Effects of conditioning and diet on the exercise physiology in mid-distance trained sled dogs

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ABSTRACT

EFFECTS OF CONDITIONING AND DIET ON THE EXERCISE PHYSIOLOGY IN MID-DISTANCED TRAINED SLED DOGS

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Aerobic exercise can affect whole body physiology. Nutritional solutions, such as dietary tryptophan and soluble fiber may be able to assist in the dynamic changes the body undergoes during exercise. This thesis investigated the effects of two dietary interventions, either an increased tryptophan: large neutral amino acid ratio or increased soluble fiber on the heart rate, respiratory rate, and internal body temperature of sled dogs. Exercise led to decreased heart rates, respiratory rate appeared to be sensitive to the training duration, and body temperature tended to decrease during an exercise bout. Increasing the tryptophan: large neutral amino acid ratio of the diet had no effect on heart rate or respiratory rate, whereas increased soluble fiber content of the diet may have led to lower working and recovery body temperature. This research could be used to support exercise performance through both improvement of exercise training regimens and diet.

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LIST OF ABBREVIATIONS

AA: amino acid

AAFCO: Association of American Feed Control Officials

ADP: Adenosine diphosphate

ALT: Alanine aminotransferase

BAT: Brown adipose tissue

BBB: Blood brain barrier

BT: Body temperature

BW: Body weight

°C: Degree Celsius

CBC: Complete blood count

CNS: Central nervous system

CO_2: Carbon dioxide

Ctl: Control

DG: Diet group

ECG: Electrocardiographic

FFAR: Free fatty acid receptors

FI: Feed intake

FM: Fat mass

g: Gram (s)

GI: gastrointestinal

h: Hour (s)

HR: heart rate

IDO: Indoleamine 2,3-dioxygenase

kcal: Kilocalorie (s)

kg: Kilogram (s)

Km: Kilometer (s)

KP: Kynurenine pathway

LMB: Lean body mass

LNAA: Large neutral amino acids

Mcal: Megacalorie (s)

ME: Metabolizable energy

min: Minute (s)

n: Sample size

NAD: Nicotinamide adenine dinucleotide

NRC: National Research Council

O_2: Oxygen

PNS: Parasympathetic nervous system

QMR: Quantitative magnetic resonance

RD: Run distance

RR: Respiratory rate

s: second (s)

SAS: Statistical analysis system

SCFA: Short chain fatty acids

SD: Standard deviation

SE(M): Standard error (of the mean)

SNS: Sympathetic nervous system

SP: Serotonergic pathway

TBW: Total body water

TDO: Tryptophan-2,3-dioxygenase

TPH: Tryptophan hydroxylase

Trp: Tryptophan

Trp: LNAA: Tryptophan to large neutral amino acid ratio

Trt: Treatment

VO_2max: maximal oxygen uptake

WAT: White adipose tissue

Wk: Week (s)

5-HT: 5- hydroxytryptamine (serotonin)

1 Literature Review

1.1 Introduction

The use of sporting dogs is becoming increasingly popular in the world today. Events such as dog sledding are on the rise with mushers training their dogs for months in preparation for a racing season. Races such as the Iditarod and the Yukon Quest, which require dogs to run more than 1500 km in less than 10 days, are the epitome of athletic performance. Exercise ultimately requires the participation of numerous physiological systems to work in combination to support the increased workload imposed on the body. The cardiovascular system, respiratory system, and thermoregulation are three processes that are affected by exercise and play a role in improved aerobic performance. In addition to the adaptations these systems undergo during training, mushers also depend on nutritional support for optimum performance. Templeman et al (2017) distributed a survey to mushers and reported that their main dietary focus is fat and protein while little attention was given to micronutrients and dietary fiber. As nutrition assists in performance outcomes alongside exercise related adaptations, nutritional interventions should be examined for their effects on health and aerobic fitness. For example, tryptophan and dietary fiber may alter physiology and better support performance. Therefore, the present literature review will explore the current knowledge about exercise, the cardiovascular system, respiratory system, thermoregulation, and how tryptophan and fiber may affect exercise related performance.

1.2 Exercise and physiological systems

The extent to which physiological changes occur due to exercise are influenced by the intensity and duration of the training program (Blomqvist & Saltin, 1983; Scrimgeor et al, 1986; Jones & Carter, 2000). With sled dogs, the ability to produce an efficient exercise regimen to support the desired performance is solely dependent on the musher/trainer and their prior knowledge on the effects of endurance training. However, if the training program does not match and/or exceed the subject's previous physical activity level, there may be no adaptive changes in physiology (Gemmill et al, 1930). Thus, establishing a well-structured exercise regimen at a given sub maximal work level, and monitoring how the body responds to exercise over time (i.e cardiorespiratory system and thermoregulation) can lead to training programs that support performance.

1.2.1 Cardiac adaptations

Of the numerous physiological adaptations that occur during the conditioning period, the cardiac effect of endurance training has been widely studied in both humans and dogs (Wyatt and Michell, 1974; Constable et al, 1994; Pluim et al, 2000; Downey et al, 2006). The term 'athlete's heart' is a common name given to the morphological and functional changes associated with endurance training. This term originates from the initial increase in oxygen and nutrient delivery to muscles during exercise causing a considerable increase in blood flow, about 13-fold, to support increased muscle metabolism (Hall, 2011). As cardiac output increases with exercise, a hemodynamic overload is induced ultimately causing the heart to adapt to increased workloads. Heart adaptations have been reported in human athletes since the late 1800's with changes including bradyarrhythmia, cardiac chamber enlargement, and overall cardiac hypertrophy (Prior & La Gerche, 2012). Cardiac hypertrophy is the most widely recognized adaptation to result from a chronic exercise regimen. This leads to increased vascular conductance, greater perfusion capacity of the muscle, increased stroke volume, and overall increased aerobic power (Saltin et al, 1968; Saltin et al, 1976l; Adeseye & Lookman, 2015). In terms of performance, these factors have been associated with faster running speeds, quicker recovery times post-exercise, and increased time to fatigue (Laughlin et al, 1989; Desai et al, 1997; Billman & Kukielka, 2007). Over time, these morphological changes can lead to alterations in various heart rate (HR) measurements and can be used to understand the adaptations of endurance exercise.

To monitor HR changes, electrocardiographic (ECG) recordings are made and used as a tool to support findings related to adaptations in cardiorespiratory fitness (Plews et al, 2013). Following cardiac adaptations, reduced HRs at both a rest and working state have been widely studied in humans (Sugawara et al, 2001), murine (Crews & Aldinger, 1967; Brum et al, 2000), and swine models (Laughlin et al, 1989). While dogs have been reported to undergo similar changes (Wyatt and Mitchell, 1974; Stone, 1977; Ritzer et al, 1980; Constable et al, 1994; Stepien et al, 1998), the ability to record HR is typically both invasive and performed in a laboratory setting. Fewer studies have been able to track the changes in HR during a bout of exercise in real-time. The ability to record HR of dogs in an in-field environment increases the ability to extrapolate to other sporting and working dogs. In addition, non-invasively recording HR at rest, during work, and post-exercise recovery states in mid-distance training sled dogs, provides an opportunity to understand how a training regimen impacts HR.

The rise in HR during exercise is controlled by the autonomic balance of sympathetic and parasympathetic activation. During an initial bout of exercise, the transition from rest to exercise is due to the inhibition of parasympathetic activity with an increase in sympathetic tone (O'Leary et al, 1997). During recovery, parasympathetic control increases, overwhelming the increased sympathetic activity causing a decrease in HR (O'Leary et al, 1997). As exercise continues, the body adapts to the increased workload resulting in the sympatho-vagal balance to become both quicker and more efficient, ultimately causing a quicker rise in HR during exercise as well as a faster recovery post-exercise (Darr et al, 1988; Sugawara et al, 2001; Buchheit et al, 2008). Following an exercise bout, the ability to recover quickly has been used as a predictor of aerobic fitness (Daanen et al, 2012). While most studies focus on changes in HR in the first minute post-exercise (Maeder et al, 2009), there is a dearth of literature pertaining to full recovery periods and changes HR encounters over time through a structured exercise regimen in dogs. If inadequate recovery time is provided, the ability to train at the desired intensity and duration is reduced and increases the risk of exercise related injury (Barnett, 2006). Dogs have been noted to recover HR to baseline levels within 30 minutes of a search and rescue type exercise (Rovira et al, 2008); however, recovery time varies depending on exercise intensity and individual differences. Heart rate recovery has also been reported following a single bout of exercise but has not been studied during a repetitive exercise program. Understanding this would provide new knowledge on canine exercise recovery, and how physiology changes with different exercise regimens.

1.2.2 Respiratory adaptations

Along with cardiac adaptations, respiratory changes also occur from exercise training. During exercise, the need for oxygen delivery to working muscles increases considerably. As such, breathing frequency, tidal volume, and minute ventilation increase and the subsequent increase in oxygen is transported throughout the body via increased blood flow. The respiratory center of the brain is located bilaterally in the medulla oblongata and pons of the brain stem. The dorsal respiratory group mainly causes inspiration, the ventral respiratory group causes expiration, and the pneumotaxic center is responsible for the rate and depth of breathing (Hall, 2011). With exercise and the accompanying increase in metabolism, O_2 consumption and CO_2 production increases approximately 20-fold from a resting state to maximal exercise (Hall, 2011). As the goal of respiration is to maintain a balance between O_2, CO_2 and hydrogen ions, the chemoreceptors in the brain are highly responsive to changes in these variables and dictate breathing rate accordingly (Nattie, 1998/1999; Ballantyne & Scheid, 2001; Hall, 2011). Increasing blood CO_2 by 25% results in a decrease in pH and significant increase in breathing frequency (Li & Nattie, 2002). However, the extent to which these gases change during exercise is minimal as the respiratory system is able to tightly regulate their concentrations in the blood (Hall, 2011). Thus, through repetitive bouts of exercise, pulmonary function ultimately shifts to a more efficient system resulting in greater diffusion capacity (Laughlin, 1985; Hall, 2011). However, the effects on dogs is understudied. Secondary to these changes, increases in VO_2max (maximal oxygen uptake), minute ventilation (amount of gas inhaled and exhaled from the lungs per minute), and tidal volume (volume of air that moves into and out of the lungs during a breathing cycle) have been reported following a structured exercise regimen indicating an adaptation to the increased workload (Musch et al, 1985; Busso et al, 2002; Downey et al, 2006; Thomas et al, 2012; Scheykhlouvand et al, 2018). However, the literature on respiratory parameters in a working dog model before, during, and following a structured exercise regimen is minimal. Measuring respiratory rate (RR) through non-invasive measurements of chest expansion and retraction, will increase our knowledge of how breathing changes during a conditioning period.

The effects of exercise on the respiratory system, specifically the breathing frequency in canines, is not well studied. As dogs must meet both metabolic and thermolytic needs during exercise, increased respiratory frequency by way of panting varies with dog and as such leads to different patterns of ventilation (Flandrois et al, 1971). Comparable studies examining breath rate in dogs show ranges of ~100-200 breaths/min during a single event using invasive recording techniques (Steiss et al, 2004; Rovira et al, 2008). Although RR hasn’t been widely studied following long-term training protocols, human athletes training 390km/ week (swimming, cycling, and running) or participating in 8 months of soccer training, experienced a decrease in breathing frequency (Bousanna et al, 2002; Di Paco et al, 2017). Thus, there is a possibility of an extended training period influencing breathing patterns. However, high frequency training can lead to potential overreaching or overtraining which can result in variations in respiratory parameters and decreases in performance (Busso et al, 2002). For example, excessive training may result in increased recovery time, whereas gain in performance (i.e. watts) and VO_2max, decrease (Tyler et al, 1996; Billat et al, 1999; Busso et al, 2002). The imbalance between training and recovery have been suggested to be the cause of these negative physiological changes. As such, the ability to monitor changes in RR may provide an indication of exercise performance, which could decrease the possibility of overtraining and improve the health and well-being of the dogs.

1.2.3 Thermoregulation

Another change occurring during exercise is the ability to regulate body temperature (BT). Thermoregulation is the ability of an organism to maintain a homeothermic state despite variable physiological changes and changes in environmental temperatures. Homeothermic animals, such as dogs, must maintain an equilibrium between heat production and heat loss, and the exchange between body surface and external environment aids in the maintenance of BT (Lewis & Foster, 1976). Canines rely on signals from the hypothalamus for temperature regulation which leads to an increase in salivation and panting (Hardy et al, 1964). During exercise there is an increased reliance on evaporation through panting for heat dissipation (Sharp et al, 1969). In contrast to panting for heat dissipation, sled dogs have adapted thick closely spaced fur (Randall et al, 2002), as well as non-shivering or adaptive thermogenesis in the skeletal muscles for maintenance of warmth (Davis, 1967). For example, shaved mongrel dogs exposed to -10°C for 8 hours daily for 6-8 weeks (Davis, 1967) developed non-shivering thermogenesis. Brown adipose tissue (BAT), commonly known for its main role in non-shivering thermogenesis in rats, is not present in adult dogs but instead white adipose tissue (WAT) dominates. White adipose tissue (which contains few mitochondria and less cytochrome oxidase) is mainly for energy storage but through chronic cold exposure, the dog's subcutaneous fat adopts more BAT characteristics (i.e. high cytochrome oxidase activity, densely populated with mitochondria, and presence of uncoupling protein) aiding in maintenance of BT (Holloway et al, 1985; Ashwell et al, 1987; NRC, 2006). During exercise, low ambient temperatures and cooling of dogs by way of ice packs has been attributed to lower rectal temperatures (Dill et al, 1932; Weibel et al, 1983; Kruk et al, 1985; Kozlowski et al, 1985). Thus, as sled dogs normally train in below freezing temperatures, attenuating the rise in rectal temperature via the environment in which they train, may positively influence exercise performance (Dill et al, 1932; Pohoska, 1979). However, as exercise reduces the need to generate heat for maintenance of BT during cold environments, the concern for losing heat by way of panting increases during increased activity level. As such, increased BT can become a limiting factor in exercise performance during physical activity (Gonzalez-Alonso et al, 1999).

