar, J.T. (2008). Ability of the Canine Brief Pain Inventory to detect response to treatment in dogs with osteoarthritis. J. Am. Vet. Med. Assoc. 233(8):1278-1283. doi: 10.2460/javma.233.8.1278

Brown, D.C., Boston, R.C., and Farrar, J.T. (2010). Use of an activity monitor to detect response to treatment in dogs with osteoarthritis. J. Am. Vet. Med. Assoc. 237:66-70. doi: 10.2460/javma.237.1.66

Brown, D.C., Boston, R.C., and Farrar, J.T. (2013). Comparison of force plate gait analysis and owner assessment of pain using the canine brief pain inventory in dogs with osteoarthritis. J. Vet. Intern. Med. 27:22-30. doi: 10.1111/jvim.12004

Brown, D.D. and Kies, M.W. (1959). The mammalian metabolism of L-histidine. I. The enzymatic formation of L-hydantion-5-propionic acid. J. Biol. Chem. 234:3182-3187.

Bujak, J.K., Kosmala, D., Szopa, I.M., Majchrzak, K., and Bednarczyk, P. (2019). Inflammation, cancer and immunity-implication of TRPV1 channel. Front. Oncol. 9:1087. doi: 10.3389/fonc.2019.01087

Burstein, S. (2015). Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. Bioorg Med Chem. 23(7):1377-85. doi: 10.1016/j.bmc.2015.01.059

Burston, J.J. and Woodhams, S.G. (2014). Endocannabinoid system and pain: An introduction. Proc. Nutr, Soc. 73(1):106-17. doi: 10.1017/S0029665113003650

Butcher, N.J. and Minchin, R.F. (2012). Arylamine N-Acetyltransferase 1: A Novel Drug Target in Cancer Development. Pharmacol. Rev. 64(1):147-165. doi: 10.1124/pr.110.004275

Campanini, B., Bettati, S., di Salvo, M.L., Mozzarelli, A., and Constestabile, R. (2013). Asymmetry of the active site loop conformation between subunits of glutamate-1-semialdehyde aminomutase in solution. Biomed. Res. Int. 2013:353270. doi: 10.1155/2013/353270

Campora, L., Miragliotta, V., Ricci, E., Cristino, L., Di Marzo, V., Albanese, F. et al. (2012). Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis. Am. J. Vet. Res. 73(7):988-95. doi: 10.2460/ajvr.73.7.988

Camporez, J.P., Wang, Y., Faarkrog, K., Chukijrungroat, N., Petersen, K.F., and Shulman, G.I. (2017). Mechanism by which arylamine N-acetyltransferase 1 ablation causes insulin resistance in mice. Proc. Natl. Acad. Sci. USA. 114(52):E11285-E11292. doi: 10.1073/pnas.1716990115

Campos, A.C., de Paula Soares, V., Carvalho, M.C., Ferreira, F.R., Vicente, M.A., Brandao, M.L., Zuardi, A.W., Zangrossi, H., and Guimaraes, F.S. (2013). Involvement of serotonin-mediated neurotransmission in the dorsal periaqueductal gray matter on cannabidiol chronic effects in panic-like responses in rats. Psychopharmacology. 226(1):13-24. doi: 10.1007/s00213-012-2878-7

Campos, A.C., Fogaça, M.V., Sonego, A.B., and Guimarães, F.S. (2016). Cannabidiol, neuroprotection and neuropsychiatric disorders. Pharmacol. Res. 112:119-127. doi: 10.1016/j.phrs.2016.01.033

Campos, A.C. and Guimarães, F.S. (2008). Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology (Berl). 199(2):223-30. doi: 10.1007/s00213-008-1168-x

Campos, A.C., Moreira, F.A., Gomes, F.V., Del Bel, E.A., and Guimarães, F.S. (2012). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 367(1607):3364-78. doi: 10.1098/rstb.2011.0389

Carlini, E.A. and Cunha, J.M. (1981). Hypnotic and antiepileptic effects of cannabidiol. J. Clin. Pharmacol. 21:417S-427S. doi: 10.1002/j.1552-4604.1981.tb02622.x

Carlisle, S.M., Trainor, P.J., and Hein, D.W. (2018). Identification of L-Asparagine as a Novel Endogenous Substrate for Human Arylamine N-Acetyltransferase 1. FASEB J. 31:821.8. doi: 10.1096/fasebj.31.1_supplement.821.8

Carrier, E.J., Auchampach, J.A., and Hillard, C.J. (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: A mechanism of cannabinoid immunosuppression. Proc. Natl. Acad. Sci. USA. 103(20):7895-7900. doi: 10.1073/pnas.0511232103

Caterina, M.J. and Pang, Z. (2016). TRP channels in skin biology and pathophysiology. Pharmaceuticals. 9(4):77. doi: 10.3390/ph9040077

Cervenka, I., Agudelo, L.Z., and Ruas, J.L. (2017). Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science. 357(6349):eaaf9794. doi: 10.1126/science.aaf9794

Chagas, M.H.N., Crippa, J.A.S., Zuardi, A.W., Hallak, J.E.C., Machado-de-Sousa, J.P., Hirotsu, C., Maia, L., Tufik, S., and Andersen, M.L. (2013). Effects of acute systemic administration of cannabidiol on sleep-wake cycle in rats. J. Psychopharmacol. 27(3):312-316. doi: 10.1177/0269881112474524

Chang, P., Huang, L., Huang, M., Tian, S., and Yang, Z. (2019). Improvement and optimization of a T-cell-dependent antibody response (TDAR) method for BALB/c mice using keyhole limpet hemocyanin (KLH) as specific antigen. J Immunotoxicol. 16(1):149-154. doi: 10.1080/1547691X.2019.1635234

Chea, B. and Giorgi, M. (2017). Trazodone: A review of its pharmacological properties and its off-label use in dogs and cats. Am. J. Anim. Vet. Sci. 12(4):188-194. doi:10.3844/ajavsp.2017.188.194

Chen, W., Kaplan, B.L.F., Pike, S.T., Topper, L.A., Lichorobiec, N.R., Simmons, S.O., et al. (2012). Magnitude of stimulation dictates the cannabinoid-mediated differential T cell response to HIVgp120. J. Leukoc. Biol. 92(5):1093-102. doi: 10.1189/jlb.0212082

Chen, Z., Brodie, M.J., Liew, D., and Kwan, P. (2018). Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: A 30-year longitudinal cohort study. JAMA Neurol. 75(3):279-286. doi: 10.1001/jamaneurol.2017.3949

Chicoine, A., Illing, K., Vuong, S., Pinto, K.R., Alcorn, J., and Cosford, K. (2020). Pharmacokinetic and Safety Evaluation of Various Oral Doses of a Novel 1:20 THC:CBD Cannabis Herbal Extract in Dogs. Front. Vet. Sci. 7:583404. doi: 10.3389/fvets.2020.583404

Chong, J., Wishart, D.S. and Xia, J. (2019). Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. Current Protocols in Bioinformatics 68:e86. doi: 10.1002/cpbi.86

Civolani, C., Barghini, P., Roncetti, A.R., Ruzzi, M., and Schiesser, A. (2000). Bioconversion of Ferulic Acid into Vanillic Acid by Means of a Vanillate-Negative Mutant of Pseudomonas fluorescens Strain BF13. Appl. Environ. Microbiol. 66(6):2311-2317. doi: 10.1128/aem.66.6.2311-2317.2000

Clark, N. and Fraser, D. (2015). Automated monitoring of resting in dogs. App. Anim. Behav. Sci. 174:99-102. doi: 10.1016/j.applanim.2015.11.019

Clifford, M.N., Kerimi, A., and Williamson, G. (2019). Bioavailability and metabolism of chlorogenic acids (acyl-quinic acids) in humans. Compr. Rev. Food Sci. Food Saf. 19:1299-1352. doi: 10.1111/1541-4337.12518

Colabroy, K.L. and Begley, T.P. (2005). Tryptophan catabolism: identification and characterization of a new degradative pathway. J. Bacteriol. 187(22):7866-9. doi: 10.1128/JB.187.22.7866-7869.2005

Condie, R., Herring, A., Koh, W.S., Lee, M., and Kaminski, N.E. (1996). J. Biol. Chem. 271(22):13175-13183. doi: 10.1074/jbc.271.22.13175

Corroon, J. and Kight, R. (2018). Regulatory status of cannabidiol in the United States: A perspective. Cannabis Cannabinoid Res. 3(1):190-194. doi: 10.1089/can.2018.0030

Corsetti, S., Borruso, S., Malandrucco, L., Spallucci, V., Maragliano, L., Perino, R. et al. (2021). Cannabis sativa L. may reduce aggressive behaviour towards humans in shelter dogs. Sci. Rep. 11(1):2773. doi: 10.1038/s41598-021-82439-2

Costa, B., Giagnoni, G., Franke, C., Trovato, A.E., and Colleoni, M. (2004). Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. Br. J. Pharmacol. 143(2):247-50. doi: 10.1038/sj.bjp.0705920

Costa, B., Trovato, A.E., Comelli, F., Giagnoni, G., and Colleoni, M. (2007). The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. Eur. J. Pharmacol, 556:75-83. doi: 10.1016/j.ejphar.2006.11.006

Craig, L., Meyers-Manor, J.E., Anders, K., Sütterlin, S., and Miller, H. (2017). The relationship between heart rate variability and canine aggression. Appl. Anim. Behav. Sci. 188:59-67. doi: 10.1016/j.applanim.2016.12.015

Crippa, J.A., Derenusson, G.N., Ferrari, T.B., Wichert-Ana, L., Duran, F.L.S., Martin-Santos, R., et al. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. J. Psychopharmacol. 25(1):121-130. doi: 10.1177/0269881110379283

Crow, J.A., Borazjani, A., Potter, P.M., and Ross, M.K. (2007). Hydrolysis of pyrethroids by human and rat tissues: examination of intestinal, liver and serum carboxylesterases. Toxicol. Appl. Pharmacol. 221(1):1-12. doi: 10.1016/j.taap.2007.03.002

Dalto, D.B. and Matte, J-J. (2017). Pyridoxine (Vitamin B6) and the Glutathione Peroxidase System; a Link between One-Carbon Metabolism and Antioxidation. Nutrients. 9:189. doi: 10.3390/nu9030189

Darling, S. and Larsen, P.O. (1961). Saccharopine, a new amino acid in baker's and brewer's yeast: I. Isolation and properties. Acta Chem. Scand. 15:743-749. doi: 10.3891/acta.chem.scand.15-0743

Davis, W.L. and Goodman, D.B. (1992). Evidence for the glyoxylate cycle in human liver. Anat. Rec. 234(4):461-468. doi: 10.1002/ar.1092340402

Dawkins, P.D. and Dickens, F. (1965). The oxidation of D- and L-glycerate by rat liver. Biochem. J. 94(2):353-67. doi: 10.1042/bj0940353

De Dilippis, D., Esposito, G., Cirillo, C., Cipriano, M., De Winter, B.Y., Scuderi, C., et al. (2011). Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. PLoS One. 6(12):e28159. doi: 10.1371/journal.pone.0028159

De Petrocellis, L. and Di Marzo, V. (2009). An introduction to the endocannabinoid system: from the early to the latest concepts. Best Pract. Res. Clin. Endocrinol. Metab. 23(1):1-15. doi: 10.1016/j.beem.2008.10.013

De Petrocellis, L., Vellani, V., Schiano-Moriello, A., Marini, P., Magherini, P.C., Orlando, P., and Di Marzo, V. (2008). Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. J. Pharmacol. Exp. Ther. 325(3):1007-1015. doi: 10.1124/jpet.107.134809

Deabold, K.A., Schwark, W.S., Wolf, S., and Wakshlag, J.J. (2019). Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp neutraceutical in healthy dogs and cats. Animals. 9:832. doi: 10.3390/ani9100832

Den, H., Robinson, W.G., and Coon, M.J. (1959). Enzymatic conversion of beta-hydroxypropionate to malonic semialdehyde. J. Biol. Chem. 234(7):1666-1671. doi: 10.1016/S0021-9258(18)69904-1

den Uijl, I., Gómez Álvarez, C.B., Bartram, D., Dror, Y., Holland, Robert, and Cook, A. (2017). External validation of a collar-mounted triaxial accelerometer for second-by-second monitoring of eight behavioral states in dogs. PLoS ONE. 12(11):e0188481. doi: 10.1371/journal.pone.0188481

Devinsky, O., Patel, A.D., Thiele, E.A., Wong, M.H., Appleton, R., Harden, C.L., et al. (2018). Randomized, dose-ranging safety trial of cannabidiol in Dravet syndrome. Neurology. 90(14):e1204-e1211. doi: 10.1212/WNL.0000000000005254

Dhital, S., Stokes, J.V., Park, N., Seo, K.S., and Kaplan, B.L.F. (2017). Cannabidiol (CBD) induces functional Tregs in response to low-level T cell activation. Cell Immunol. 312:25-24. doi: 10.1016/j.cellimm.2016.11.006

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S.A., et al. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. Schizophr. Bull. 40(6):1509-17. doi: 10.1093/schbul/sbt181

Dow, C., Michel, K.E., Love, M., and Brown, D.C. (2009). Evaluation of optimal sampling interval for activity monitoring in companion animals. Am. J. Vet. Res. 70(4):444-448. doi: 10.2460/ajvr.70.4.444

Dreschel, N.A. (2010). The effects of fear and anxiety on health and lifespan in pet dogs. Appl. Anim. Behav. Sci. 125:157-162. doi: 10.1016/j.applanim.2010.04.003

Dreschel, N.A. and Granger, D.A. (2005). Physiological and behavioral reactivity to stress in thunderstorm-phobic dogs and their caregivers. Appl. Anim. Behav. Sci. 95:153-168. doi: 10.1016/j.applanim.2005.04.009

D'Souza, D.C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y-T., et al. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology. 29(8):1558-72. doi: 10.1038/sj.npp.1300496

Duran, M., Perez, E., Abanades, S., Vidal, X., Saura, C., Majem, M., et al. (2010). Preliminary efficacy and safety of an oromucosal standardized cannabis extract in chemotherapy-induced nausea and vomiting. Br. J. Clin. Pharmacol. 70(5):656-63. doi: 10.1111/j.1365-2125.2010.03743.x

Durg, S., Lobo, M., Venkatachalam, L., Rao, G., and Bhate, J. (2019). A systematic review and meta-analysis of oxaceprol in the management of osteoarthritis: An evidence from randomized parallel-group controlled trials. Pharmacol. Rep. 71(2):374-383. doi: 10.1016/j.pharep.2018.12.010

Dvorak, M., Watkinson, A., McGlone, F., and Rukwied, R. (2003). Histamine induced responses are attenuated by a cannabinoid receptor agonist in human skin. Inflamm. Res. 52(6):238-245. doi: 10.1007/s00011-003-1162-z

El-Gohary, M. and Eid, M.A. (2004). Effect of cannabinoid ingestion (in the form of bhang) on the immune system of high school and university students. Hum. Exp. Toxicol. 23(3):149-56. doi: 10.1191/0960327104ht426oa

El-Kalyoubi, S.A., Fayed, E.A., and Abdel-Razek, A.S. (2017). One pot synthesis, antimicrobial and antioxidant activities of fused uracils: pyrimidodiazepines, lumazines, triazolouracil and xanthines. Chem. Cent. J. 11(1):66. doi: 10.1186/s13065-017-0294-0

Elikkottil, J., Gupta, P., and Gupta, K. (2009). The analgesic potential of cannabinoids. J. Opioid Manag. 5(6):341-357.

Elmes, M.W., Kaczocha, M., Berger, W.T., Leung, K., Ralph, B.P., Wang, L., et al. (2015). Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). J. Biol. Chem. 290(14):8711-21. doi: 10.1074/jbc.M114.618447

Engler, W.J. and Bain, M. (2017). Effect of different types of classical music played at a veterinary hospital on dog behavior and owner satisfaction. J. Am. Vet. Med. Assoc. 251(2):195-200. doi: 10.2460/javma.251.2.195

Englund, A., Morrison, P.D., Nottage, J., Hague, D., Kane, F., Bonaccorso, S., et al. (2013). Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. J. Psychopharmacol. 27(1):19-27. doi: 10.1177/0269881112460109

Eskander, B.S., Barbar, M., Evans, R.B., Enomoto, M., Lascelles, B.D.X., and Conzemius, M.G. (2020). Correlation of activity data in normal dogs to distance traveled. Can. J. Vet. Res. 84(1):44-51.

Evans, M.D. and Cooke, M.S. (2004). Factors contributing to the outcome of oxidative damage to nucleic acids. Bioessays. 26(5):533-42. doi: 10.1002/bies.20027

Ewing, L.E., Skinner, C.M., Quick, C.M., Kennon-McGill, S., McGill, M.R., Walker, L.A., et al. (2019). Hepatotoxicity of a cannabidiol-rich cannabis extract in the mouse model. Molecules. 24:1694. doi:10.3390/molecules24091694

Falck, J.R., Reddy, L.M., Byun, K., Campbell, W.B., and Yi, X-Y. (2007). Epoxygenase eicosanoids: synthesis of tetrahydrofuran-diol metabolites and their vasoactivity. Bioorg. Med. Chem. Lett. 17(9):2634-8. doi: 10.1016/j.bmcl.2007.01.096

FDA. (2020). What you need to know (and what we're working to find out) about products containing cannabis or cannabis-derived compounds, including CBD. Washington DC.

