Effect of grains on satiety in dogs

– investigated using a behavioural approach

Effekten av spannmål på mättnad hos hund – undersöktes med hjälp av beteendemetoder

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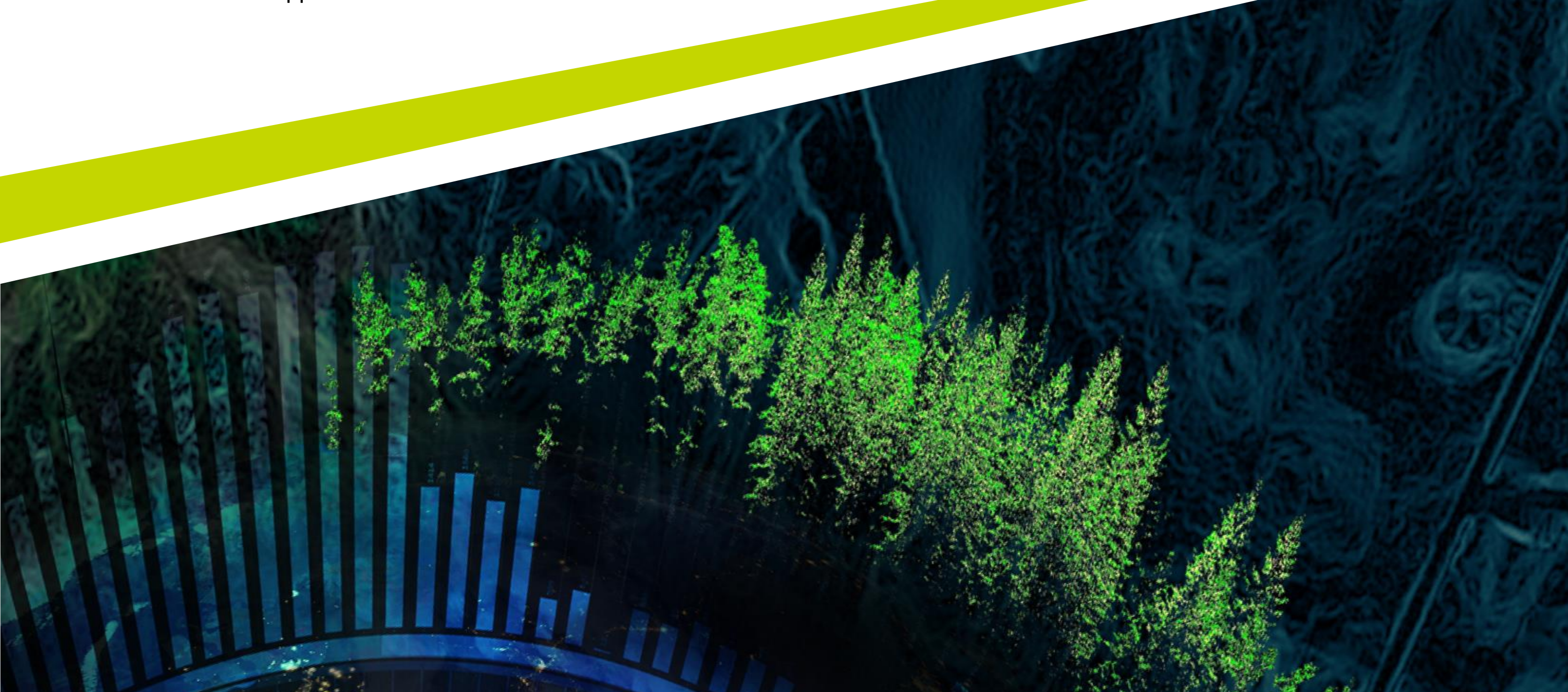

Effect of grains on satiety in dogs – based on behavioural indicators

Effekt av spannmål på mättnad hos hund – undersöktes med hjälp av beteendemetoder