Greater BT can have serious physiological consequences (Lewis & Foster, 1976) and the ability to dissipate heat during exercise is key to minimizing damage and maintaining training status. At body temperatures exceeding 42°C (107.5 °F) in dogs, deleterious events begin to take place (Adolph, 1947; Young et al, 1959; Lewis & Foster, 1976). Measuring BT has been recorded using various techniques including arterially (Pan et al, 1986), internal devices (Angle & Gillette, 2011), and whole-body imaging (Zangi, 2016). Conversely, absolute BT by way of rectal probes is the standard measurement for research in clinical and field studies. The average BT for a dog at rest is ~37-39°C (Baker & Miller, 2013) and the variability of BT at rest and during exercise is dependent on the individual dog. Numerous studies have recorded BT in dogs prior to and following exercise (Nold et al, 1991; Gillette et al, 2011; Piccione et al, 2012; Zangi, 2016). However, few studies follow the BT changes in sled dogs during exercise and with the completion of a conditioning period. Phillips et al (1981) recorded BT pre-run and post-run following a 3-month training program in sled dogs but did not report the BT changes over those 3 months. In addition, in this study, the dogs were running to a max distance of 16km, which does not represent typical sled dogs participating in mid-long-distance races (>50km). As a result, a training program of 16km in 3 months adds limited information to the true conditions of sled dog training. As well, the conditioning period has been reported to increase the efficiency of thermoregulation ultimately leading to a lower exercising BT (Piwonka et al, 1965; Shvartz et al, 1974). For example, as cardiac output increases during exercise, 17% of this increased blood volume is directed towards respiratory muscles (Hales ad Dampney, 1975) for support of heat exchange in dogs (Robbins et al, 2017). Through continual measurement of BT during exercise, both transient and dynamic physiological changes can be monitored compared to intermittent recordings.

1.3 Effects of tryptophan on physiological systems

In addition to exercise-induced physiological changes, nutritional interventions can both support and alter functional changes during exercise. Tryptophan (Trp) is an indispensable amino acid (AA) for dogs and used in the biosynthesis of proteins. It is metabolized into various active substances (i.e NAD^+, tryptamine) via two major pathways: the kynurenine pathway (KP), and the serotonergic pathway (SP). As the SP can influence whole body physiology, Trp supplemented in excess of its dietary requirement should increase production of central serotonin (5-hydroxytryptamine, 5-HT) (Fernstrom, 2013). Serotonin is a neurotransmitter that plays an important role in numerous physiological processes that may lead to modulation of HR and RR (Jacobs et al, 1971; Mitchell et al, 1984). However, for Trp to be used in the SP for production of 5-HT, Trp must cross the blood brain barrier (BBB). In doing so, Trp competes with large neutral amino acids (LNAA; valine, leucine, isoleucine, phenylalanine, and tyrosine) for access to the BBB transporters (Ruddick et al, 2006). Once in the brain, Trp is initially converted into 5-hydroxytryptophan by tryptophan hydroxylase (TPH), a rate-limiting enzyme that under normal physiological conditions is not fully saturated (O’Mahony et al, 2015). Therefore, increasing the Trp concentration in the brain should improve the likelihood of synthesizing central serotonin which, in turn, may alter HR and RR.

1.3.1 Effects of tryptophan and serotonin on heart rate

Exercise has been shown to induce increases in serotonin levels in the brain of both human and murine models (Brown & Huss, 1973; Brown et al, 1979; Dey et al, 1999; Zimmer et al, 2016). With increases in serotonin, the possibility to alter cardiovascular functions becomes increasingly possible as changes to 5-HT and 5-HT receptors change with endurance training (Jakeman et al, 1994). As many studies examine the effects of supraphysiological injections of serotonin on HR, the consensus in the literature pertaining to the cardiovascular effects is minimal as changes such as bradycardia, tachycardia, hypotension, hypertension, vasodilation, and vasoconstriction have all been reported (reviewed by Saxena & Villalon, 1990). A combination of various factors, including species and dose, contribute to the response observed following serotonin administration (Kottegoda et al, 1955; Write & Angus, 1989; Davis, 2017; Ionvino et al, 2019). In the dog model, following serotonin injections, changes to HR also do not follow a consistent pattern as both increases (Rudolph & Paul, 1957; Eckstein et al, 1971), decreases (Zucker et al, 198), and no change (Bove & Dewey, 1985; Bache et al, 1992) have all been reported. However, as sled dogs are highly active endurance athletes, there is a lack of literature pertaining to the effects of serotonin on HR during exercise. It has been proposed that certain 5-HT receptors in the central nervous system are downregulated and less excitable during exercise (Jakeman et al, 1994; Chennaoui et al, 2000). In addition, as sympathetic and parasympathetic activity shift with conditioning, the effect at which 5-HT creates a response is dependent on the sympathetic activity (Haddy et al., 1959; Emerson et al., 1973). Therefore, as sympathetic tone increases, 5-HT exerts a vasodilator effect while a decreased sympathetic tone results in a vasoconstriction effect. Thus, 5-HT can potentially affect HR during a conditioning period. However, to our knowledge, no research has previously examined dietary Trp supplementation during intensive exercise or throughout a conditioning period. Providing a dietary Trp supplement to progressively increase serotonin concentrations during a training season allows for a unique environment to non-invasively measure if cardiovascular functions are altered. As well, it allows for the opportunity to compare how serotonin levels differ in response to exercise itself and through Trp supplementation. As a decreased HR is commonly used as a marker of aerobic fitness, studying how serotonin - a neurotransmitter involved in cardiovascular development (Bouwknecht et al, 2000; Barrett et al, 2012) - can influence HR during exercise, provides the opportunity to determine if serotonin can support an animal during intense exercise while having no deleterious effects on performance.

1.3.2 Effects of tryptophan and serotonin on respiratory rate

Serotonin is a known regulator of a variety of brain functions including respiration (for review, see Jacobs & Azmitia, 1992; Hodges & Richerson, 2008: Hilaire et al, 2010). Although both stimulatory (Corcoran et al, 2014; Iovino et al, 2019) and inhibitory (Mitchell et al, 1984) effects of 5-HT have been described to influence respiratory motor output, the extent to which they are expressed depends upon the combined activation of pre and postsynaptic 5-HT receptors within neural structures of the respiratory control center (Hodges & Richerson, 2008, 2010; Dutschmann et al, 2009). For example, activation of the pre-synaptic 5-HT receptors results in an inhibition of other neurotransmitters which then decrease breathing frequency while activation of the post-synaptic receptors has a stimulatory effect (Hodges & Richerson, 2008). However, the mechanism behind which receptor will be activated to influence breathing frequency is still unknown. Serotonin injections in a sedentary and/or anesthetized dog model have resulted in increased respiratory depth and breathing rates (Douglas & Toh, 1952; Zucker et al, 1980). Although oversupplying Trp and its effects have not been widely studied, Paradis et al (1991) reported that an oversupply of Trp (0.6g of Trp/kg of body weight) led to respiratory distress in ponies. Therefore, studying the effects of dietary Trp supplementation on physiological parameters such as RR in an exercising model may provide insight into how 5-HT may affect breathing.

1.3.3 Future research directions

Overall, the effects of tryptophan and 5-HT on HR and RR of exercising dogs is a fairly new topic. Previous research suggests that 5-HT has varying effects on HR and RR in different animal models. Moreover, most studies used a supraphysiological concentration of serotonin and fail to compare how the subjects responded during a training bout. Future research is warranted to determine the effects of dietary Trp supplementation through increasing the Trp:LNAA ratio in exercising dogs to understand if serotonin may improve training and performance or if it leads to negative outcomes during exercise.

1.4 Effects of soluble fiber on physiological systems

Another dietary intervention that may influence whole-body physiology of sled dogs is dietary fiber. Dietary fiber can be divided into two categories depending on their solubility in water (i.e. soluble and insoluble fiber). Some soluble fibers have greater fermentation by gastrointestinal microbes, increase intestinal transit time, and slow nutrient absorption (NRC, 2006). Additionally, when fermented, soluble fibers produce short-chain-fatty-acids (SCFAs; acetate, propionate, butyrate) which act as energy substrates for cell growth and metabolism by the host. Some examples of soluble fibers include oat soluble fiber, flaxseed meal, chicory root, and isolated prebiotics. Prebiotics by definition are “nondigestible oligosaccharides that selectively stimulate the growth and/or activity of a limited number of resident colonic beneficial bacteria” (Gibson & Roberfroid, 1995). Insoluble fibers are not as extensively fermented by gastrointestinal microbiota but exert stool-bulking effects leading to decreased transit time as well as increased fecal mass (Meyer and Tungland, 2001). Given the variety of effects dietary fiber provides, it has also been used as a tool to improve exercise performance in athletes (Wu et al, 2013; Okamoto et al, 2019). For example, increased running times in mice has been reported following 6 weeks of exercise while consuming a high fiber diet containing hemi-cellulose and lignin (Okamoto et al, 2019). However, research on the extent of performance-related outcomes while consuming a soluble fiber enriched diet in exercising dogs is limited.

1.4.1 Effects of exercise and soluble fiber on the gut microbiome of dogs

The gastrointestinal microbiota of canines is a highly complex environment that influences the overall physiology of the individual through both immune status and nutritional support. The microbiota of a dog’s hindgut is dominated by anaerobic species of the *Bacteroidetes*, *Firmicutes*, and *Fusobacteria* family (Middelbos et al, 2010; Hand et al, 2013). One way of altering colonic bacteria is through the use of fermentative substrates. For example, prebiotics can improve the microbial population by increasing beneficial bacteria (*Bifidobacteria*, *Lactobacilli*, and *Eubacteria*) (Gibson & Wang, 1994; Araya-Kojima et al, 1995). However, if pathogenic bacteria dominate causing a microbial dysbiosis, instances of diarrhea and other unfavorable outcomes may occur (Campbell et al, 2015; Guard et al, 2015). In sporting dogs, diarrhea has been reported to accompany higher levels of enteropathogens (Long, 1993; McKenzie et al, 2010) and the leading cause of exercise discontinuation and performance loss (Gagné *et al*., 2013, Mackenzie *et al*., 2010). Although mushers rank dietary fiber low in importance when feeding their dogs (Templeman et al, 2017), provision of soluble fiber has led to fewer instances of diarrhea (Leib, 2000) as well as increases in beneficial bacteria (*Lactobacillus* and *Bifidobacterium*) (Gagné *et al*., 2013; Garcia-Mazcorro et al, 2017) which may support improved exercise performance. As such, increasing beneficial bacteria and minimizing diarrheal outcomes may contribute to improved functionality of physiological systems due to the gut-brain axis (Carbotti et al, 2015). For instance, the possibility of improved thermoregulation and respiratory capacity by way of an optimized gut environment and the subsequent improved ability to maintain hydration status would improve health and performance of sporting dogs. Thus, by examining the effects of dietary soluble fiber on gut microbes, BT, and RR in an exercising dog model, allows for a greater understanding of how the body responds and if performance can be improved.

1.4.2 Effects of soluble fiber on respiratory rate

The effects of dietary fiber on RR has not been extensively studied. The potential for microbiota to contribute to cardiorespiratory control is gaining more traction as respiratory dysregulation has been reported to alter microbiota (Golubeva et al. 2015;

Moreno-Indias et al, 2015, 2016). In a hypercapnia environment, rats given antibiotics experience decreased breathing frequency compared to control (O'Connor et al, 2019). In addition, during hypercapnia, rats with a higher abundance of *Clostridiales* have higher breathing frequencies (Golubeva et al, 2015). Together, these studies suggest that the gut microbiota may influence RR, however the mechanism behind such physiological change is not well understood. As such, improving the gut microbiota via increasing beneficial bacteria by way of soluble fiber may assist in breathing frequencies and may support exercise induced respiratory adaptations. As well, provision of soluble fiber results in SCFA production which function as major signaling mediators in maintaining physiological homeostasis (Stilling et al, 2014; Canfora et al, 2015). Short chain fatty acids can stimulate water absorption through the transport of anions across the apical membrane of the epithelial cells lining the gut (Herschel et al, 1981). As dogs rely primarily on panting and salivation for heat dissipation, decreased RR have been reported following dehydration in dogs (Kozlowski et al, 1980). This is due to an adaptative response of reduced breathing frequency to conserve water content (Baker et al, 1983; Horowitz & Nadel, 1984). However, there is a dearth of literature surrounding the effects of fiber, SCFA, gut microbiota, hydration status, and respiratory parameters in an exercising dog model.

1.4.3 Effects of soluble fiber on body temperature

Regulation of internal temperature during endurance exercise has been reported to be a limiting factor to performance (Gonzalez-Alonso et al, 1999). The effects of soluble fiber on BT have not been widely studied; however, potential benefits from provision of soluble fiber may have secondary outcomes influencing temperature regulation. For example, hydration status of an athlete has been reported to affect thermoregulation (Ramanzin et al, 1994; Bhatti and Firkins, 1995). Soluble fibers have greater water holding capacity and it has been suggested that the increased fermentation allows for the bound water to be available for absorption (McBurney et al, 1985). In addition, as fermentation of soluble fiber results in SCFAs, transport of these SCFA anions across the apical membrane of the epithelial cells lining the gut stimulates water absorption (Herschel et al, 1981). However, the main heat dissipation technique utilized in dogs is evaporative cooling through panting, and the loss of water from salivation. Over time, this can lead to signs of hypertonic dehydration (Templeman et al, 2020b) which can lead to increased BT (Kozlowski et al, 1980). For example, hydrated dogs have lower BT compared to dehydrated dogs (Kozlowski et al, 1980; Baker et al, 1983; Baker & Turlejska, 1989). However, as sled dogs run for long distances without water, maintaining a eu-hydrated state by increased soluble fiber intake may support thermoregulation during exercise (Zanghi et al, 2018). For example, horses fed diets high in soluble fiber had greater total body water and lower exercising BT (Spooner et al, 2008); however, it should be noted that unlike dogs, horses dissipate heat primarily through active sweating. Even so, the previous study suggests that the increased total body water content contributed to the thermoregulatory abilities leading to the decreased BT (Spooner et al, 2008).

