Journal of Animal Science, 2020, Vol. 98, No. 5, 1–12

doi:10.1093/jas/skaa128
Advance Access publication April 21, 2020
Received: 14 January 2020 and **Accepted:** 18 April 2020
Companion Animal Nutrition

COMPANION ANIMAL NUTRITION

Effects of incremental exercise and dietary tryptophan supplementation on the amino acid metabolism, serotonin status, stool quality, fecal metabolites, and body composition of mid-distance training sled dogs

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Abstract

Exercise improves the health of dogs; however, the extreme exertion experienced by sled dogs may lead to variable metabolic and fecal characteristics. Nutritional interventions, such as dietary tryptophan (**Trp**), may reduce the prevalence of these exercise-induced disturbances. Sporting diets tend to have high crude protein concentrations in contrast to adult maintenance diets and this results in less Trp relative to other amino acids (**AA**). Therefore, sporting dogs represent an ideal cohort to assess the effects of supplemental Trp. The objective was to evaluate the effects of supplemental dietary Trp and an incremental training regimen on AA and serotonin status, fecal scores and metabolites, and body composition in client-owned Siberian huskies. Sixteen dogs (nine females and seven males) were used, with a mean age of 4.8 ± 2.5 yr and body weight (**BW**) of 24.3 ± 4.3 kg. Dogs were blocked for sex, age, and BW and randomly allocated into two groups with eight fed a dry extruded control diet (**Ctl**) and eight fed Ctl supplemented with Trp to reach a Trp:large-neutral AA (**LNAA**) ratio of 0.075:1 (treatment, Trt). The exercise regimen was designed to increase in distance each week, but weather played a role in setting the daily distance. Each week BW was recorded and food allotments were adjusted to maintain initial BW. Pre and post-exercise blood samples were taken every 3 wk, dogs then received a meal followed by 1, 2, and 4 h post meal blood collections (serum AA, serotonin). Stool collection and scoring occurred each week and body composition was measured on weeks –1 and 11. Serotonin, AA, fecal metabolite, and body composition data were analyzed using PROC MIXED of SAS with dog as a random effect and week and Trt as fixed effects. Stool score data were analyzed using PROC FREQ to compare stool score and Trt, and PROC CORR was used to analyze associations between fecal score, temperature, humidity, and run distance. Dogs on Trt had greater fasted Trp compared with baseline, greater post-meal Trp and serotonin compared with baseline, greater post-meal Trp compared with fasted, and greater post-meal Trp and serotonin compared with Ctl ($P < 0.05$). Fecal data indicated that Trp improved stool scores ($P < 0.05$) yet had no effect on fecal metabolites. An overall increase in lean and decrease in fat mass was found ($P < 0.05$), but Trt had no effect on body composition. Optimization of the dietary Trp:LNAA ratio may help to improve GI health without compromising performance in actively training sled dogs.

Key words: amino acids, body composition, exercise, fecal quality, sled dogs, tryptophan

Abbreviations

A:G	albumin to globulin ratio
AA	amino acid
AAFCO	Association of American Feed Control Officials
ALP	alkaline phosphatase
ALT	alanine transaminase
BBB	blood–brain barrier
BCAA	branched-chain AA
BCFA	branched-chain fatty acid
BW	body weight
CB	conjugated bilirubin
CBC	complete blood count
CK	creatinine kinase
Ctl	control
DG	diet group
DM	dry matter
ECT	eosinophil count
FB	free bilirubin
FFA	free fatty acid
FI	food intake
FM	fat mass
GCT	glucose challenge test
GI	gastrointestinal
GIT	gastrointestinal tract
HCT	hematocrit
HGB	hemoglobin
5-HIAA	5-hydroxyindoleacetic acid
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine
LBM	lean body mass
LCT	lymphocyte count
LNAA	large-neutral-amino-acid
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCT	monocyte count
MCV	mean corpuscular volume
MPV	mean platelet volume
NCT	neutrophil count
NRC	National Research Council
PLTCRT	plateletcrit
PLTS	platelets
QMR	quantitative magnetic resonance
RBC	red blood cell
RD	run distance
RDW	red cell distribution width
SCFA	short-chain fatty acid
SIALP	steroid induced alkaline phosphatase
STP	sampling time point
TB	total bilirubin
TBW	total body water
TDO	tryptophan-2,3-dioxygenase
TP	total protein
Trt	treatment
TSP	total serum protein
UPLC	ultra-performance liquid chromatography
WBC	white blood cell

Introduction

Regular exercise can improve the physiological and psychological well-being of domestic dogs (Laros et al., 1971; Coppinger and Zuccotti, 1999; Menor-Campos et al., 2011); however, extreme exertion, such as that experienced by sled dogs, can lead to musculoskeletal injuries, variable metabolic and fecal characteristics, and disturbances in the gastrointestinal (**GI**) environment, including microbial transformations, compromised intestinal permeability, and alterations in gut motility and transit rate (Coffman, 2000; van Nieuwenhoven et al., 2004; Royer et al., 2005; McKenzie et al., 2007, 2010; Cook et al., 2016; Davis et al., 2016). Nutritional solutions, such as tryptophan (**Trp**) supplementation, may assist in reducing the prevalence of GI disturbances related to exercise. Additionally, by utilizing sled dogs, a cohort that is highly motivated to exercise and similar with regard to genetics, diet, housing, and training, we believe that we can investigate the effects of nutritional intervention and/or exercise using far fewer dogs than would be possible if similar experiments were carried out using an alternative cohort of healthy, adult dogs at maintenance.

Tryptophan is an indispensable amino acid (**AA**) for dogs, and supplementation of Trp in excess of its dietary requirement has been explored as a means to increase production of serotonin (5-hydroxytryptamine, **5-HT**), a cerebrospinal fluid neurotransmitter involved in the regulation of mood as well as the modulation of the GI environment (Lucki, 1998; Gainetdinov et al., 1999; Mohammad-Zadeh et al., 2008; O'Mahony et al., 2015). However, Trp competes with the large neutral AA (**LNAA**; tyrosine [Tyr], phenylalanine [Phe], leucine [Leu], isoleucine [Ile], and valine [Val]) for transport across the blood–brain barrier (**BBB**). Therefore, increasing the ratio of dietary Trp:LNAA may improve the likelihood of Trp accessing BBB transporters so as to potentially synthesize serotonin.

Tryptophan-enriched diets have been additionally investigated for their effects on gut health due to Trp-catabolizing bacterial strains (e.g., *Lactobacillus* spp.) playing a role in protecting against and alleviating GI tract (**GIT**) inflammation (Etienne-Mesmin et al., 2017). Changes in Trp metabolism can alter the severity and prevalence of GI symptoms in subjects with diarrhea-predominant inflammatory bowel disease; however, neither acute Trp depletion nor increased Trp status affected the healthy, control subjects (Shufflebotham et al., 2006). As such, much remains unknown regarding the manner by which Trp and/or serotonin may modulate the GI environment in a healthy population.