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Abstract

High-quality foods have become abundant for our pet animals and with that, obesity has become a major threat to the health and welfare of our companion dogs. The use of dietary fibres in animal feeds to prolong satiety is well discussed in the literature for several species, but scarce when it comes to dogs. Therefore, the aim of this study was to investigate the effect of different grains on behavioural indicators of satiety in dogs. It was hypothesised that satiety would decrease with time post-meal consumption, and that wheat would be the least satiating grain. Eleven privately owned dogs received three diets: a wheat based diet, an oat based diet and a rye based diet, in three periods in a cross-over design and a random order. The feeds were formulated to be as similar as possible in composition and energy content. At 15 min, 3 h and 6 h post-meal consumption feeding motivation was assessed in a runway test, where dogs should run through a short track to find a small food reward at the end. At 15 min and 6 h post-meal consumption attention bias towards a food-related cue was assessed by placing the dogs own bowl and their favourite toy at a distance from the dog and then setting it free for 60 seconds. The results from the runway showed that dogs reduced the latency to reach the food reward with time post-meal consumption, showing that we indeed measured satiety in the runway test. Dogs on the wheat diet generally had a higher feeding motivation than dogs on either of the other diets, with no difference between the rye and oats diet. Dogs got quicker to approach the bowl at 6 h compared to 15 min post-meal consumption in the attention bias test. Although not significant, the dogs showed a pattern of being more likely to choose the bowl at 6 hours compared to 15 minutes post-meal consumption that was in line with the hypothesised outcome. Diet had mixed effects in the attention bias test but with no clear pattern. In conclusion, dogs became more feeding motivated with time and the wheat diet was less satiating across time and test days. The rye and oat diet both seem equally satiating. Therefore, including these grains in commercial dog foods may promote satiety and therefore may help combat obesity. There is, however, still a need of further research into the effect of long-term intake of specific dietary fibres on the microbiota and subsequent satiety and health effects, to promote welfare in our four-legged canine companions.

Keywords: dog, dogs, satiety, feeding motivation, dietary fibres, grain, whole grain, wheat, oats, rye, runway, attention bias

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1. Introduction

Obesity has become a major threat to the welfare of our companion dogs. Obesity predisposes our dogs to decreased longevity and a variety of diseases (German, 2006; Weber *et al.*, 2007). Weight loss management commonly entails energy restriction together with increased exercise level (Weber *et al.*, 2007). The main issue with this management is that it causes hunger, which in turn increases scavenging and begging behaviours (Weber *et al.*, 2007; Bosch *et al.*, 2009b). These behaviours can be tough to deal with for the owners, and it can also result in that the owners feed their dogs above the energy requirement maintaining or even increasing the obesity (Bosch *et al.*, 2009b). Not only obese dogs scavenge and beg for more food. Pet dogs, that are fed the right level of nutrients and energy, may still feel hunger during parts of the day (Bosch *et al.*, 2009b). This might suggest that even though feeds satisfy the dogs nutritional and energy requirements, some feeds might be lacking components that stimulate a sufficient satiety response. Another explanation is that throughout their evolution, mammals have had to spend a lot of time feeding to build up energy reserves (i.e. fat) for times when food and energy was scarce (Morrison and Berthoud, 2007; Bellisari, 2008; Verbeek *et al.*, 2012). In the words of Morrison and Berthoud (2007), "*Procurement of food and water is one of the most important behaviours for any life form*". Today high-quality feeds that are high in energy are abundant. These energy dense foods should not be fed ad-libitum, as the amount must be restricted to prevent obesity. This limited amount of food might not satisfy the animals high need to express feeding behaviour (Bellisari, 2008; Verbeek *et al.*, 2012), and when this limited amount of high-quality food is consumed the food seeking continues with the risk of becoming stereotypic (D'Eath *et al.*, 2009). Consumptions of food can be rewarding and pleasurable, and preference of or 'liking' a specific food also controls food intake (Saper *et al.*, 2002; Morrison and Berthoud, 2007; Verbeek *et al.*, 2012). Diets containing high amounts of fibre may have negative impact on palatability, depending on inclusion level in the feed and the source of fibre (Weber *et al.*, 2007; Hours *et al.*, 2016).