Another possible mechanism influencing thermoregulation is the change in gut microbiota (Chevalier et al, 2015; Le Sciellour et al, 2019; Li et al, 2019). Specifically, mice lacking gut microbiota have been reported to have impaired thermogenic capacity during a cold-exposure test (Li et al, 2019) whereas transplanting cold-exposed microbiota (higher *Firmicute* abundance) has been reported to aid in maintenance of BT (Chevalier et al, 2015). As literature suggests soluble fiber increases beneficial microbial bacteria (ex. *Bifidobacterium*; Middlebos et al, 2007; Scott et al, 2008) and as sled dogs regularly train in below freezing environments, the combination of diet and environment may influence the dogs' thermoregulatory ability. However, there is a dearth of literature pertaining to the effects of dietary soluble fiber and the possible change in gut microbiota influencing the BT of cold-exposed exercising dogs. As such, monitoring the fluctuations in internal BT while providing an increased soluble fiber diet during a conditioning period can provide a basis for future research.

1.4.4 Future research directions

Overall, the effects of dietary fiber on physiological systems such as RR and BT has not been studied in an exercising dog model. Previous research suggests that soluble fiber, influencing hydration status and gut microbiota composition has the potential to influence breathing frequency and BT. As such, exercise performance may be influenced. Thus, future research is warranted regarding the effect of optimized dietary soluble fiber diets during incremental exercise on RR and BT in canine athletes.

1.5 Overall conclusions

While the effects of exercise on HR, RR, and internal temperature have been studied in humans, there are no current data on sled dogs and how these parameters change before, during and after a training season. Understanding how the body adapts to exercise could be a useful tool for mushers and trainers to maximize the adaptations in their dogs to support mid-distance racing. Additionally, both Trp and soluble fiber have the potential to modulate the cardiorespiratory system and thermoregulation of dogs training in cold environments. While the effects of supraphysiological injections of serotonin have been studied in dogs, the effects of increased dietary Trp supplementation on physiological parameters has not. In addition, dietary soluble fiber may be beneficial in actively training sled dogs by improving gut health and supporting physiological adaptations during exercise. Thus, provision of different nutritional interventions may support exercise induced adaptations and ultimately influence the performance of sporting dogs.

1.6 Thesis objectives and hypotheses

The research objectives of this thesis are to examine the effects of (1) repetitive endurance exercise, (2) Trp supplementation, and (3) increased dietary soluble fiber, on pre, mid, and post-exercise HR, RR, and internal BT in mid-distance training sled dogs. It is hypothesized that (1) increasing the duration of exercise and (2) providing supplemental dietary Trp will reduce HR and recovery time of heart and RR following a bout of exercise due to the effects of serotonin on the autonomic nervous system, (3) increased soluble fiber supplementation will decrease BT and RR due to alleviation of GI issues minimizing water loss through exercise-induced diarrhea and salivary losses.

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2 Exercise but not supplemental dietary tryptophan influences heart rate and respiratory rate in sled dogs

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2.1 Abstract

Tryptophan (Trp), an indispensable amino acid for dogs, is the precursor of serotonin, a neurotransmitter with a variety of effects throughout the body, including the ability to modulate cardiac and pulmonary activity. This study aimed to investigate the effects of a 12-week incremental exercise regimen and supplemental dietary Trp on heart rate (HR) and respiratory rate (RR) in client-owned sled dogs. Sixteen Siberian huskies were randomly allocated to either treatment or control diet groups. Both groups were fed a control diet (Trp to large neutral amino acid ratio of 0.047:1); however, treatment dogs received a Trp supplement to achieve a Trp to large neutral amino acid ratio of 0.075:1. Every three weeks, external telemetry equipment was used to non-invasively measure and record HR and RR at a resting, working, and post-exercise state in a controlled exercise challenge. A mixed model was used to test differences between diet, activity parameter, and week. Dietary Trp supplementation had no effect on HR or RR. Independent of diet, resting, working, post-exercise HR, and time to recover post-exercise HR decreased from week -1 to week 11 ($p < 0.05$). Resting HR had the greatest reduction from week -1 to week 11 (21%, $p < 0.05$). Working RR did not change with exercise ($p > 0.10$), but rRR and postRR decreased from week -1 to week 11 ($p < 0.05$). These data suggest that the exercise regimen the dogs were subjected to may have positively impacted the dogs' capacity to sustain aerobic exercise, whereas Trp supplementation had no effect on HR or RR.

2.2 Introduction

Sled dogs regularly participate in resistance and aerobic exercise in preparation for, and throughout, their competitive racing season. Their exercise performance can be affected by numerous factors, including ambient temperature (Robbins et al, 2017), feed intake (Orr, 1966), and intensity of exercise (Taylor, 1957). During prolonged exercise, as the workload increases, the oxygen and energy requirements of the muscles increase considerably. As such, heart rate (HR) and respiratory rate (RR) increase to sustain oxygen delivery and help maintain aerobic energy production (Rovira et al, 2008). Given the integral role of oxygen delivery in aerobic exercise performance, it is well accepted that the cardiorespiratory system is one of the limiting factors for extended bouts of exercise (Sonetti et al, 2001; Hall, 2011; Dunham & Harms, 2012). Thus, it is important to understand how the cardiorespiratory system can be augmented in order to maximize aerobic potential. In humans and dogs, the respiratory and cardiovascular systems respond to aerobic conditioning through the strengthening of the diaphragm and cardiac hypertrophy, respectively (Constable et al, 1994; Stepien et al, 1998; Pluim et al, 2000; Downey et al, 2007). These adaptations allow for the generation of greater force during inspiration and expiration, as well as ventricular contraction to maximize oxygen intake and delivery (Hodgson, 2014). This supports reductions in HR and improvements in respiratory capacity that have been reported following a structured exercise regimen (Sugawara et al, 2001; Huang et al, 2016). Therefore, by measuring specific changes in parameters such as HR and RR that occur in the dog in response to exercise, we can elucidate how a controlled training regimen may support cardiorespiratory adaptations and performance during a training season.

Dietary adaptations have also been reported to positively affect exercise performance in dogs. Dogs fed diets high in protein and fat (dietary protein > 30%, dietary fat > 40%) have been reported to outperform dogs fed a low-protein, low-fat, and high-carbohydrate diet (dietary protein < 30%, dietary fat < 40%) in areas such as hunting (more successful finds) (Davenport et al, 2001), stamina (Kronfeld, 1973), and decreased risk of injury (8 times lower) (Reynolds et al, 1999) during endurance type exercise. The goal of mushers and trainers is to maximize the performance of their dogs, and nutritional solutions may assist and support adaptations to the cardiorespiratory system.

Tryptophan (Trp) is an indispensable amino acid (AA) for dogs and the sole precursor for serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter that can trigger various physiological responses within the body. The binding of serotonin to specific receptors can elicit effects on HR and RR by either stimulatory or inhibitory mechanisms. However, the central biosynthesis of 5-HT to evoke a response in HR and RR is dependent on the Trp concentration in the central nervous system (CNS) (Dalton, 1986; Lalley et al, 1994; Berger et al, 2009). In order to be used for central serotonin synthesis, Trp must compete with the large neutral AA (LNAA; valine, leucine, isoleucine, phenylalanine, and tyrosine) for access to blood brain barrier (BBB) transporters (Ruddick et al, 2006). Once in the brain, Trp is initially converted into 5-hydroxytryptophan by tryptophan hydroxylase (TPH), a rate-limiting enzyme that under normal physiological conditions is not fully saturated (O'Mahony et al, 2015). Therefore, increasing the Trp:LNAA ratio should increase the likelihood of synthesizing central serotonin which, in turn, may alter cardiac and pulmonary function. The effects of serotonin on pulmonary and vascular responses have been reported in various animal models; however, responses vary depending on the species. Following supraphysiological serotonin injections, cats experienced a decreased HR (Jacobs et al, 1971) and dogs an increased HR (Eckstein et al, 1971), whereas rats experienced significant changes to RR in the form of apnoea (Mitchell et al, 1984), and rabbits had an elevated RR (Iovino et al, 2019). These responses are a result of direct injections of serotonin, and, to our knowledge, the effect of changes in serotonin status by way of increased dietary Trp supplementation on cardiovascular and respiratory outcomes has not been evaluated in dogs. By utilizing sled dogs, a cohort that is routinely subjected to prolonged bouts of strenuous exercise and who share similar genetics, dietary management, housing, and training regimens, we can examine the effects of Trp supplementation and exercise on HR and RR using fewer animals than if an alternative cohort of healthy adult dogs at maintenance was used. Thus, the objectives of this study was to investigate the effects of 12 weeks of incremental exercise conditioning and an increased Trp:LNAA ratio via supplemental dietary Trp on pre, mid, and post-exercise HR and RR in actively training sled dogs.

2.3 Materials and Methods

2.3.1 Animals and housing

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 years (mean ± standard deviation, SD) 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, Canada) that had been approved by the University of Guelph's Animal Care Services. During the study, dogs were housed in free-run, outdoor kennels (3.5–80 square meters) containing anywhere from 2 to 10 dogs each, in which one house per dog was provided for rest and shelter. The study was approved by the Animal Care Committee of the University of Guelph (Animal Use Protocol #4008).

2.3.2 Diet and Study Design

For complete diet and study design, refer to Templeman et al. (2020). In brief, dogs were blocked for age, sex, and BW before being randomly allocated to one of two diet 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 weeks prior to the study period (weeks -2 and -1), all dogs were acclimated to the dry, extruded Ctl diet (dry matter basis: 4074 kcal/kg metabolizable energy (ME), 94% dry matter (on an as-fed basis), 16% nitrogen-free extract (NFE), 47% crude protein, 25% fat, 0.46% Trp, 1.14 g Trp/Mcal; Champion Petfoods LT., Morinville, AB, Canada) 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. For additional details regarding the ingredient and nutrient composition of the Ctl diet, refer to Templeman et al. (2020). Dogs in the Ctl group were fed the Ctl diet throughout the entire study period, while Trt dogs were fed the Ctl diet supplemented with dietary Trp (ADM Animal Nutrition, Woodstock, ON, Canada) so as to achieve a Trp:LNAA ratio of 0.075:1 (dry matter basis: 0.73% Trp, 1.80 g Trp/Mcal; Trp:LNAA ratio of Ctl diet was 0.047:1). This ratio was determined with the goal of exceeding both the minimum dietary Trp:LNAA ratios as derived from the National Research Council's (NRC, 2006) suggested minimum amino acid requirements for adult dogs at maintenance (Trp:LNAA ratio of 0.061:1) as well as the highest Trp:LNAA ratio used when feeding medium or large breed dogs their estimated Trp requirements as determined using indicator amino acid techniques (Trp:LNAA ratio of 0.074:1, medium breed dogs) (Templeman et al, 2019). All dogs were fed once daily, and 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, dogs had ad libitum access to fresh water.

2.3.3 Exercise regimen

For the complete exercise regimen, refer to Templeman et al. (2020). In brief, a 12-week exercise regimen was implemented, with exercise distance increasing incrementally throughout the trial period. However, weather played a role in setting the daily distance. Training consisted of all dogs running on a standard 16-dog gangline that was attached to an all-terrain vehicle (ATV). A pace of approximately 15 km/h was averaged throughout the training period. Every three weeks, starting on week -1 (weeks

-1, 2, 5, 9, 11), one off-day in the dogs' training schedule (no running) was replaced by an exercise challenge. Dogs were run at a consistent distance at a pace of ~15 km/h in four-dog teams. At week -1, the team of 4 dogs that the owners deemed the least experienced and physically fit were run at a pace of ~15 km/h until any one dog presented with a HR exceeding 300 beats per min (bpm) (Van Citters & Franklin, 1969) or displayed any of the predetermined fatigue-associated signs, such as lack of motivation, loose tug line or tight neckline, leaning on gang line, increased salivation, or any other changes deviating from normal exercise behavior. Thereafter, all exercise challenges for all groups of dogs throughout the study (weeks -1, 2, 5, 11) were run at this distance, which was set at 4 km and at a pace of 15 km/h. During each exercise challenge, all dogs were equipped with external telemetry jackets to non-invasively record both HR and RR (emka TECHNOLOGIES, Falls Church, VA, USA).

2.3.4 Telemetry Jackets

Jacketed non-invasive telemetry devices (emkaPACK 4G, emka TECHNOLOGIES, Falls Church, VA, USA) were used to collect the HR and RR sensor data. For non-invasive recordings of HR, electrocardiographic (ECG) readings were obtained from the placement of 4 electrodes on each dog: 2 placed axillar and 2 placed posterior to their right and left scapula (Figure S1). Before placement of electrodes, each attachment site was shaved, cleaned using isopropyl alcohol to remove any lipids on the skin surface, and wiped with skin adhesion wipes (Smith & Nephew, London, UK). To minimize signal noise during a session, electrodes and wires were taped and securely wrapped around the dog's body to prevent any movement during recording. In addition, an undershirt and jacket were worn by each dog to help secure electrodes and wires into position and to reduce exposed wires which were likely to be chewed, caught on objects, or induce noise. Heart rate data were recorded every 10 s during the exercise challenges, using the IOX software from emka TECHNOLOGIES (emka TECHNOLOGIES, Falls Church, VA, USA). Only HRs exceeding an 80% success rate as well as appropriate ECG signals through examination of the ECG trace were used in the current study, to distinguish heartbeats from artifacts. This technique has been previously validated in dogs (Prior et al, 2009). In addition, discrete stages of HR were recorded, which comprised resting HR (rHR), working HR (wHR), post-exercise HR (postHR), and totalHR (average of all activity levels combined). Each HR stage was recorded for all dogs at each exercise challenge. In addition, the time required to reach a post-exercise HR (tpostHR) was also determined.