To the best of our knowledge, the effect of incremental exercise, with or without Trp supplementation, on body composition of sled dogs has not been directly investigated. In humans, 9 mo of incremental aerobic exercise resulted in a reduction in total fat mass and an increase in leg lean mass (Evans et al., 2001). However, in a study by Antonio et al. (2000), humans were subjected to 6 wk of exercising training while being provided with either an essential AA supplement or a placebo and no differences in body composition were reported between the two groups. Thus, while changes to body composition are expected due to the duration and degree of aerobic conditioning, the extent to which Trp supplementation might effect changes to body composition in this cohort of dogs remains unknown.

To the authors' knowledge, no literature exists that has evaluated the effects of Trp supplementation on sled dogs participating in controlled bouts of aerobic training. Thus,

the objective of this study was to investigate the effects of supplemental dietary Trp and an incremental training regimen on the outcomes of serum Trp and the Trp:LNAA ratio, serum serotonin, fecal quality and metabolites, and body composition in mid-distance (<150 km) training Siberian huskies. We hypothesized that serum AA and serotonin concentrations would increase post-exercise and postprandially, and that these increases as well as the changes to the Trp:LNAA ratio would be most pronounced in dogs receiving dietary Trp supplementation. As well, we hypothesized that 12 wk of conditioning would result in an improvement in body composition (increase lean and decrease fat mass), an increase in fecal protein-related metabolites, and a reduction in stool quality, but that Trp-supplemented dogs would have higher quality defecations.

Materials and Methods

Animals and housing

The present experiment was approved by the University of Guelph's Animal Care Committee (Animal Use Protocol # 4008). Sixteen client-owned domestic Siberian huskies (9 females: 4 intact, 5 spayed; 7 males: 2 intact, 5 neutered), with an average age of 4.8 ± 2.5 yr and body weight (**BW**) of 24.3 ± 4.3 kg, were used in the study. Dogs were housed and trained at an off-site facility (Rajenn Siberian Huskies, Ayr, ON) that had been visited and approved by the University of Guelph's Animal Care Services. During the study, dogs were pair or group-housed in free-run, outdoor kennels that ranged in size from 3.5 to 80 square meters and contained between 2 and 10 dogs each. Two dogs were removed from the trial (one on week 7, Ctl; one on week 9, Trt) due to exercise-related injuries; all data collected from them up until their respective points of removal were included in this report.

Diets and study design

Dogs were blocked for age, sex, BW, and gangline position before being randomly allocated to one of two groups: control (**Ctl**) (n = 8; 4 males, 3 neutered, 1 intact; 4 females, 2 spayed, 2 intact) or treatment (**Trt**) (n = 8; 3 males, 2 neutered, 1 intact; 5 females, 3 spayed, 2 intact). For 2 wk prior to the study period (weeks –2 and –1), all dogs were acclimated to the dry extruded Ctl diet (Table 1; Champion Petfoods LT, Morinville, AB) that met or exceeded all National Research Council (NRC, 2006) and Association of American Feed Control Officials (AAFCO, 2016) nutrient recommendations for adult dogs at maintenance. During the acclimation period and throughout the trial, dogs were fed once daily at 1700 hours. Dogs were initially fed at intakes determined from historical feeding records. BWs were recorded at week –1 and thereafter, BW was measured each week and food allotments were adjusted to maintain the dogs' week –1 BW. Dogs in the Ctl group were fed the Ctl diet throughout the entire trial while Trt dogs were fed the Ctl diet supplemented with dietary Trp (ADM Animal Nutrition, Woodstock, ON). Treatment diets consisted of the Ctl diet top-dressed with Trp solutions to reach a Trp to LNAA ratio of 0.075:1 (Trp:LNAA ratio of the Ctl diet was 0.047:1). This ratio was determined with the goal of exceeding both the minimum dietary Trp:LNAA ratio of 0.061:1 as derived from NRC (2006) suggested minimum AA requirements for adult dogs at maintenance as well as the highest Trp:LNAA ratio used when feeding medium or large breed dogs to their Trp requirement as determined by indicator AA oxidation (Trp:LNAA of 0.074:1, medium breed dogs; Templeman et al., 2019). Tryptophan solutions were made by dissolving crystalline Trp into deionized water heated to 30 °C to reach a concentration of 10 g/L. Once fully dissolved, the solution was brought to room temperature (22 °C) and then stored at 4 °C until use. New solutions were made each week so as to reduce the risks of bacterial contamination associated with prolonged storage. Solutions were added to each ration and mixed for 10 min to ensure a homogenous incorporation into the kibble. At feeding, all dogs were tethered and fed individually to allow accurate monitoring of food consumption. Any orts were weighed and recorded daily. Throughout the entire trial period, all dogs were allowed ad libitum access to fresh water.

Table 1. Diet nutrient content on dry matter basis and ingredient composition[1] of the control diet

Nutrient contents	Analyzed content
Metabolizable energy, kcal/kg (calculated)[2]	4,074.35
Dry matter[3], %	94.15
Crude Protein, %	47.92
Ether extract, %	24.61
Crude Fiber, %	2.43
Ash, %	9.08
Arg, %	2.91
Cys, %	0.40
His, %	0.95
Ile, %	1.66
Leu, %	3.03
Lys, %	2.77
Met, %	0.91
Phe, %	1.71
Thr, %	1.64
Trp, %	0.46
Tyr, %	1.17
Val, %	2.12

[1]Ingredient composition: Pea starch, pork meal, fresh chicken, low ash herring meal, chicken fat, chicken meal, chicken skin meal, fresh pork, fresh pork organ blend (blend of 51% to 52% fresh pork meat and 48% to 49% fresh pork organs [liver, kidney, and spleen]), chicken and turkey giblets, spray-dried pork liver, pork-based palatant (liquid), hydrolyzed poultry protein, herring oil, pork-based palatant (dry), sodium chloride, dried kelp, choline chloride, poultry-based palatant, alpha tocopherol, natural antioxidant (liquid), thiamine, pantothenic acid, potassium chloride, selenium ye, zinc proteinate, natural antioxidant (dry), copper proteinate.
[2]Calculated metabolizable energy based on modified Atwater values and presented on an as-fed basis.
[3]Dry matter presented on an as-fed basis.

Exercise regimen

A 12-wk exercise regimen was proposed whereby exercise intensity and duration would increase incrementally (refer to anticipated run distances, Table 2). However, decisions regarding the distance ran each day and number of stops (e.g., for water) were made with consideration of the ambient temperature and humidity. Inclement weather also caused the removal of the exercise challenge proposed to take place on week 8. Training consisted of dogs running on a standard 16-dog gangline with staggered, pair-wise groupings of Trt and Ctl dogs (Supplementary Figure S1A). The gangline was attached to an all-terrain vehicle with one rider who controlled the machine in its lowest gear. A pace of ~15 km/h was averaged throughout the training period. Running pace and distance traveled were measured using a speedometer and odometer on the all-terrain vehicle.