Dietary fibres have been shown to prolong satiety, not only in dogs, but in humans and other animals as well (Slavin and Green, 2007; Bosch *et al.*, 2009a). Dietary fibres are resistant to digestion in the small intestine and commonly reaches the colon substantially unmodified (EFSA, 2010). Some dietary fibres that are fermented in the colon, yields short-chain fatty acids (SCFA) that affect the release of gastrointestinal satiety related hormones into the blood (Bosch *et al.*, 2009a). The total dietary fibre content in common grains like wheat, oats and rye are different and rye contain almost double the amount of total dietary fibres than wheat and oats, while wheat has higher total dietary fibre content than oats (Andersson *et al.*, 2009; Frølich *et al.*, 2013). There is also a difference in level of different types of dietary fibres in the grains, e.g. difference in level of cellulose, arabinoxylans and β-glucans (Frølich *et al.*, 2013). Arabinoxylans, highest in rye, are largely fermented by the microbial population in the colon which stimulates microbial growth and yields SCFA (Mendis *et al.*, 2016). Oat has the highest content of β-glucans (Frølich *et al.*, 2013). β-glucans, apart from also being fermented by the colonic bacteria, are soluble and viscous fibres that slow gastric emptying, digestion rate and might possess some bulking properties due to binding of water (Slavin, 2013; Tosh and Miller, 2016). These properties of arabinoxylans and β-glucans have been suggested to increase satiety effect, even though the process is not yet fully established (Tosh and Miller, 2016).

Although there is evidence that dietary fibres can prolong satiety, there is still little research on how feeding motivation, physiological satiety parameters or behaviour is affected by dietary fibres in dogs specifically (Bosch *et al.*, 2007). In a previous study in humans, a comparison of a whole grain wheat and whole grain rye diet showed no differences in post-consumption appetite but a reduced energy intake with regular intake of with the rye-based diet (Shur *et al.*, 2017), suggesting that whole wheat is less satiating throughout the day than whole rye.

Voluntary feed intake (VFI) has been widely used as a measurement of satiety in dogs. VFI has been shown to depend on the level of dietary fibres in the diet (Jackson *et al.*, 1997; Bosch *et al.*, 2009a), kibble density, size (Serisier *et al.*, 2014) and shape (Sagols *et al.*, 2019). However, testing the feeling of satiety in dogs throughout the day using VFI would not be possible since allowing the dog to consume unlimited amounts of food several times a day would yield confounding results where there is hard to differ between effect of treatment or amount of food consumed during the day.

An early study using a runway test to assess feeding motivation in rats was able to show a difference in feeding motivation depending on the age of the rats using running speed as their measurement (Blokland and Raaijmakers, 1993), and their findings were supported by a later study by García-San Frutos *et al.* (2012) that also reported a difference in feeding motivation in rats due to age. Two studies using runway tests to assess feeding motivation in pigs eating different sources of dietary fibres found that dietary fibres promoted satiety and reduced feeding motivation (Souza da Silva *et al.*, 2012; 2013). Runway tests have been used to assess subjective states in a number of animal species (Lansade *et al.*, 2008; Gibbons *et* *al.*, 2010; Pelhaitre *et al.*, 2012; Doyle *et al.*, 2015), making runways a promising tool for assessing motivation in dogs.

The use of attention bias tests is becoming more common in research to assess the affective state of an animal with the aim of improving animal welfare (Verbeek *et al.*, 2014; Lee *et al.*, 2016; Crump *et al.*, 2018; Monk *et al.*, 2018). In these tests the animals are presented with one or more stimuli. Time for initial engagement (i.e. latency to approach) or discovery, sustained attention towards one stimulus and also shifting attention towards another stimulus are different aspects that can be used as measurements of attention (Lee *et al.*, 2016; Crump *et al.*, 2018).

In this study satiety through behaviour was assessed in privately owned dogs that were fed three different feeds, each containing either wheat, oat or rye as their source of grain. The aim was to investigate if type of grain in the feed would affect the feeling of satiety expressed by behaviour in dogs. Repeated runway and attention bias tests were used to assess the feeling of satiety. It was expected that wheat would be the least satiating grain, but there is not enough evidence to make predictions about whether rye and oats are equally satiating or not. Latency to reach the bowl containing a food reward at the end of a runway will decrease with time post-meal consumption and since rye and oats were expected to be most satiating it was hypothesised that the decrease in latency with time would be lowest for dogs on those diets and highest for the dogs on the wheat diet. Further it was hypothesised that, when given the choice, the dogs will be more likely to choose a food-related cue (i.e. dogs own empty food bowl) over a favourite toy in the afternoon (6 hours post-) compared to directly following (15 minutes post-) meal consumption. Total duration of attention towards their bowl will increase with time post-meal consumption and since rye and oats was expected to be most satiating it was hypothesised that the increase would be lowest for dogs on those diets and highest for the wheat diet.