Non-invasive measurements and recordings of RR were obtained using respiration inductance plethysmography (RIP), which utilizes custom fit respiratory bands placed around the dogs' thoraxes to measure the expansion and retraction of the thorax during a breathing cycle (Figure S1). Respiratory rate data were recorded, and mean values were derived for every 5 breaths. Breath detection thresholds were established for each subject by observing the signal amplitudes of the RIP sensors during peak inspiration and expiration and setting the detection thresholds to a level sufficiently above the noise floor. Additionally, the data were filtered using a digital bandpass filter with a 0.1 to 30 Hz @-3 dB. The band pass filter was used to allow breathing frequencies between 0.1 and 30 Hz to be detected and recorded, while any other frequencies not within this range were attenuated. In addition, only RRs exceeding an 80% success rate were used in the current study, to distinguish breaths from artifacts. Resting RR (rRR), working RR (wRR), post exercise RR (postRR), and totalRR (average of all activity levels combined) were recorded at each challenge. In addition, the time required to reach a post-exercise RR (tpostRR) was recorded.

All resting data were collected when the dogs were lying quietly in a secluded room with limited dog-to-dog or human-to-dog interaction for approximately 1 h to allow their HR and RR to decrease to resting. Following resting data collection, dogs were put into harnesses and placed on the 4-dog gangline, where the challenge run commenced and where wHR and wRR were recorded. Once the challenge ceased, dogs were immediately watered and remained on the gangline for 1 h to continue gathering HR and RR data until postHR and postRR were obtained, from which tpostHR and tpostRR data were determined.

2.3.5 Statistical analyses

All statistical analyses were performed with the Statistical Analysis System (SAS v. 9.4; SAS Institute Inc., Cary, NC, USA). Heart rate and RR data were analyzed for outliers by removing the data points that exceeded a HR greater than 350 bpm and lower than 10 bpm. For RR, data points greater than 200 breaths per min and lower than 5 breaths per min were also considered outliers and subsequently removed. In addition, the periods during the exercise challenges when a 4-dog team was stopped were removed as these interruptions interfered with the dogs’ ability to maintain a wHR or wRR. These stops included instances of defecation and urination, as well as distractions such as wildlife which caused the dogs to alter their course and/or stop the run briefly. In addition, as exercise challenges were run on back roads, stops were made when crossing streets and turning around at the halfway point of the run. Following the outlier removal, a TRANSREG procedure was used to transform the data before HR and RR were analyzed as a repeated measure over time using PROC GLIMMIX. When fixed effects were significant, means were compared using the Tukey Honestly Significant Difference (HSD) test. Dog was treated as a random effect, and activity (resting, working, post exercise, and time to post-exercise), week, and diet group were treated as fixed effects. Time to post-exercise HR and RR was determined from the point at which exercise stopped following an exercise challenge until a plateaued HR and RR was measured using a TRANSREG procedure. The time point from immediate cessation of exercise up until the plateau was achieved was used, and a mean time was calculated for recovery. A PROC CORR was used where appropriate to assess the strength of a linear relationship between all activity parameters (rHR, rRR, wHR, wRR, postHR, postRR, tpostHR, tpostRR), temperature (°C), and week. Statistical significance was declared at $p < 0.05$ and trends at $p \leq 0.10$.

2.4 Results

During data analysis, 17% (43/256) of the HR observations and 15% of RR observations (29/192) were removed due to either software malfunctions, which caused inaccurate readings, or interruptions during the challenge run, which altered with wHR or wRR. Two dogs were removed from the trial (one Ctl on week 7; one Trt on week 9) due to exercise-related injuries. All data collected from these dogs until their removal were included in the analyses. Due to inclement weather, adjustments were made to the proposed 12-week incremental training regimen, including the removal of the week 9 exercise challenge and a descaling of the daily running duration by approximately half (maximum distance run was 34 km by week 12). Environmental temperatures for the four exercise challenges were recorded using a digital thermometer (Gain Express, Hong Kong, China). The mean ambient temperature for each of the four challenge days were as follows: 5 °C (week -1), 4 °C (week 2), -1 °C (week 5), 2 °C (week 11). For information regarding food intake, run distance, BW, and additional details regarding the training schedule, refer to Templeman et al. (2020). In brief, food intake, run distance, and BW did not differ between diet groups, but all differed between weeks. Run distance followed an incremental increase until week 7, when we were forced to return to distances equivalent to week 4. Food intake was greatest at weeks 6 and 7, before our runs were descaled and diet intakes were correspondingly reduced to week 5 levels. Body weight was lowest from week 3 to week 7, but baseline body weights were maintained from weeks 8 to 11.

2.4.1 Heart Rate

No differences were found between the diet groups for rHR, wHR, postHR, or tpostHR ($p > 0.10$); therefore, these data were pooled to report the effect of exercise. For all activity parameters (resting, working, and recovery post-exercise), HR significantly decreased by week 11 when compared to week -1 values ($p < 0.05$, **Table 2.1).** Resting HR had the greatest reduction in proportion to baseline values over time

(21%, $p < 0.05$), while wHR had the smallest reduction, at 17% ($p < 0.05$; **Table 2.2**). Time required to recover HR post-exercise was significantly decreased by week 5 ($p < 0.05$; **Figure 2.1**) but had no difference thereafter ($p > 0.10$). Environmental temperature was not correlated with any activity parameter ($p > 0.10$). Run distance was negatively correlated with rHR ($r = -0.441$, $p < 0.05$) and postHR ($r = -0.361$, $p < 0.05$) but had a tendency to be correlated with wHR ($r = -0.274$, $p = 0.07$). No differences were found when comparing sex, BW, or age with rHR, wHR, and postHR over weeks ($p > 0.10$).

Table 2.1 Least square mean heart rate (beats per minute) at rest, work, and post-exercise on week -1, 2, 5, and 11 challenges in sled dogs running at a pace of 15km/h for 4km.

Activity Parameter [1]	Exercise Challenge				SEM [2]	*p* Value		
	Week −1	Week 2	Week 5	Week 11		Week	Diet	Week x Diet [3]
totalHR, bpm [4]	194 [a]	182 [b]	179 [b,c]	168 [c]	3	<0.01	0.10	0.57
rHR, bpm	91 [a]	79 [b]	71 [b]	68 [b]	4	<0.01	0.38	0.57
wHR, bpm	270 [a]	249 [a,b]	261 [a,b]	234 [b]	9	<0.01	0.10	0.17
postHR, bpm	110 [a]	101 [a,b]	95 [b]	92 [b]	4	<0.01	0.55	0.85

[1]TotalHR, total mean heart rate; rHR, resting heart rate; wHR, working heart rate; postHR, post-exercise heart rate.
[2] SEM, standard error of the mean, $n = 16$ for rHR (weeks −1, 2, and 5), $n = 14$ for rHR (week 11), $n = 16$ for wHR (week −1), $n = 12$ for wHR (week 2), $n = 7$ for wHR (week 5), $n = 10$ for wHR (week 11), $n = 16$ for postHR (week −1), $n = 14$ for postHR (weeks 2, 5, and 11).
[3] Interaction effect between week and diet.
[4]bpm, beats per minute. [a,b,c] Values in a row with different superscripts are different ($p < 0.05$).

Table 2.2 Change from baseline (week -1) as proportion of heart rate, work, adnd post-exercise on week -1, 2, 5, and 11 challenges in sled dogs running at a pace of 15 km/h for 4 km.

Activity Parameter [1]	Exercise Challenge				SEM [2]	*p* Value		
	Week −1	Week 2	Week 5	Week 11		Week	Diet	Week x Diet [3]
totalHR, % of total	194	−10 *	−12 *	−17 *	2	<0.01	0.10	0.57
rHR, %	91	−8	−20 *	−21 *	4	<0.01	0.30	0.81
wHR, %	270	−15 *	−5	−17 *	3	<0.01	0.18	0.06
postHR, %	110	−11	−16	−19 *	4	<0.01	0.87	0.88

[1]TotalHR, total mean heart rate; rHR, resting heart rate; wHR, working heart rate; postHR, post-exercise heart rate.

[2] SEM, standard error of the mean, *n* = 16 for rHR (weeks −1, 2, and 5), *n* = 14 for rHR (week 11), *n* = 16 for wHR (week −1), *n* = 12 for wHR (week 2), *n* = 7 for wHR (week 5), *n* = 10 for wHR (week 11), *n* = 16 for postHR (week −1), *n* = 14 for postHR (weeks 2, 5, and 11).

[3] Interaction effect between week and diet. * Values in a row with an asterisk are different from baseline ($p \leq 0.05$).

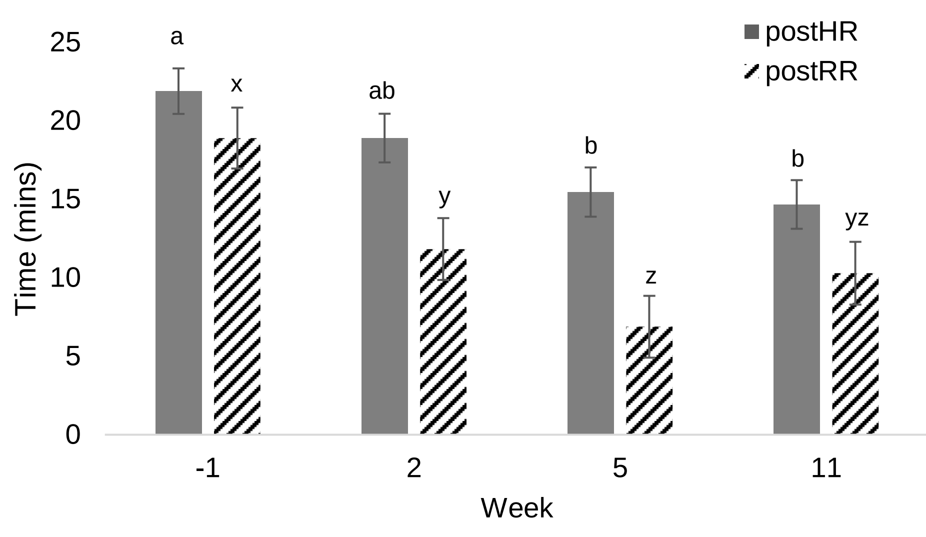

Figure 2.1 Total mean time ± standard error (SEM) to reach post-exercise heart rate (postHR) and respiratory rate (postRR) after exercise challenge on weeks −1, 2, 5, 11 in sled dogs running at a pace of 15 km/h for 4 km. [a,b,c] HR or RR values with different superscripts are different among weeks ($p < 0.05$).

2.4.2 Respiratory Rate

No differences were found between diet group and rRR, wRR, or postRR ($p > 0.10$); therefore, these data were pooled to report the effects of exercise. Control dogs had a more rapid recovery from baseline ($p < 0.05$), while Trt dogs took 3 weeks longer to recover ($p < 0.05$; **Table 2.3**); however, there was no diet effect ($p > 0.10$; **Table 2.3**). No other differences were observed between diet groups and tpostRR ($p > 0.10$); as such, the diet groups were pooled to examine the effects of training. Respiratory rate was greatest at week −1 compared to week 11 for both resting and post-exercise activity levels ($p < 0.05$; **Table 2.4**). There was no change in wRR over time ($p > 0.10$; **Table 2.4**). Time to post-exercise RR decreased over time and was negatively correlated with week (r = −0.354, $p < 0.05$). No differences were observed in tpostRR between weeks 2 and 11 or weeks 5 and 11 ($p > 0.10$; **Figure 2.1**). Environmental temperature was positively correlated postRR (r = 0.457, $p < 0.05$) but was not correlated with wRR ($p >0.10$). Run distance was negatively correlated with rRR (r = −0.409, $p < 0.05$) and postRR (r = −0.481, $p < 0.05$) but was not correlated with wRR ($p > 0.10$). No differences were found when comparing age, BW, or sex with rRR, wRR, or postRR over weeks ($p > 0.10$).

Table 2.3 Least square mean time to recovery respiratory rate (mins) post-exercise for both diet groups on week −1, 2, 5, and 11 challenges in sled dogs running at a pace of 15 km/h for 4 km.

Diet Group [1]	Exercise Challenge				SEM [2]	*p* Value		
	Week −1	Week 2	Week 5	Week 11		Week	Diet	Week x Diet [3]
Trt, mins	19 [a]	16 [a]	6 [b]	12 [a,b]	2	<0.01	0.37	0.06
Ctl, mins	18 [a]	7 [b]	7 [b]	8 [b]	2	<0.01		

[1] Trt, treatment; Ctl, control.
[2] SEM, standard error of the mean, *n* = 7 for Trt (weeks −1, 2, and 11), *n* = 8 for Trt (week 5); *n* = 8 for Ctl (week −1), *n* = 7 for Ctl (week 2), *n* = 6 for Ctl (weeks 5 and 11).
[3] Interaction effect between week and diet. [a,b,c] Values in a row with different superscripts are different ($p \leq 0.05$).

Table 2.4 Least square mean respiratory rate (breathing frequency) at rest, work, and post-exercise on week −1, 2, 5, and 11 challenges in sled dogs running at a pace of 15 km/h for 4 km.