Table 2. Mean daily RD, FI, and BW data for all dogs from week 0 to 11, body composition data (LBM, FM, TBW) at weeks −1 and 11, and anticipated RD for the proposed exercise regimen

	Week											P-value		
	0	1	2	3	4	5	6	7	8	9	10	11	SEM[1]	DG[2]	Wk	DG × Week[3]
RD, km/d[4]	6.9[e]	12.9[de]	17.5[cde]	23.8[bcd]	31.0[abc]	37.2[a]	42.2[a]	30.0[abc]	31.3[abc]	34.5[ab]	31.6[abc]	34.2[ab]	4.4	0.97	≤0.01	0.98
ARD[5], km/d	6.0	12.0	18.0	24.0	30.0	36.0	42.0	48.0	54.0	60.0	66.0	72.0	—	—	—	—
FI, g/d	253.7[f]	267.9[f]	271.1[f]	292.2[f]	322.5[ef]	387.5[de]	533.8[a]	586.5[a]	523.48[ab]	511.17[abc]	458.7[bcd]	425.5[cd]	23.6	0.95	≤0.01	0.96
BW, kg	24.2[a]	23.8[a]	23.6[ab]	22.9[bcd]	22.5[cde]	22.2[e]	22.1[e]	22.8[cde]	23.5[ab]	23.8[a]	24.2[a]	24.3[a]	1.2	0.94	≤0.01	0.84
LBM, %[6]	65.7[b]	—	—	—	—	—	—	—	—	—	—	76.9[a]	2.2	0.30	≤0.01	0.53
FM, %	16.8[a]	—	—	—	—	—	—	—	—	—	—	12.3[b]	2.8	0.43	≤0.01	0.44
TBW, %	48.4[b]	—	—	—	—	—	—	—	—	—	—	56.8[a]	2.0	0.16	≤0.01	0.52

[1]BW, FI with $n = 16$ for weeks 0 to 7, $n = 15$ for week 8, and $n = 14$ for weeks 9 to 11; RD with $n = 4$ for weeks 0 to 10, and $n = 2$ for week 11 (n = number of days running that week); body composition with $n = 13$ for weeks −1 and 11.
[2]Trt or Ctl.
[3]Interaction effect between DG and week.
[4]Mean daily run distance determined as average of the 4 d of each week the dogs trained.
[5]Anticipated RD for proposed incremental exercise regimen (distance proposed to be run 4 d/wk of training).
[6]Baseline evaluation of body composition (LBM, FM, and TBW) occurred at week −1.
[a–f]Values in a row with different superscript are significantly different ($P \leq 0.05$).

On weeks −1, 2, 5, and 11, one off-day (no training) was replaced by an exercise challenge whereby dogs would run a consistent distance at ~15 km/h as a team of four dogs. Four-dog teams were predetermined based on each dog's position in the 16-dog gangline (Supplementary Figure S1B). At week 0, the team of four dogs that the owners deemed the least motivated and physically fit were run at 15 km/h until one dog presented with a heart rate that exceeded 300 beats/ min (van Citters and Franklin, 1969; heart rate evaluated using noninvasive, real-time, external telemetry equipment; emka TECHNOLOGIES, Falls Church, VA) or displayed one of the predetermined fatigue-associated behaviors, including lack of motivation, loose tug line or tight neckline, leaning on gang line, or increased salivation. At this point, the challenge stopped, dogs were immediately watered, and the final distance traveled was recorded (4 km). Thereafter, all challenge groups for all weeks (−1, 2, 5, and 11) ran for 4 km at 15 km/h.

Blood sample collection and analysis

Fasting blood samples were collected on weeks −1, 1, 3, 6, 9, and 11 to assess the standard serum veterinary diagnostic measurements and markers of nutritional and health status (Supplementary Tables S1 and S2). Dogs were fasted for 12 h overnight and 5 mL samples were collected by way of cephalic venipuncture with a serum Vacutainer system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Whole blood samples (1 mL) were kept on ice prior to being analyzed for hematological indices (e.g., complete blood cell count, **CBC**) using a Siemens ADVIA 2120 hematology analyzer (Siemens Healthcare LTD., Oakville, ON). Separate samples (4 mL) were centrifuged at 2,000 × *g* for 20 min at 4 °C using a Beckman J6-MI centrifuge (Beckman Coulter, Indianapolis, IN), then serum aliquots were collected, frozen, and kept at −80 °C prior to the analysis. These samples were then analyzed for serum biochemical components using a Roche Cobas 6000 c501 biochemistry analyzer (Roche Diagnostics, Indianapolis, IN). Due to an aversion to the restraint and collection procedure, no blood samples were collected from one dog throughout the trial period; however, this dog was considered healthy based on a general health evaluation by a licensed veterinarian prior to and during the trial.

On exercise challenge days, pre-exercise blood samples were taken via cephalic venipuncture with a serum Vacutainer system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) 15 min prior to performing the exercise challenge. Once the challenge commenced, dogs were immediately watered and then had a post-exercise blood sample taken as described above. All dogs then received a meal consisting of 75% of their daily ration followed by postprandial blood collections at 1, 2, and 4 h post-meal (Eccleston et al., 1968) as described above. All samples were centrifuged at 2,000 × *g* for 20 min at 4 °C using a Beckman J6-MI centrifuge (Beckman Coulter, Indianapolis, IN), then serum aliquots were collected, frozen, and kept at −80 °C prior to the analysis. Serum samples were analyzed for serotonin using a canine 5-HT enzyme-linked immunosorbent assay (MyBioSource Inc., San Diego, CA) and for AA using an ultra-performance liquid chromatography system (**UPLC**; Waters Corporation, Milford, MA). Briefly, 100 µL of serum was deproteinated by adding 100 µL of 10% sulfosalicylic acid (Sigma Aldrich, Oakville, ON) and centrifuged using a Fisherbrand accuSpin Micro 17 (Thermo Fisher Scientific, Waltham, MA) at 12,000 rpm for 5 min. After centrifuging, 10 µL of supernatant was sampled for derivatization. AA standards and serum were derivatized using an AccQ-Tag Ultra derivatization kit (Waters Corporation, Milford, MA, USA). Derivatized AA (1 µL injection

volume) were separated in a column (2.1 × 200 mm, 1.7 µL) maintained at 55 °C using UPLC (Waters Corporation, Milford, MA) with ultraviolet detection at a wavelength of 260 nm. Amino acid peak areas were compared with the calibration standard and analyzed using Waters Empower 2 Software (Waters Corporation, Milford, MA, USA).

Fecal sample collection and analysis

Fecal collection and stool scoring were conducted a minimum of twice weekly. Fecal scores were assessed using a 5-point visual scoring system (1 = hard/dry and 5 = watery diarrhea) (Moxham, 2001). A score of 2.5 was considered ideal and represents a well-formed stool. Defecations were identified, scored, photographed, and collected within 15 min of being voided. For fecal scoring, an additional evaluator used the photographs to re-score all defecations, and the average of the two scores was used for further analysis. The primary (on-site) and secondary (photograph) evaluators remained the same throughout the study. Interobserver reliability on fecal scoring was conducted by calculating Kappa statistics using the PROC FREQ procedure in SAS (v. 9.4; SAS Institute Inc., Cary, NC). For fecal collections, whole samples were collected, all visible contaminants (e.g., grass and hair) and portions of samples that were in contact with the ground were removed, and then samples were transferred into a Whirl-pak bag (Thermo Fisher Scientific, Waltham, MA) to be homogenized. Homogenized samples were then stored in sterile 50 mL centrifuge tubes (Thermo Fisher Scientific, Waltham, MA), frozen, and kept at –80 °C until further analysis. Samples were analyzed for short-chain fatty acids (**SCFA**; acetic acid, propionic acid, and butyric acid), branched-chain fatty acids (**BCFA**; isobutyric acid and isovaleric acid), and lactic acid using an Agilent HP1000 series high-performance liquid chromatography system (**HPLC**; Agilent Technologies, Santa Clara, CA).