2. Literature review

2.1. Satiety, feeding motivation and dietary fibre – definition and welfare importance

When studying satiety with a behavioural approach it is hard to do so without also talking about feeding motivation since they are connected. Kirkden and Pajor (2006) defines motivation as "*a construct used to describe the strength or willingness with which an animal engages in behaviour*". We usually describe ourselves as hungry when we are motivated to engage in feeding behaviour, and the term feeding motivation involves the feeling hunger (Kirkden and Pajor, 2006). Feeling hungry, thus motivated to feed, and feeling satiated would then be considered opposites. Jewell *et al.* (2000) define satiety as "*the lack of desire to eat and usually results from the consequences of ingestion*". Green and Delgary (1997) defines satiety similarly, while they also define satiation "*as the process which develops during eating and brings an episode of eating to a close*". Satiety and satiation are affected differently depending on several properties of foods (Green and Delgary, 1997). As previously stated, weight loss management commonly entails energy restriction together with increased exercise level, causing hunger, which in turn increases scavenging and begging behaviours (Weber *et al.*, 2007; Bosch *et al.*, 2009b). Macronutrient content of feeds can be altered to address this issue, and protein, fibres, water and carbohydrates are macronutrients that reportedly provides the most satiation (Weber *et al.*, 2007; Sagols *et al.*, 2019). Diets high in dietary fibres have been proved to increase satiety and might provide certain health benefits (Weber *et al.*, 2007; Frølich *et al.*, 2013). The European Food Safety Authority (EFSA, 2010) define dietary fibre as "*non-digestible carbohydrates plus lignin*", which encompasses non-starch polysaccharides (NSP), resistant starch (RS), resistant oligosaccharides (ROS) and lignin associated with the NSP. Cellulose, hemicellulose, pectin and β-glucans are examples of NSP (EFSA, 2010).

Most of these dietary fibres escape the enzymatic digestion of the upper gastro-intestinal tract and ends up in the colon where gut microbes digest what the animal itself cannot (EFSA, 2010; Hur and Lee, 2015). Dietary fibres can either be soluble or insoluble, and microbial enzymes can digest soluble fibres into SCFA (Hur and Lee, 2015). These SCFA are absorbed and used as energy by the animal and functions as a regulator for food intake and satiety (Hur and Lee, 2015). If the microbial composition is altered, it can lead to a number of diseases (Hur and Lee, 2015). Studies done in humans suggest that increased consumptions of dietary fibres and whole grain foods lowers the risk of not only obesity, but also cardiovascular diseases (CVD), type 2 diabetes and possibly even some cancers (Frølich *et al.*, 2013). As well as in obese humans, cardiorespiratory diseases (CRD), certain cancers and diabetes mellitus (among many other diseases) have been reported in obese dogs (German, 2006).

2.1.1. Dietary fibre – content differences in grains

In this study we compare the effects of including wheat, rye or oats in the diet on satiety in dogs. It is therefore important to understand the differences of these common grains and how these differences might result in different satiety effects. Dietary fibre, defined and specified in the section above, is composed of several different types of non-digestible carbohydrates and the lignin associated with them (EFSA, 2010). Wheat, rye and oats all contain dietary fibres such as cellulose, lignin, arabinoxylan (i.e. a form of hemicellulose), β-glucans and fructan, however, they contain different levels of them (Table 1; Frølich *et al.*, 2013; Fadel *et al.*, 2018).