Ferro, R., Adamska, A., Lattanzio, R., Mavrommati, I., Edling, C.E., Arifin, S>A., Fyffe, C.A., Sala, G., Sacchetto, L., Chiorino, G., De Laurenzi, V., Piantelli, M., Sansom, O.J., Maffucci, T., and Falasca, M. (2018). GPR55 signalling promotes proliferation of pancreatic cancer cells and tumor growth in mice, and its inhibition increases effects of gemcitabine. Oncogene. 37(49):6368-6382. doi: 10.1038/s41388-018-0390-1

Fiehn, O., Kopka, J., Trethewey, R.N., and Willmitzer, L. (2000). Identification of uncommon plant metabolites based on calculation of elemental compositions using gas chromatography and quadrupole mass spectrometry. Anal. Chem. 72(15):3573-3580. doi: 10.1021/ac991142i

Finco-Kent, D. and Kawabata, T.T. (2005). Development and Validation of a Canine T-Cell-Dependent Antibody Response Model for Immunotoxicity Evaluation. J. Immunotoxicol. 2:197-201. doi: 10.1080/15476910500362853

Fons, M., Cami, B., and Chippaux, M. (1991). Possible involvement of a L-Δ1-pyrroline-5-carboxylate (P5C) reductase in the synthesis of proline in Desulfovibrio desulfuricans Norway. Biochem. Biophys. Res. Commun. 179(2):1088-1094. doi: 10.1016/0006-291x(91)91931-2

Foster, B.C., Abramovici, H., and Harris, C.S. (2019). Cannabis and cannabinoids: Kinetics and interactions. Am. J. Med. doi: 10.1016/j.amjmed.2019.05.017

Franklin, I.K. and Wollheim, C.B. (2004). GABA in the Endocrine Pancreas: Its Putative Role as an Islet Cell Paracrine-signalling Molecule. J. Gen. Physiol. 123(3):185-190. doi: 10.1085/jgp.200409016

Franzini de Souza, C.C., Maccariello, C.E.M., Dias, D.P.M., Almeida, N.A.D.S., de Medeiros, M.A. (2017). Autonomic, endocrine and behavioural responses to thunder in laboratory and companion animals. Physiol. Behav. 169:208-215. doi: 10.1016/j.physbeh.2016.12.006

Franzini de Souza, C.C., Dias, D.P.M., Nascimento de Souza, R., and de Medeiros, M.A. (2018). Use of behavioural and physiological responses for scoring sound sensitivity in dogs. PLoS ONE. 13(8):e0200618. doi: 10.1371/journal.pone.0200618

Gacsi, M., Maros, K., Sernkvist, S., Farago, T., and Miklosi, A. (2013). Human analogue safe haven effect of the owner: Behavioral and heart rate response to stressful social stimuli in dogs. PLoS One. 8(3):e58475. doi: 10.1371/journal.pone.0058475

Gaffal, E., Cron, M., Glodde, N., and Tuting, T. (2013). Anti-inflammatory activity of topical THC in DNFB-mediated mouse allergic contact dermatitis independent of CB1 and CB2 receptors. Allergy. 68(8):994-1000. doi: 10.1111/all.12183

Gagne, S.J., Stout, J.M., Liu, E., Boubakir, Z., Clark, S.M., and Page, J.E. (2012). Identification of olivetolic acid cyclase from *Cannabis sativa* reveals a unique catalytic route to plant polyketides. Proc. Natl. Acad. Sci. USA. 109(31):12811-12816. doi: 10.1073/pnas.1200330109

Galiazzo, G., Giancola, F., Stanzani, A., Fracassi, F., Bernardini, C., Forni, M., Pietra, M., and Chiocchetti, R. (2018). Localization of cannabinoid receptors CB1, CB2, GPR55, and PPARα in the canine gastrointestinal tract. Histochem. Cell Biol. 150(2):187-205. doi: 10.1007/s00418-018-1684-7

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, Carayon, P., Bouaboula, M., Shire, D., le Fur, G., and Casellas, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur. J. Biochem. 232(1):54-61. doi: 10.1111/j.1432-1033.1995.tb20780.x

Gamble, L.J., Boesch, J.M., Frye, C.W., Schwark, W.S., Mann, S., Wolfe, L., et al. (2018). Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. Front. Vet. Sci. 5:165. doi: 10.3389/fvets.2018.00165

Gangadharan, V., Selvaraj, D., Kurejova, M., Njoo, C., Gritsch, S., Skoricova, D., et al. (2013). A novel biological role for the phospholipid lysophosphatidylinositol in nociceptive sensitization via activation of diverse G-protein signalling pathways in sensory nerves in vivo. Pain. 154, 2801–2812. doi: 10.1016/j.pain.2013.08.019

Giang, D.K. and Cravatt, B.F. (1997). Molecular characterization of human and mouse fatty acid amide hydrolase. Proc. Natl. Acad. Sci. USA. 94(6):2238-42. doi: 10.1073/pnas.94.6.2238

Gibb, D.J., Shah, M.A., Mir, P.S., and McAllister, T.A. (2005). Effect of full-fat hemp seed on performance and tissue fatty acids of feedlot cattle. Can. J. Anim. Sci. 85(2): 223–230. doi: 10.4141/A04-078

Gilbert-Gregory, S.E., Stull, J.W., Rice, M.R., and Herron, M.E. (2016). Effects of trazodone on behavioral signs of stress in hospitalized dogs. J. Am. Vet. Med. Assoc. 249(11):1281-1291. doi: 10.2460/javma.249.11.1281

Ginguay, A., Cynober, L., Curis, E., and Nicolis, I. (2017). Ornithine Aminotransferase, an Important Glutamate-Metabolizing Enzyme at the Crossroads of Multiple Metabolic Pathways. Biology (Basel). 6(1):18. doi: 10.3390/biology6010018

Giuffride, A., Leweke, F.M., Gerth, C.W., Schreiber, D., Koethe, D., Faulhaber, J., et al. (2004). Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 29(11):2108-14. doi: 10.1038/sj.npp.1300558

Goldansaz, S.A., Markus, S., Berjanskii, M., Rout, M., Guo, A.C., Wang, Z., et al. (2020). Candidate serum metabolite biomarkers of residual feed intake and carcass merit in sheep. J. Anim. Sci. 98(10):1-15. doi: 10.1093/jas/skaa298

Gomes, F.V., Reis, D.G., Alves, F.H.F., Corrêa, F.M.A., Guimarães, F.S., and Resstel, L.B.M. (2012). Cannabidiol injected into the bed nucleus of the stria terminalis reduces the expression of contextual fear conditioning via 5-HT1A receptors. J. Psychopharmacol. 26(1):104-13. doi: 10.1177/0269881110389095

Good, P., Haywood, A., Gogna, G., Martin, J., Yates, P., Greer, R., and Hardy, J. (2019). Oral medicinal cannabinoids to relieve symptom burden in the palliative care of patients with advanced cancer: A double-blind, placebo controlled, randomized clinical trial of efficacy and safety of cannabidiol (CBD). BMC Palliat. Care. 18(1):110. doi: 10.1186/s12904-019-0494-6

Graczyk, M., Łukowicz, M., and Dzierzanowski, T. (2021). Prospects for the use of cannabinoids in psychiatric disorders. Front. Psychiatry. 12:620073. doi: 10.3389/fpsyt.2021.620073

Gram, D.X., Ahren, B., Nagy, I., Olsen, U.B., Brand, C.L., Sundler, F., et al. (2007). Capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in Zucker diabetic rat, an animal model for some aspects of human type 2 diabetes. Eur. J. Neurosci. 25(1):213-23. doi: 10.1111/j.1460-9568.2006.05261.x

Gray, M.J. and Escalante-Semerena, J.C. (2010). A new pathway for the synthesis of α-ribazole-phosphate in Listeria innocua. Mol. Microbiol. 77(6):1429-38. doi: 10.1111/j.1365-2958.2010.07294.x

Gray, R.A. and Whalley, B.J. (2020). The proposed mechanisms of action of CBD in epilepsy. Epileptic Disord. 22(S1):10-15. doi: 10.1684/epd.2020.1135

Griffies J.D., J. Zutty, M. Sarzen, and S. Soorholtz. (2018). Wearable sensor shown to specifically quantify pruritic behaviors in dogs. BMC Vet. Res. 14:124. doi: 10.1186/s12917-018-1428-x

Grimison, P., Mersaides, A., Kirby, A., Lintzeris, Morton, R., Haber, P., et al. (2020). Oral THC:CBD cannabis extract for refractory chemotherapy-induced nausea and vomiting: a randomised, placebo-controlled, phase II crossover trial. Ann. Oncol. 31(11):1553-1560. doi: 10.1016/j.annonc.2020.07.020

Grof, C.P.L. (2018). Cannabis, from plant to pill. Br J Clin Pharmacol. 84:2463-2467. doi: 10.1111/bcp.13618

Grossman, P. and Taylor, E.W. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. Biol. Psychol. 74:263-285. doi: 10.1016/j.biopsycho.2005.11.014

Gruden, G., Barutta, F., Kunos, G., and Pacher, P. (2016). Role of the endocannabinoid system in diabetes and diabetic complications. Br. J. Pharmacol. 173(7):1116-27. doi: 10.1111/bph.13226

Gruen, M.E. and Sherman, B.L. (2008). Use of trazodone as an adjunctive agent in the treatment of canine anxiety disorders: 56 cases (1995-2007). J. Am. Vet. Med. Assoc. 233(12):1902-1907. doi: 10.2460/javma.233.12.1902

Gruen, M.E., Roe, S.C., Griffith, E., Hamilton, A., and Sherman, B.L. (2014). The use of trazodone to facilitate post-surgical confinement in dogs. J. Am. Vet. Med. Assoc. 245(3):296-301. doi:10.2460/javma.245.3.296

Guerrero-Alba R., Barragán-Iglesias, P., González-Hernández, A., Valdez-Moráles, E.E., Granados-Soto, V., Condés-Lara, M., Rodríguez, M.G., and Marichal-Cancino, B.A. (2019) Some prospective alternatives for treating pain: The endocannabinoid system and its putative receptors GPR18 and GPR55. Front. Pharmacol. 9:1496. doi: 10.3389/fphar.2018.01496

Guo, J-H., Hexige, S., Chen, L., Zhou, G-J., Wang, X., Jiang, J-M., et al. (2006). Isolation and characterization of the human D-glyceric acidemia related glycerate kinase gene GLYCTK1 and its alternatively splicing variant GLYCTK2. DNA Seq. 17(1):1-7. doi: 10.1080/10425170500476665

Guo, K. and Li, L. (2009). Differential 12C-/13C-isotope dansylation labeling and fast liquid chromatography/mass spectrometry for absolute and relative quantification of the metabolome. Anal. Chem. 81(10):3919-3932. doi: 10.1021/ac900166a

Gupta, N.K. and Vennesland, B. (1964). Glyoxylate carboligase of Escherichia coli: A flavoprotein. J. Biol. Chem. 239:3787-3789.

Gusho, C.A. and Court, T. (2020). Cannabidiol: A brief review of its therapeutic and pharmacologic efficacy in the management of joint disease. Cureus. 12(3):e7375. doi: 10.7759/cureus.7375

Guterman, I., Masci, T., Chen, X., Negre, F., Pichersky, E., Dudareva, N., et al. (2006). Generation of phenylpropanoid pathway-derived volatiles in transgenic plants: rose alcohol acetyltransferase produces phenylethyl acetate and benzyl acetate in petunia flowers. Plant Mol. Biol. 60(4):555-63. doi: 10.1007/s11103-005-4924-x

Haggerty, H.G. (2007). Immunotoxicity testing in non-rodent species. J Immunotoxicol. 4:165-169. doi: 10.1080/15476910701337241

Hahn, S.M. (2020). FDA advances work related to cannabidiol products with focus on protecting public health, providing market clarity. Report published by the US Food and Drug Administration. Washington DC.

Han, K.H., Lim, S., Ryu, J., Lee, C-W., Kim, Y., Kang, J-H., et al. (2009). B1 and CB2 cannabinoid receptors differentially regulate the production of reactive oxygen species by macrophages. Cardiovasc. Res. 84(3):378-86. doi: 10.1093/cvr/cvp240

Hansen, B.D., Lascelles, B.D.X., Keene, B.W., Adams, A.K., and Thomson, A.E. (2007). Evaluation of an accelerometer for at-home monitoring of spontaneous activity in dogs. Am. J. Vet. Res. 68(5):468-475. doi: 10.2460/ajvr.68.5.468

Hanŭs L.O., Tchilibon, S., Ponde, D.E., Breuer, A., Fride, E. and Mechoulam, R. (2005). Enantiomeric cannabidiol derivatives: synthesis and binding to cannabinoid receptors. Org. Biomol. Chem. 3(6):1116-1123. doi: 10.1039/b416943c

Harvey, D.J., Samara, E., and Mechoulam, R. (1991a). Urinary metabolites of cannabidiol in dog, rat and man and their identification by gas chromatography – mass spectrometry. J. Chromatogr. 562(1-2):299-322. doi: 10.1016/0378-4347(91)80587-3

Harvey, D.J., Samara, E., and Mechoulam, R. (1991b). Comparative metabolism of cannabidiol in dog, rat, and man. Pharmacol. Biochem. Behav. 40(3):523-32. doi: 10.1016/0091-3057(91)90358-9

Hassall, H. and Greenberg, D.M. (1963). Studies on the enzymic decomposition of urocanic acid. V. The formation of 4-oxoglutaramic acid, a nonenzymic oxidation product of 4(5)-imidazolone-5(4)-propionic acid. J. Biol. Chem. 238:1423-1431.

Hawes, J.W., Jaskiewicz, J., Shimomura, Y., Huang, B., Bunting, J., Harper, E.T., et al. (1996). Primary structure and tissue-specific expression of human beta-hydroxyisobutyryl-coenzyme A hydrolase. J. Biol. Chem. 271(42):26430-4. doi: 10.1074/jbc.271.42.26430

Hegde, V.L., Singh, U.P., Nagarkatti, P.S., and Nagarkatti, M. (2015). Critical role of mast cells and peroxisome proliferator-activated receptor γ in the induction of myeloid-derived suppressor cells by marijuana cannabidiol in vivo. J. Immunol. 194(11):5211-22. doi: 10.4049/jimmunol.1401844

Heinz, F., Lamprecht, W., and Kirsch, J. (1968). Enzymes of fructose metabolism in human liver. J. Clin. Invest. 47(8):1826–1832. doi: 10.1172/JCI105872

Hemp Industry Daily. (2019). Annual Hemp and CBD Industry Factbook. 2nd edition. Ann Holland Ventures, Inc. Lakewood, CO.

Henriet, E., Jager, S., Tran, C., Bastien, P., Michelet, J-F., Minondo, A-M., et al. (2017). A jasmonic acid derivative improves skin healing and induces changes in proteoglycan expression and glycosaminoglycan structure. Biochim. Biophys. Acta. Gen. Subj. 1861(9):2250-2260. doi: 10.1016/j.bbagen.2017.06.006

Henrotin, Y., Mobasheri, A., and Marty, M. (2012). Is there any scientific evidence for the use of glucosamine in the management of human osteoarthritis? Arthritis Res. Ther. 14(1): 201. doi: 10.1186/ar3657

Herron, M.E. and Shreyer, T. (2014). The pet-friendly veterinary practice: a guide for practitioners. Vet. Clin. North Am. Small Anim. Pract. 44(3):451-481. doi: 10.1016/j.vcsm.2014.01.010

Hildebrandt, T., Knuesting, J., Berndt, C., Morgan, B., and Scheibe, R. (2015). Cytosolic thiol switches regulating basic cellular functions: GAPDH as an information hub? Biol. Chem. 396(5):523-537. doi: 10.1515/hsz-2014-0295

Hill, M.N. and Patel, S. (2013). Translational evidence for the involvement of the endocannabinoid system in stress-related psychiatric illnesses. Biol. Mood Anxiety Disord. 3(1):19. doi: 10.1186/2045-5380-3-19

Hillard, C.J. and Jarrahian, A. (2000). The movement of N-arachidonoylethanolamine (anandamide) across cellular membranes. Chem. Phys. Lipids. 108(1-2):123-34. doi: 10.1016/s0009-3084(00)00191-2

Hirabayashi, T., Ochiai, H., Sakai, S., Nakajima, K., and Terasawa, K. (1994). Inhibitory effect of ferulic acid and isoferulic acid on murine interleukin-8 production in response to influenza virus infections in vitro and in vivo. Planta Med. 61(3):221-6. doi: 10.1055/s-2006-958060

Hobbs, J.M., Vazquez, A.R., Remijan, N.D., Trotter, R.E., McMillan, T.V., Freedman, K.E., Wei, Y., Woelfel, K.A., Arnold, O.R., Wolfe, L.M., Johnson, S.A., and Weir, T.L. (2020). Evaluation of pharmacokinetics and acute anti-inflammatory potential of two oral cannabidiol preparations in healthy adults. Phytother. Res. 34(7):1696-1703. doi: 10.1002/ptr.6651

Hoffman, C.L., Ladha, C., and Wilcox, S. (2019). An actigraphy-based comparison of shelter dogs and owned dogs activity patterns. J. Vet. Behav. 34:30-36. doi: 10.1016/j.jveb.2019.08.001

Hong, H.A., Khaneja, R., Tam, N.M.K., Cazzato, A., Tan, S., Urdaci, M., Brisson, A., Gasbarrini, A., Barnes, I., and Cutting, S.M. (2009). Bacillus subtilis isolated from the human gastrointestinal tract. Res. Microbiol. 160(2):134-43. doi: 10.1016/j.resmic.2008.11.002

Hosoda, H., Yokohama, H., and Nambara, T. (1984). Synthesis of cortol 3-glucuronides and cortolone 3-glucuronides. Chem. Pharm. Bull. 32(10):4023-8. doi: 10.1248/cpb.32.4023

Hou, Y., Moreau, F., and Chadee, K. (2012). PPARγ is an E3 ligase that induces the degradation of NFκB/p65. Nat. Commun. 3:1300. doi: 10.1038/ncomms2270

Hsiao, Y-T., Yi, P-L., Li, C-L., and Chang, F-C. (2012). Effect of cannabidiol on sleep disruption induced by the repeated combination tests consisting of open field and elevated plus-maze in rats. Neuropharmacology. 62(1):373-84. doi: 10.1016/j.neuropharm.2011.08.013