Activity Parameter [1]	Exercise Challenge				SEM [2]	*p* Value		
	Week −1	Week 2	Week 5	Week 11		Week	Diet	Week x Diet [3]
totalRR, bf [4]	75 [a]	73 [a,b]	69 [b]	68 [b]	1	<0.01	0.36	0.81
rRR, bf	23 [a]	19 [a,b]	15 [c]	16 [b,c]	1	<0.01	0.66	0.12
wRR, bf	176 [a]	172 [a]	174 [a]	169 [a]	4	0.50	0.86	0.74
postRR, bf	27 [a]	26 [a]	18 [b]	20 [b]	1	<0.01	0.17	0.55

[1] totalRR, total mean respiratory rate; rRR, resting respiratory rate; wRR, working respiratory rate; postRR, post-exercise respiratory rate.
[2] SEM, standard error of the mean, *n*= 14 for rRR (week −1), *n* = 16 for rRR (weeks 2, 5), *n* = 13 for rRR (week 11), *n* = 15 for wRR (week −1), *n* = 14 for wRR (week 2), *n* = 8 for wRR (week 5), *n* = 9 for wRR (week 11), *n* = 15 for postRR (week −1), *n* = 14 for postRR (weeks 2 and 5), *n* = 13 for postRR (week 11).
[3] Interaction effect between week and diet.
[4] bf, breathing frequency (breaths per minute). [a,b,c] Values in a row with different superscripts are different ($p \leq 0.05$).

2.5 Discussion

The data presented herein indicate that for mid-distance training sled dogs, 12 weeks of dietary Trp supplementation had little to no effect on HR or RR. However, the conditioning regimen that the dogs were subjected to may have resulted in a reduction in HR and RR as well as a more rapid recovery to a resting state following exercise.

Our laboratory previously reported that Trp supplementation, at levels necessary to achieve a dietary Trp:LNAA ratio of 0.075:1, resulted in an increased 5-HT level in Trt dogs by week 11 (Templeman et al, 2020). In the current study, dogs fed the Ctl diet presented with more rapid RR recovery times by week 2, whereas dogs fed the diet supplemented with Trp recovered to the same extent by week 5. However, there was no diet effect on RR recovery, suggesting that Trp supplementation did not affect respiratory parameters during the exercise regimen. As previous research reports serotonin increases HR and RR in dogs (Eckstein et al, 1971; Zucker & Cornish, 1980), the minimal effects presented in the current study suggest that the increased Trp concentrations identified in serum by week 5 (Templeman et al, 2020) may not have been to such an extent as to influence 5-HT or cardiac and respiratory rates. In addition, no changes were observed in HR or RR during week 11 when 5-HT concentrations were increased (Templeman et al, 2020). It has been previously hypothesized that the intensity of aerobic exercise influences the sensitivity and downregulation of 5-HT receptors in various animal models (Jakeman et al, 1994; Chennaoui et al, 2000). As the dogs in the current study were subjected to a training regimen that accumulated a total run distance of ~1200 km, the possibility of the downregulation of 5-HT receptors by week 11 of the current study could support the limited changes seen. Therefore, as 5-HT levels in serum were not increased until the final week of the study (week 11), future research is warranted to investigate the effects of HR and RR over an extended period of time. In addition, Trp metabolism is influenced by two major pathways, the kynurenine and the serotonergic pathways. The kynurenine pathway, which consumes more of the available free Trp than the serotonergic pathway (Bender, 1983), is involved in ATP production, with oxidation as the main outcome and nicotinamide adenine dinucleotide (NAD) as a minor outcome (Hodgson, 2014). Upregulation of the kynurenine pathway by various factors during exercise (i.e., steroid hormones and inflammatory cytokines) (Tremblay et al, 2004; Suzuki et al, 2006; Comassi et al, 2015) could explain the lack of treatment effect on HR and RR, where the majority of supplemented Trp was used for protein synthesis and energy production through the kynurenine pathway rather than for cardiorespiratory modifications. However, the current study did not measure kynurenine pathway metabolites, steroid hormones, or inflammatory cytokines and, as such, cannot definitively say that Trp metabolism was largely involved in the kynurenine pathway over the serotonergic pathway. Regardless, independent of Trp supplementation, the reduction in HR and RR following a conditioning regimen is consistent with previous studies in dogs, indicating that whole-body physiological adaptations may have occurred (Ordway et al, 1982; Stepien et al, 1998; Billman et al, 2015) .

The reductions in resting, working, and post-exercise HR observed in the present study are comparable to previous research examining both sled dogs and mongrel dogs (Wyatt & Mitchell, 1974; Constable et al, 1994; Stepien et al, 1998). For example, Stepien and colleagues (1998) noted a 15% reduction in rHR in Alaskan huskies following 5 months of aerobic training, while Tipton et al. (1974) reported a 40-bpm difference in wHR following 10 weeks of aerobic training in mongrel dogs. In addition, Billman and Kukielka (2007) examined the post-exercise HR of 10 dogs before and after 10 weeks of aerobic training and reported an overall decrease in HR and more rapid recovery to resting HR following exercise. These changes were attributed to adaptations in autonomic control and cardiac morphology. As a 21% decrease in rHR, 35-bpm reduction in wHR, and quicker recovery times post-exercise were observed in the current study, it is likely that these dogs also underwent physiological modifications.

Previous studies investigating cardiorespiratory measurements following various training levels (8 months to 5 years) in humans, reported decreases in breathing frequency when compared to sedentary and lesser trained counterparts (Boussana et al, 2002; Di Paco et al, 2017). In the current study, decreases in RR were observed at both a resting and post-exercise state, whereas no changes were reported in wRR. However, due to adjustments that were made to the training regimen in the current study, the lack of differences observed in wRR may have been attributed to a reduction in training load. As descaling of the training regimen took place at week 7, the maximum distance run by week 11 was equivalent to the distance run by week 4 (34 km). As such, the incremental training regimen was no longer implemented from week 7 onward. Therefore, the duration of the training program may not have been to the extent required to induce physiological adaptations (Blomqvist & Saltin, 1983). However, as wHR decreased, our data may suggest that the respiratory system requires consistent training in order to adapt to exercise, as it appears to be more sensitive to a reduced training workload. In support of this, Amory et al. (1988) reported that, following a 4-week training program in ponies, 2 weeks of detraining caused RR to vary in breathing frequency at both exercise and recovery states, while no such changes were reported in HR. This study, as well as the current study, underpin the importance of continuous training for the maintenance of training-induced adaptations. However, even though wRR did not change following a conditioned state, rRR, postRR, and tpostRR decreased with 12 weeks of training, suggesting a possible training effect. However, independent of exercise-induced decreases in HR and RR, habituation to novel objects can also affect cardiorespiratory parameters (Leiner & Fendt, 2011). As no adaptation period was provided to the dogs in the current study for exercise challenge days, the decreases in HR and RR could indicate a possible habituation to the novel equipment and set-up over time, rather than to the exercise regimen itself. Thus, providing a period of adaptation to the equipment prior to the study measures, as well as investigating the effects of habituation to the equipment, in both exercised and sedentary control groups of dogs could be beneficial in providing further insight.

Since sled dogs are known to train and race in cold climates, both the environment in which their exercise takes place and their internal temperature could influence cardiorespiratory outcomes (Kruk et al, 1985; Saursaunet, 2010). Dogs do not dissipate heat in the same manner as humans; rather, dogs rely primarily on panting for heat dissipation during exercise (Lewis & Foster, 1976). In the current study, both HR and RR were found to be correlated with temperature, indicating that as ambient temperature increased, so too did the beat and breathing frequency. However, as the warmest weather was measured at week −1 of the study (5 °C), the correlation between HR and RR with temperature could also be attributed to the unconditioned state of the dogs. This can also be seen as week 5 was recorded with the lowest temperature at −1 °C, whereas week 11 was 2 °C, and no differences between these two exercise challenges were observed, suggesting that environmental temperature may have had little effect on HR and RR.

The authors do acknowledge that there were limitations to this study that should be noted. Foremost, with regard to cardiorespiratory parameters, only HR and RR were measured; however, other cardiorespiratory parameters, such as heart rate variability, stroke volume, maximal oxygen consumption, minute ventilation, and tidal volume, could be monitored alongside HR and RR to attempt to elucidate the changes occurring during a training period. In addition, no adaptation period to the equipment was given prior to the start of the study or collection of body temperatures during exercise challenges, which may have had a possible influence on HR and RR. In addition, the descaling of the incremental training regimen and removal of an exercise challenge day may have impacted our measured parameters. Future studies are warranted to investigate the influence of an incremental training regimen and dietary Trp supplementation on sled dogs, using additional cardiorespiratory outcomes while also considering the effects of body temperature, environmental temperature, and habituation responses on HR and RR.

2.6 Conclusions

Overall, the aim of this study was to evaluate the effects of dietary Trp supplementation as well as a 12-week training regimen on the outcomes of HR and RR in client-owned Siberian huskies training for mid-distance races. The findings of the current study suggest that repetitive endurance exercise potentially promotes the adaptation of both cardiac and pulmonary systems, as evident through reductions in mean HR, RR, and the time required to achieve a recovery state post-exercise. These data indicate that after 12 weeks of exercise conditioning, independent of Trp supplementation, rHR, rRR, wHR, postHR, and postRR decreased from week −1. In addition, within the constraints of this study design, there were no effects of Trp supplementation on HR and RR, but future research regarding Trp supplementation, serotonin status, an incremental training regimen, and various cardiorespiratory parameters is warranted. In addition, familiarity with the equipment as well as body temperature could also have influenced our results and should be considered. However, this research suggests that when developing a training regimen, maintenance of incremental increases in intensity and duration may support performance in sled dogs during mid-distance races.

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3 Effects of exercise and supplemental fiber on the respiratory rate, body temperature, and body composition of mid-distance training sled dogs

3.1 Abstract

Dietary fiber can affect physiology in a variety of ways, such as increasing colonic absorption of water and improving overall gut health, which may positively impact exercise performance. The objectives of this study were to investigate the effects of increased dietary soluble fiber (insoluble: soluble fiber ratio of 3:1 vs an insoluble: soluble fiber ratio of 4:1) and an incremental training regimen on the outcomes of respiratory rate (RR), internal body temperature (BT), body composition, and markers of health and nutritional status in mid-distance training sled dogs. Fourteen dogs (12 Siberian and 2 Alaskan huskies) with a mean age of 3.7 ± 2.7 years and body weight (BW) of 21.5 ± 2.8 kg were blocked by age, sex, and BW, and randomly allocated into one of two diet groups. Seven dogs were fed a dry extruded control diet (Ctl; 4 males, 3 females) with an insoluble: soluble fiber ratio of 4:1 (1% soluble fiber on an as-fed basis), and seven dogs were fed a dry extruded treatment diet (Trt; 5 females, 2 males) with an insoluble: soluble fiber ratio of 3:1 (2% soluble fiber on an as-fed basis). Each week, BW was measured, and feed allotments were adjusted to maintain baseline BW. Fasting blood samples were taken every three weeks to assess markers of nutritional and health status. All dogs underwent 9 weeks of incremental exercise conditioning where the exercise regimen was designed to increase in distance each week. Every three weeks, external telemetry equipment was used to non-invasively measure and record RR and internal BT at a resting, working, and post-exercise recovery state during a controlled exercise challenge. Body composition was measured on weeks -1 and 9 using quantitative magnetic resonance. Body composition, RR, BT, fasted blood samples, feed intake, BW, and environmental temperature were analyzed using a mixed model with dog as a random effect and week and diet group as fixed effects. Treatment dogs had lower working and post-exercise BT compared to Ctl ($P < 0.05$). In addition, Trt dogs had a lower recovery BT at week 2 and 5 compared to Ctl dogs ($P < 0.05$). Diet had no effect on RR or body composition ($P > 0.10$). Nine weeks of incremental exercise resulted in a 7% increase in lean and 3.5% decrease in fat mass ($P < 0.05$). These data suggest that increasing dietary soluble fiber may positively influence BT but does not affect RR or body composition. Overall, increased soluble fiber could be included in diets of actively training sled dogs without compromising their health or performance.

3.2 Introduction

Exercise has the capacity to affect whole body physiology; however, the level of high intensity endurance training, such as that experienced by sled dogs, can lead to gastrointestinal disturbances, heat stress, and possible dehydration (Mackenzie et al, 2010; Gagne et al, 2013). Nutritional solutions, such as increased dietary soluble fiber inclusion, may support exercise performance through mitigation of these deleterious effects of exercise (Gibson & Wang, 1994; Araya-Kojima et al, 1995; Wu et al, 2013; Okamoto et al, 2019).

Dietary fiber can be divided into two categories based on its solubility in water (i.e. soluble and insoluble fiber) (Howarth et al, 2001; Bronkowska et al, 2018). Soluble fibers typically have an increased extent of fermentation by gastrointestinal microbes yielding short-chain fatty acids (SCFA; mainly acetate, propionate, and butyrate) (Howarth et al, 2001). Short chain fatty acids have various physiological effects, including regulation of satiety (Bosch et al, 2009), improvement of gut health (Swanson et al., 2002), and increased water absorption (Herschel et al, 1981). As sled dogs primarily thermoregulate via respiratory evaporation (i.e. panting), water loss by way of salivation increases considerably during exercise leading to signs of hypertonic dehydration (Templeman et al, 2020a). Hypertonic dehydration can affect a variety of physiological outcomes, including lowered respiratory rate (RR) and increased internal body temperatures (BT) in dogs (Kozlowski et al, 1980; Horowitz & Nadel, 1984; Barker & Turleiska, 1989). As an increased BT greater than 42°C (107°F) can have serious physiological consequences in dogs (Lewis & Foster, 1976), the ability to dissipate heat via panting during exercise is key to minimizing deleterious outcomes and maintaining performance. Therefore, as SCFA can increases water absorption in the colon of dogs, providing a diet with optimized soluble fiber may prevent dehydration and aid in heat dissipation which could influence RR and BT during exercise (Zanghi et al, 2018).