The different physiological effects dietary fibres will have on the body differs with the characteristics of these dietary fibres, such as solubility, viscosity and fermentability (Slavin, 2013). Fibres that are viscous have the property that they form gel in the intestinal tract and fermentable fibres promote colonic bacteria activity and growth (Slavin, 2013). Cellulose and lignin are classified as insoluble and non-fermentable fibres, β-glucans are classified as viscous fibres that are both soluble (i.e. water-extractable) and fermentable and arabinoxylans are classified as viscous and soluble fibres that vary in the level of fermentability (Andersson *et al.*, 2009; Frølich *et al.*, 2013; Slavin, 2013). The outer parts of most naked grains (husk fallen off or dehulled) consists of the insoluble and less fermentable dietary fibres (Frølich *et al.*, 2013). In these outer parts the cell walls are lignified and are in wheat and

Table 1. *Dietary fibre content and composition of whole grain wheat, whole grain rye and dehulled oats in % of dry matter.*

Component	Wheat	Oats	Rye
Total DF	13.5	10.2	19.9
Arabinoxylan	5.6	2	8.9
Cellulose	2.5	1.3	2.9
b-Glucan	0.8	5	1.5
Fructan	1.3	0.2	4.1
Lignin	0.8	1.4	1.1

The values were adapted from a review by Frølich *et al.* (2013). DF = dietary fibre.

rye mainly associated with high cellulose and arabinoxylan content that will yield high bulking effect and influence the large intestine passage rate (Frølich *et al.*, 2013). Oats, low in total arabinoxylan content, have been reported to have an approximate of 85 % of β-glucan content in their cell walls (Tosh and Miller, 2016). In the grains' endosperm there are more of the viscous fibres, i.e. soluble arabinoxylans and β-glucans, which have been shown to sometimes influence ileal absorption of nutrients negatively (Rakha *et al.*, 2011; Frølich *et al.*, 2013). β-glucans can modify cholesterol and blood glucose concentrations by their interference with digestion of cholesterols, bile acids and carbohydrates (EFSA, 2010). Fructan, being of a low molecular and a highly water-soluble and fermentable fibre, are believed to not generally influence ileal absorptions of nutrients (Andersson *et al.*, 2009; Fadel *et al.*, 2018).

As mentioned, arabinoxylans can be soluble and insoluble and it is due to a structural diversity among arabinoxylans themselves that vary between grains and therefore fermentability of this fibre varies depending on its' molecular structure (Mendis *et al.*, 2016; Fadel *et al.*, 2018). The higher arabinoxylan in rye compared to both wheat and oats (Table 1) is due to the fact that rye has a larger cell wall proportion in the starchy endosperm (Frølich *et al.*, 2013). The soluble proportion of arabinoxylans in rye grains are greater (2.4 – 4.1 %) compared to the proportion found in wheat grains (0.5 – 0.8 %, Andersson *et al.*, 2009; Fadel *et al.*, 2018). Because rye has a lager soluble and thus fermentable proportion of arabinoxylans, rye would stimulate a larger yield of SCFA, increased bulking effect and slow down large intestinal passage rate to a larger extent than wheat (Frølich *et al.*, 2013; Hur and Lee, 2015).

Thus, depending on consumed grain type the physiological response to dietary fibres will differ (Frølich *et al.*, 2013). The exact truth about what positive physiological effects grains exert is still not resolved because of the interactions between their components, not only dietary fibre components but also various bioactive components of grains (Frølich *et al.*, 2013).

2.2. Behavioural measures – why runway and attention bias

Since satiety and feeding motivation both are subjective affective states (i.e. feelings) of the animals, they are not directly measurable (Kirkden and Pajor, 2006; Verbeek *et al.*, 2011), and unfortunately, they cannot tell us when they are feeling sufficiently satiated. However, some methods to indirectly measure satiety have previously been used for this purpose in dogs and other animals.