For HPLC, samples were prepared by combining 0.1 g of sample with 1 mL of 0.005 N sulfuric acid (concentrated sulfuric acid in Milli-Q water; Sigma Aldrich, Oakville, ON). Samples were vortexed for 60 s and then centrifuged using a Fisherbrand accuSpin Micro 17 (Thermo Fisher Scientific, Waltham, MA) at 13,300 rpm for 15 min. Supernatant was drawn into an HPLC vial and 0.005 N sulfuric acid was added to achieve a two-times dilution. For the HPLC system, the column temperature was 60 °C, the refractive index detector temperature was 40 °C, and injection volume was 20 µL. A standard curve was developed for each SCFA and BCFA with the following serial dilutions: 0.25, 0.50, 1.00, 2.00, and 4.00 mmol/L. OpenLAB CDS ChemStation edition software was used for system control and data acquisition (Agilent Technologies, Santa Clara, CA).

Body composition assessment

Body composition (fat mass, **FM**; lean body mass, **LBM**; total body water, **TBW**) was measured using quantitative magnetic resonance (**QMR**) imaging technology (Cancog Tech., Fergus, ON) on weeks –1 and 11. Using QMR technology, imaging of fat and lean tissues was done efficiently (<4 min per animal) and dogs did not need to be anesthetized (Mitchell et al., 2011). Due to issues related to physical confinement, one dog did not undergo body composition imaging. As well, the two dogs that were removed from the trial did not undergo week 11 body composition imaging, so their data were removed from this report.

Statistical analysis

Amino acid and serotonin data were analyzed using PROC MIXED of SAS (v. 9.4; SAS Institute Inc., Cary, NC). Dog was treated as a random effect and week, diet group (**DG**; Trt or Ctl), and sampling time point were treated as fixed effects. Fasted blood sample (CBC, serum biochemistry), fecal metabolite, and body composition data were analyzed using PROC MIXED of SAS (v. 9.4; SAS Institute Inc., Cary, NC). Dog was treated as a random effect and week and DG (Trt or Ctl) were treated as fixed effects. The effect of DG and week as well as their interaction were evaluated. Week was treated as a repeated measure, BW was used as a covariate, and means were separated using the Tukey adjustment. Fecal score data were analyzed using PROC FREQ of SAS (v. 9.4; SAS Institute Inc., Cary, NC) with a Chi-square test to compare fecal scores and DGs. To assess the strength of a linear relationship between fecal score, ambient temperature, humidity, RD, and week, data were analyzed using PROC CORR of SAS (v. 9.4; SAS Institute Inc., Cary, NC). Significance was declared at a $P \leq 0.05$ and trends at a $P \leq 0.10$.

Results

Mean daily RD, food intake, BW, and body composition

Mean daily RD, food intake (**FI**), and BW did not differ between Trt or Ctl ($P > 0.10$), but all differed by week. RD for weeks 5 and 6 was greater than weeks 0 to 3, for weeks 9 and 11 was greater than 0 to 2, and for weeks 4, 7, 8, and 10 was greater than weeks 0 and 1 ($P \leq 0.05$; Table 2). As well, daily RD was positively correlated with week ($r = 0.79$, $P \leq 0.05$), negatively correlated with ambient temperature ($r = –0.78$, $P \leq 0.05$) and humidity ($r = –0.24$, $P \leq 0.05$), and tended to be positively correlated with fecal score ($r = 0.17$, $P \leq 0.10$; data not shown). FI at weeks 6 and 7 was greater than weeks 0 to 5 and 10 to 11, at week 8 was greater than weeks 0 to 5 and 11, and at week 9 was greater than weeks 0 to 5 ($P \leq 0.05$; Table 2). BW at weeks 5 and 6 was lower than weeks 0 to 3 and 8 to 11, at weeks 4 and 7 was lower than weeks 0 to 2 and 8 to 11, and at week 3 was lower than for weeks 0 to 1 and 9 to 11 ($P \leq 0.05$; Table 2).

Body composition (LBM, FM, and TWB) did not differ between Trt and Ctl dogs ($P > 0.10$); however, when data from all dogs were pooled, LBM and TBW increased while FM decreased by week 11 when compared with week –1 ($P \leq 0.05$; Table 2).

Fasted and postprandial serum Trp, LNAA, Trp:LNAA, and serotonin

At baseline (week –1), no differences were observed between Trt and Ctl dogs for serum Trp, LNAA, the Trp:LNAA ratio, or serotonin at fasted or at any postprandial sampling time point ($P > 0.10$; Table 3). Within DG, Trt and Ctl dog serum LNAA was greater at 4 h postprandial compared with 1 h postprandial and when fasted ($P \leq 0.05$; Table 3). For Trt dogs, the Trp:LNAA ratio was greater at fasting compared with 1, 2, and 4 h postprandial ($P \leq 0.05$); however, no differences were observed for the serum Trp:LNAA ratio with Ctl dogs or for concentrations of serum Trp with either Trt or Ctl dogs ($P > 0.10$; Table 3).

At week 2, Trt dogs had greater serum Trp at 1 and 2 h postprandial, a greater Trp:LNAA ratio at 1 h postprandial, and greater serum serotonin at 4 h postprandial compared with Ctl at the same sampling time points ($P \leq 0.05$; Table 3). Within DG, Trt dog serum Trp and Trp:LNAA ratio were greater at 1 h postprandial than when fasted or at 2 and 4 h postprandial, and LNAA was lower when fasted compared with any postprandial time point ($P \leq 0.05$; Table 3). As well, Trt dog serum Trp at 1 and 2 h postprandial and Trp:LNAA ratio at 1 h postprandial for

Table 3. Fasted and postprandial (1, 2, and 4 h) serum concentrations of selected AA and serotonin in control dogs and Trp-supplemented (treatment) dogs at weeks −1, 2, 5, and 11