Soluble fiber may also impact body composition of actively training sled dogs. For example, sedentary humans provided increased soluble fiber have lower body weights (BW), body mass index, and fat mass (FM) compared to those ingesting maltodextrin (control) (Guerin-Deremaux et al, 2011). Fat mass and lean body mass

(LBM) are common measurements obtained while examining body composition and have been reported to change with exercise (Evans et al, 2001; Allen et al, 2018; Templeman et al., 2020b). However, the effects of an increased soluble fiber diet on body composition in an exercising dog model has not yet been directly studied.

Therefore, the objective of this study was to investigate the effects of an increased soluble fiber diet and an incremental training regimen on the outcomes of RR, BT, and body composition in mid-distance training Siberian and Alaskan huskies. We hypothesized that increased soluble fiber supplementation would decrease exercise induced internal BT, RR, FM and increase LBM in actively training sled dogs.

3.3 Materials and Methods

3.3.1 Animals and housing

The study was approved by the University of Guelph's Animal Care Committee (Animal Use Protocol #4008). Twelve client-owned domestic Siberian Huskies (8 females: 8 intact; 4 males: 1 intact, 3 neutered) and 2 Alaskan Huskies (2 neutered males) with an average age of 3.75 ± 2.7 yr (mean ± SD) and body weight (BW) of 21.54 ± 2.83 kg were used in the study. Dogs resided and trained at an off-site facility (Rajenn Siberian Huskies, Ayr, ON) that had been previously visited and approved by the University of Guelph's Animal Care Services. During the study, dogs were group-housed in free-run, outdoor kennels (3.5-80 square meters) containing anywhere from 2-10 dogs each.

3.3.2 Diets and study design

Dogs were blocked for age, sex, and BW before being randomly allocated into one of two diet groups: control (Ctl; n=7; 3 females: 3 intact; 4 males: 1 intact, 3 neutered) or treatment (Trt; n=7; 5 females: 5 intact; 2 males: 2 neutered). For 2 weeks prior to the study period, all dogs were acclimated to a dry extruded Ctl diet (Champion Petfoods LT., Morinville, AB; for ingredient and nutrient composition refer to Templeman et al., 2020) 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 both the acclimation and study period, dogs were consistently fed once daily at 15:00h. Feed allowance was first determined using historical feeding records and the calculated metabolizable energy (ME) content of the diet. Body weight was measured at baseline and each week following, and diet intake was adjusted to maintain baseline BW. Dogs in the Ctl group were fed the Ctl diet with an insoluble: soluble fiber ratio of 4:1 (dry matter basis: 4074 kcal/kg metabolizable energy (ME), 94% dry matter (on an as-fed basis), 47% crude protein, 25% fat, 0.74% soluble fiber), while Trt dogs were fed a diet with an insoluble: soluble fiber ratio of 3:1 (dry matter basis: 4120 kcal/kg ME, 94% dry matter (on an as-fed basis), 47% crude protein, 26% fat, 2.12% soluble fiber) (Champion Petfoods LT., Morinville, AB). BioMOS®, a MOS derived strain of Sacchoromyces cervisiae yeast, oat soluble fiber, flaxseed meal, yucca, and chicory root are included as soluble fiber sources in the Trt diet, in place of pea starch. These ingredients were chosen and added to reach an inclusion level previously reported to positively influence canine gastrointestinal metabolites (Swanson et al, 2002; Grieshop et al, 2004; Barry et al, 2009; Ferreira et al, 2018), while yucca was added to control fecal odor (Cheeke, 2000). Daily meals were mixed with 1 cup of water before feeding; diets were mixed for 10 mins to allow for a homogenous mixture. At feeding time, all dogs were tethered and individually fed to allow for monitoring of food consumption. Dogs were allowed 30 minutes to eat their allotted food, and any orts were weighed and recorded daily. Throughout the entirety of the study, dogs had *ad libitum* access to fresh water.

3.3.3 Exercise regimen

A 9-wk exercise regimen (weeks 0-8) was implemented with exercise distance increasing incrementally throughout the trial period. The dogs were anticipated to run 6 km a day (4 days a week) at week 0 and reach 56 km a day (4 days a week) by week 8, but ambient conditions played a role in determining the daily run distance (RD; Table 3.1). Training consisted of all dogs running on a 14-dog gangline with staggered, pair-wise groupings of Trt and Ctl dogs. The gangline was attached to an all-terrain vehicle with one rider who controlled the machine in its lowest gear. A pace of approximately 15km/h was averaged throughout the training period. Additionally, running speed and distance were measured using a speedometer and odometer on the all-terrain vehicle.

3.3.4 Exercise challenges

On wks -1, 2, 5, and 8, one off-day in the dog’s training schedule (no running) was replaced by an exercise challenge. During each exercise challenge, dogs were run for 3km at a consistent distance at a pace of ~20km/h in four-dog teams. The distance run during exercise challenges was based on a previous study from our laboratory

(Chapter 2 of thesis; Thornton et al, 2020) as well as trail access and availability. Each group contained 2 Trt and 2 Ctl dogs. Following the 3km run, dogs were watered immediately. During each exercise challenge, all dogs were equipped with external telemetry jackets to non-invasively record both RR and BT (emka TECHNOLOGIES., Falls Church, VA, USA).

3.3.5 Telemetry jackets

Jacketed non-invasive telemetry devices (emka TECHNOLOGIES, Inc. emkaPACK 4G) were used to collect RR and BT sensor data. Collection of RR data was based on a previous study by our laboratory (Chapter 2 of thesis; Thornton et al., 2020).

For BT measurements, which is regarded as pilot work, a rectal probe (emka TECHNOLOGIES, Inc) was inserted 2 inches into the rectum of each dog. Each probe was individually calibrated twice; once prior to the start of the study following manufacturing and the second minutes before insertion into the dogs. Internal BT was measured every second at a resting (rBT), working (wBT), and post-exercise state (postBT). Once the challenge ceased, dogs were immediately watered and remained on the gangline for 30 minutes to continue gathering RR and BT data until postRR and postBT were obtained.

3.3.6 Blood sample collection and analysis

Blood samples were collected and analyzed as described by Templeman et al., 2020. In brief, fasting blood samples were collected on wks -1, 2, 5, and 8 to assess standard serum veterinary diagnostic measurements and markers of nutritional and health status (Tables 3.4 and 3.5). 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 LT., Oakville, ON). Separate samples (4 ml) were centrifuged at 2,000 x g for 20 min at 4 °C using a Beckman J6-MI centrifuge (Beckman Coulter, Indianapolis, IN). Serum aliquots were collected and analyzed for serum biochemical components using a Roche Cobas 6000 c501 biochemistry analyzer (Roche Diagnostics, Indianapolis, IN).

3.3.7 Body composition assessment

Body composition (fat mass, FM; lean body mass, LBM; and total body water, TBW) were measured using quantitative magnetic resonance (QMR) technology (Cancog Tech, Fergus, ON) on weeks -1 and 9. Using QMR technology, dogs were able to be imaged for FM and LBM while avoiding anesthetization (Mitchell et al, 2011). Due to the removal of 2 dogs from the study, only twelve of the original fourteen underwent week 9 body composition imaging. As such, the two dog’s data are not included in this report.

3.3.8 Statistical analyses

Statistical analyses were performed with Statistical Analysis System (SAS) (v. 9.4; SAS Institute Inc., Cary, NC). Respiratory rate and BT data were analyzed for outliers by removing the data points that exceeded a RR greater than 200 breaths/min and lower than 5 breaths/min. For BT, data points higher than 42°C and lower than 36° C were also considered outliers. As well, the periods during the exercise challenges when a 4-dog team was stopped were removed as these interruptions reduced the dogs' ability to maintain a wRR. These stops included instances of defecation and urination. Following data cleanup, a TRANSREG procedure was used to optimally transform the RR data. Body temperature was calculated using the means of each 1-minute interval from the start of each exercise challenge until 30 minutes post-exercise. Variances in RR, BT, fasted blood analytes (CBC, serum biochemistry), feed intake, BW, and body composition data were analyzed using PROC GLIMMIX of SAS (v. 9.4; SAS Institute Inc., Cary, NC). Dog was treated as a random effect, and activity level (resting, working, and post exercise), week, and diet group (DG; Trt or Ctl) were treated as fixed effects. Activity level was analyzed against diet group and week as well as week against diet group. Respiratory rate means were compared using the Tukey HSD whereas BT means were compared using a Fishers LSD. Statistical significance was declared at $P \leq 0.05$ and trends at P $0.05 < P \leq 0.10$.

3.4 Results

During data analysis, 18% (27/153) of the RR observations and 31% (433/1378) of BT observations were removed due to either software malfunctions, which caused inaccurate readings, or movement of rectal probes during the challenge run. Two dogs were removed from the trial (one on wk 2; Ctl, one on wk 4; Ctl) due to exercise related injuries; all data collected from these dogs until their removal were included in the results. Due to receiver limitations, only 8 dogs were monitored for BT throughout the study (3 males; Trt= 1, Ctl= 2, 5 females; Trt= 3, Ctl= 2).

3.4.1 Mean daily food intake, BW, and body composition

No differences were observed between mean daily feed intake (FI), BW, and RD with diet group ($P > 0.10$), but all variables differed by week ($P < 0.05$; **Table 3.1**). Feed intake was greatest at weeks 4 to 8 ($P < 0.05$; **Table 3.1**) with no differences between weeks 4 to 8 ($P > 0.10$; **Table 3.1**). Body weight decreased from baseline at weeks 2 and 3 ($P < 0.05$); however, BW did not differ from baseline (week -1) for weeks 0 and1 ($P > 0.10$) and from weeks 4 to 8 ($P > 0.10$; **Table 3.1**).

Body composition (FM, LBM, TBW) at weeks -1 and 9 did not differ between diet groups ($P > 0.10$); however, when data were pooled to evaluate the effects of exercise all variables differed by week ($P < 0.05$; **Table 3.1**). Lean body mass increased by 7%, TBW increased by 5.3%, and FM decreased by 3.5% from week -1 to week 8 ($P < 0.05$; **Table 3.1**).

Table 3.1 Mean daily run distance, feed intake, and BW data for all dogs from weeks -1 to 8, body composition data (lean body mass, fat mass, total body water) at weeks -1 and week 8, and anticipated run distance for the proposed exercise regimen with mean temperature and humidity for each week.

	Week											P-Value		
	-1	0	1	2	3	4	5	6	7	8	SEM	DG[1]	Wk	DGxWk[2]
RD, km/d[3]	3.0^{f}	5.8^{de}	11.2^{c}	17.0^{de}	23.8^{e}	30.1^{d}	25.4^{c}	20.4^{b}	27.0^{a}	37.1^{a}	1.08	0.99	<0.01	1.00
ARD, km/d[4]	3	6	12	18	24	30	36	42	48	54				
FI, g/d	273.4^{c}	325.9^{d}	367.5^{d}	427.3^{c}	480.6^{b}	570.9^{a}	563.9^{a}	560.4^{a}	560.4^{a}	560.4^{a}	30.9	0.44	<0.01	0.93
BW, kg	21.7^{abc}	21.3^{bc}	21.3^{bc}	21.1^{c}	21.0^{c}	21.7^{abc}	22.1^{ab}	22.1^{ab}	22.3^{a}	22.5^{a}	0.34	0.34	<0.01	0.57
LBM, %	73.1^{b}									80.1^{a}	2.80	0.97	<0.01	0.92
FM, %	16.2^{a}									12.7^{b}	1.90	0.85	<0.01	0.15
TBW, %	54.7^{b}									60.0^{a}	2.02	0.96	<0.01	0.69
T, °C	-3.8^{dc}	2.2^{a}	2.4^{a}	-2.0^{cd}	0.1^{b}	-7.8^{f}	-0.6^{bc}	2.3^{a}	-6.0^{cf}	-3.5^{d}	0.6	0.82	<0.01	1.00
H, %	81.4^{e}	95.0^{a}	89.7^{abc}	86.2^{bcde}	84.8^{cde}	89.3^{abc}	87.4^{bcd}	91.0^{ab}	80.7^{de}	81.3^{e}	1.6	0.89	<0.01	1.00

[1]DG, Trt: Treatment Ctl: Control
[2]Intercation effect between DG and week
[3]RD, mean daily run distance determined as an average of the 4 days of each week the dogs trained.
[4]ARD, anticipated run distance for proposed incremental exercise regimen (distance proposed to be run 4d/wk of training)
^{abc}Values in a row with different superscript are significantly different (P<0.05)

3.4.2 Body temperature

No differences in rBT were observed between dietary treatments (P > 0.10; **Table 3.2)**; however, Trt dogs presented with a lower mean wBT and postBT when compared to Ctl dogs (P < 0.05, **Table 3.2**). Working BT tended to decrease from week-1 to week 8 (P = 0.07; **Table 3.2**) and was lower at week 2 for dogs on Trt compared to Ctl dogs (P < 0.05, **Table 3.2**). Post exercise BT was lower at week 2 and 5 for dogs on Trt vs dogs on Ctl (P < 0.05; **Table 3.2**). No other differences were observed between diet group; therefore, internal BT was pooled to evaluate the effect of exercise. No differences were observed between rBT over weeks (P > 0.10; **data not shown**). Working BT was significantly lower at week 8 compared to week 5 (P < 0.05; **data not shown**) and tended to be lower than week -1 (P < 0.10; **data not shown**). Post exercise BT was greater at week 5 compared to weeks 2 and 8 (P < 0.05; **data not shown**) but was not different than week -1 (P > 0.10; **data not shown**).