Jackson *et al.* (1997) argues that VFI is an objective method that might be useful as a measurement of satiety. Jackson *et al.* (1997) used thirty small breed dogs that were fed 50% of their individual body weight each morning of either a low fibre diet or a high fibre diet, and approximately 6 hours later given unrestricted access to the control feed. Both meals were available to the dogs for 15-20 minutes. They found that dogs fed the low fibre diet consumed more calories each day than dogs fed the high fibre diet. However, the total intake of food each day was not significantly different. Hence, the lower calorie intake on the high fibre diet might be attributed to that the high fibre diet contained fewer calories than the low fibre diet, which Jackson *et al.* (1997) also mentions. Bosch *et al.* (2009a) also compared a low and a high fibre using sixteen beagle dogs. After eating the experimental diets for 7 weeks the dogs were offered 1 kg of the control diet 6 hours after the morning meal and were allowed to eat for 20 minutes. Dogs on the high fibre diet tended to eat less than dogs on the low fibre diet, suggesting either increased feeling of satiation on the high fibre diet or that a high fibre diet is less palatable. As mentioned, palatability also determines food intake (Saper *et al.*, 2002) so it is important to control for this when investigating satiety. Apart from differences in level and type of dietary fibres in the feeds, VFI has also been used to assess the effect of commercial weight loss diets, kibble density and shape on food intake, palatability and meal duration (Serisier *et al.*, 2014; Hours *et al.*, 2016; Sagols *et al.*, 2019). Even though VFI has been useful in assessing satiety in dogs previously, it seemed to crude of a measurement to suit the needs for this study. Measuring satiety using VFI as a measurement does not consider if the dogs like a food more and thus will consume more of it even past feeling sated. One common factor in all the studies mentioned above (apart from Jackson *et al.* [1997]) is that the experimental feeds differ in large aspects such as composition and kibble density and/or shape. The feeds that we were used in this study are all very similar in composition and kibble size and shape are approximately the same. Therefore, better methods are needed that can detect small differences in the current experimental feeds.

According to Kirkden and Pajor (2006), assessing motivations and feelings, such as satiety, has previously been done using choice, operant and preference tests depending on which research question aims to be answered. Operant tests, where the animals are trained to perform tasks such as turning a wheel, pressing a button or a lever to obtain a food reward, have been used to assess satiety and feeding motivation of other monogastric animals, such as pigs (Kirkden and Pajor, 2006; Souza da Silva *et al.*, 2012; 2013). No study was found where operant tasks were used to assess satiety or feeding motivation in dogs', and even though the use of operant tests seems to be useful in detecting a satiety effect from dietary fibres of different kind and levels (Souza da Silva *et al.*, 2012; 2013) the pre-training for these kinds of studies is time-consuming and possibly tiring for the subject animals (Thompson *et al.*, 2016). This can lead to negative impact on the animals' ability to perform during subsequent tests (Thompson *et al.*, 2016). Runway tests have successfully used for assessing motivation tests, and they require little to no pre-training (Gibbons *et al.*, 2010; Souza da Silva *et al.*, 2012; 2013). No studies were found where feeding motivation in dogs had been assessed using a runway test.

Attention bias tests are being increasingly used to assess affective states in animals, which also require little to no pre-training (Lee *et al.*, 2016; Crump *et al.*, 2018).

For this study there was little time available for any pre-training and VFI seemed like a too crude a measurement to achieve the current aim. There was no previous research done on satiety using a runway or attention bias test on dogs specifically, but they had proven useful investigating other animals and their affective states. If these methods were shown useful in measuring feeding motivation, this study could produce new information to the field.

2.2.1. Runway test – previous research

Since feeding motivation is linked to satiety it has proven a useful tool in indirectly measuring satiety. Runways have been used to assess social motivation in several species of animals, e.g. cows (Gibbons *et al.*, 2010), broilers (Pelhaitre *et al.*, 2012) and horses (Lansade *et al.*, 2008), anxiety in sheep (Doyle *et al.*, 2015), walking performance in broilers (Bokkers and Koene, 2004) and feeding motivation in pigs (Souza da Silva *et al.*, 2012; 2013) and rats (Blokland and Raaijmakers, 1993; García-San Frutos *et al.*, 2012).

In a study on feeding motivation in pigs, Souza da Silva *et al.* (2012) found that feeding motivation did not differ between a diet containing lignocellulose and diet containing resistant starch as dietary fibre sources compared to a control diet with high starch and none of the fibres using a runway test. Both treatment feeds had lower metabolizable energy than the control diet suggesting that the treatment feeds had higher satiety effect that made up for the lower available energy (Souza da Silva *et al.*, 2012). However, the same study reported an increased feeding motivation in a third treatment diet containing pectin as the fibre source, showing that pectin was the least satiating fibre. Another study, assessing dietary fibres effect on feeding motivation in pigs, by Souza da Silva *et al.* (2012) they investigated if differences in the fermentability of fibres would affect feeding motivation using a runway test and found a reduced feeding motivation in pigs fed a high level of fermentable fibres. Blokland and Raaijmakers (1993) were able to show a difference in feeding motivation depending on the age of the rats using runway speed as their measurement. Similarly, García-San Frutos *et al.* (2012) reported a difference in feeding motivation in rats due to age, where older rats showed a reduced motivation for sweet rewards. The study where Bokkers and Koene, (2004) investigated walking performance in food deprived slow- versus fast-growing broilers using a runway test, they placed a food reward. Utilizing the broilers' feeding motivation to evaluate their ability to walk. The slow-growing broilers' walking performance were affected by their motivation for food while the fast-growing broilers were not, but rather affected by their ability to walk because of their body weight.