Hu, C.A., Lin, W.W., and Valle, D. (1996). Cloning, characterization, and expression of cDNAs encoding human delta 1-pyrroline-5-carboxylate dehydrogenase. J. Biol. Chem. 271(16):9795-800. doi: 10.1074/jbc.271.16.9795

Huan, T., and Li, L. (2015). Quantitative metabolome analysis based on chromatographic peak reconstruction in chemical isotope labeling liquid chromatography mass spectrometry. Anal. Chem. 87:7011–7016. doi: 10.1021/acs.analchem.5b01434

Huchelmann, A., Boutry, and Hachez, C. (2017). Plant glandular trichomes: Natural cell factories of high biotechnological interest. Plant Physiol. 175:6-22. doi: 10.1104/pp.17.00727

Hudson, J.T., Slater, M.R., Taylor, L., Scott, H.M., and Kerwin, S.C. (2004). Assessing repeatability and validity of a visual analogue scale questionnaire for use in assessing pain and lameness in dogs. Am. J. Vet. Res. 65(12):1634-1643. doi: 10.2460/ajvr.2004.65.1634

Huemer, H.P., Himmelreich, A., Honlinger, B., Pavlic, M., Eisendle, K., Hopfl, R., Rabl, W., and Czerny, C.P. (2007). “Recreational” drug abuse associated with failure to mount a proper antibody response after a generalized orthopoxvirus infection. Infection. 35(6):469-473. doi: 10.1007/s15010-007-6194-9

Huemer, H.P., Lassnig, C., Bernhard, D., Sturm, S., Nowotny, N., Kitchen, M., and Pavlic, M. (2011). Cannabinoids lead to enhanced virulence of the smallpox vaccine (vaccina) virus. Immunobiology. 216:670-677. doi: 10.1016/j.imbio.2010.11.001

Huestis, M.A. (2007). Human cannabinoid pharmacokinetics. Chem. Biodivers. 4(8):1770-1804. doi: 10.1002/cbdv.200790152

Hurd, Y.L., Spriggs, S., Alishayev, J., Winkel, G., Gurgov, K., Kudrich, C., et al. (2019). Cannabidiol for the reduction of cue-induced craving and anxiety in drug-abstinent individuals with heroin use disorder: A double-blinded randomized placebo-controlled trial. Am. J. Psychiatry. doi: 10.1176/appi.ajp.2019.18101191

Hydbring-Sandberg, E, von Walter, LW, Hoglund, K, Svartberg, K, Swenson, L, and Forkman, B. (2004). Physiological reactions to fear provocation in dogs. J Endocrinol. 180:439-448. doi: 10.1677/joe.0.1800439

Iffland, K. and Grotenhermen, F. (2017). An update on safety and side effects of cannabidiol: A review of clinical data and relevant animal studies. Cannabis Cannabinoid Res. 2(1):139-154. doi: 10.1089/can.2016.0034

Igarashi, K. and Kashiwagi, K. (2010). Cellular function by polyamines. Int. J. Biochem. Cell Biol. 42(1):39-51. doi: 10.1016/j.biocel.2009.07.009

Imamachi, N., Park, G.H., Lee, H., Anderson, D.J., Simon, M.I., Basbaum, A.I., and Han, S-K. (2009). TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. Proc. Natl. Acad. Sci. USA. 106(27):11330-11335. doi: 10.1073/pnas.0905605106

Iseger, T.A. and Bossong, M.G. (2015). A systematic review of the antipsychotic properties of cannabidiol in humans. Schizophr. Res. 162(1-3):153-61. doi: 10.1016/j.schres.2015.01.033

Jadoon, K.A., Ratcliffe, S.H., Barrett, D.A., Thomas, E.L., Stott, C., Bell, J.D., et al. (2016). Efficacy and Safety of Cannabidiol and Tetrahydrocannabivarin on Glycemic and Lipid Parameters in Patients With Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled, Parallel Group Pilot Study. Diabetes Care. 39(10):1777-1786. doi: 10.2337/dc16-0650

Jan, T.R., Rao, G.K., and Kaminski, N.E. (2002). Cannabinol enhancement of interleukin-2 (IL-2) expression by T cells is associated with an increase in IL-2 distal nuclear factor of activated T cell activity. Mol. Pharmacol. 61(2):446-54. doi: 10.1124/mol.61.2.446

Jan, T.R., Su, S.T., Wu, H.Y., and Liao, M.H. (2007). Suppressive effects of cannabidiol on antigen-specific antibody production and functional activity of splenocytes in ovalbumin-sensitized BALB/c mice. Int Immunopharmacol. 7(6):773-780. doi: 10.1016/j.intimp.2007.01.015

Jankowski, M.P., Rau, K.K., and Koerber, H.R. (2017). Cutaneous TRPM8-expressing sensory afferents are a small population of neurons with unique firing properties. Physiol. Rep. 5(7):e13234. doi: 10.14814/phy2.13234

Jastrząb, A., Gęgotek, A., and Skrzydlewska, E. (2019). Cannabidiol regulates the expression of keratinocyte proteins involved in the inflammation process through transcriptional regulation. Cells. 8(8):827. doi: 10.3390/cells8080827

Jay, A.R., Krotscheck, U., Parsley, E., Benson, L., Kravitz, A., Mulligan, A., et al. (2013). Pharmacokinetics, bioavailability, and hemodynamic effects of trazodone after intravenous and oral administration of a single dose to dogs. Am. J. Vet. Res. 74(11):1450-1456. doi: 10.2460/ajvr.74.11.1450

Jenny, M., Santer, E., Pirich, E., Schennach, H., and Fuchs, D. (2009). Δ9-tetrahydrocannabinol and cannabidiol modulate mitoegn-induced tryptophan degradation and neopterin formation in peripheral blood mononuclear cells in vitro. J. Neuroimmunol. 207:75-82. doi: 10.1016/j.jneuroim.2008.12.004

Jesus, C.H.A., Redivo, D.D.B., Gasparin, A.T., Sotomaior, B.B., de Carvalho, M.C., Genaro, K., Zuardi, A.W., Hallak, J.E.C., Crippa, J.A., Zanoveli, J.M., and da Cunha, J.M. (2019). Cannabidiol attenuates mechanical allodynia in streptozotocin-induced diabetic rats via serotonergic system activation through 5-HT1A receptors. Brain Res. 1715:156-164. doi: 10.1016/j.brainres.2019.03.014

Jeurissen, A., Ceuppens, J.L., and Bossuyt, X. (2004). T lymphocyte dependence of the antibody response to 'T lymphocyte independent type 2' antigens. Immunology. 111(1):1-7. doi: 10.1111/j.1365-2567.2003.01775.x

Jiang, R., Yamaori, S., Takeda, Shuso, Yamamoto, I., and Watanabe, K. (2011). Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. Life Sci. 89:165-170. doi: 10.1016/j.lfs.2011.05.018

Jin, E.S., Browning, J.D., Murphy, R.E., and Malloy, C.R. (2018). Fatty liver disrupts glycerol metabolism in gluconeogenic and lipogenic pathways in humans. J. Lipid Res. 59(9):1685-1694. doi: 10.1194/jlr.M086405

Jin, Z., Mendu, S.K., and Birnir, B. (2013). GABA is an effective immunomodulatory molecule. Amino Acids. 45(1):87-94. doi: 10.1007/s00726-011-1193-7

Jo, J-E., Raj, S.M., Rathnasingh, C., Selvakumar, E., Jung, W-C., and Park, S. (2008). Cloning, expression, and characterization of an aldehyde dehydrogenase from Escherichia coli K-12 that utilizes 3-Hydroxypropionaldehyde as a substrate. Appl. Microbiol. Biotechnol. 81(1):51-60. doi: 10.1007/s00253-008-1608-x

Johnsen, U., Dambeck, M., Zaiss, H., Fuhrer, T., Soppa, J., Sauer, U., and Schonheit, P. (2009). D-xylose degradation pathway in the halophilic archaeon Haloferax volcanii. J. Biol. Chem. 284(40):27290-27303. doi: 10.1074/jbc.M109.003814

Johnson, J.R., Burnell-Nugent, M., Lossignol, D., Ganae-Motan, E.D., Potts, R., and Fallon, M.T. (2010). Multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of THC:CBD extract and THC extract in patients with intractable cancer-related pain. J. Pain Symptom Manage. 39(2):167-79. doi: 10.1016/j.jpainsymman.2009.06.008

Johnson, R. (2019). Defining hemp: A fact sheet. Congressional Research Service report R4472.

Johnston, D.G., Alberti, K.G., Wright, R., and Blain, P.G. (1982). Glycerol clearance in alcoholic liver disease. Gut. 23(4):257-64. doi: 10.1136/gut.23.4.257

Juknat, A., Pietr, M., Kozela, E., Rimmerman, N., Levy, R., Gao, F., et al. (2013). Microarray and pathway analysis reveal distinct mechanisms underlying cannabinoid-mediated modulation of LPS-induced activation of BV-2 microglial cells. PLoS One. 8(4):e61462. doi: 10.1371/journal.pone.0061462

Kaiser, R., Tremblay, P-B., Sezer, O., Possinger, K., Roots, I., and Brockmöller, J. (2004). Investigation of the association between 5-HT3A receptor gene polymorphisms and efficiency of antiemetic treatment with 5-HT3 receptor antagonists. Pharmacogenetics. 14(5):271-8. doi: 10.1097/00008571-200405000-00001

Kanazawa, K., Ashida, H., Minamoto, S., and Natake, M. (1986). The effect of orally administered secondary autoxidation products of linoleic acid on the activity of detoxifying enzymes in the rat liver. Biochim. Biophys. Acta. 879(1):36-43. doi: 10.1016/0005-2760(86)90263-8

Kang, S.S., Mun, K-C., Seo, J.H., Choe, M., and Ha, E. (2018). Cyanate improves insulin sensitivity and hepatic steatosis in normal and high fat-fed mice: Anorexic and antioxidative effects. Chem. Biol. Interact. 279:121-128. doi: 10.1016/j.cbi.2017.10.026

Kaplan, J.S., Stella, N., Catterall, W.A., and Westenbroek, R.E. (2017). Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc. Natl. Acad. Sci. USA. 114(42):11229-11234. doi: 10.1073/pnas.1711351114

Kargl, J., Andersen, L., Hasenohrl, C., Feuersinger, D., Stancic, A., Fauland, A., Magnes, C., El-Heliebi, A., Lax, S., Uranitsch, S., Haybaeck, J., Heinemann, A., and Schicho, R., (2016). GPR55 promotes migration and adhesion of colon cancer cells indicating role in metastasis. Br. J. Pharmacol. 173(1):142-154. doi: 10.1111/bph.13345

Karmaus, P.W.F., Wagner, J.G., Harkema, J.R., Kaminski, N.E., and Kaplan, B.L.F. (2013). Cannabidiol (CBD) enhances lipopolysaccharide (LPS)-induced pulmonary inflammation in C57BL/6 mice. J. Immunotoxicol. 10(3):321-8. doi: 10.3109/1547691X.2012.741628

Katayama, M., Kubo, T., Mogi, K., Ikeda, K., Nagasawa, M., and Kikusui, T. (2016). Heart rate variability predicts emotional state in dogs. Behav. Processes. 128:108-112. doi: 10.1016/j.beproc.2016.04.015

Katz, J. and Tayek, J.A. (1998). Gluconeogenesis and the Cori cycle in 12-, 20-, and 40-h-fasted humans. Am. J. Physiol. 275(3):E537-42. doi: 10.1152/ajpendo.1998.275.3.E537

Kawai, R., Aida, T., Hattori, H., Furukawa, T., Mori, K., Takasaki, W., Takahashi, N., and Kawada, T. (2013). Evaluation of canine T-cell dependent antibody response to the primary and secondary immunization with keyhole limpet hemocyanin. J Toxicol Sci. 38(4):571-579. doi: 10.2131/jts.38.571

Kesner, A.J. and Lovinger, D.M. (2020). Cannabinoids, endocannabinoids and sleep. Front. Mol. Neurosci. 13:125. doi: 10.3389/fnmol.2020.00125

Khan, B.A., Warner, P., and Wang, H. (2014). Antibacterial properties of hemp and other natural fibre plants: A review. Bioresources. 9(2):3642-3659. doi: 10.15376/biores.9.2.3642-3659

Khomyakova, M., Bukmez, O., Thomas, L.K., Erb, T.J., and Berg, I.A. (2011). A methylaspartate cycle in haloarchaea. Science. 331(6015):334-7. doi: 10.1126/science.1196544

Kim, H.G., Cheon, E.J., Bai, D.S., Lee, Y.H., and Koo, B.H. (2018). Stress and Heart Rate Variability: A Meta-Analysis and Review of the Literature. Psychiatry Investig. 15(3):235-245. doi: 10.30773/pi.2017.08.17

Kis, B., Ifrim, F.C., Buda, V., Avram, S., Pavel, I.Z., Antal, D., Paunescu, V., Dehelean, C.A., Ardelean, F., Diaconeasa, Z., Soica, C., and Danciu, C. (2019). Cannabidiol – from plant to human body: A promising bioactive molecule with multi-target effects in cancer. Int. J. Mol. Sci. 20(23):5905. doi: 10.3390/ijms20235905

Kirkland, J.B. (2009). Niacin status, NAD distribution and ADP-ribose metabolism. Curr. Pharm. Des. 15(1):3-11. doi: 10.2174/138161209787185823

Kisaki, T. and Tolbert, N.E. (1969). Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. Plant Physiol. 44(2):242-50. doi: 10.1104/pp.44.2.242

Klein, C., Karanges, E., Spiro, A., Wong, A., Spencer, J., Huynh, T., et al. (2011). Cannabidiol potentiates Δ^9-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. Psychopharmacology (Berl). 218(2):443-57. doi: 10.1007/s00213-011-2342-0

Knapik, A.A., Petkowski, J.J., Otwinowski, Z., Cymborowski, M.T., Cooper, D.R., Chruszcz, M., et al. (2012). Structure of Escherichia coli RutC, a member of the YjgF family and putative aminoacrylate peracid reductase of the rut operon. Acta. Crystallogr. Sect. F. Struct. Biol. Cryst. Commun. 68(Pt 11):1294-9. doi: 10.1107/S1744309112041796

Kogan, L.R., Hellyer, P.W., and Robinson, N.G. (2016). Consumers' perceptions of hemp products for animals. J. Am. Holist. Vet. Med. Assoc. 42:40-48.

Kogan, L.R., Hellyer, P.W., and Schoenfeld-Tacher, R. (2018). Dog owners' use and perceptions of cannabis products. J. Am. Holist. Vet. Med. Assoc. 51:26-33.

Kogan, L.R., Hellyer, P.W., Silcox, S., and Schoenfeld-Tacher, R. (2019b). Canadian dog owners' use and perceptions of cannabis products. Can. Vet. J. 60(7):749-755

Kogan, L., Schoenfeld-Tacher, R., Hellyer, P., and Rishniw, M. (2019a). US veterinarians' knowledge, experience, and perception regarding the use of cannabidiol for canine medical conditions. Front. Vet. Sci. 5:338. doi: 10.3389/fvets.2018.00338

Koketsu, K., Mitsuhashi, S., and Tabata, K. (2013). Identification of homophenylalanine biosynthetic genes from the cyanobacterium Nostoc punctiforme PCC73102 and application to its microbial production by Escherichia coli. Appl. Environ. Microbiol. 79(7):2201-2208. doi: 10.1128/AEM.03596-12

Kolevská, J., Brunclík, V., and Svoboda, M. (2003). Circadian rhythm of cortisol secretion in dogs of different daily activities. Acta Veterinaria Brno. 72(4):599-605: doi: 10.2754/avb200372040599

Kordowska-Wiater, M. (2015). Production of arabitol by yeasts: Current status and future prospects. J. Appl. Microbiol. 119(2):303-314. doi: 10.1111/jam.12807

Kornel, L., Patel, S.K., Ezzeraimi, E., and Shackleton, C.H. (1995). Corticosteroids in human blood: IX. Evidence for adrenal secretion of sulfate-conjugated cortisol, 11 beta,17 alpha-dihydroxy-4-pregnene-3,20-dione-21-yl-sulfate. Steroids. 60(12):817-23. doi: 10.1016/0039-128x(95)00141-c

Korpivaara, M., Laapas, K., Huhtinen, M., Schöning, B., and Overall, K. (2017). Dexmedetomidine oromucosal gel for noise-associated acute anxiety and fear in dogs — a randomised, double-blind,placebo-controlled clinical study. Vet. Rec. 180(14):356. doi: 10.1136/vr.104045

Kozela, E., Juknat, A., Gao, F., Kaushansky, N., Coppola, G., and Vogel, Z. (2016). Pathways and gene networks mediating the regulatory effects of cannabidiol, a nonpsychoactive cannabinoid, in autoimmune T cells. J. Neuroinflammation. 13(1):136. doi: 10.1186/s12974-016-0603-x

Kozela, E., Lev, N., Kaushansky, N., Eilam, R., Rimmerman, N., Levy, R., et al. (2011). Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6 mice. Br. J. Pharmacol. 163(7):1507-19. doi: 10.1111/j.1476-5381.2011.01379.x

Krehl, W.A., Torbet, N., de la Huerga, J., and Elvehjem, C.A. (1946). Relation of synthetic folic acid to niacin deficiency in dogs. Arch. Biochem. 11:363-369.