Table 3.2 Mean body temperature (°C) at resting, working, and post-exercise state for control (insolube: soluble fiber ratio of 4:1) and treatment groups (insoluble: soluble fiber ratio of 3:1) in sled dogs running at a pace of 20km/h for 3km.

		Exercise Challenge				SEM	Mean	SEM	P-value		
		Week -1	Week 2	Week 5	Week 8				DG	Wk	DGxWk
rBT[2], °C	Trt	38.4	38.7	38.7	38.7	1.53	38.6	0.16	0.556	0.830	0.992
	Ctl	38.6	38.9	38.9	38.8	0.39	38.8	0.17			
wBT, °C	Trt	$39.7^{\#}$	39.2^{*}	39.7	$39.3^{\#}$	0.20	39.4^{*}	0.14	0.006	0.159	0.108
	Ctl	39.8	39.9^{*}	39.8	39.6	0.19	39.8^{*}	0.13			
postBT, °C	Trt	39.6^{b}	38.9^{a*}	39.3^{ab*}	39.1^{ab}	0.23	39.2^{*}	0.13	0.009	0.055	0.012
	Ctl	39.3^{b}	39.7^{ab*}	40.1^{a*}	39.3^{b}	0.24	39.6^{*}	0.13			

[1]Trt, treatment dogs; Ctl, control dogs; SEM, standard error of the mean, n= 3 for rBT (Trt: n=2; Ctl: n=1, week -1 and 2), n=7 for rBT (Trt: n=4; Ctl: n=3, week 5), n=4 for rBT (Trt: n= 3; Ctl: n=1, week 8); n=5 for wBT (Trt: n=2; Ctl: n=3, week -1), n=7 for wBT (Trt: n=3; Ctl: n=4, week 2), n=7 for wBT (Trt: n=4; Ctl: n=3, week 5) n=6 for wBT (Trt: n=3; Ctl: n=3, week 8); n=5 for

postBT (Trt: n=2,; Ctl: n=3, week -1), n=7 for postBT (Trt: n=3: Ctl: n=4, week 2), n=7 for postBT (Trt: n=4: Ctl: n=3, week 5), n=6 for postBT (Trt: n=3: Ctl: n=3, week 8)
[2]rBT, resting body temperature; wBT, working body temperature; postBT, post-exercise body temperature
[a,b]Values in a row with different superscripts are significantly different (P < 0.05; within treatment)
[#]Mean value for body temperature that tended to differ from weeks within the same row (P < 0.10)

3.4.3 Respiratory rate

No differences in rRR, wRR, and postRR were observed between dietary treatments (P > 0.10; **Table 3.3**); therefore, these data were pooled to evaluate effect of exercise. No differences in activity level (rRR, wRR, postRR) were observed between weeks throughout the study duration (P > 0.10; **Table 3.3**).

Table 3.3 Mean respiratory rate (breaths per minute) at rest, work, and post-exercise during weeks -1, 2, 5 and 8 for sled dogs running at a pace of 20km/h for 3km.

		Exercise challenge				SEM	Mean[1]	SEM	P-value		
		Wk -1	Wk 2	Wk 5	Wk 8				Wk	DG	DGxWk
rRR[2], bf[3]	Trt[4]	18	16	17	18	1.53	17	0.60	0.481	0.262	0.790
	Ctl	19	18	17	18	1.71	18	0.67			
wRR, bf	Trt	173	171	170	172	2.89	172	2.69	0.934	0.143	0.354
	Ctl	165	166	166	165	3.39	165	2.96			
postRR, bf	Trt	22	28	26	26	2.43	26	1.93	0.723	0.809	0.382
	Ctl	25	25	24	25	3.18	25	2.06			

[1]Trt, treatment dogs; Ctl, control dogs; SEM, standard error of the mean, n=11 for rRR (week -1), n=13 for rRR (week 2), n=10 for rRR (week 5 and 8); n=13 for wRR (week -1), n=11 for wRR (week 2), n=10 for wRR (week 5 and 8); n=9 for postRR (week -1, 2 and 5), n=11 for postRR (week 8)
[2]rRR, resting respiratory rate; wRR, working respiratory rate; postRR, post-exercise respiratory rate
[3]bf, breathing frequency (breaths per minute)

3.4.4 Complete blood count and serum biochemistry

Treatment dogs had greater concentrations of white blood cells, leukocytes, and alanine aminotransferase (ALT; $P < 0.05$; **Table 3.4, 3.5**) and lower concentrations of creatinine ($P < 0.05$; **Table 3.5**) compared to Ctl dogs; however, all mean CBC and serum biochemistry values were within standard reference range for dogs of both diet groups (as determined by the Animal Health Laboratory, University of Guelph, Guelph, ON). With the exception of five dogs that presented with high eosinophils at week -1 and 2, all dogs remained healthy throughout the study period (**Table 3.4**).

Table 3.4 Complete blood count values at weeks -1, 2, 5 and 8 for all dogs, and mean values (± SE[1]) across all weeks for control or increased soluble fiber (treatment) dogs taken at rest

Parameter[2]	Reference Range	Sampling Wk				Diet Group		SEM		P-value		
		-1	2	5	8	Trt[3]	Ctl	Wk	DG	DG	Wk	DGxWk[4]
WBC, x10^9/L	4.9-15.4	11.40	12.00	11.96	10.56	12.74*	10.23	0.69	0.75	0.03	0.15	0.38
RBC, x10^12/L	5.8-8.5	7.36[a]	7.31[a]	6.69[b]	7.12[a]	7.17	7.07	0.15	0.15	0.65	≤0.01	0.79
HGB, g/L	133-197	174.86[a]	172.29[a]	158.34[b]	167.36[a]	167.07	169.35	3.54	3.44	0.64	≤0.01	0.80
HCT, L/L	0.39-0.56	0.51[a]	0.50[ab]	0.44[c]	0.47[d]	0.49	0.48	0.01	0.01	0.46	≤0.01	0.85
MCV, fL	66-75	69.64[a]	68.78[a]	**65.87**[b]	66.32[b]	67.50	67.81	0.65	0.59	0.71	≤0.01	0.15
MCH, pg	21-25	23.71	23.64	23.79	23.57	23.68	23.68	0.19	0.23	0.98	0.41	0.72
MCHC, g/L	321-360	341.71[b]	342.93[b]	360.30[a]	354.93[a]	349.58	350.36	2.74	1.67	0.74	≤0.01	0.07
RDW, %	11-14	12.31	12.41	12.07	12.08	12.22	12.21	0.20	0.18	0.98	0.42	0.05
PLTS, x10^9/L	117-418	276.57[b]	275.93[b]	317.25[a]	308.44[a]	295.54	293.00	25.6	33.58	0.97	≤0.01	0.84
MPV, fL	7-14	9.46[b]	9.96[a]	9.31[b]	9.44[b]	9.46	9.63	0.2	0.25	0.62	≤0.01	0.60
PLTCRT, %	0.14-0.47	0.26	0.27	0.29	0.29	0.27	0.28	0.02	0.03	0.87	0.05	0.77
TSP, g/L	55-75	75.14[a]	70.71[bc]	72.18[b]	68.81[c]	73.6	71.36	0.72	0.58	0.15	≤0.01	0.66
NCT, x10^9/L	2.9-10.6	6.17[b]	6.65[ab]	7.18[a]	5.87[b]	7.08	5.86	0.48	0.55	0.14	≤0.05	0.17
LCT, x10^9/L	0.8-5.1	2.97	3.21	2.84	2.91	3.47*	2.49	0.23	0.27	0.02	0.32	0.37
MCT, x10^9/L	0-1.1	0.59	0.67	0.76	0.71	0.78	0.58	0.09	0.08	0.10	0.50	0.09
ECT, x10^9/L	0.08-1.33	**1.63**	**1.45**	1.04	1.04	1.36	1.28	0.29	0.28	0.85	0.29	0.37

[1]Standard error of the mean, n = 15 for wks -1 to 6 and n = 13 for wks 9 and 11, n = 8 for treatment diet group, n = 7 for control diet group.
[2]WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLTS, platelets; MPV, mean platelet volume; PLTCRT, plateletcrit; TSP, total serum protein; NCT, neutrophil count; LCT, lymphocyte count; MCT, monocyte count; ECT, eosinophil count.
[3]Trt, treatment; Ctl, control; DG, diet group.
[4]Interaction effect between diet group and wk.
[a,b,c] Values in a row with different superscript are different ($P \leq 0.05$).
*Mean value for treatment dogs significantly differs from control dogs within the same row ($P \leq 0.05$). **Bolded values** indicate complete blood count values that fall outside of standard reference range (as determined by Animal Health Laboratories, University of Guelph, Guelph, ON.

Table 3.3 Serum biochemistry values at weeks -1, 2, 5, and 8 for all dogs, and mean value (± SE[1]) across all weeks for control or increased soluble fiber (treatment) dogs taken at rest.

Parameter[2]	Reference range	Sampling Wk				Diet Group		SEM		P-value		
		-1	2	5	8	Trt[3]	Ctl	Wk	DG	DG	Wk	DG x Wk[4]
Ca, mmol/L	2.5-3	**2.29**	**2.44**	**2.44**	**2.46**	2.35	2.47	0.09	0.07	0.26	0.53	0.45
P, mmol/L	0.9-1.85	1.28[a]	1.26[ac]	1.18[b]	1.20[bc]	1.24	1.23	0.04	0.05	0.96	≤0.05	0.83
Mg, mmol/L	0.7-1	0.83[a]	0.84[ab]	0.76[c]	0.80[d]	0.81	0.81	0.02	0.02	0.92	≤0.01	0.39
Na, mmol/L	140-154	148.20[c]	146.77[a]	145.48[b]	146.48[ab]	146.75	146.72	0.42	0.36	0.95	≤0.01	0.59
K, mmol/L	3.8-5.4	4.81[bc]	5.03[a]	4.71[b]	4.89[ac]	4.79	4.92	0.06	0.05	0.10	≤0.01	0.27
Cl, mmol/L	104-119	112.86[a]	113.70[a]	111.79[b]	111.34[b]	112.29	112.56	0.41	0.35	0.59	≤0.01	0.08
CO_2, mmol/L	15-25	17.40	17.01	15.93	17.33	17.04	16.79	0.65	0.51	0.73	0.28	0.50
Anion gap, mmol/L	13-24	22.81[a]	22.11[b]	22.66[a]	22.75[a]	22.42	22.25	0.44	0.43	0.79	≤0.01	0.38
Na: K	29-37	30.86[ab]	29.38[b]	30.93[ac]	29.80[bc]	30.71	29.77	0.37	0.33	0.06	≤0.01	0.25
TP, g/L	55-74	62.32[a]	61.61[ab]	60.09[b]	61.43[ab]	61.89	60.83	0.67	0.68	0.29	≤0.05	0.88
Albumin, g/L	29-43	38.99[a]	36.95[b]	37.59[ab]	37.72[ab]	38.21	37.41	0.54	0.57	0.34	≤0.01	0.34
Globulin, g/L	21-42	23.33[ab]	24.66[a]	22.49[b]	23.70[ab]	23.68	23.42	0.68	0.71	0.79	≤0.05	0.78
A: G	0.7-1.8	1.69[a]	1.52[b]	1.69[a]	1.60[ab]	1.63	1.62	0.06	0.06	0.96	≤0.05	0.49
Urea, mmol/L	3.5-10	7.01[c]	8.48[b]	**10.48**[a]	**10.34**[a]	8.72	9.44	0.40	0.37	0.19	≤0.01	0.66
Creatinine, μmol/L	20-150	64.59	66.94	63.21	66.06	60.22*	70.17	3.28	2.45	0.01	0.82	0.23
Glucose, mmol/L	3.3-7.3	5.68	5.41	5.68	5.36	5.50	5.55	0.11	0.08	0.67	0.05	0.81
Cholesterol, mmol/L	3.6-10.2	4.19[b]	4.65[bc]	5.13[ac]	5.01[ac]	4.65	4.84	0.22	0.27	0.63	≤0.01	0.06
TB, μmol/L	0-4	1.07	1.34	0.97	1.16	1.25	1.02	0.16	0.14	0.27	0.27	0.64
CB, μmol/L	0-1	0.36[b]	0.65[ab]	0.99[a]	0.60[ab]	0.68	0.62	0.14	0.09	0.67	≤0.05	0.69
FB, μmol/L	0-3	0.71[a]	0.71[a]	0.0 [b]	0.59[ab]	0.57	0.43	0.18	0.13	0.47	≤0.05	0.86
ALP, U/L	22-143	24.73[b]	25.87[ab]	35.11[ab]	39.31[a]	38.52	24.00	6.53	8.03	0.21	≤0.05	0.48
SIALP, U/L	0-84	9.89	11.41	19.77	20.16	6.59	24.02	7.12	9.11	0.19	0.10	0.35
GCT, U/L	0-7	0.73[c]	1.78[ab]	2.45[a]	1.24[bc]	1.54	1.56	0.35	0.33	0.95	≤0.01	0.24
ALT, U/L	19-107	29.43	33.85	37.74	35.84	42.57*	25.86	5.35	4.91	0.03	0.55	0.06
CK, U/L	40-255	106.22	159.72	102.22	107.52	122.43	115.41	26.61	20.18	0.80	0.27	0.99
Amylase, U/L	299-947	348.03[a]	318.46[ab]	**284.31**[b]	**272.21**[b]	291.07	320.44	25.09	28.93	0.47	≤0.01	0.25
Lipase, U/L	25-353	80.43	77.45	73.68	75.01	84.89	68.39	6.06	7.33	0.13	0.53	0.60

[1]Standard error of the mean, n = 15 for wks -1 to 6 and n = 13 for wks 9 and 11, n = 8 for treatment diet group, n = 7 for control diet group.
[2]Ca, calcium; P, phosphorus; Mg, magnesium; Na, sodium; K, potassium; Cl, chloride; CO_2, carbon dioxide; Na: K, sodium to potassium ratio; TP, total protein; A: G, albumin to globulin ratio; TB, total bilirubin; CB, conjugated bilirubin; FB, free bilirubin; ALP, alkaline phosphatase; SIALP, steroid induced alkaline phosphatase; GCT, glucose challenge test; ALT, alanine transaminase; CK, creatinine kinase. [3]Trt, treatment; Ctl, control; DG, diet group.[4]Interaction effect between diet group and wk.[a,b,c] Values in a row with different superscript are different ($P \leq 0.05$). *Mean value for treatment dogs significantly differs from control dogs within the same row ($P \leq 0.05$). **Bolded values** indicate serum biochemistry values that fall outside of standard reference range (as determined by Animal Health Laboratories, University of Guelph, Guelph, ON.