Thus, runway tests have proven to be useful in determining animals' motivation to feed, among other useful areas. Since no study was found where a runway test had been used for measuring motivation of any sort in dogs, this study may serve as a pilot study towards validating the use of runway tests for this purpose.

2.2.2. Attention bias – previous research

The affective state of an individual influence cognitive processes such as judgement, memory and/or attention (Mendl *et al.*, 2009; Burman *et al.*, 2011). Methods and theories on how to investigate the relationship between these cognitive processes are well established in humans (Crump *et al.*, 2018).

It has been shown that, depending on their affective states', animals tend to respond more positively or negatively to ambiguous stimuli using a form of cognitive bias methods called judgement bias tests (Mendl *et al.*, 2009; Lee *et al.*, 2016). Verbeek *et al.* (2014b) found that sheep that consumed a food reward were more optimistic in a judgement bias test and that opioid drugs can further strengthen this positive effect on affective state after consumption of a palatable food reward. The same was not found in dogs, however, due to methodological issues (Burman *et al.*, 2011). Burman *et al.* (2011) argues that "*Such an apparent discrepancy between predicted and observed outcomes in cognitive bias studies has occurred previously and appears to be more likely to occur when a short-term induction of affect is stopped just prior to the cognitive bias test…*". Similar discrepancies were found in a study on sheep by Doyle *et al.* (2010). Crump *et al.* (2018) argues that judgement bias tests are limited by the time-consuming pre-training need, impracticality in applied settings, and that it leads to tiering of the subjects.

Previous research done in humans found that attentional bias toward food-related stimuli is associated to the motivational state of hunger (Leland and Pineda, 2006; Castellanos *et al.*, 2009). Castellanos *et al.* (2009) found that obese subjects are biased toward food cues even after eating, while individuals with normal weight are not. Suggesting that gaze direction and duration in attention bias tests might be a useful tool in assessing hunger and/or satiety. Attention bias is another form of cognitive bias that, unlike judgement bias, is not dependent of interpreting pessimistic and optimistic responses of the subject animal (Crump *et al.*, 2018). The usage of attention biases as a tool to assess affective states of non-human animals have grown, and starlings (Brilot *et al.*, 2009), sheep (Verbeek *et al.*, 2014; Lee *et al.*, 2016; Monk *et al.*, 2018), cattle (Lee *et al.*, 2018), rhesus macaques (Bethell *et al.*, 2012) and capuchin monkeys (Boggiani *et al.*, 2018) are some of the animals that have been subjected to these types of methods up to date. In a study by Verbeek *et al.* (2014a), they used an attention bias test to assess the biased attention towards a food-related cue in sheep following a short period of food restriction. Food restricted sheep were shown to express more feeding motivation, were slower to disengage attention and spent more time interacting with the food-related cue, the cue in this case was an empty familiar food bucket.

On the basis of these findings, attention bias tests seem to be the most promising method of choice for the aim of this study. The pre-training is low to nothing and based on previous research it seems like a good method for measuring internal motivations such as satiety in animals. Since there are no previous studies assessing satiety in dogs using attention bias tests, this study may serve as a pilot study towards validating the use of attention bias tests for this purpose.