		Fasted		1 h postprandial		2 h postprandial		4 h postprandial			*P*-value		
		Trt[1]	Ctl	Trt	Ctl	Trt	Ctl	Trt	Ctl	SEM[1]	DG[2]	STP	DG × STP[3]
Week −1	Trp, μM	77.8	79.1	86.9	88.0	87.1	91.0	100.0	101.24	11.7	0.84	0.12	0.71
	LNAA[4], μM	552.3^{d}	554.3^{d}	759.2^{bc}	694.0^{c}	850.6^{ab}	774.1^{bc}	929.6^{a}	873.8^{ab}	49.2	0.19	<0.01	0.39
	Trp:LNAA[5]	0.14^{a}	0.14^{a}	0.11^{bc}	0.12^{abc}	0.10^{c}	0.12^{abc}	0.11^{bc}	0.12^{abc}	0.015	0.41	<0.01	0.75
	5-HT, ng/mL	109.55	110.34	113.95	106.00	117.13	111.10	117.32	111.50	7.81	0.46	0.09	0.79
Week 2	Trp, μM	84.9^{c}	71.8^{c}	175.7^{a*}	96.3^{bc}	119.9^{b*}	86.1^{c}	116.8^{b}	88.2^{bc}	12.8	<0.01	<0.01	0.07
	LNAA, μM	568.2^{c}	549.7^{c}	814.1^{ab}	746.9^{b}	903.8^{a}	801.5^{ab}	911.1^{a}	807.0^{ab}	55.6	0.27	<0.01	0.75
	Trp:LNAA	0.15^{b}	0.14^{bc}	0.22^{a*}	0.13^{bc}	0.13^{bc}	0.11^{c}	0.13^{bc}	0.11^{c}	0.016	0.03	<0.01	0.13
	5-HT, ng/mL	112.79^{ab}	108.44^{b}	117.76^{ab}	108.59^{b}	122.86^{ab}	114.37^{ab}	128.97^{a}	105.06^{b}	8.11	0.02	0.03	0.08
Week 5	Trp, μM	113.3^{bc*}	85.8^{c}	175.1^{a*}	105.1^{bc}	123.7^{b*}	91.1^{c}	125.5^{b}	98.3^{bc}	13.1	<0.01	<0.01	0.12
	LNAA, μM	746.8^{b*}	738.6^{b*}	819.5^{ab}	802.0^{ab}	763.0^{b}	710.1^{b}	914.6^{a}	792.0^{ab}	59.2	0.27	0.03	0.73
	Trp:LNAA	0.15^{b}	0.13^{b}	0.22^{a*}	0.13^{b}	0.16^{b}	0.13^{b}	0.14^{b}	0.13^{b}	0.016	0.04	<0.01	0.08
	5-HT, ng/mL	121.14^{ab}	103.65^{b}	122.19^{ab}	102.66^{b}	123.29^{ab}	103.63^{b}	128.51^{a}	100.32^{b}	8.58	0.04	0.03	0.11
Week 11	Trp, μM	99.9^{bc*}	88.1^{c}	142.1^{a*}	92.1^{bc}	125.4^{ab*}	84.2^{c}	107.4^{bc}	86.4^{c}	14.7	0.01	0.03	0.10
	LNAA, μM	790.5*	809.8*	816.5	929.5*	839.2	839.0	907.4	847.0	63.9	0.59	0.08	0.25
	Trp:LNAA	0.13^{bc}	0.11^{c}	0.17^{a}	0.10^{c}	0.15^{ab}	0.10^{c}	0.12^{bc}	0.10^{c}	0.017	<0.01	0.03	0.07
	5-HT, ng/mL	127.05^{a}	105.52^{b}	133.20^{a}	103.48^{b}	139.18^{a}	103.43^{b}	143.99^{a*}	102.23^{b}	8.91	<0.01	<0.01	0.07

[1]Trt, treatment dogs; Ctl, control dogs; SEM, $n = 8$ for treatment weeks −1, 2, and 5; $n = 7$ for treatment week 11, control weeks −1, 2, and 5; $n = 6$ for control week 11.
[2]Trt or Ctl; Sampling time point (fasted, 1, 2, and 4 h postprandial).
[3]Interaction effect between DG and sampling time point.
[4]LNAA: Val, Phe, Ile, Leu, Tyr
[5]Ratio of Trp to LNAA.
[a–d]Values in a row with different superscript are significantly different ($P \leq 0.05$).
*Significantly different ($P \leq 0.05$) when compared with the same sampling time point at week −1 (within treatment).

week 2 were greater than the same time points at week –1 ($P \leq 0.05$; Table 3). For Ctl dogs, serum LNAA was lower when fasted compared with all postprandial time points ($P \leq 0.05$; Table 3).

At week 5, Trt dogs had greater serum Trp concentrations at 1 and 2 h postprandial, a greater Trp:LNAA ratio at 1 h postprandial, and greater serum serotonin at 4 h postprandial compared with Ctl at the same sampling time points ($P \leq 0.05$; Table 3). Within DG, Trt dog serum Trp and Trp:LNAA ratio were greater at 1 h postprandial compared with fasted, 2, and 4 h postprandial, and LNAA was greater at 4 h postprandial compared with fasted or 2 h postprandial ($P \leq 0.05$; Table 3). Week 5 serum Trp at fasting, 1 and 2 h postprandial, LNAA when fasted, and Trp:LNAA ratio at 1 h postprandial for Trt dogs were greater than for week –1 at the same time points ($P \leq 0.05$; Table 3). As well, for Ctl dogs, fasted serum LNAA at week 5 was greater than for fasted at week –1 ($P \leq 0.05$; Table 3).

At week 11, Trt dogs had greater serum Trp concentrations, Trp:LNAA ratios at 1 and 2 h postprandial, and greater serum serotonin at fasted, 1, 2, and 4 h postprandial compared with Ctl dogs at the same sampling time points ($P \leq 0.05$; Table 3). Within DG, serum Trp and the Trp:LNAA ratio for Trt dogs were greater at 1 h postprandial compared with fasted and 4 h postprandial ($P \leq 0.05$; Table 3). When compared with the same sampling time points at week –1, Trt dog serum Trp at fasting, 1 and 2 h postprandial time points, and serum LNAA when fasted were greater at week 11 ($P \leq 0.05$; Table 3). Control dog serum LNAA at fasting and 1 h postprandial on week 11 was greater than for week –1 at the same sampling time points ($P \leq 0.05$; Table 3). As well, Trt dog serum serotonin at 4 h postprandial on week 11 was greater than 4 h postprandial concentrations at week –1 ($P \leq 0.05$; Table 3).

Pre- and post-exercise serum Trp, LNAA, Trp:LNAA, and serotonin

No differences were observed between pre and post-exercise serum Trp, LNAA, Trp:LNAA ratio, or serotonin within any week for either Trt or Ctl dogs ($P > 0.10$; Table 4). However, differences were observed when comparing serum concentrations of Trp or LNAA across all wks: 1) serum Trp for Trt dogs was greatest post-exercise at week 5; 2) serum LNAA for Trt dogs was increased post-exercise at weeks 5 and 11; and 3) serum LNAA for Ctl dogs was greatest post-exercise at week 11 ($P \leq 0.05$; Table 4). No diet differences were observed for pre- or post-exercise concentrations of Trp, LNAA, or Trp:LNAA at any week ($P > 0.10$; Table 4); however, mean pre- and post-run serotonin on weeks 5 and 11 were greater for Trt than Ctl ($P \leq 0.05$; Table 4). As well, when data from all pre- and post-exercise sampling time points were pooled, mean serum serotonin for Trt dogs was greater when compared with Ctl ($P \leq 0.05$; data not shown).

Fecal scores and fecal metabolites

Treatment dogs had proportionately more defecations scored between 2 and 3.5 (Trt, 64%; Ctl, 33%) and fewer scored between 4 and 5 (Trt, 36%; Ctl, 67%) as compared with Ctl dogs ($\chi^2 = 15.88$, $P \leq 0.05$; Table 5). When pooled across all weeks, the mean fecal score for Trt dogs (3.3) tended to be lower than for Ctl (3.8) ($P \leq 0.10$); however, week and DG*week interaction had no effect on mean fecal score ($P > 0.10$, data not shown). Fecal score tended to be positively correlated with RD ($r = 0.17$, $P \leq 0.10$; data not shown); though, no linear relationships were observed between fecal score and temperature or humidity ($P > 0.10$; data not shown).