Kreimeyer, A., Perret, A., Lechaplais, C., Vallenet, D., Medigue, C., Salanoubat, M., et al. (2007). Identification of the last unknown genes in the fermentation pathway of lysine. J. Biol. Chem. 282(10):7191-7. doi: 10.1074/jbc.M609829200

Kress, D., Alhapel, A., Pierik, A.J., and Essen, L-O. (2008). The crystal structure of enamidase: a bifunctional enzyme of the nicotinate catabolism. 384(4):837-847. doi: 10.1016/j.jmb.2008.09.002

Kudo, F., Yamamoto, Y., Yokoyama, K., Eguchi, T., and Kakinuma, K. (2005). Biosynthesis of 2-deoxystreptamine by three crucial enzymes in Streptomyces fradiae NBRC 12773. 58(12):766-774. doi: 10.1038/ja.2005.104

Kuhathasan, N., Dufort, A., MacKillop, J., Gottschalk, R., Minuzzi, L., and Frey, B.N. (2019). The use of cannabinoids for sleep: A critical review on clinical trials. Exp. Clin. Psychopharmacol. 27(4):383-401. doi: 10.1037/pha0000285

Kunos, G. and Tam, J. (2011). The case for peripheral CB_1 receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. Br. J. Pharmacol. 163(7):1423-31. doi: 10.1111/j.1476-5381.2011.01352.x

Kurihara, S., Oda, S., Kato, K., Kim, H.G., Koyanagi, T., Kumagai, H., et al. (2005). A Novel Putrescine Utilization Pathway Involves γ-Glutamylated Intermediates of Escherichia coli K-12*. J. Biol. Chem. 280(6):4602-4608. doi: 10.1074/jbc.M411114200

Kwack, M.H., Ahn, J.S., Kim, M.K., Kim, J.C., and Sung, Y.K. (2010). Preventable effect of L-threonate, an ascorbate metabolite, on androgen-driven balding via repression of dihydrotestosterone-induced dickkopf-1 expression in human hair dermal papilla cells. BMB Rep. 43(10):688-92. doi: 10.5483/BMBRep.2010.43.10.688

Ladha, C. and Hoffman, C.L. (2018). A combined approach to predicting rest in dogs using accelerometers. Sensors. 18:2649. doi: 10.3390/s18082649

Laflamme D. (1997). Development and validation of a body condition score system for dogs. Canine Pract. 22:10–15.

Land, E.J., Ramsden, C.A., and Riley, P.A. (2003). Tyrosinase autoactivation and the chemistry of ortho-quinone amines. Acc. Chem. Res. 36(5):300-8. doi: 10.1021/ar020062p

Landa, L., Sulcova, A., and Gbelec, P. (2016). The use of cannabinoids in animals and therapeutic implications for veterinary medicine: a review. Veterinární Medicína. 61(3):111-122. doi: 10.17221/8762-VETMED

Landsberg, G.M., Mougeot, I., Kelly, S., and Milgram, N.W. (2015). Assessment of noise-induced fear and anxiety in dogs: Modification by a novel fish hydrolysate supplemented diet. J. Vet. Behav. 10:391-398. doi:10.1016/j.jveb.2015.05.007

Lanuti, M., Talamonti, E., Maccarrone, M., and Chiurchiu, V. (2015). Activation of GPR55 receptors exacerbates oxLDL-induced lipid accumulation and inflammatory responses, while reducing cholesterol efflux from human macrophages. PLoS One. 10(5):e0126839. doi: 10.1371/journal.pone.0126839

Laprairie, R.B., Bagher, A.M., Kelly, M.E.M., and Denovan-Wright, E.M. (2015). Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. Br. J. Pharmacol. 172(20):4790-805. doi: 10.1111/bph.13250

Larqué, E., Sabater-Molina, M., and Zamora, S. (2007). Biological significance of dietary polyamines 23(1):87-95. doi: 10.1016/j.nut.2006.09.006

Lattanzi, S., Trinka, E., Striano, P., Zaccara, G., Del Giovane, C., Nardone, R., et al. (2020). Cannabidiol efficacy and clobazam status: A systematic review and meta-analysis. Epilepsia. 61(6):1090-1098. doi: 10.1111/epi.16546

Lauckner, J. E., Jensen, J. B., Chen, H. Y., Lu, H. C., Hille, B., and Mackie, K. (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. Proc. Natl. Acad. Sci. U.S.A. 105, 2699–2704. doi: 10.1073/pnas.0711278105

Lee, J.C., Bertoglio, L.J., Guimarães, F.S., and Stevenson, C.W. (2017). Cannabidiol regulation of emotion and emotional memory processing: relevance for treating anxiety-related and substance abuse disorders. Brit. J. Pharmacol. 174:3242-3256. doi: 10.1111/bph.13724

Lee, X.C., Werner, E., and Falasca, M. (2021). Molecular mechanism of autophagy and its regulation by cannabinoids in cancer. Cancers. 13:1211. doi: 10.3390/cancers13061211

Legrand, J., Bouchez, C., Mimouni, C., N'Guyen, A., Bouchard, J., Ameller, T., and Descotes, J. (2012). Immunotoxic effects of cyclophosphamide and cyclosporine in the dog. J. Immunotoxicol. 10(1):90-95. doi: 10.3109/1547691X.2012.723766

Lehmann, C., Fisher, N.B., Tugwell, B., Szczesniak, A., Kelly, M., and Zhou, J. (2016). Experimental cannabidiol treatment reduces early pancreatic inflammation in type 1 diabetes. Clin. Hemorheol. Microcirc. 64(4):655-662. doi: 10.3233/CH-168021

Lehmann, T. and Pollmann, S. (2009). Gene expression and characterization of a stress-induced tyrosine decarboxylase from Arabidopsis thaliana. FEBS Letters. 583:1895-1900. doi: 10.1016/j.febslet.2009.05.017

Leonard, W., Zhang, P., Ying, D., and Fang, Z. (2020). Tyramine-derived hydroxycinnamic acid amides in plant foods: sources, synthesis, health effects and potential applications in food industry. Crit. Rev. Food Sci. Nutr. 1-18. doi: 10.1080/10408398.2020.1845603

Leweke, F.M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., et al. (2012). Cannabidiol enhances anadamide signaling and alleviates psychotic symptoms of schizophrenia. Transl. Psychiatry. 2(3):e94. doi: 10.1038/tp.2012.15

Ley, J.P., Engelhart, K., Bernhardt, J., Bertram, H-J. (2002). 3,4-Dihydroxymandelic acid, a noradrenalin metabolite with powerful antioxidative potential. J. Agric. Food Chem. 50(21):5897-902. doi: 10.1021/jf025667e

Li, L., Li, R., Zhou, J., Zuniga, A., Stanislaus, A.E., Wu, Y., et al. (2013). MyCompoundID:using an evidence-based metabolome library for metabolite identification. Anal. Chem. 85:3401–3408. doi:10.1021/ac400099b

Li, X., Yu, C., Cai, Y., Liu, G., Jia, J., and Wang, Y. (2005). Simultaneous determination of six phenolic constituents of danshen in human serum using liquid chromatography/tandem mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 820(1):41-7. doi: 10.1016/j.jchromb.2005.03.005

Lim, S.Y., Sharan, S., and Woo, S. (2020). Model-based analysis of cannabidiol dose-exposure relationship and bioavailability. Pharmacotherapy. 40(4):291-300. doi: 10.1002/phar.2377

Linares, I.M.P., Guimarães, F.S., Eckeli, A., Crippa, A.C.S., Zuardi, A.W., Souza, J.D.S., Hallak, J.E., and Crippa, J.A.S. (2018). No Acute Effects of Cannabidiol on the Sleep-Wake Cycle of Healthy Subjects: A Randomized, Double-Blind, Placebo-Controlled, Crossover Study. Front. Pharmacol. 9:315. doi: 10.3389/fphar.2018.00315

Linster, C.L. and Schaftingen, E.V. 2007. Vitamin C. Biosynthesis, recycling and degradation in mammals. FEBS J. 274(1):1-22. doi: 10.1111/j.1742-4658.2006.05607.x

Liou, G.I., Auchampach, J.A., Hillard, C.J., Zhu, G., Yousufzai, B., Mian, S., et al. (2008). Mediation of cannabidiol anti-inflammation in the retina by equilibrative

nucleoside transporter and A2A adenosine receptor. Invest. Ophthalmol. Vis. Sci. 49(12):5526-31. doi: 10.1167/iovs.08-2196

Lippincott, J.A., Lippincott, B.B., and Chang, C-C. (1972). Promotion of crown-gall tumor growth by lysopine, octopine, nopaline, and carnosine. Plant. Physiol. 49(2):131-137. doi: 10.1104/pp.49.2.131

Liu, I-M., Hsu, F-L., Chen, C-F., and Cheng, J-T. (2000). Antihyperglycemic action of isoferulic acid in streptozotocin-induced diabetic rats. Br. J. Pharmacol. 129(4):631-636. doi: 10.1038/sj.bjp.0703082

Loke, W.M., Jenner, A.M., Proudfoot, J.M., McKinley, A.J., Hodgson, J.M., Halliwell, B., et al. (2009). A metabolite profiling approach to identify biomarkers of flavonoid intake in humans. J. Nutr. 139(12):2309-14. doi: 10.3945/jn.109.113613

Louis, P. and Flint, H.J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol. Lett. 294(1):1-8. doi: 10.1111/j.1574-6968.2009.01514.x

Lu, H-C. and Mackie, K. (2015). An introduction to the endogenous cannabinoid system. Biol. Psychiatry. 79(7):516-25. doi: 10.1016/j.biopsych.2015.07.028

Lus, G., Cantello, R., Danni, M.C., Rini, A., Sarchielli, P., Tassinari, T., and Signoriello, E. (2018). Palatability and oral cavity tolerability of THC:CBD oromucosal spray and possible improvement measures in multiple sclerosis patients with resistant spasticity: A pilot study. Neurodegener. Dis. Manag. 8(2):105-113. doi: 10.2217/nmt-2017-0056

Ma, Q., Zhang, X., and Qu, Y. (2018). Biodegradation and biotransformation of indole: advances and perspectives. Front. Microbiol. 9:2625. doi: 10.3389/fmicb.2018.02625

Maccariello, C.E.M., Franzini de Souza, C.C., Morena, L., Dias, D.P.M., and de Medeiros, M.A. (2018). Effects of acupuncture on the heart rate variability, cortisol levels, and behavioural response induced by thunder sound in beagles. Physiol. Behav. 186:37-44. doi: 10.1016/j.physbeh.2018.01.006

Maccarrone, M., Bab, I., Biro, T., Cabral, G.A., Dey, S.K., Di Marzo, V., Konje, J.C., Kunos, G., Mechoulam, R., Pacher, P., Sharkey, K.A., and Zimmer, A. (2015). Endocannabinoid signaling at the periphery: 50 years after THC. Trends Pharmacol. Sci. 36(5):277-96. doi: 10.1016/j.tips.2015.02.008

Macfarlane, S. and Macfarlane, G.T. (2003). Regulation of short-chain fatty acid production. Proc. Nutr. Soc. 62(1):67-72. doi: 10.1079/PNS2002207

Mahajan, D.K., Anderson, G., Poole, W.K., Billar, R.B., and Little, B. (1983). Changes in the concentration of 17 alpha,20 alpha-dihydroxypregn-4-en-3-one during pregnancy, labor, and delivery and the effect of dexamethasone treatment during the third trimester of pregnancy. J. Clin. Endocrinol. Metab. 57(3):585-91. doi: 10.1210/jcem-57-3-585

Mahendran, Y., Cederberg, H., Vangipurapu, J., Kangas, A.J., Soininen, P., Kuusisto, J., et al. (2013). Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. Diabetes Care. 36(11):3732-8. doi: 10.2337/dc13-0800

Malenka, R.C., Nestler, E.J., and Hyman, S.E. (2009). Chapter 6: Widely Projecting Systems: Monoamines, Acetylcholine, and Orexin. In Sydor A, Brown RY (eds.). Molecular Neuropharmacology: A Foundation for Clinical Neuroscience (2nd ed.). New York, USA: McGraw-Hill Medical. pg. 157.

Manini, A.F., Yiannoulos, G., Bergamaschi, M.M., Hernandez, S., Olmedo, R., Barnes, A.J., et al. (2015). Safety and pharmacokinetics of oral cannabidiol when administered concomitantly with intravenous fentanyl in humans. J. Addict. Med. 9(3):204-210. doi: 10.1097/ADM.0000000000000118

Marcal, D., Rego, A.T., Carrondo, M.A., and Enguita, F.J. (2009). 1,3-Propanediol dehydrogenase from Klebsiella pneumoniae: Decameric quaternary structure and possible subunit cooperativity. J. Bacteriol. 191(4):1143-51. doi: 10.1128/JB.01077-08

Marinho, A.L.Z., Vila-Verde, C., Fogaca, M.V., and Guimaraes, F.S. (2015). Effects of intra-infralimbic prefrontal cortex injections of cannabidiol in the modulation of emotional behaviors in rats: contribution of 5HT1A receptors and stressful experiences. Behav. Brain Res. 286:49-56. doi: 10.1016/j.bbr.2015.02.023

Marsch, F., Foeller, E., Rammes, G., Bunck, M., Kössl, M., Holsboer, F., et al. (2007). Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. J. Neurosci. 27(4):832-9. doi: 10.1523/JNEUROSCI.3303-06.2007

Massi, P., Vaccani, A., Bianchessi, S., Costa, B., Macchi, P., and Parolaro, D. (2006). The non-psychoactive cannabidiol triggers caspase activation and oxidative stress in human glioma cells. Cell Mol. Life Sci. 63(17):2057-2066. doi: 10.1007/s00018-006-6156-x

Massimini, M., Vedove, E.D., Bachetti, B., Di Pierro, F., Ribecco, C., D'Addario, C., et al. (2021). Polyphenols and cannabidiol modulate transcriptional regulation of Th1/Th2 inflammatory genes related to canine ttopic dermatitis. Front. Vet. Sci. 8:606197. doi: 10.3389/fvets.2021.606197

Mates, A. and Hinton, N.A. (1976). Immune response in dog. 3. Antibody formation in dogs and rabbits to human serum proteins and keyhole limpet haemocyanin. Microbios. 17(70):175-187.

McCabe, M. and Cital, S. (2021). Cannabinoids for Gastrointestinal Health. In: Cital, S., Kramer, K., Hughston, L., and Gaynor, J.S. (eds) Cannabis Therapy in Veterinary Medicine. Springer, Cham. doi: 10.1007/978-3-030-68317-7_8

McCarty, R.M., Somogyi, A., Lin, G., Jacobsen, N.E., and Bandarian, V. (2009). The Deazapurine Biosynthetic Pathway Revealed: In Vitro Enzymatic Synthesis of PreQ0 from Guanosine 5′-Triphosphate in Four Steps. Biochem. 48(18):3847-3852. doi: 10.1021/bi900400e

McCraty, R. and Shaffer, F. (2015). Heart rate variability: New perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. Glob. Adv. Health. Med. 4(1):46-61. doi: 10.7453/gahmj.2014.073

McDougall, J.J. (2006). Arthritis and pain: Neurogenic origin of joint pain. Arthritis Res. Ther. 8:220. doi: 10.1186/ar2069

McGrath, S., Bartner, L.R., Rao, S., Kogan, L.R., and Hellyer, P.W. (2018). A report of adverse effects associated with the administration of cannabidiol in healthy dogs. Amer. Holistic Vet. Med. Assoc. 52:34-38.

McGrath, S., Bartner, L.R., Rao, S., Packer, R.A., and Gustafson, D.L. (2019). Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. J. Am. Vet. Med. Assoc. 254(11):1301-1308. doi: 10.2460/javma.254.11.1301

Mead, A. (2017). The legal status of cannabis (marijuana) and cannabidiol (CBD) under U.S. law. Epilepsy Behav. 70:288-291. doi: 10.1016/j.yebeh.2016.11.021

Mechoulam, R., Parker, L.A., and Gaillily, R. (2002). Cannabidiol: An overview of some pharmacologial aspects. J. Clin. Pharmacol. 42:11S-19S. doi: 10.1177/0091270002238789

Mechoulam, R. and Shvo, Y. (1963). The structure of cannabidiol. Tetrahedron. 19:2073-2078. doi: 10.1016/0040-4020(63)85022-x

Meeprom, A., Sompong, W., Chan, C.B., and Adisakwattana, S. (2013). Isoferulic Acid, a New Anti-Glycation Agent, Inhibits Fructoseand Glucose-Mediated Protein Glycation in Vitro. Molecules. 18(6):6439-54. doi: 10.3390/molecules18066439

Meeprom, A., Sompong, W., Suantawee, T., Thilavech, T., Chan, C.B., and Adisakwattana, S. (2015). Isoferulic acid prevents methylglyoxalinduced protein

glycation and DNA damage by free radical scavenging activity. BMC Complement. Altern. Med. 15:346. doi: 10.1186/s12906-015-0874-2

Meister, A. (1994). Glutathione-ascorbic acid antioxidant system in animals. J. Biol. Chem. 269(13):9397-9400.

Mersaides, A.J., Tognela, A., Haber, P.S., Stockler, M., Lintzeris, N., Simes, J., et al. (2018). Oral cannabinoid-rich THC/CBD cannabis extract for secondary prevention of chemotherapy-induced nausea and vomiting: a study protocol for a pilot and definitive randomised double-blind placebo-controlled trial (CannabisCINV). BMJ Open. 8(9):e020745. doi: 10.1136/bmjopen-2017-020745

Michel, K.E. and Brown, D.C. (2011). Determination and application of cut points for accelerometer-based activity counts of activities with differing intensity in pet dogs. Am. J. Vet. Res. 72(7):866-870. doi: 10.2460/ajvr.72.7.866

Millar, S.A., Stone, N.L., Yates, A.S., and O'Sullivan, S.E. (2018). A Systematic Review on the Pharmacokinetics of Cannabidiol in Humans. Front. Pharmacol. 9:1365. doi: 10.3389/fphar.2018.01365

Miller, J.E. and Litwack, G. (1971). Purification, properties, and identity of liver mitochondrial tyrosine aminotransferase. J. Biol. Chem. 246(10):3234-3240.