3.5 Discussion

This study was the first to evaluate the effects of an increased soluble fiber diet on internal BT, RR, and body composition in mid-distance training sled dogs. The data presented herein indicate that for actively training sled dogs, 9 weeks of an increased soluble fiber diet may have contributed to a decrease in the working and recovery state internal BT but had no effect on RR or body composition. Additionally, 9 weeks of exercise training led to an overall increase in LBM and decrease in FM.

3.5.1 Body temperature

Given that dogs main form of heat dissipation is through evaporative panting, water loss by way of salivation increases considerably during exercise, contributing to an increased risk of dehydration and in turn influencing thermoregulatory capabilities (Hardy et al, 1964). For example, dehydrated sedentary dogs by way of water restriction or induced hypertonic dehydration have been previously reported to have elevated BTs when compared to hydrated dogs (Kozlowski et al, 1980; Baker et al, 1983; Baker & Turlejska, 1989). Furthermore, the Trt dogs in the current study presented with an overall reduction in internal BT at both a working and post-exercise state when compared to the Ctl dogs, suggesting that supplementation of soluble fiber so as to achieve an inclusion level of 2.12% may have contributed to a more efficient regulation of internal BT during exercise. As fermentable soluble fiber has a greater water holding capacity (Ramanzin et al, 1994; Mudgil & Marak, 2013) compared to less fermentable fibers such as cellulose (McBurney et al, 1984; Zentek et al, 1996), the possibility of increased hydration status via increased dietary soluble fiber may have influenced BT.

For example, exercising horses fed diets high in soluble fiber reported greater TBW and lower exercising BT (Spooner et al, 2008); however, it should be noted that unlike dogs, horses dissipate heat primarily through active sweating. As the diet in the current study had no impact on changes in TBW content, and since no other measurements of hydration status were taken (i.e. salivary or urinary loss or changes in osmolarity), we cannot definitely say that improved dietary soluble fiber delivery improved hydration which caused the change in BT. However, as products of fiber fermentation result in SCFA production, these major anions have been reported to be responsible for osmotic water absorption in the colon of the dog (Herschel et al, 1981). Our laboratory previously reported that fecal SCFA concentrations were greater in Trt dogs receiving an increased soluble fiber diet compared to Ctl dogs (Unpublished). Therefore, future research is warranted regarding SCFA concentrations, water absorption, exercise, and thermoregulation of dogs as these pilot data may provide important findings regarding how diet may affect possible exercise performance via thermoregulatory capabilities.

Independent of diet, conditioning has been reported to lower exercising BT (Piwonka et al, 1965; Shvartz et al, 1974). In the current study, wBT at week 8 tended to decrease from baseline suggesting a possible improvement in the thermoregulatory ability as training progressed. As exercise training leads to an increased cardiac output with increased blood volumes directed towards respiratory muscles (Hales and Dampney, 1975), the improved regulation of BT is thought to be due to the increased blood flow to areas of heat exchange supporting thermoregulation in dogs (Robbins et al, 2017). Therefore, as previous studies report improved cardiorespiratory capacity with exercise (Sugawara et al, 2001; Huang et al, 2016), the likelihood of such an event occurring in the current study is high. However, as only RR was measured in the current study, with no other cardiorespiratory parameters measured, the ability to relate thermoregulation to improved exercise conditioning in exercising dogs requires further investigation. However, as exercise usually takes place in colder environments, this reduction in BT could also be attributed to ambient temperature. However, as temperature was so variable throughout the current training period, future research should be conducted to investigate if there is an association between ambient temperature and internal BT.

3.5.2 Respiratory rate

Hydration status has also been reported to influence RR, as dehydrated dogs have lower RR (Kozlowski et al, 1980) in attempt to conserve body water content while panting (Baker et al, 1983; Horowitz & Nadel, 1984). Although the current study reported no changes in RR following an increased soluble fiber diet, the level of dehydration may not have been great enough to influence RR. For example, a previous study from our lab reported that sled dogs running 5 km led to signs of hypertonic dehydration (Templeman et al, 2020), whereas the dogs in the current study were running just over 3km. This suggests that unlike BT, RR may not be affected by slight changes in hydration. Future research is warranted to investigate changes in hydration status at shorter distances with an insoluble: soluble ratio of 3:1 in relation to RR in an exercising dog model.

For all dogs, 9 weeks of endurance exercise training had no effect on RR during any given activity level. Our laboratory previously reported that 12 weeks of aerobic exercise training resulted in a decreased RR at both a resting and post-exercise state with changes starting at week 5 (Thornton et al, 2020). As the dogs in the current study were running 25.4 km/day during week 5 and the previous study's run distance was 37.2 km/day at week 5, this 11.8 km/day difference could be behind the lack of exercise effects. In addition, the current study began descaling the training regimen at week 4 whereas Thornton et al (2020) began descaling at week 7. As a result, the changes in duration could have influenced RR, as our lab previously reported that RR may be sensitive to a training regimen and require a continuous incremental training regimen to elicit changes (Thornton et al, 2020).

3.5.3 Body composition

Participation in regular aerobic exercise positively affects body composition resulting in reductions in FM and increases in LBM in humans (Mosher et al, 1994; Evans et al, 2001). In dogs, 12 weeks of endurance training has resulted in an increased LBM of 11% and decreased FM of 4.5% (Templeman et al, 2020). The current study reported an increase in LBM of 7%, whereas FM decreased by 3.5% with diet having no effect on body composition. As previous studies examine the effects of fiber supplementation in overweight, sedentary humans (Guerin-Deremaux et al, 2011), the effects of exercise in the current study may have been greater than that of increased soluble fiber. For example, as BW was maintained and FI was adjusted to maintain energy requirements for increased exercise, the changes seen in LBM and reduction in FM can likely be attributed to the exercise regimen itself.

3.6 Conclusion

The findings in the current study suggest that the addition of 2% soluble fiber to achieve an insoluble: soluble fiber ratio of 3:1 contributed to a reduction in internal BT at both a working and recovery post-exercise state. Supplemental soluble fiber had no effect on RR or body composition; however, a 9-week training regimen resulted in increased LBM and decreased FM. Future research is warranted to investigate how a diet with an insoluble: soluble fiber ratio of 3:1 influences the hydration status, and various cardiorespiratory measurements (i.e. heart rate, VO_2max) in exercising dogs. Overall, these results can be used to improve training regimens and diets that may influence exercise physiology, health and performance of sled dogs.

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4 General discussion and conclusions

Dogs have a long history in sport, work and in service. As mushers and trainers condition their dogs for specific activities (i.e. mid distance races), specific physiological changes that occur during training have been widely studied. For example, cardiovascular, respiratory, and thermoregulatory changes have been previously reported (Wyatt and Mitchell, 1974; Piccione et al, 2012; Thornton et al, 2020). However, there is still a dearth of literature pertaining to the effects of an incremental training regimen and examining how the body responds to different levels of training. The results presented in this thesis characterize how heart rate (HR), respiratory rate (RR), and internal body temperature (BT) change throughout a conditioning period in sled dogs, as well as how these parameters change in the context of two dietary interventions, either improved soluble fiber delivery or an increased Trp: LNAA ratio.

In both Chapter 2 and 3, a similar incremental training regimen was implemented, however Chapter 2 had accumulated a total run distance of ~1200km with the max run distance completed in a day to be 50km, whereas Chapter 3 had accumulated a total run distance ~825km with a max run distance of 37km/day. As such, results differed between studies. For example, in Chapter 2, both HR and RR were measured and reported pre-run, during, and post-run for each exercise challenge (four in total). Decreases in both HR and RR as well as quicker recovery times post-exercise were reported herein. Chapter 3 had no differences in RR, and HR was not measured due to owner's request. Instead, an additional parameter of measuring internal BT by way of rectal probes was used. Previous studies have suggested that RR can be influenced via internal BT (Baker & Turlejska, 1989) and as such, Chapter 3 explored the relationship between BT and RR. However, as RR decreased in Chapter 2 but not Chapter 3, the variation in these physiological measurements could likely be due to the training regimen implemented. Chapter 2 described an incremental training program that increased weekly until weather required the run distance to be reduced in week 7. Chapter 3 was also designed to follow an incremental training regimen, however weather led to the reduction of run distance by week 4. Changes in HR and RR began occurring around week 5, which in Chapter 2, was a mean run distance of 37.2km/day whereas in week 5 of Chapter 3, when the descaling already occurred, a mean run distance of 25.4km/day was achieved. As Chapter 2 indicated that RR may be more sensitive to training, the premature descaling of the training regimen could be a contributing factor to the minimal effects observed. In Chapter 3, we presented data to suggest a trend towards a decreased mean BT during a working state. As week 8 led to the accumulated run distance of ~825km, the exercise regimen, despite the failed incremental training regimen, resulted in a trend towards a lower exercising BT from week -1. While, internal BT generally decreases with increased conditioning (Nold et al, 1991; Piwonka et al, 1965; Shvartz et al, 1974; Zangi, 2016) this was the first study, to the authors knowledge, that measured and recorded BT throughout the entirety of a run (pre, during, and post) as well as various times throughout a training regimen (week -1, 2, 5, 8) to monitor changes over time. However, depending on various circumstances (i.e. hydration status, environmental temperature) the ability to thermoregulate may become impaired. If temperature exceeds 42 °C in the dog, deleterious effects may begin to take place (Lewis & Foster, 1980). As well, if hydration status decreases, dogs adapt with decreased RR to conserve body water content leading to greater increases in BT (Baker et al, 1983; Horowitz & Nadel, 1984). However, a limitation of the present study is that we did not monitor hydration status or control ambient temperature and as such, future research is warranted to examine the relationship between hydration status, BT, RR, and environmental temperature. Maintaining a eu-hydrated state while training in appropriate temperatures can improve endurance performance and mitigate the detrimental increase in working BT (Dill et al, 1932; Weibel et al, 1983).

This thesis additionally investigated the effects of two dietary interventions: tryptophan (Trp) and soluble fiber. Diet has previously been reported to positively effect exercise performance in dogs (Kronfeld, 1973; Reynolds et al, 1999; Davenport et al, 2001). As discussed in Chapter 2, Trp supplementation so as to achieve a Trp: LNAA ratio of 0.075:1, had no effect on HR or RR. Although serotonin (5-HT) has been previously investigated to increase HR and RR in dogs (Eckstein et al, 1971; Zucker et al, 1980), these studies were conducted following a supraphysiological injection of 5-HT. As well, it has been previously hypothesized that the intensity of aerobic exercise influences the sensitivity and downregulates 5-HT receptors in various animal models (Jakeman et al, 1994; Chennaoui et al, 2000). As supplemental Trp was provided, a run accumulation of ~1200km was completed, and serum 5-HT levels did not increase until the final week of study, future research is warranted regarding Trp supplementation or a pre-load phase before training and greater than 12 weeks of exercise training on HR and RR measurements.

In Chapter 3, the inclusion of BioMOS, oat soluble fiber, flaxseed meal, yucca, and chicory root to decrease the insoluble: soluble fiber ratio from 4:1 to 3:1 resulted in decreased exercising and recovery BT with no changes reported in RR following a 3km run. However, as the effects of soluble fiber have not been directly studied in an exercising dog model, previous studies have demonstrated that the gut microbiome has influenced RR outcomes in rats (Golubeva et al, 2015). In addition, mice lacking gut microbiota have impaired thermogenic capacity and become hypothermic during a cold-exposure test (Li et al, 2019). It is possible that the soluble fiber may not have influenced the gut microbiota of the dogs to a significant extent to influence RR, but enough to support thermoregulation. Our laboratory previously demonstrated that the increased soluble fiber (2% soluble fiber on an as-fed basis) increased products of fermentation (i.e. SCFA) which suggest a shift in the gut microbiota (unpublished). Future research should aim to determine how increased soluble fiber impacts the gut microbiome and in turn RR and BT during an exercise bout. In addition, as sled dogs are known to demonstrate hypertonic dehydration during exercise (Templeman et al, 2020), the ability for greater water loss influencing thermoregulatory capacity is possible. As dogs receiving the Trt diet had lower exercising BT, the possibility of increased soluble fiber influencing hydration could be present resulting in changes in internal BT. As such, future research is warranted regarding various hydration measurements (i.e. urinary and salivary losses, changes in osmolarity) and increased exercise with an insoluble: soluble fiber ratio of 3:1.

Overall, the results presented within this thesis underpin the importance of continuous exercise training for maintenance of exercise performance. During training, mushers and trainers should maintain an incremental training period to support those exercise induced adaptations as failing to do so can influence training induced progress as seen in Chapter 2. Additionally, Trp may influence HR and RR, however longer training regimens are needed (>12 weeks) to elucidate the effects on performance. Dietary soluble fiber may be used to lower exercising BT which could lead to improved performance as increased BT is seen as a limiting factor during training and racing (Gonzalez-Alonso et al, 1999). These results are the first to characterize the effects of different nutritional interventions on exercise-induced HR, RR, and BT in mid-distance training sled dogs, as well as furthering the knowledge on how diet can influence physiology and performance in working dogs.

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