No differences were observed between Trt and Ctl dogs for any of the mean fecal SCFA or BCFA values ($P > 0.10$). Week had no effect on propionic acid ($P > 0.10$), tended to affect isovaleric acid and acetic acid ($P \leq 0.10$), and had a significant effect on all other metabolites ($P \leq 0.05$, Table 6). Concentrations of fecal isobutyric acid were at their highest at week 11, fecal lactic acid was highest at week 10, and butyric acid was highest at week 6 (Table 6).

Complete blood count and serum biochemistry

With the exception of the two dogs that sustained exercise-related injuries and were removed from the trial, all dogs remained healthy throughout the trial period. Data related to the standard serum veterinary diagnostic measurements and markers of nutritional and health status are presented as supplementary material (see Supplementary Tables S1 and S2). Compared with Ctl, Trt dogs had higher red blood cell concentrations and mean platelet volume, lower red cell

Table 4. Pre- and post-training run serum concentrations of selected AA and serotonin in control and Trp-supplemented (treatment) dogs at weeks –1, 2, 5, and 11

	Week –1	Week 2	Week 5	Week 11	SEM[1]				
	Pre-run	Post-run	Pre-run	Post-run	Pre-run	Post-run	Pre-run	Post-run	
Treatment									
Trp, μM	67.8[b]	73.8[ab]	74.1[ab]	87.2[ab]	90.3[ab]	107.0[a]	83.0[ab]	97.9[ab]	9.0
LNAA[2], μM	524.9[b]	552.4[b]	538.4[b]	566.7[b]	652.5[ab]	744.5[a]	673.2[ab]	771.0[a]	38.5
Trp:LNAA[3]	0.13	0.13	0.14	0.14	0.14	0.15	0.12	0.13	0.018
5-HT, ng/mL	109.99	113.55	107.70	116.79	121.14*	122.09*	124.11*	127.12*	7.51
Control									
Trp, μM	65.3	79.8	67.7	74.3	68.3	85.8	72.1	85.8	9.8
LNAA[2], μM	521.3[b]	554.3[b]	529.0[b]	570.5[ab]	605.9[ab]	678.8[ab]	689.6[ab]	816.3[a]	42.1
Trp:LNAA[3]	0.13	0.14	0.13	0.13	0.11	0.13	0.10	0.11	0.019
5-HT, ng/mL	105.54	110.34	106.66	108.44	103.65	104.26	102.10	105.41	8.11

[1]Treatment, $n = 8$ (weeks –1, 2, and 5), $n = 7$ for treatment (week 11) and control (weeks –1, 2, and 5), $n = 6$ for control (week 11).
[2]LNAA: Val, Phe, Ile, Leu, Tyr.
[3]Ratio of Trp to LNAA.
[a,b]Values in a row with different superscript are different ($P \leq 0.05$).
*Significantly different ($P \leq 0.05$) when compared with the equivalent control at the same sampling time point within week.

Table 5. Relative frequency (%) of fecal scores for defecations from control dogs (n = 68) and Trp-supplemented (treatment) dogs (n = 69) over 12 wk

	Fecal score[1]								
	1	2	2.5	3	3.5	4	4.5	5	P-value	Chi-square
Treatment	0	7	19	27	11	19	7	10	0.01	15.88
Control	0	3	15	9	6	30	18	19		

[1]Fecal score ranged from 1 (hard/dry) to 5 (watery diarrhea).

distribution width values ($P \leq 0.05$), and tended to have higher hemoglobin and hematocrit ($P \leq 0.10$; Supplementary Table S1). Treatment dogs had lower serum glucose ($P \leq 0.05$) and tended to have higher creatinine and conjugated bilirubin than Ctl ($P \leq 0.10$; Supplementary Table S2). All mean complete blood count and serum biochemistry values were within standard reference range (as determined by the Animal Health Laboratory, University of Guelph, Guelph, ON).

Discussion

When compared with Ctl dogs, dogs receiving Trp supplementation had higher concentrations of postprandial serum Trp, Trp:LNAA ratio, and serotonin, and were more likely to have well-formed defecations. As well, an overall increase in LBM and decrease in FM were observed following 12 wk of exercise conditioning in mid-distance training Siberian huskies; however, Trp supplementation had no effect on changes to body condition.

Considering the high dietary protein and fat content in diets intended for performance dogs and the aerobic nature of the endurance exercise performed, sled dogs represent an ideal model to investigate the physiological and metabolic outcomes of supplemental Trp. To start, a portion of circulating Trp is bound to albumin, which further limits the availability for BBB transport. However, free fatty acids (**FFA**) also bind to albumin and it is believed that increased concentrations of circulating FFA (induced by aerobic exercise or a high fat diet) can dissociate albumin-bound Trp, increase concentrations of free Trp, and improve the potential for BBB transport (Knott and Curzon, 1972; Jenkins et al., 2016). The authors acknowledge that serum FFA were not measured and that serum albumin was not affected by Trp supplementation (Supplemental Table S2). However, it should be noted that these serum data are representative of dogs in a fasted state, and that fasting decreases the synthesis of albumin (Busher, 1990). In healthy individuals, the increased availability of circulating AA, either in response to a protein-rich meal or via exercise-induced mobilization of AA, will result in an increase in albumin synthesis (Busher, 1990). As such, future research examining the relationship between circulating AA, FFA, and albumin (or prealbumin) in actively exercising dogs receiving high-protein diets is warranted.

In addition to the potential influence of FFA, in most protein-containing ingredients, Trp is present in much lower concentrations than the competing LNAA, relative to dietary requirements (Bosch et al., 2009; Fernstrom, 2012). Therefore, in cases where the protein component of a diet is greater than in average adult maintenance diets, such as in diets intended for performance dogs, the Trp:LNAA ratio is lower and the potential for Trp to access the BBB and synthesize serotonin in the brain is reduced. For example, a number of commercially available dry extruded diets formulated for sporting dogs (e.g., dietary fat and protein contents > 18% and 28%, respectively, on a dry matter basis) and with a label describing its intended use for 'sporting,' 'performance', or 'endurance' dogs (n = 14) were analyzed for Trp, LNAA, and the Trp:LNAA ratio (Templeman et al., unpublished data). Of those diets, all met or exceeded the NRC (2006) suggested minimum requirements for LNAA for adult dogs, all but one diet met or exceeded the suggested requirement for Trp, yet none of the diets met the minimum dietary Trp:LNAA ratio of 0.061:1 as derived from NRC (2006) suggested minimum Trp and LNAA requirements (highest at 0.057:1, lowest at 0.032:1, and mean at 0.043:1). In the future, it may prove beneficial to further explore whether or not animals with increased metabolic demands would benefit from supplementation of dietary Trp at levels described herein.

Body composition

Treatment had no effect on changes in body composition; however, when treatments were pooled, LBM and TWB increased by approximately 11% and 8.5%, respectively, while FM decreased by approximately 4.5% after 12 wk of conditioning. An inverse relationship between TBW and FM (and proportional relationship between TBW and LBM) has been demonstrated in humans (Mukherjee et al., 2005). While LBM increased and FM decreased over the 12-wk conditioning period, these changes occurred without any change in BW. This indicates that aerobic conditioning resulted in the development of LBM and reduction of FM, as has been previously demonstrated in humans (Mosher et al., 1994; Evans et al., 2001), while BW maintenance was supported with appropriate increases in energy intake to match the increase in exercise and thermoregulatory-induced energy expenditure.