Mills, D.S., Ramos, D., Estelles, M.G., and Hargrave, C. (2006). A triple blind placebo-controlled investigation into the assessment of the effect of Dog Appeasing Pheromone (DAP) on anxiety related behaviour of problem dogs in the veterinary clinic. App. Anim. Behav. Sci. 98:114-126. doi: 10.1016/j.applanim.2005.08.012

Minamoto, S., Kanazawa, K., Ashida, H., and Natake, M. (1988). Effect of orally administered 9-oxononanoic acid on lipogenesis in rat liver. Biochim. Biophys. Acta. 958(2):199-204. doi: 10.1016/0005-2760(88)90177-4

Minke, B. (2006). TRP channels and Ca2+ signaling. Cell Calcium. 40(3):261-75. doi: 10.1016/j.ceca.2006.05.002

Miyazaki, T., Honda, A., Ikegami, T., Iwamoto, J., Monma, T., Hirayama, T. et al. (2015) Simultaneous quantification of salivary 3-hydroxybutyrate, 3-hydroxyisobutyrate, 3-hydroxy-3-methylbutyrate, and 2-hydroxybutyrate as possible markers of amino acid and fatty acid catabolic pathways by LC–ESI–MS/MS. SpringerPlus 4:494. doi: 10.1186/s40064-015-1304-0

Molnár, G.A., Kun, S., Selley, E., Kertesz, M., Szelig, L., Csontos, C., et al. (2016). Role of Tyrosine Isomers in Acute and Chronic Diseases Leading to Oxidative Stress - A Review. Curr. Med. Chem. 23(7):667-685. doi: 10.2174/0929867323666160119094516

Molnár, G.A., Wagner, Z., Marko, L., Szegi, T.K., Mohas, M., Kocsis, B., et al. (2005). Urinary ortho-tyrosine excretion in diabetes mellitus and renal failure: evidence for hydroxyl radical production. Kidney Int. 68(5):2281-2287. doi: 10.1111/j.1523-1755.2005.00687.x

Monteleone, P. (1991). Effects of trazodone on plasma cortisol in normal subjects. A study with drug plasma levels. Neuropsychopharmacology. 5(1):61-64.

Moore, L.W., Chilton, W.S., and Canfield, M.L. (1997). Diversity of opines and opine-catabolizing bacteria isolated from naturally occurring crown gall tumors. Appl. Environ. Microbiol. 63(1):201-207. doi: 10.1128/AEM.63.1.201-207.1997

Moreira, F.A., Aguiar, D.C., and Guimaraes, F.S. (2006). Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. Prog. Neuropsychopharmacol. Biol. Psychiatry. 30(8):1466-1471. doi: 10.1016/j.pnpbp.2006.06.004

Morena, M., Patel, S., Bains, J.S., and Hill, M.N. (2016). Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 41(1):80-102. doi: 10.1038/npp.2015.166

Morgan, C.J.A., Gardener, C., Schafer, G., Swan, S., Demarchi, C., Freeman, T.P., et al. (2012). Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. Psychol. Med. 42(2):391-400. doi: 10.1017/S0033291711001322

Morgan, C.J.A., Schafer, G., Freeman, T.P., and Curran, H.V. (2010). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study. Br. J. Psychiatry. 197(4):285-90. doi: 10.1192/bjp.bp.110.077503

Morris, E.M., Kitts-Morgan, S.E., Spangler, D.M., Gebert, J., Vanzant, E.S., McLeod, K.R., and Harmon, D.L. (2021). Feeding cannabidiol (CBD) containing treats did not affect canine daily voluntary activity. Front. Vet. Sci. 8:645667. doi: 10.3389/fvets.2021.645667

Morris, E.M., Kitts-Morgan, S.E., Spangler, D.M., McLeod, K.R., Costa, J.H.C., and Harmon, D.L. (2020). The impact of feeding cannabidiol (CBD) containing treats on canine response to a noise-induced fear response test. Front. Vet. Sci. 7:569565. doi: 10.3389/fvets.2020.569565

Morrison, P.D., Nottage, J., Stone, J.M., Bhattacharyya, S., Tunstall, N., Brenneisen, R., et al. (2011). Disruption of frontal θ coherence by Δ9-tetrahydrocannabinol is associated with positive psychotic symptoms. Neuropsychopharmacology. 36(4):827-36. doi: 10.1038/npp.2010.222

Morrison, R., Reilly, J.J., Penpranze, V., Pendlebury, E., and Yam, P.S. (2014). A 6-month observational study of changes in objectively measured physical activity during weight loss in dogs. J. Small Anim. Pract. 55:566-570. doi: 10.1111/jsap.12273

Mu, W., Hassanin, H.A.M., Zhou, L., and Jiang, B. (2018). Chemistry behind rare sugars and bioprocessing. J. Agric. Food Chem. 66(51):13343-13345. doi: 10.1021/acs.jafc.8b06293

Muller, C., Gines, J.A., Conzemius, M., Meyers, R., and Lascelles, B.D.X. (2018). Evaluation of the effect of signalment and owner-reported impairment level on accelerometer-measured changes in activity in osteoarthritic dogs receiving a non-steroidal anti-inflammatory. Vet. J. 242:48-52. doi: 10.1016/j.tvjl.2018.10.005

Muller, C., Gines, J.A., Conzemius, M., Meyers, R., and Lascelles, B.D.X. (2018). Evaluation of the effect of signalment and owner-reported impairment level on accelerometer-measured changes in activity in osteoarthritic dogs receiving a non-steroidal anti-inflammatory. Vet. J. 242:48-52. doi: 10.1016/j.tvjl.2018.10.005

Muller, C., Morales, P., and Reggio, P.H. (2019). Cannabinoid ligands targeting TRP channels. Front. Mol. Neurosci. 11:487. doi: 10.3389/fnmol.2018.00487

Mung, D., and Li, L. (2017). Development of chemical isotope labeling LC-MS for milk metabolomics: comprehensive and quantitative profiling of the Amine/Phenol submetabolome. Anal. Chem. 89:4435–4443. doi: 10.1021/acs.analchem.6b03737

Murillo-Rodríguez, E. (2008). The role of the CB1 receptor in the regulation of sleep. Prog. Neuropsychopharmacol. Biol. Psychiatry. 32(6):1420-7. doi: 10.1016/j.pnpbp.2008.04.008

Murillo-Rodríguez, E., Blanco-Centurion, C., Sanchez, C., Piomelli, D., and Shiromani, P.J. (2003). Anandamide enhances extracellular levels of adenosine and induces sleep: an in vivo microdialysis study. Sleep. 26(8):943-7. doi: 10.1093/sleep/26.8.943

Murillo-Rodríguez, E., Machado, S., Rocha, N.B., Budde, H., Yuan, T-F., and Arias-Carrion, O. (2016). Revealing the role of the endocannabinoid system modulators, SR141716A, URB597 and VDM-11, in sleep homeostasis. Neuroscience. 339:433-449. doi: 10.1016/j.neuroscience.2016.10.011

Murillo-Rodríguez, E., Millan-Aldaco, D., Palomero-Rivero, M., Mechoulam, R., and Drucker-Colin, R. (2006). Cannabidiol, a constituent of Cannabis sativa, modulates sleep in rats. FEBS Lett. 580(18):4337-45. doi: 10.1016/j.febslet.2006.04.102

Murillo-Rodríguez, E., Millan-Aldaco, D., Palomero-Rivero, M., Mechoulam, R., and Drucker-Colin, R. (2008). The nonpsychoactive Cannabis constituent cannabidiol is a wake-inducing agent. Behav. Neurosci. 122(6):1378-82. doi: 10.1037/a0013278

Murillo-Rodríguez, E., Sarro-Ramirez, A., Sanchez, D., Mijangos-Moreno, S., Tejeda-Padron, A., Poot-Ake, A., et al. (2014). Potential effects of cannabidiol as a wake-promoting agent. Curr. Neuropharmacol. 12(3):269-72. doi: 10.2174/1570159X11666131204235805

Nabissi, M., Morelli, M.B., Amantini, C., Liberati, S., Santoni, M., Ricci-Vitiani, L., Pallini, R., and Santoni, G. (2015). Cannabidiol stimulates Aml-1a-dependent glial differentiation and inhibits glioma stem-like cells proliferation by inducing autophagy in a TRPV2-dependent manner. Int. J. Cancer. 137(8):1855-69. doi: 10.1002/ijc.29573

Nagashima, K., Lopez, C., Donovan, D., Ngai, C., Fontanez, N., Bensadoun, A., et al. (2005). Effects of the PPARgamma agonist pioglitazone on lipoprotein metabolism in patients with type 2 diabetes mellitus. J. Clin. Invest. 115(5):1323-32. doi: 10.1172/JCI23219

Naka, K., Jomen, Y., Ishihara, K., Kim, J., Ishimoto, T., Bae, E-J., et al. (2015). Dipeptide species regulate p38MAPK–Smad3 signaling to maintain chronic myelogenous leukaemia stem cells. Nat. Commun. 6:8039. doi: 10.1038/ncomms9039

Nakato, J., Aoki, H., Tokuyama, Y., Yamamoto, Y., Iwakura, H., Matsumura, S., et al. (2019). FEBS Lett. 593(18):2637-2645. doi: 10.1002/1873-3468.13522

Nam, Y., Kim, Y., and Lee, J. (2016). Sleep monitoring based on a tri-axial accelerometer and a pressure sensor. Sensors. 16:750. doi: 10.3390/s16050750

Nazıroğlu, M. (2015). TRPV1 channel: A potential drug target for treating epilepsy. Curr. Neuropharmacol. 13(2):239-47. doi: 10.2174/1570159x13666150216222543

Nazıroğlu, M. and Demirdaş, A. (2015). Psychiatric disorders and TRP channels: Focus on psychotropic drugs. Curr. Neuropharmacol. 13(2):248-57. doi: 10.2174/1570159x13666150304001606

Nicholson, A.N., Turner, C., Stone, B.M., and Robson, P.J. (2004). Effect of Δ-9-tetrahydrocannabinol and cannabidiol on nocturnal sleep and early-morning behavior in young adults. J. Clin. Psychopharmacol. 24(3):305-313. doi: 10.1097/01.jcp.0000125688.05091.8f

Nikiforova, V., Giesbertz, P., Wiemer, J., Bethan, B., Looser, R., Liebenberg, V., et al.

(2014). Glyoxylate, a new marker metabolite of type 2 diabetes. J. Diabetes Res. 2014:685204. doi: 10.1155/2014/685204

Nishizuka, Y., Ichiyama, A., Gholson, R.K., and Hayaishi, O. (1965). Studies on the Metabolism of the Benzene Ring of Tryptophan in Mammalian Tissues: I. Enzymic formation of glutaric acid from 3-hydroxy anthranilic acid. J. Biol. Chem. 240:733-739. doi: 10.1016/S0021-9258(17)45237-9

Nuttall, F.Q., Ngo, A., and Gannon, M.C. (2008). Regulation of hepatic glucose production and the role of gluconeogenesis in humans: Is the rate of gluconeogenesis constant? Diabetes Metab. Res. Rev. 24(6):438-58. doi: 10.1002/dmrr.863

Ogunade, I. M., Jiang, Y., Adeyemi, J., Oliveira, A., Vyas, D., and Adesogan, A. T. (2018). Biomarker of aflatoxin ingestion: 1H NMR-based plasma metabolomics of dairy cows fed aflatoxin B1 with or without sequestering agents. Toxins. 10:545. doi: 10.3390/toxins10120545

Ogunade, I., Oyebade, A., Osa-Andrews, B., and Peters, S. (2021). Plasma carboxyl-metabolome is associated with average daily gain divergence in beef steers. Animals. 11(1):67. doi: 10.3390/ani11010067

Ohta, A. and Sitkovsky, M. (2001). Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature. 414(6866):916-20. doi: 10.1038/414916a

O'Kell, A.L., Garrett, T.J., Wasserfall, C., and Atkinson, M.A. (2017). Untargeted metabolomic analysis in naturally occurring canine diabetes mellitus identifies similarities to human Type 1 Diabetes. Sci. Rep. 7(1):9467. doi: 10.1038/s41598-017-09908-5

Olivares, C., Jimenez-Cervantes, C., Lozano, J.A., Solano, F., and Garcia-Borron, J.C. (2001). The 5,6-dihydroxyindole-2-carboxylic acid (DHICA) oxidase activity of human tyrosinase. Biochem. J. 354(Pt 1):131-139. doi: 10.1042/0264-6021:3540131

Olivas-Aguirre, M., Torres-Lopez, L., Valle-Reyes, J.S., Hernandez-Cruz, A., Pottosin, I., and Dobrovinskaya, O. (2019). Cannabidiol directly targets mitochondria and disturbs calcium homeostasis in acute lymphoblastic leukemia. Cell Death Dis. 10:779. doi: 10.1038/s41419-019-2024-0

O'Sullivan, S.E. (2016). An update on PPAR activation by cannabinoids. Br. J. Pharmacol. 173(12):1899-910. doi: 10.1111/bph.13497

Otte, K.B., Kirtz, M., Nestl, B.M., and Hauer, B. (2013). Synthesis of 9-oxonanonic acid, a precursor for biopolymers. ChemSusChem. 6(11):2149-56. doi: 10.1002/cssc.201300183

Overall, K.L. (2013). Manual of Clinical Behavioral Medicine for Dogs and Cats. St. Louis, MO: Elsevier Health Sciences.

Overall, K.L., Dunham, A.E., and Frank, D. (2001). Frequency of nonspecific clinical signs in dogs with separation anxiety, thunderstorm phobia, and noise phobia, alone or in combination. J. Am. Vet. Med. Assoc. 219(4):467-473. doi: 10.2460/javma.2001.219.467

Pacher, P., Batkai, S., and Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol. Rev. 58(3):389-462. doi: 10.1124/pr.58.3.2

Padberg, I., Peter, E., Gonzalez-Maldonado, S., Witt, H., Mueller, M., Weis, T., et al. (2014). A new metabolic signature in type-2 diabetes mellitus and its pathophysiology. PLoS One. 9(1):e85082. doi: 10.1371/journal.pone.0085082

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., and Pasquali, R. (2006). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr. Rev. 27(1):73-100. doi: 10.1210/er.2005-0009

Pahwa, R., Goyal, A., Bansal, P., and Jialal, I. (Updated Nov. 2020). Chronic Inflammation. In: StatPearls [Internet]. StatPearls Publishing; Treasure Island, FL. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493173/

Pakin, C., Bergaentzle, M., Aoude-Werner, D., and Hasselmanna, C. (2005). Alpha-ribazole, a fluorescent marker for the liquid chromatographic determination of vitamin B12 in foodstuffs. J. Chromatogr. A. 1081(2):182-9. doi: 10.1016/j.chroma.2005.05.066

Palestrini, C., Minero, M., Cannas, S., Berteselli, G., Scaglia, E., Barbieri, S., et al. (2010). Efficacy of a diet containing caseinate hydrolysate on signs of stress in dogs. J. Vet. Behav. 5(6):309-317. doi: 10.1016/j.jveb.2010.04.004

Pan, H., Mukhopadhyay, P., Rajesh, M., Patel, V., Mukhopadhyay, B., Gao, B., et al. (2009). Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. J. Pharmacol. Exp. Ther. 328(3):708-14. doi: 10.1124/jpet.108.147181

Panoutsopoulos, G.I. and Beedham, C. (2005). Metabolism of isovanillin by aldehyde oxidase, xanthine oxidase, aldehyde dehydrogenase and liver slices. Pharmacology. 73(4):199-208. doi: 10.1159/000082860

Parimoo, B. and Tanaka, T. (1993). Structural organization of the human isovaleryl-CoA dehydrogenase gene. Genomics. 15(3):582-90. doi: 10.1006/geno.1993.1111

Park, J.W., Park, S.R., Nepal, K.K., Han, A.R., Ban, Y.H., Yoo, Y.J., et al. (2011). Discovery of parallel pathways of kanamycin biosynthesis allows antibiotic manipulation. Nat. Chem. Biol. 7(11):843-852. doi: 10.1038/nchembio.671

Parker, L.A., Kwiatkowska, M., Burton, P., and Mechoulam, R. (2004). Effect of cannabinoids on lithium-induced vomiting in the Suncus murinus (house musk shrew). Psychopharmacology (Berl). 171(2):156-61. doi: 10.1007/s00213-003-1571-2

Part, C.E., Kiddie, J.L., Hayes, W.A., Mills, D.S., Neville, R.F., Morton, D.B., and Collins, L.M. (2014). Physiological, physical and behavioural changes in dogs (Canis familiaris) when kennelled: Testing the validity of stress parameters. Phys. Behav. 133(22):260-271. doi: 10.1016/j.physbeh.2014.05.018

Peachee, V.L., Smith, M.J., Beck, M.J., Stump, D.G., and White, K.L. (2013). Characterization of the T-dependent antibody response (TDAR) to keyhole limpet hemocyanin (KLH) in the Göttingen minipig. J. Immunotoxicol. 11(4):376-382. doi: 10.3109/1547691X.2013.853716

Pertwee, R.G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br. J. Pharmacol. 153(2):199-215. doi: 10.1038/sj.bjp.0707442

Petrosino, S., Verde, R., Vaia, M., Allara, M., Iuvone, T., and Di Marzo, V. (2018). Anti-inflammatory properties of cannabidiol, a nonpsychotropic cannabinoid, in experimental allergic contact dermatitis. J. Pharmacol. Exp. Ther. 365(3):652-663. doi: 10.1124/jpet.117.244368

Philpott, H.T., O'Brien, M., and McDougall, J.J. (2017). Attenuation of early phase inflammation by cannabidiol prevents pain and nerve damage in rat osteoarthritis. Pain. 158:2442-2451. doi: 10.1097/j.pain.0000000000001052

Picard, F. and Auwerx, J. (2002). PPAR(gamma) and glucose homeostasis. Annu. Rev. Nutr. 22:167-97. doi: 10.1146/annurev.nutr.22.010402.102808

Pickup, E., German, A.J., Blackwell, E., Evans, M., and Westgarth, C. (2017). Variation in activity levels amongst dogs of different breeds: Results of a large online survery of dog owners from the UK. J. Nutr. Sci. 6:e10. doi: 10.1017/jns.2017.7

Podebrad, F., Heil, M., Beck, T., Mosandl, A., Sewell, A.C., and Bohles, H. (2000). Stereodifferentiation of 3-hydroxyisobutyric- and 3-aminoisobutyric acid in human urine by enantioselective multidimensional capillary gas chromatography-

mass spectrometry. Clin. Chim. Acta. 292(1-2):93-105. doi: 10.1016/s0009-8981(99)00210-7

Pojić, M., Mišan, A., Sakač, M., Hadnadev, T.D., Šarić, B., Milovanović, I., et al. (2014). Characterization of Byproducts Originating from Hemp Oil Processing. J. Agric. Food Chem. 62(51):12436-42. doi: 10.1021/jf5044426

Politano, V.T., Diener, R.M., Christian, M.S., Hawkins, D.R., Ritacco, G., and Api, A.M. (2013). The pharmacokinetics of phenylethyl alcohol (PEA): safety evaluation comparisons in rats, rabbits, and humans. Int. J. Toxicol. 32(1):39-47. doi: 10.1177/1091581812471688

Polko, J.K. and Kieber, J.J. (2019). 1-Aminocyclopropane 1-carboxylic acid and its emerging role as an ethylene-independent growth regulator. Front. Plant Sci. 10:1602. doi: 10.3389/fpls.2019.01602

Preston, T., Baltzer, W., and Trost, S. (2012). Accelerometer validity and placement for detection of changes in physical activity in dogs under controlled conditions on a treadmill. Res. Vet. Sci. 93:412-416. doi: 10.1016/j.rvsc.2011.08.005

Prud'homme, G., Glinka, Y., and Wang, Q. (2013). GABA exerts anti-inflammatory and immunosuppressive effects. J. Immunol. 190(1 Supplement):68.15.

Purwana, I., Zheng, J., Li, X., Deurloo, M., Son, D.O., Zhang, Z., et al. (2014). GABA Promotes Human b-Cell Proliferation and Modulates Glucose Homeostasis. Diabetes. 63(12):4197-205. doi: 10.2337/db14-0153

Raj, H. and Poelarends, G.J. (2013). The roles of active site residues in the catalytic mechanism of methylaspartate ammonia-lyase. FEBS Open Bio. 3:285-290. doi: 10.1016/j.fob.2013.07.002

Rajesh, M., Mukhopadhyay, P., Batkai, S., Haskó, G., Liaudet, L., Drel, V.R., et al. (2007). Cannabidiol attenuates high glucose-induced endothelial cell inflammatory response and barrier disruption. Am. J. Physiol. Heart Circ. Physiol. 293(1):H610-9. doi: 10.1152/ajpheart.00236.2007

Rajesh, M., Mukhopadhyay, P., Batkai, S., Patel, V., Saito, K., Matsumoto, S., et al. (2010). Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. J. Am. Coll. Cardiol. 56(25):2115-25. doi: 10.1016/j.jacc.2010.07.033

Rakshan, F., Day, T.A., Blakely, R.D., and Barker, E.L. (2000). Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. J. Pharmacol. Exp. Therp. 292(3):960-7.

Randall, D.D. and Tolbert, N.E. (1971). 3-Phosphoglycerate phosphatase in plants. I. Isolation and characterization from sugarcane leaves. J. Biol. Chem. 246(17):5510-7. doi: 10.1016/S0021-9258(18)61935-0

Ranjith, N.K., Sasikala, C., and Ramana, C.V. (2007). Catabolism of L-phenylalanine and L-tyrosine by Rhodobacter sphaeroides OU5 occurs through 3,4-dihydroxyphenylalanine. Res. Microbiol. 158(6):506-11. doi: 10.1016/j.resmic.2007.04.008

Ray, P., Torck, A., Quigley, L., Wangzhou, A., Neiman, M., Rao, C., Lam, T., Kim, J-Y., Kim, T.H., Zhang, M.Q., Dussor, G., and Price, T.J. Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an RNA-seq-based resource for pain and sensory neuroscience research. Pain. 159(7):1325-1345. doi: 10.1097/j.pain.0000000000001217

Razavi, R., Chan, Y., Afifiyan, F.N., Liu, X.J., Wan, X., Yantha, J., et al. (2006). TRPV1+ sensory neurons control beta cell stress and islet inflammation in autoimmune diabetes. Cell. 127(6):1123-35. doi: 10.1016/j.cell.2006.10.03

Reddy, D.S. (2006). Physiological role of adrenal deoxycorticosterone-derived neuroactive steroids in stress-sensitive conditions. Neuroscience. 138(3):911-20. doi: 10.1016/j.neuroscience.2005.10.016

Reddy, D.S. and Rogawski, M.A. (2002). Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J. Neurosci. 22(9):3795-805. doi: 10.1523/JNEUROSCI.22-09-03795.2002

Ren, R., Hashimoto, T., Mizuno, M., Takigawa, H., Yoshida, M., Azuma, T., et al. (2013). A lipid peroxidation product 9-oxononanoic acid induces phospholipase A2 activity and thromboxane A2 production in human blood. J. Clin. Biochem. Nutr. 52(3):228-33. doi: 10.3164/jcbn.12-110

Richardson, D., Pearson, R.G., Kurian, N., Latif, M.L., Garle, M.J., Barrett, D.A., Kendall, D.A., Scammell, B.E., Reeve, A.J., and Chapman, V. (2008). Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. Arthritis Res. Ther. 10(2):R43. doi: 10.1186/ar2401

Rigo, L.U., Marechal, L.R., Vieira, M.M., and Veiga, L.A. (1985). Oxidative pathway for L-rhamnose degredation in Pullularia pullulans. Can. J. Microbiol. 31(9):817-822. doi: 10.1139/m85-153

Roberts, L.D., Souza, A.L., Gerszten, R.E., and Clish, C.B. (2012). Targeted metabolomics. Curr. Protoc. Mol. Biol. 98(1):30.2.1-30.2.24. doi: 10.1002/0471142727.mb3002s98

Robinson, E., Templeman, J.R., Thornton, E., Croney, C.C., Niel, L., and Shoveller, A.K. (2020). Investigating the effects of incremental conditioning and supplemental dietary tryptophan on the voluntary activity and behaviour of mid-distance training sled dogs. PLoS ONE. 15(8):e0232643. doi: 10.1371/journal.pone.0232643

Robinson, E., Thornton, E., Templeman, J.R., Croney, C.C., Niel, L., and Shoveller, A.K. (2021). Changes in Behaviour and Voluntary Physical Activity Exhibited by Sled Dogs throughout Incremental Exercise Conditioning and Intermittent Rest Days. Animals. 11(1):118. doi: 10.3390/ani11010118

Rock, E.M., Bolognini, D., Limebeer, C.L., Cascio, M.G., Anavi-Goffer, S., Fletcher, P.J., Mechoulam, R., Pertwee, R.G., and Parker, L.A. (2012). Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behavior via indirect agonism of 5-HT_{1A} somato-dendritic autoreceptors in the dorsal raphe nucleus. Br. J. Pharmacol. 165(8):2620-34. doi: 10.1111/j.1476-5381.2011.01621.x

Rock, E.M., Sullivan, M.T., Collins, S.A., Goodman, H., Limebeer, C.L., Mechoulam, R., and Parker, L.A. (2020). Evaluation of repeated or acute treatment with cannabidiol (CBD), cannabidiolic acid (CBDA) or CBDA methyl ester (HU-580) on nausea and/or vomiting in rats and shrews. Psychopharmacology (Berl). 237(9):2621-2631. doi: 10.1007/s00213-020-05559-z

Rockwell, C.E. and Kaminski, N.E. (2004). A cyclooxygenase metabolite of anandamide causes inhibition of interleukin-2 secretion in murine splenocytes. J. Pharmacol. Exp. Ther. 311(2):683-90. doi: 10.1124/jpet.104.065524

Romagnoli, G., Verhoeven, M.D., Mans, R., Rey, Y.F., Bel-Rhlid, R., van den Broek, M., et al. (2014). An alternative, arginase-independent pathway for arginine metabolism in Kluyveromyces lactis involves guanidinobutyrase as a key enzyme. Mol. Microbiol. 93(2):369-89. doi: 10.1111/mmi.12666

Rorsman, P., Berggren, P-O., Bokvist, K., Ericson, H., Mohler, H., Ostenson, C-G., et al. (1989). Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. Nature. 341:233-236. doi: 10.1038/341233a0

Rosado, B., González-Martínez, Á., Pesini, P., García-Belenguer, S., Palacio, J., Villegas, A., Suárez, M.-L. Santamarina, G., and Sarasa, M. (2012). Effect of age and severity of cognitive dysfunction on spontaneous activity in pet dogs – Part 1: Locomotor and exploratory behavior. Vet. J. 194:189-195. doi: 10.1016/j.tvjl.2012.03.025

Rosenberg, E.C., Tsien, R.W., Whalley, B.J., and Devinsky, O. (2015). Cannabinoids in epilepsy. Neurotherapeutics. 12:747-768. doi: 10.1007/s13311-015-0375-5

Rosenkrantz, H., Fleischman, R.W., and Grant, R.J. (1981). Toxicity of short-term administration of cannabinoids to rhesus monkeys. Toxicol. Appl. Pharamcol. 58(1):118-31. doi: 10.1016/0041-008x(81)90122-8

Rotzinger, S., Fang, J., and Baker, G.B. (1998). Trazodone is metabolized to m-chlorophenylpiperazine by CYP3A4 from human sources. Drug Metab. Dispos. 26(6):575-575.

Russo, E.B. (2007). History of cannabis and its preparations in saga, science, and sobriquet. Chem. Biodivers. 4(8):1614-48. doi: 10.1002/cbdv.200790144

Russo, E.B. (2016). Beyond cannabis: Plants and the endocannabinoid system. Trends Pharmacol. Sci. 37(7):594-605. doi: 10.1016/j.tips.2016.04.005

Russo, E.B., Burnett, A., Hall, B., and Parker, K.K. (2005). Agonistic properties of cannabidiol at 5-HT_{1a} receptors. Neurochem. Res. 30(8):1037-1043. doi: 10.1007/s11064-005-6978-1

Russo, E.B., Jiang, H.E., Li, X., Sutton, A., Carboni, A., del Bianco, F., Mandolino, G., Potter, D.J., Zhao, Y.X., Bera, S., Zhang, Y.B., Lü, E.G., Ferguson, D.K., Hueber, F., Zhao, L.C., Liu, C.J., Wang, Y.F., and Li, C.S. (2008). Phytochemical and genetic analyses of ancient cannabis from Central Asia. J. Exp. Bot. 59:4171–4182. doi:10.1093/jxb/ern260

Ryberg, E., Larsson, N., Sjögren, S., Hjorth, S., Hermansson, N-O., Leonova, J., Elebring, T., Nilsson, K., Drmota, T., and Greasley, P.J. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. Br. J. Pharmacol. 152(7):1092-101. doi: 10.1038/sj.bjp.0707460

Sahr, T., Ravanel, S., Basset, G., Nichols, B.P., Hanson, A.D., and Rebeille, F. (2006). Folate synthesis in plants: purification, kinetic properties, and inhibition of aminodeoxychorismate synthase. Biochem. J. 396:157-162. doi: 10.1042/BJ20051851

Salido, E., Pey, A.L., Rodriguez, R., and Lorenzo, V. (2012). Primary hyperoxalurias: Disorders of glyoxylate detoxification. Biochim. Biophys. Acta. 1822(9):1453-1464. doi: 10.1016/j.bbadis.2012.03.004

Salonen, J.H., Rimpilainen, M., Lehtonen, L., Lehtonen, O.P., and Nikoskelainen, J. (2001). Measurement of the D-arabinitol/L-arabinitol ratio in urine of neutropenic patients treated empirically with amphotericin B. Eur. J. Clin. Microbiol. Infect. Dis. 20(3):179-84. doi: 10.1007/s100960100472

Samara, E., Bialer, M., and Harvey, D.J. (1991). Metabolism of cannabidiol by the rat. Eur. J. Drug Metab. Pharmacokineti. 16(4):305-313. doi: 10.1007/BF03189976

Samara, E., Bialer, M., and Harvey, J. (1990a). Pharmacokinetics of urinary metabolites of cannabidiol in the dog. Biopharm. Drug Dispos. 11(9):785-795. doi: 10.1002/bdd.2510110906

Samara, E., Bialer, M., and Harvey, J. (1990b). Identification of urinary metabolites of cannabidiol in the dog. Drug Metab. Dispos. 18(5):571-579.

Samara, E., Bialer, M., and Mechoulam, R. (1988). Pharmacokinetics of cannabidiol in dogs. Drug Metab. Dispos. 16(3):469-472.

Sanford, A.E., Castillo, E., and Gannon, R.L. (2008). Cannabinoids and hamster circadian activity rhythms. Brain Res. 1222:141-8. doi: 10.1016/j.brainres.2008.05.048

Santos, P.M.P. and Vieira, A.J.S.C. (2013). Antioxidising activity of cinnamic acid derivatives against oxidative stress induced by oxidising radicals. J. Phys. Org. Chem. 26:432-439. doi: 10.1002/poc.3104

Saracyn, M., Zdanowski, R., Brytan, M., Kade, G., Nowak, Z., Patera, J., Dyrla, P., Gil, J., and Wankowicz, Z. (2015). D-galactosamine intoxication in experimental animals: Is it only an experimental model of acute liver failure? Med. Sci. Monit. 21:1469-77. doi: 10.12659/MSM.893291

Sass, J.O., Fischer, K., Wang, R., Christensen, E., Scholl-Burgi, S., Chang, R., et al. (2010). D-glyceric aciduria is caused by genetic deficiency of D-glycerate kinase (GLYCTK). Hum. Mutat. 31(12):1280-5. doi: 10.1002/humu.21375

Sato, F., Inui, T., and Takemura, T. (2007). Metabolic Engineering in Isoquinoline Alkaloid Biosynthesis. Curr. Pharm. Biotechnol. 8(4):211-218. doi: 10.2174/138920107781387438

Schmid, K., Schonlebe, J., Drexler, H., and Mueck-Weymann, M. (2010). The effects of cannabis on heart rate variability and well-being in young men. Pharmacopsychiatry. 43(4):147-150. doi: 10.1055/s-0030-1248314

Schneider, K., Kienow, L., Schmelzer, E., Colby, T., Bartsch, M., Miersch, O., et al. (2005). A new type of peroxisomal acyl-coenzyme A synthetase from Arabidopsis thaliana has the catalytic capacity to activate biosynthetic precursors of jasmonic acid. J. Biol. Chem. 280(14):13962-72. doi: 10.1074/jbc.M413578200

Scholem, R.D. and Brown, G.K. (1983). Metabolism of malonic semialdehyde in man. Biochem. J. 216(1):81-5. doi: 10.1042/bj2160081

Schroeder, F., McIntosh, A.L., Martin, G.G., Huang, H., Landrock, D., Chung, S., et al. (2016). Fatty acid binding protein-1 (FABP1) and the human FABP1 T94A

variant: Roles in the endocannabinoid system and dyslipidemias. Lipids. 51(6):655-76. doi: 10.1007/s11745-016-4155-8

Schuelert, N. and McDougall, J.J. (2008). Cannabinoid-mediated antinociception is enhanced in rat osteoarthritis knees. Arthritis Rheum. 58(1):145-53. doi: 10.1002/art.23156

Sethi, S., Brietzke, E. (2015). Omics-based biomarkers: application of metabolomics in neuropsychiatric disorders. Int. J. Neuropsychopharmacol. 19(3):1-13. doi: 10.1093/ijnp/pyv096.

Shaffer, F. and Ginsberg, J.P. (2017). An overview of heart rate variability metrics and norms. Front. Public Health. 5:258. doi: 10.3389/fpubh.2017.00258

Shaffer, F., McCraty, R., and Zerr, C.L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Front. Psychol. 5:1040. doi: 10.3389/fpsyg.2014.01040

Shannon, S., Lewis, N., Lee, H., and Hughes, S. (2019). Cannabidiol in anxiety and sleep: A large case series. Perm. J. 23:18-041. doi: 10.7812/TPP/18-041

Sharma, M., Hudson, J.B., Adomat, H., Guns, E. and Cox, M.E. (2014). *In vitro* anticancer activity of plant-derived cannabidiol on prostate cancer cell lines. Pharmacol. Pharm. 5(8):806-820. doi: 10.4236/pp.2014.58091

Shaw, W., Kassen, E., and Chaves, E. (1995). Increased urinary excretion of analogs of Krebs cycle metabolites and arabinose in two brothers with autistic features. Clin. Chem. 41(8 Pt 1):1094-104.