AA metabolism and serotonin status

Supplementation of dietary Trp at the concentrations described herein successfully led to an increase in fasted and postprandial serum Trp concentrations in Trt dogs. Evaluation of postprandial serum AA concentrations is essential when investigating the effect of a nutritional intervention on AA metabolism, as these data are largely indicative of diet, with factors such as AA appearance rate playing a decisive role in determining postprandial AA utilization. Even when ingested concurrently, AA derived either from free AA or from an intact protein-based source are assimilated independently, resulting in differences in rate and/or degree of retention in body protein or irreversible catabolism (Nolles, 2006). In the current study, increased serum concentrations of Trp following a meal for Trt dogs were evident at each of the sampling weeks past baseline. However, the most drastic increases in serum Trp occurred at 1-h postprandial, indicating a greater and more rapid appearance of Trp in serum of Trt dogs, likely due to the free/crystalline nature of the supplemented Trp. This 1-h post-administration spike in serum Trp has been observed previously following Trp dosing in dogs.

Table 6. Lactic acid, SCFA, and BCFA data for weeks 1 to 11 as well as for Trp-supplemented (treatment) and control dogs

	Weeks											DG[1]			P-value		
	1	2	3	4	5	6	7	8	9	10	11	SEM	Trt	Ctl	SEM	DG	Wk	DG × Week[2]
Lactic acid[3]	9.01^{ab}	5.42^{b}	8.36^{ab}	5.20^{b}	7.77^{ab}	11.56^{ab}	7.19^{ab}	15.46^{ab}	7.38^{ab}	21.22^{a}	20.35^{ab}	3.91	10.34	11.28	1.85	0.70	0.01	0.72
Acetic acid	90.32	67.71	86.65	59.51	66.79	91.56	88.60	80.60	96.13	90.93	74.63	10.37	83.64	78.80	4.91	0.46	0.10	0.81
Propionic acid	27.54	20.95	27.46	16.85	20.08	28.51	27.11	22.63	26.14	27.21	23.43	3.22	24.15	24.56	1.66	0.85	0.20	0.86
Butyric acid	15.18^{ab}	12.93^{ab}	16.77^{ab}	10.86^{b}	11.18^{ab}	18.14^{a}	13.04^{ab}	11.84^{ab}	14.80^{ab}	13.77^{ab}	11.28^{ab}	1.86	13.56	13.67	0.84	0.92	0.01	0.53
Isobutyric acid	3.43^{ab}	3.50^{ab}	2.83^{ab}	2.45^{b}	2.57^{b}	2.67^{b}	2.80^{ab}	4.62^{a}	3.45^{ab}	3.96^{ab}	4.78^{a}	0.47	3.18	3.56	0.24	0.23	≤0.01	0.58
Isovaleric acid	5.74	4.38	4.51	4.18	4.02	5.87	5.80	4.67	5.77	5.25	4.61	0.72	4.99	4.97	0.64	0.95	0.07	0.46

[1]Trt or Ctl.
[2]Interaction effect between DG and week.
[3]All data presented in micromoles per gram (μmol/g); lactic acid; SCFA: acetic acid, propionic acid, butyric acid; BCFA: isobutyric acid and isovaleric acid.
[a,b]Values in a row (within week) with different superscript are different ($P \leq 0.05$).

Eccleston et al. (1968) administered Trp intravenously to adult mongrel dogs before taking blood, cerebrospinal fluid, and brain region samples at 1, 2, and 4 h following Trp dosing. Eccleston et al. (1968) reported that concentrations of Trp in whole blood were greatest at 1 h post-administration before declining to near-baseline concentrations by 4 h().

Furthermore, Eccleston et al. (1968) reported that concentrations of serotonin in all regions of the brain were greatest at 1 h post-Trp-administration while concentrations of 5-hydroxyindoleacetic acid (**5-HIAA**), the primary metabolite of serotonin, reached maximal concentrations at 2 h post-Trp administration. In the present study, the increase in serum Trp at 1 h postprandial was accompanied by a comparable 1 h postprandial increase in the ratio of Trp:LNAA in serum with Trt dogs. Considering the competitive aspect of BBB access, this increased Trp:LNAA ratio should, in theory, improve the likelihood of serotonin synthesis. However, it was not until 4 h postprandial where increases in serum serotonin were reported for Trp-supplemented Trt dogs in the current study. It should be noted though that in the Eccleston et al.'s (1968) study, Trp was administered intravenously, thus eliminating the variability associated with GIT absorption, and administration occurred following an 8 h fast, thus reducing the competition with the LNAA for BBB access(. These features likely improved the potential for, and rate of, serotonin synthesis in the brain following Trp dosing. In the current study, by week 11 postprandial serum serotonin concentrations for Trt dogs had increased from baseline and all postprandial concentrations were greater than Ctl. This indicates that in healthy, adult Siberian huskies being fed to maintain BW throughout a 12-wk conditioning period, supplementing dietary Trp can lead to increases in serum serotonin status; however, the increase in serum serotonin concentration appears to occur at a slower rate than the changes in serum Trp.

An increase in circulating AA, specifically branched-chain AA (**BCAA**; Ile, Leu, and Val), in response to endurance exercise has been well defined in exercising subjects. BCAA supply skeletal muscle with energy, contribute to the suppression of lactic acid accumulation during exercise, and attenuate delayed onset skeletal muscle soreness following exercise; all of which are desirable outcomes for athletes participating in extended bouts of exercise (Felig and Wahren, 1971; She et al., 2010; Ra et al., 2013). In a 2014 study investigating the effects of exercise on AA profiles in adult Foxhounds, de Godoy et al., (2014) reported significant increases in serum Trp and BCAA in response to a ~2.5-h bout of unstructured exercise, and these increases were most pronounced in the dogs fed a nutrient-fortified diet). The lack of response in serum AA in the current study may have been due to the degree of exercise the dogs were subjected to in this study when samples were taken (during exercise challenges). The distance ran (4 km) for each of the exercise challenges may not have been sufficient enough to elicit the magnitude of change in serum AA concentration reported in de Godoy et al. (2014), even though it was adequate enough to have a dog reach one of the predescribed thresholds for ceasing the initial exercise challenge. Moreover, degree of conditioning may have played a role in the differences in response of serum AA between the current study and that of de Godoy et al. (2014). In horses, the level of training was shown to affect circulating AA concentrations, with the more conditioned subjects having significantly greater concentrations of all BCAA as well as Phe and Tyr prior to a bout of exercise (Klein et al., 2020). However, due to the differences in animals used (e.g., breed, hormonal status, conformation) and exercise regimens implemented

(e.g., duration, number of events, unstructured vs. structured) between the current study and that of de Godoy et al. (2014), it is not possible to conclude whether one group of animals was any more or less conditioned than the other.