Sheppard, G. and Mills, D.S. (2003). Evaluation of dog-appeasing pheromone as a potential treatment for dogs fearful of fireworks. Vet. Rec. 152:432-436. doi: 10.1136/vr.152.14.432

Sherman, B.L. and Mills, D.S. (2008). Canine anxieties and phobias: An update on separation anxiety and noise aversions. Vet. Clin. Small Anim. 38:1081-1106. doi: 10.1016/j.cvsm.2008.04.012

Shikita, M., Inano, H., and Tamaoki, B. (1967). Further studies on 20-alpha-hydroxysteroid dehydrogenase of rat testes. Biochemistry. 6(6):1760-4. doi: 10.1021/bi00858a026

Shrivastava, A., Kuzontkoski, P.M., Groopman, J.E., and Prasad, A. (2011). Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. Mol. Cancer Ther. 10(7):1161-1172. doi: 10.1158/1535-7163.MCT-10-1100

Siegel, R.L., Miller, K.D., and Jemal, A. (2020). Cancer statistics, 2020. CA Cancer J. Clin. 70(1):7-30. doi: 10.3322/caac.21590

Silva, G.D., Del Guerra, F.B., de Oliveira Lelis, M., and Pinto, L.F. (2020). Cannabidiol in the treatment of epilepsy: A focused review of evidence and gaps. Front. Neurol. 11:531939. doi: 10.3389/fneur.2020.531939

Silver, R.J. (2019). The endocannabinoid system of animals. Animals. 9(9):686. doi: 10.3390/ani9090686

Silvestri, C. and Di Marzo, V. (2013). The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. Cell Metab. 17(4):475-90. doi: 10.1016/j.cmet.2013.03.001

Silvestri, C., Paris, D., Martella, A., Melck, D., Guadagnino, I., Cawthorne, M., et al. (2015). Two non-psychoactive cannabinoids reduce intracellular lipid levels and inhibit hepatosteatosis. J. Hepatol. 62(6):1382-90. doi: 10.1016/j.jhep.2015.01.001

Silvestro, S., Mammana, S., Cavalli, E., Bramanti, P., and Mazzon, E. (2019). Use of cannabidiol in the treatment of epilepsy: Efficacy and security in clinical trials. Molecules. 24(8):1459. doi: 10.3390/molecules24081459

Siwak, C.T., Tapp, P.D., and Milgram, N.W. (2001). Effect of age and level of cognitive function on spontaneous and exploratory behaviors in the Beagle dog. Learn. Mem. 8(6):317-25. doi: 10.1101/lm.41701

Skrivan, M., Englmaierova, M., Taubner, T., and Skrivanova, E. (2020). Effects of dietary hemp seed and flaxseed on growth performance, meat fatty acid compositions, liver tocopherol concentration and bone strength of cockerels. Animals. 10:458. doi: 10.3390/ani10030458

Smith, G.K., Duch, D.S., Edelstein, M.P., and Bigham, E.C. (1992). New inhibitors of sepiapterin reductase. Lack of an effect of intracellular tetrahydrobiopterin depletion upon in vitro proliferation of two human cell lines. J. Biol. Chem. 267(8):5599-5607.

Smith, L.A., Azariah, F., Lavender, V.T.C., Stoner, N.S., and Bettiol, S. (2015). Cannabinoids for nausea and vomiting in adults with cancer receiving chemotherapy. Cochrane Database Syst. Rev. 2015(11):CD009464. doi: 10.1002/14651858.CD009464.pub2

Smith, N., Longo, N., Levert, K., Hyland, K., and Blau, N. (2019). Phase I clinical evaluation of CNSA-001 (sepiapterin), a novel pharmacological treatment for phenylketonuria and tetrahydrobiopterin deficiencies, in healthy volunteers. Mol. Genet. Metab. 126(4):406-412. doi: 10.1016/j.ymgme.2019.02.001

Solinas, M., Massi, P., Cantelmo, A.R., Cattaneo, M.G., Cammarota, R., Bartolini, D., Cinquina, V., Valenti, M., Vicentini, L.M., Noonan, D.M., Albini, A. and Parolaro, D. (2012). Cannabidiol inhibits angiogenesis by multiple mechanisms. Br. J. Pharmacol. 167(6):1218-31. doi: 10.1111/j.1476-5381.2012.02050.x

Solinas, M., Massi, P., Cinquina, V., Valenti, M., Bolognini, D., Gariboldi, M., Monti, E. Rubino, T., and Parolaro, D. (2013). Cannabidiol, a non-psychoactive cannabinoid compound, inhibits proliferation and invasion in U87-MG and T98G glioma cells through a multitarget effect. PLoS One. 8(10):e76918. doi: 10.1371/journal.pone.0076918

Song, S. (2000). Can the glyoxylate pathway contribute to fat-induced hepatic insulin resistance? Med. Hypotheses. 54(5):739-747. doi: 10.1054/mehy.1999.0943

Spindle, T.R., Cone, E.J., Goffi, E., Weerts, E.M., Mitchell, J.M., Winecker, R.E., Bigelow, G.E., Flegel, R.R., and Vandrey, R. (2020). Pharmacodynamic effects of vaporized and oral cannabidiol (CBD) and vaporized CBD-dominant cannabis in infrequent cannabis users. Drug Alcohol Depend. 211:107937. doi: 10.1016/j.drugalcdep.2020.107937

Sreevalsan, S., Joseph, S., Jutooru, I., Chadalapaka, G., and Safe, S.H. (2011). Induction of apoptosis by cannabinoids in prostate and colon cancer cells is phosphatase dependent. Anticancer Res. 31(11):3799-3807.

Srivastava, M.D., Srivastava, B.I., and Brouhard, B. (1998). Δ9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. Immunopharmacol. 40(3):179-185. doi: 10.1016/s0162-3109(98)00041-1

Stok, J.E., Huang, H., Jones, P.D., Wheelock, C.E. Morisseau, C., and Hammock, B.D. (2004). Identification, expression, and purification of a pyrethroid-hydrolyzing carboxylesterase from mouse liver microsomes. J. Biol. Chem. 279(28):29863-9. doi: 10.1074/jbc.M403673200

Stout, J.M., Boubakir, Z., Ambrose, S.J., Purves, R.W., and Page, J.E. (2012). The hexanoyl-CoA precursor for cannabinoid biosynthesis is formed by an acyl-activating enzyme in *Cannabis sativa* trichomes. Plant J. 71:353-365. doi: 10.1111/j.1365-313X.2012.04949.x

Strand, L.P. and Scheline, R.R. (1975). The metabolism of vanillin and isovanillin in the rat. Xenobiotica. 5(1):49-63. doi: 10.3109/00498257509056093

Sun, S., Hu, F., Wu, J., and Zhang, S. (2017). Cannabidiol attenuates OGD/R-induced damage by enhancing mitochondrial bioenergetics and modulating glucose metabolism via pentose-phosphate pathway in hippocampal neurons. Redox Biol. 11:577-585. doi: 10.1016/j.redox.2016.12.029

Sun, Q., Weinger, J.G., Mao, F., and Liu, G. (2016). Regulation of structural and functional synapse density by L-threonate through modulation of intraneuronal magnesium concentration. Neuropharmacology. 108:426-39. doi: 10.1016/j.neuropharm.2016.05.006

Sunda, F. and Arowolo, A. (2020). A molecular basis for the anti-inflammatory and anti-fibrosis properties of cannabidiol. FASEB J. 34(11):14083-14092. doi: 10.1096/fj.202000975R

Swaminathan, A., Lucas, R.M., Dear, K., and McMichael, A.J. (2014). Keyhole limpet haemocyanin – a model antigen for human immunotoxicological studies. Brit. J. Clin. Pharmacol. 78(5):1135-1142. doi: 10.1111/bcp.12422

Tambaro, S. and Bortolato, M. (2012). Cannabinoid-related agents in the treatment of anxiety disorders: current knowledge and future perspectives. Recent Pat. CND Drug Discov. 7(1):25-40. doi: 10.2174/157488912798842269

Tarpey, M.M. (2002). Sepiapterin treatment in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 22(10):1519-21. doi: 10.1161/01.atv.0000038144.37823.bf

Taura, F., Sirikantaramas, S., Shoyama, Y., Yoshikai, K., Shoyama, Y., and Morimoto, S. (2007). Cannabidolic-acid synthase, the chemotype-determining enzyme in the fiber-type *Cannabis sativa*. FEBS Lett. 581(16):2929-2934. doi: 10.1016/j.febslet.2007.05.043

Thayer, J.F., Yamamoto, S.S., and Brosschot, J.F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int. J. Cardiol. 141(2):122-131. doi: 10.1016/j.ijcard.2009.09.543

Theodore, W.H. (2003). Does serotonin play a role in epilepsy? Epilepsy Curr. 3(5):173-177. doi: 10.1046/j.1535-7597.2003.03508.x

Thiele, I., Swainston, N., Fleming, R.M.T., Hoppe, A., Sahoo, S., Aurich, M.K., et al., (2013). A community-driven global reconstruction of human metabolism. Nat. Biotechnol. 31(5):419-25. doi: 10.1038/nbt.2488

Tiira, K., Sulkama, S., and Lohi, H. (2016). Prevalence, comorbidity, and behavioral variation in canine anxiety. J. Vet. Behav. 16:36-44. doi: 10.1016/j.jveb.2016.06.008

Tiranti, V., D'Adamo, P., Briem, E., Ferrari, G., Mineri, R., Lamantea, E., et al. (2004). Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. Am. J. Hum. Genet. 74(2):239-52. doi: 10.1086/381653

Tobie, C., Péron, F., and Larose, C. (2015). Assessing food preferences in dogs and cats: A review of the current methods. Animals. 5(1):126-137. doi: 10.3390/ani5010126

Thomas, A., Baillie, G.L., Phillips, A.M., Razdan, R.K., Ross, R.A., and Pertwee, R.G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br. J. Pharmacol. 150(5):613-23. doi: 10.1038/sj.bjp.0707133

Torres, S., Lorente, M., Rodriguez-Fornes, F., Hernandez-Tiedra, S., Salazar, M., Garcia-Taboada, E., Barcia, J., Guzman, M., and Velasco, G. (2011). A combined preclinical therapy of cannabinoids and termozolomide against glioma. Mol. Cancer Ther. 10(1):90-103. doi: 10.1158/1535-7163.MCT-10-0688

Tóth, K.F., Adam, D., Biro, T., and Olah, A. (2019). Cannabinoid Signaling in the Skin: Therapeutic Potential of the "C(ut)annabinoid" System. Molecules. 24:918. doi: 10.3390/molecules24050918

Trevaskis, N.L., Shackleford, D.M., Charman, W.N., Edwards, G.A., Gardin, A., Appel-Dingemanse, S., et al. (2009). Intestinal lymphatic transport enhances the post-prandial oral bioavailability of a novel cannabinoid receptor agonist via avoidance of first-pass metabolism. Pharm. Res. 26(6):1486-1495. doi: 10.1007/s11095-009-9860-z

Ueda, N., Puffenbarger, R.A., Yamamoto, S., and Deutsch, D.G. (2000). Chem. Phys. Lipids. 108(1-2):107-21. doi: 10.1016/s0009-3084(00)00190-0

USDA. (2000). Industrial Hemp in the United States: Status and Market Potential. Washington DC.

Vaia, M., Petrosino, S., De Filippis, D., Negro, L., Guarino, A., Carnuccio, R., et al. (2016). Palmitoylethanolamide reduces inflammation and itch in a mouse model of contact allergic dermatitis. Eur. J. Pharmacol. 791:669-674. doi: 10.1016/j.ejphar.2016.10.005

van de Donk, T., Niesters, M., Kowal, M.A., Olofsen, E., Dahan, A., and van Velzen M. (2018). An experimental randomized study on the analgesic effects of pharmaceutical-grade cannabis in chronic pain patients with fibromyalgia. Pain. 160(4):860-869. doi: 10.1097/j.pain.0000000000001464

Vanhooren, P.T. and Vandamme, E.J. (1999). L-fucose: Occurrence, physiological role, chemical, enzymatic and microbial synthesis. J. Chem. Technol. Biotechnol. 74(6):479-497. doi: 10.1002/(SICI)1097-4660(199906)74:6<479::AID-JCTB76>3.0.CO;2-E

Vásquez-Vivar, J., Duquaine, D., Whitsett, J., Kalyanaraman, B., and Rajagopalan, S. (2002). Altered tetrahydrobiopterin metabolism in atherosclerosis: implications for use of oxidized tetrahydrobiopterin analogues and thiol antioxidants. Arterioscler. Thromb. Vasc. Biol. 22(10):1655-61. doi: 10.1161/01.atv.0000029122.79665.d9

Vaughn, D., Kulpa, J., and Paulionis, L. (2020). Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. Front. Vet. Sci. 7:51. doi: 10.3389/fvets.2020.00051

Vaughn, L.K., Denning, G., Stuhr, K.L., de Wit, H., Hill, M.N., and Hillard, C.J. (2010). Endocannabinoid signalling: has it got rhythm? Br. J. Pharmacol. 160(3):530-43. doi: 10.1111/j.1476-5381.2010.00790.x

Vella, G.J., Hill, R.E., Mootoo, B.S., and Spenser, I.D. (1980). The status of glycolaldehyde in the biosynthesis of vitamin B6. J. Biol. Chem. 255(7):3042-3048.

Verhoeven, N.M., Huck, J.H., Roos, B., Struys, E.A., Salomons, G.S., Douwes, A.C., van der Knaap, M.S., and Jakobs, C. (2001). Transaldolase deficiency: Liver cirrhosis associated with a new inborn error in the pentose phosphate pathway. Am. J. Hum. Genet. 68(5):1086-92. doi: 10.1086/320108

Verrico, C.D., Wesson, S., Konduri, V., Hofferek, C.J., Vazquez-Perez, J., Blair, E., Dunner, K. Jr., Salimpour, P., Decker, W.K., and Halpert, M.M. (2020). A randomized, double-blinded, placebo-controlled study of daily cannabidiol for the treatment of canine osteoarthritis pain. Pain. 161(9):2191-2202. doi: 10.1097/j.pain.0000000000001896

Vertuani, S., Angusti, A., and Manfredini, S. (2004). The antioxidants and pro-antioxidants network: an overview. Curr. Pharm. Des. 10(14):1677-94. doi: 10.2174/1381612043384655

Visse, K., Blome, C., Phan, N.Q., Augustin, M., and Ständer, S.(2017). Efficacy of Body Lotion Containing N-palmitoylethanolamine in Subjects with Chronic Pruritus due to Dry Skin: A Dermatocosmetic Study. Acta. Derm. Venereol. 97(5):639-641. doi: 10.2340/00015555-2593

Visser, W.F., Verhoeven-Duif, N.M., and de Koning, T.J. (2012). Identification of a human trans-3-hydroxy-L-proline dehydratase, the first characterized member of a novel family of proline racemase-like enzymes. J. Biol. Chem. 287(26):21654-62. doi: 10.1074/jbc.M112.363218

Vogt, N.A., Sargeant, J.M., Stevens, C.P.G., and Dunn, J.N. (2019). A survey of

veterinary student attitudes concerning whether marijuana could have therapeutic value for animals. PLoS One. 14(7):e0219430. doi: 10.1371/journal.pone.0219430

Vogt, T. (2010). Phenylpropanoid biosynthesis. Mol. Plant. 3(1):2-20. doi: 10.1093/mp/ssp106

Vučković, S., Srebro, D., Vujovic, K.S., Vucetic, C., and Prostran, M. (2018). Cannabinoids and pain: New insights from old molecules. Front. Pharmacol. 9:1259. doi: 10.3389/fphar.2018.01259

Wakshlag, J.J., Schwark, W.S., Deabold, K.A., Talsma, B.N., Cital, S., Lyubimov, A., Iqbal, A., and Zakharov, A. (2020). Pharmacokinetics of cannabidiol, cannabidiolic acid, Δ9-tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. Front. Vet. Sci. 7:505. doi: 10.3389/fvets.2020.00505

Wallace, J.E., Kogan, L.R., Carr, E.C.J., and Hellyer, P.W. (2020). Motivations and expectations for using cannabis products to treat pain in humans and dogs: a mixed methods study. J. Cannabis Res. 2(1):36. doi: 10.1186/s42238-020-00045-x

Wan, Y., Wang, Q., and Prud'homme, G.J. (2015). GABAergic system in the endocrine pancreas: A new target for diabetes treatment. Diabetes Metab. Syndr. Obes. 8:79-87. doi: 10.2147/DMSO.S50642

Wang, F. and Multhoff, G. (2021). Repurposing cannabidiol as a potential drug candidate for anti-tumor therapies. Biomolecules. 11(4):582. doi: 10.3390/biom11040582

Wang, H., Hu, P., and Jiang, J. (2011). Pharmacokinetics and safety of calcium L-threonate in healthy volunteers after single and multiple oral administrations. Acta. Pharmacol. Sin. 32(12):1555-60. doi: 10.1038/aps.2011.138

Wang, H., Yang, B., Hao, G., Feng, Y., Chen, H., Feng, L., et al. (2011). Biochemical characterization of the tetrahydrobiopterin synthesis pathway in the oleaginous fungus Mortierella alpine. Microbiology (Reading). 157(Pt 11):3059-3070. doi: 10.1099/mic.0.051847-0

Wang, J., Hodes, G.E., Zhang, H., Zhang, S., Zhao, W., Golden, S.A., et al. (2018). Epigenetic modulation of inflammation and synaptic plasticity promotes resilience against stress in mice. Nat. Commun. 9(1):477. doi: 10.1038/s41467-017-02794-5

Wang, W., Baker, P., and Seah, S.Y.K. (2010). Comparison of Two Metal-Dependent

Pyruvate Aldolases Related by Convergent Evolution: Substrate Specificity, Kinetic Mechanism, and Substrate Channeling. Biochem. 49(17):3774-3782. doi: 10.1021/bi100251u

Wang, X., Zhao, X., Chou, J., Yu, J., Yang, T., Liu, L., et al. (2018). Taurine, glutamic acid and ethylmalonic acid as important metabolites for detecting human breast cancer based on the targeted metabolomics. Cancer Biomark. 23(2):255-268. doi: 10.3233/CBM-181500

Watanabe, S., Morimoto, D., Fukumori, F., Shinomiya, H., Nishiwaki, H., Kawano-Kawada, M., et al. (2012). Identification and characterization of D-hydroxyproline dehydrogenase and Delta1-pyrroline-4-hydroxy-2-carboxylate deaminase involved in novel L-hydroxyproline metabolism of bacteria: metabolic convergent evolution. J. Biol. Chem. 287(39):32674-88. doi: 10.1074/jbc.M112.374272

Watanabe, S., Shimada, N., Tajima, K., Kodaki, T., and Makino, K. (2006). Identification and characterization of L-arabonate dehydratase, L-2-keto-3-deoxyarabonate dehydratase, and L-arabinolactonase involved in an alternative pathway of L-arabinose metabolism. Novel evolutionary insight into sugar metabolism. J. Biol. Chem. 281(44):33521-33536. doi: 10.1074/jbc.M606727200

Watanabe, S., Tajima, K., Fujii, S., Fukumori, F., Hara, R., Fukuda, R., et al. (2016). Functional characterization of aconitase X as a cis-3-hydroxy-L-proline dehydratase. Sci. Rep. 6:38720. doi: 10.1038/srep38720

Wegkamp, A., van Oorschot, W., de Vos, W.M., and Smid, E.J. (2007). Characterization of the Role of para-Aminobenzoic Acid Biosynthesis in Folate Production by Lactococcus lactis. Appl. Environ. Microbiol. 73(8):2673-2681. doi: 10.1128/AEM.02174-06

Welling, P.G. (1996). Effects of food on drug absorption. Annu. Rev. Nutr. 16:383-415. doi: 10.1146/annurev.nu.16.070196.002123

Weltha, L., Reemmer, J., and Boison, D. (2019). The role of adenosine in epilepsy. Brain Res. Bull. 151:46-54. doi: 10.1016/j.brainresbull.2018.11.008

Wen, L., Zang, L., Huang, K., Li, S., Wang, R., and Wang, P.G. (2016). Efficient enzymatic synthesis of L-rhamnulose and L-fuculose. Bioorg. Med. Chem. Lett. 26(3):969-972. doi: 10.1016/j.bmcl.2015.12.051

Wernimont, S.M., Thompson, R.J., Mickelsen, S.L., Smith, S.C., Alvarenga, I.C., and Gross, K.L. (2018). Use of accelerometer activity monitors to detect changes in pruritic behaviors: Interim clinical data on 6 dogs. Sensors. 18:249. doi: 10.3390/s18010249

White, M.R. and Garcin, E.D. (2017). D-Glyceraldehyde-3-Phosphate Dehydrogenase Structure and Function. Subcell. Biochem. 83:413-453. doi: 10.1007/978-3-319-46503-6_15

Whitling, P.F., Wolff, R.F., Deshpande, S., Di Nisio, M., Duffy, S., Hernandez, A.V., et al. (2015). Cannabinoids for medical use: A systematic review and meta-analysis. JAMA. 313(24):2456-73. doi: 10.1001/jama.2015.6358

Wolfe L, Jethva R, Oglesbee D, and Vockley J. (2011). Short-Chain Acyl-CoA Dehydrogenase Deficiency. In: GeneReviews, eds. M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, G. Mirzaa, and A. Amemiya. (Seattle, WA: University of Washington, Seattle), 1993–2021.

Wormald, D., Lawrence, A.J., Carter, G., and Fisher, A.D. (2017). Reduced heart rate variability in pet dogs affected by anxiety-related behaviour problems. Phys. Behav. 168:122-127. doi: 10.1016/j.physbeh.2016.11.003

Wright, K.L., Duncan, M., and Sharkey, K.A. (2008). Cannabinoid CB_2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. Br. J. Pharmacol. 153(2): 263–270. doi: 10.1038/sj.bjp.0707486

Wu, Y., and Li, L. (2012). Determination of total concentration of chemically labeled metabolites as a means of metabolome sample normalization and sample loading optimization in mass spectrometry-based metabolomics. Anal. Chem. 84:10723–10731. doi: 10.1021/ac3025625

Wu, Y-H., Hu, S-Q., Liu, J., Cao, H-C., Xu, W., Li, Y-J., and Li, L-J. (2014). Nature and mechanisms of hepatocyte apoptosis induced by D-glalactosamine/lipopolysaccharide challenge in mice. Int. J. Mol. Med. 33(6):1498-506. doi: 10.3892/ijmm.2014.1730

Xia, J., Broadhurst, D., Wilson, M., and Wishart, D. (2013). Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics. 9(2):280-299. doi: 10.1007/s11306-012-0482-9

Xie, Z. and Hu, H. (2018). TRP channels as drug targets to relieve itch. Pharmacauticals. 11(4):100. doi: 10.3390/ph11040100

Yam, P.S., Penpranze, V., Young, D., Todd, M.S., Cloney, A.D., Houston-Callaghan, K.A., and Reilly, J.J. (2011). Validity, practical utility and reliability of Actigraph accelerometry for the measurement of habitual physical activity in dogs. J. Small Anim. Pract. 52(2):86-91. doi: 10.1111/j.1748-5827.2010.01025.x

Yamaori, S., Ebisawa, J., Okushima, Y., Yamamoto, I., and Watanabe, K. (2011). Potent

inhibition of human cytochrome P450 3A isoforms by cannabidiol: role of phenolic hydroxyl groups in the resorcinol moiety. Life Sci. 88(15-16):730-736. doi: 10.1016/j.lfs.2011.02.017

Yang, G., Ge, S., Singh, R., Basu, S., Shatzer, K., Zen, M., et al. (2017). Glucuronidation: driving factors and their impact on glucuronide disposition. Drug Metab. Rev. 49(2):105-138. doi: 10.1080/03602532.2017.1293682

Yang, K-H., Galadari, S., Isaev, D., Petroianu, G., Shippenberg, T.S., and Oz, M. (2010). The nonpsychoactive cannabinoid cannabidiol inhibits 5-hydroxytryptamine3A receptor-mediated currents in Xenopus laevis oocytes. J. Pharmacol. Exp. Ther. 333(2):547-54. doi: 10.1124/jpet.109.162594

Yang Y., Dong, G., Wang, Z., Wang, J., Zhang, Z., and Liu, J. (2018). Rumen and plasma metabolomics profiling by UHPLC-QTOF/MS revealed metabolic alterations associated with a high-corn diet in beef steers. PLoS ONE. 13(11):e0208031. doi: 10.1371/journal.pone.0208031

Yassin, M., Oron, A. and Robinson, D. (2017). Effect of Adding Medical Cannabis Treatment (MCT) to Analgesic Treatment in Patients with Low Back Pain related to Fibromyalgia: An Observational Cross-over Single Center Study. Clin. Exp. Rheumatol. 116(1):13-20. doi: 10.21767/2471-982X.100016

Yazaki, K., Fukui, H., and Tabata, M. (1987). Dihydroshikonofuran, an unusual metabolite of quinone biosynthesis in Lithospermum cell cultures. Chem. Pharm. Bull. 35(2):898-901. doi: 10.1248/cpb.35.898

Yemis, G.P., Pagotto, F., Bach, S., and Delaquis, P. (2011). Effect of Vanillin, Ethyl Vanillin, and Vanillic Acid on the Growth and Heat Resistance of Cronobacter Species. J. Food Prot. 74(12):2062-2069. doi: 10.4315/0362-028X.JFP-11-230

Yokochi, N., Yoshikane, Y., Matsumoto, S., Fujisawa, M., Ohnishi, K., and Yagi, T. (2009). Gene identification and characterization of 5-formyl-3-hydroxy-2-methylpyridine 4-carboxylic acid 5-dehydrogenase, an NAD+-dependent dismutase. J. Biochem. 145(4): 493-503. doi: 10.1093/jb/mvp007

Yorifuji, T., Koike, K., Sakurai, T., and Yokoyama, K. (1986). 4-Aminobutyraldehyde and 4-Guanidinobutyraldehyde Dehydrogenases for Arginine Degradation in Pseudomonas putida. Agr. Biol. Chem. 50(8):2009-2016. doi: 10.1271/bbb1961.50.2009

Yoshida, K-I., Yamaguchi, M., Morinaga, T., Kinehara, M., Ikeuchi, M., Ashida, H., and Fujita, Y. (2008). myo-Inositol catabolism in Bacillus subtilis. J. Biol. Chem. 283(16):10415-24. doi: 10.1074/jbc.M708043200

Yuan, C., Wang, X-M., Guichard, A., Tan, Y-M., Qian, C-Y., Yang, L-J., and Humbert,

P. (2014). N-palmitoylethanolamine and N-acetylethanolamine are effective in asteatotic eczema: results of a randomized, double-blind, controlled study in 60 patients. Clin. Interv. Aging. 9:1163-1169. doi: 10.2147/CIA.S65448

Zarzycki, J., Brecht, V., Muller, M., and Fuchs, G. (2009). Identifying the missing steps of the autotrophic 3-hydroxypropionate CO2 fixation cycle in Chloroflexus aurantiacus. Proc. Natl. Acad. Sci. USA. 106(50):21317-22. doi: 10.1073/pnas.0908356106

Zendulka, O., Dovrtelova, G., Noskova, K., Turjap, M., Sulcova, A., Hanus, L., et al. (2016). Cannabinoids and cytochrome P450 interactions. Curr. Drug Metab. 17(3). doi: 10.2174/1389200217666151210142051

Zgair, A., Lee, J.B., Wong, J.C.M., Taha, D.A., Aram, J., DiVirgilio, D., McArthur, J.W., Cheng, Y-K., Hennig, I.M., Barrett, D.A., Fischer, P.M., Constantinescu, C.S., and Gershkovich, P. (2017). Oral administration of cannabis with lipids leads to high levels of cannabinoids in the intestinal lymphatic system and prominent immunomodulation. Sci. Rep. 7:14542. doi: 10.1038/s41598-017-15026-z

Zgair, A., Wong, J.C.M., Lee, J.B., Mistry, J., Sivak, O., Wasan, K.M., Henning, I.M., Barrett, D.A., Constantinescu, C.S., Fischer, P.M., and Gershkovich, P. (2016). Dietary fats and pharmaceutical lipid excipients increase systemic exposure to orally administered cannabis and cannabis-based medicines. Am. J. Transl. Res. 8(8):3448-3459.

Zhang, J-M. and An, J. (2007). Cytokines, inflammation, and pain. Int. Anesthesiol. Clin. 45(2):27-37. doi: 10.1097/AIA.0b013e318034194e

Zhang, Q., Chen, X., Hongyan, G., Trindade, L.M., Salentijn, E.M.J., Guo, R., Guo, M., Xu, Y., and Yang, M. (2018). Latitudinal adaptation and genetic insights into the origins of *Cannabis sativa* L. Front. Plant Sci. 9:1876. doi: 10.3389/fpls.2018.01876

Zhang, X., Qin, Y., Pan, Z., Li, M., Liu, X., Chen, X., Qu, G., Zhou, L., Xu, M., Zheng, Q., and Li, D. (2019). Cannabidiol induces cell cycle arrest and cell apoptosis in human gastric cancer SGC-7901 cells. Biomolecules. 9(8):302. doi: 10.3390/biom9080302

Zhao, S., Dawe, M., Guo, K., and Li, L. (2017). Development of high-performance chemical isotope labeling LC-MS for profiling the carbonyl metabolome. Anal. Chem. 89(12):6758-6765. doi: 10.1021/acs.analchem.7b01098

Zhao, S., Li, H., Han, W., Chan, W., and Li, L. (2019). Metabolomic coverage of chemical-group-submetabolome analysis: Group classification and four-channel chemical isotope labeling LC-MS. Anal. Chem. 91(18):12108-12115. doi: 10.1021/acs.analchem.9b03431

Zhao, S., Luo, X., and Li, L. (2016). Chemical isotope labeling LC-MS for high coverage and quantitative profiling of the hydroxyl submetabolome in metabolomics. Anal. Chem. 88(21):10617-10623. doi: 10.1021/acs.analchem.6b02967

Zhao, Z. and Moghadasian, M.H. (2008). Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. Food Chem. 109(4):691-702. doi:10.1016/j.foodchem.2008.02.039

Zhu, G., Yin, C., Tian, Z., Wang, T., Sun, W., Xiang, Q., and Guo, G. (2015). Metabolomic analysis of plasma from patients with acute mountain sickness using chromatography-mass spectrometry. Medicine. 94(45):e1988. doi: 10.1097/MD.0000000000001988

Zimniewska, M., Rozańska, W., Gryszczynska, A., Romanowska, B., and Kicinska-Jakuowska, A. (2018). Antioxidant Potential of Hemp and Flax Fibers Depending on Their Chemical Composition. Molecules. 23(8):1993. doi: 10.3390/molecules23081993

Zou, Z., Tao, T., Li, H., and Zhu, X. (2020). mTOR signaling pathway and mTOR inhibitors in cancer: Progress and challenges. Cell Biosci. 10:31. doi: 10.1186/s13578-020-00396-1

Zorzenon, M.R.T., Santiago, A.N., Mori, M.A., Piovan, S., Jansen, C.A., Padilha, M.E.P., et al. (2019). Cannabidiol improves metabolic dysfunction in middle-aged diabetic rats submitted to a chronic cerebral hypoperfusion. Chem. Biol. Interact. 312:108819. doi: 10.1016/j.cbi.2019.108819

Zuardi, A.W., Crippa, J.A.S., Hallak, J.E.C., Pinto, J.P., Chagas, M.H.N., Rodrigues, G.G.R., et al. (2009). Cannabidiol for the treatment of psychosis in Parkinson's disease. J. Psychopharmacol. 23(8):979-83. doi: 10.1177/0269881108096519

Zuardi, A.W., Guimarães, F.S., and Moreira, A.C. (1993). Effect of cannabidiol on plasma prolactin, growth hormone and cortisol in human volunteers. Braz. J. Med. Biol. Res. 26(2):213-217.

VITA

ELIZABETH MILLER MORRIS

EDUCATION:

Sam Houston State University (SHSU), Huntsville, TX
- Master of Science, Agriculture; emphasis in Equine Nutrition
- May 2017
- Advisor, Marcy Beverly, Ph.D.
- Thesis: The effect of CLA supplementation on fat deposition and lean muscle mass in horses

Truman State University (TSU), Kirksville, MO
- Bachelor of Science, Biology; Summa Cum Laude
- May 2015

PROFESSIONAL EXPERIENCE:

Scientist, Hill's Pet Nutrition, Global Clinical Nutrition and Claims
- 2021 – present

Graduate Research Assistant, University of Kentucky, Department of Animal and Food Sciences
- 2018-2021

Elementary Coordinator, Center Point Church
- 2020-2021

Specialist – Veterinary Technical Solutions, Boehringer Ingelheim
- 2017-2018

Research Intern, Kentucky Equine Research
- 2016

Graduate Assistant, SHSU, Department of Agricultural Sciences & Engineering Technology
- 2015-2016

TEACHING EXPERIENCE:

Adjunct Faculty, Georgetown College, Department of Biology
- 2020

Graduate Teaching Assistant, SHSU, Department of Agricultural Sciences & Engineering Technology

- 2016-2017

Undergraduate Teaching Assistant, TSU, Department of Agricultural Sciences

- 2013-2015

PEER-REVIEWED PUBLICATIONS:

Journal Articles

Morris, E.M., S.E. Kitts-Morgan, D.M. Spangler, I.M. Ogunade, K.R. McLeod, and D.L. Harmon. 2021. Canine carboxyl- and hydroxyl- containing metabolites altered after a three-week supplementation of cannabidiol (CBD)-containing treats in an exploratory study. J. Anim. Sci. Res. 5(2). doi: 10.16966/2576-6457.153

Morris, E.M., S.E. Kitts-Morgan, D.M. Spangler, I.M. Ogunade, K.R. McLeod, and D.L. Harmon. 2021. Alteration of the canine metabolome after a three-week supplementation of cannabidiol (CBD) containing treats: An exploratory study of healthy animals. Front. Vet. Sci. 8:685606. doi: 10.3389/fvets.2021.685606

Morris, E.M., S.E. Kitts-Morgan, D.M. Spangler, J. Gebert, E.S. Vanzant, K.R. McLeod, and D.L. Harmon. 2021. Feeding cannabidiol (CBD) containing treats did not affect canine daily voluntary activity. Front Vet Sci. 8:645667. doi: 10.3389/fvets.2021.645667

Mrugala, D., J. Leatherwood, **E. Morris**, E. Dickson, C. Latham, R. Owen, M. Beverly, S. Kelley, and S. White-Springer. 2021. Dietary conjugated linoleic acid supplementation may alter skeletal muscle mitochondria and antioxidant status in young horses. J Anim Sci. 99(2):skab037. doi: 10.1093/jas/skab037

Morris, E.M., S.E. Kitts-Morgan, D.M. Spangler, K. McLeod, J.H.C. Costa, and D.L. Harmon. 2020. The impact of feeding cannabidiol (CBD) containing treats on canine response to a noise-induced fear response test. Front Vet Sci. 7:569565. doi: 10.3389/fvets.2020.569565

Miller, E.F., J.L. Leatherwood, M.J. Anderson, M.M. Beverly. 2017. Evaluation of conjugated linoleic acid (CLA) supplementation on equine body composition. Appro Poult Dairy & Vet Sci. 3(3). doi: 10.31031/APDV.2018.03.000565

Abstracts

Morris, E.M., S.E. Kitts-Morgan, D.M. Spangler, K.R. McLeod, J.H.C. Costa, and D.L. Harmon. 2020. The impact of feeding treats containing cannabidiol (CBD) on the canine fear response to a noise-induced fear response test. Abstract 81. 2020 ASAS-CSAS-WSASAS Virtual Annual Meeting & Trade Show.

Morris, E.M., S.E. Kitts-Morgan, D.M. Spangler, K.R. McLeod, and D.L. Harmon. 2020. The impact of feeding treats containing cannabidiol (CBD) on the daily activity level of dogs. Abstract 82. 2020 ASAS-CSAS-WSASAS Virtual Annual Meeting & Trade Show.

Miller, E.F., J.L. Leatherwood, M.J. Anderson, M.M. Beverly. 2017. The effect of CLA supplementation on fat deposition and lean muscle mass in horses. Abstract 80. 2017 ASAS-CSAS Annual Meeting & Trade Show MS Graduate Student Poster Competition. Baltimore, Maryland.

Miller, E.F., F.R. Melgar, T.D. Morgan, S.L. Ivey, C.L. Loest, L.M. White, and K.W. Walter. 2015. Evaluation of inter-day variation of horses on total fecal collection. J. Anim. Sci. 93, E-suppl 2:340.

SCHOLARSHIPS AND AWARDS:

NACTA Graduate Teaching Award
- 2017

SHSU College of Sciences Special Graduate Scholarship Award
- 2016-2017
- $1,500 per semester

Allen and Joan Triplett Scholarship, SHSU
- 2016-2017
- $500 per semester

TruScholar Research Grant
- 2014
- $3,000

Missouri Bright Flight Scholarship
- 2011-2015
- $3,000 annually