Along with an increase in circulating AA, exercise is known to elicit an increase in circulating FFA as well. In 1986, Chaouloff et al. subjected rats to 1 h of low-intensity exercise and reported that the exercise-induced increase in circulating FFA was associated with an increase in free (unbound) Trp, an increase in Trp concentrations in the brain, and an increase in 5-HIAA in the brain (Chaouloff et al. 1986). Circulating FFA will compete with Trp for the ability to bind albumin; as such, an increase in FFA will result in the displacement of Trp from albumin, ultimately increasing circulating free Trp and the proportion of Trp competing for BBB access (Knott and Curzon, 1972). Considering that Trp hydroxylase, the enzyme responsible for the initial and rate-limiting step in synthesis of serotonin, is not fully saturated under normal conditions, an increase in Trp entering the brain should, theoretically, result in an increase in serotonin synthesis (Fernstrom and Wurtman, 1971). For the current study, though, the anticipated increase in post-exercise serotonin was not observed. This may again be a case of the distance ran during the exercise challenges not being sufficient enough to elicit the magnitude of change in serum FFA reported by Chaouloff et al. (1986).

However, the influence of exercise and the associated stress and catecholamine response on the kynurenine pathway may also have played a role. For example, tryptophan-2,3-dioxygenase (**TDO**) is an enzyme that contributes to the initiation of Trp catabolism via the kynurenine pathway which, in turn, can reduce the availability of Trp for serotonin synthesis (Neumeister, 2003; Badawy, 2017). Exercise, and even the anticipation of exercise, can upregulate the production of cortisol and catecholamines (Dimsdale and Moss, 1980; Angle et al., 2009), both of which have an agonistic effect on TDO activity (Badawy and Evans, 1975). As such, the stress and hormonal response to exercise may have limited the potential of serotonin synthesis in the current by promoting the utilization of Trp via the kynurenine pathway. In the future, an evaluation of kynurenine pathway metabolites under similar experimental conditions may help to confirm this hypothesis.

Fecal scores

In the current study, as the duration of exercise increased, the quality of the defecations tended to subsequently decrease. Although there is limited non-anecdotal information available as to the prevalence of loose stools and other GI issues in active working dogs, McKenzie et al. (2010) reported that 36% of the sled dog population monitored during their study experienced diarrhea during a long-distance endurance race, compared with only 12% prior to the race. Intensive aerobic exercise is associated with accelerated colonic transit time, typically resulting in abnormal patterns of defecation in exercising humans (van Nieuwenhoven et al., 2004). As well, rigorous physical activity can compromise intestinal barrier function, an outcome that is commonly accompanied with an increased prevalence of diarrhea, and defects in GI barrier function have been demonstrated with sled dogs undergoing strenuous bouts of exercise (Royer et al., 2005). However, in the current study, dogs receiving Trp supplementation were more likely to have defecations scored between 2 and 3.5, using a visual scoring system where a score of 2.5 is considered ideal, suggesting that Trp supplementation resulted in an improvement in stool quality. While much is still being uncovered with regard to the functions of serotonin in the GIT, the increased concentrations of circulating serotonin in the Trp-supplemented dogs may have played a role in improving fecal quality and/or altering the metabolite profile. In humans, nearly 90% of the serotonin in the body is synthesized by enterochromaffin cells in the GI mucosa (Gainetdinov et al., 1999; Gershon and Tack, 2007; Gershon, 2013). These cells respond to various stimuli by releasing serotonin which, by way of activation of serotonin-specific receptors, can then mediate a number of outcomes within the GI environment that may affect stool quality, such as gut motility, epithelial secretions, inflammation, and vasodilation (Mawe and Hoffman, 2013). While additional work is required to elucidate what exactly is causing Trp supplementation to result in improved stool quality, given the data presented herein, any environmental intervention that improves Trp and/or serotonin status should be considered to improve stool quality under conditions of stress, such as intensive exercise.

Fecal metabolites

As exercise can cause variable gut transit times and stool characteristics, it is likely that the substrate load and overall environment for hindgut microbiota are altered. The specific changes to the gut microbiota in response to exercise and their effects on the canine host are not well understood; however, Gagné et al. (2013) carried out a study that investigated the effects of synbiotics on gut microbial metabolism of actively training sled dogs. Synbiotic supplementation significantly increased *Lactobacillus* spp., improved fecal scores, and reduced the daily instances of diarrhea over a 6-wk training period in sled dogs (Gagné et al., 2013). Unfortunately, though, while this study provided interesting data, the study design did not allow for the interpretation of responses specific to exercise or dietary treatment alone. In the future, evaluations of microbial diversity and abundance would provide more clarification with regard to how microbial shifts may be affected by exercise and/or nutritional intervention in training sled dogs.

Moreover, as exercise increased in intensity, greater protein intake, as a result of greater FI to satisfy the increasing energy requirements, can increase the amount of protein that escapes hydrolytic-enzymatic digestion, resulting in greater substrate in the hindgut for proteolytic microbes to thrive (Hooda et al., 2013). The fermentation of protein leads to the production of some SCFA, but primarily putrefactive compounds such BCFA and sulfur-containing compounds associated with GI diseases and fecal odor (Magee et al., 2000). In the current study, concentrations of fecal BCFA were elevated when FI increased, supporting the notion that as dietary protein intake increases, there is a greater potential for proteolytic fermentation.

Conclusions and Implications

The aim of this study was to evaluate the effects of supplemental dietary Trp as well as a 12-wk incremental training regimen on the outcomes of serum Trp and LNAA, serum serotonin, fecal quality, fecal metabolites, and body composition in client-owned Siberian huskies training for a competitive season of mid-distance races (<150 km). This study is the first to demonstrate that Trp supplementation at inclusion levels necessary to achieve a dietary Trp:LNAA ratio of 0.075:1 does improve Trp status; although, the changes in serum Trp occur prior to changes in serum serotonin status. This suggests that an extended adaptation period is necessary in order to adapt dogs to supplemental dietary Trp before serum serotonin status will improve. Tryptophan supplementation also improved

stool quality, which is an important finding considering that as the duration and intensity of exercise increased during the study, the overall quality of stools tended to decrease. Finally, regardless of dietary treatment, this 12-wk exercise regimen resulted in a profound change in body composition, with an increase in LBM of greater than 10% and a reduction in FM of nearly 5%, all without a change in mean BW. It should be noted, though, that while Trp supplementation did not have an effect on some of the outcomes reported (e.g., body composition), dietary Trp fed at the levels described herein did not negatively impact any of the parameters evaluated. As such, when developing a training and dietary regimen for sporting or working dogs, supplementation of dietary Trp, or any environmental intervention that improves the Trp status, can be beneficial if the objectives are to improve GI function and stool quality under stressful conditions, such as intensive exercise; however, careful consideration must be paid to the dosage and duration of dietary supplementation.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Acknowledgment

The work was funded by Champion Petfoods LT (Morinville, AB) and Mitacs (Toronto, ON).

Authors' Contributions

J.R.T. and A.K.S. designed the experiment. J.R.T, E.T., and C. C-F. conducted the research, J.R.T analyzed the data, and all authors contributed to the writing of the manuscript. A.K.S. had primary responsibility for the final content. All authors read and approved the final manuscript.

Conflict of interest statement

J.R.T, E.T., C.C-F., E.J.S., K.S.S., and A.K.S. have no conflicts of interest.

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