3. Material & Methods

This study was approved by Uppsala djurförsöksetiska nämnd (ID-No: 5.8.18-18808/2017) and had signed consents from the owners of the dogs used in the study. In this study satiety was assessed through behaviour in eleven privately owned dogs that were fed three different feeds each containing a different type of grain. A crossover design was applied where each dog was fed each of the three feeds, one at a time, during three different but succeeding treatment periods. Four of the dogs started on either the wheat diet or oat diet and three dogs started on the rye diet (n=11). The feed order was then alphabetical resulting in three different orders, Wheat-Oats-Rye, Oats-Rye-Wheat and Rye-Wheat-Oats. Each treatment period was between 21-35 (25 ± 3.5 standard deviation) days. The owners, handlers and experimenters were blinded to which feed the dogs were fed during each period. The dogs ate each feed in a randomized but balanced order.

3.1. Experimental feeds

The three experimental feeds tested in this study had different grains and thereby different main sources of dietary fibre. The three feeds contained meal from either whole wheat (wheat diet), rolled oats (oat diet) or whole rye (rye diet). The wheat diet was the control feed, because wheat is most commonly used in dog food while oats is less common, and rye is not used at all. Apart from the grains, the feeds were formulated to be as similar as possible in composition and energy content so that the main difference in satiety between feeds would depend on the type of grain in the feed. In Table 2 only the raw analysis is shown. Crude fibre (CF) content is not a fair representation of all the dietary fibres in the feeds, because the CF method only recovers incomplete fractions of total lignin, hemicellulose and cellulose, varying from 90 % to as low as 10 % of total content of these fibres (de-Oliveira *et al.*, 2012). Cellulose and small amounts of lignin are the main constituents of CF in pet foods (de-Oliveira *et al.*, 2012). This study was part of a larger study that will be published later on with a deeper analysis of the dietary fibre content in the feeds. Unfortunately, those values are not available to us at this time.

Table 2. *Composition and proximate analysis in percentage (%) or per kilo (/kg) of provided sample of the wheat diet, oat diet and rye diet.*

Proximate analysis			
Components	Wheat diet	Oat diet	Rye diet
Crude Fibre (%)	1.88	1.98	1.89
EG-Fat (%)	15.61	15.29	15
CP (%)	27.5	28	27.2
Ash (%)	6.48	6.57	6.62
DM	93.07	91.88	92.03
MJ/kg	19.87	19.44	19.65
ME/kg*	3913.1	3807.48	3866.17
Composition			
Ingredients (%)	Wheat diet	Oat diet	Rye diet
Whole wheat	25		
Whole rye		25	
Rolled oats			25
Corn	15	15	15
Rice	11.674	10.82	12.5
Lignocellulose	1.5	1.5	1.5
Chicken (dried)	29.673	30.39	29.903
Chicken (fresh)	5	5	5
Chicken broth	3	3	3
Pig fat	7.493	7.63	6.437
Premix	1.66	1.66	1.66

* Calculated using an equation from the National Research Council (NRC, 2006) for metabolizable energy (ME) in prepared dog foods. CP = crude protein, DM = dry matter, MJ = megajoule.

3.2. Subjects & Management

Eleven privately owned dogs of different age (5.6 ± 2.8 standard deviation), weight (19.7 ± 7.3 standard deviation) and breed (Table 3) were used in this study. The dogs were fed a daily amount that would keep them stable in weight throughout the study. The dogs were fed twice per day. Each dog was also weighed once every week to make sure that the daily amount was enough to keep the weight stable. The dogs were all living with their owners in their home environment during the whole experiment. Owners were told to eliminate or at least minimize the amount of stuff the dogs ate apart from the experimental feeds. If the dogs managed to eat something outside on walks or the owners gave the dogs treats the owners were also told to make notes about what the dogs ate and how much. The owners also had to make notes if the dogs did not eat their daily amount of food and if the dog had any arising gastro-intestinal issues because of the feeds.

Table 3. *All dogs ID, sex, breed, age (years) and average weight (kg) throughout the study.*

Dog ID	Sex	Breed	Age	Weight
1	Female	Mixed breed (Labrador / Working Labrador / Rhodesian ridgeback)	7	22.2
2	Female	Working Labrador	3	19.8
3	Male	Nova scotia duck tolling retriever	2	20.2
4	Male	Lhasa Apso	9	8.5
5	Female	Border Collie	5	15.1
6	Female	Unknown	8	20.4
7	Male	Welsh Corgi Cardigan	3	16.9
8	Female	Staffordshire Bull Terrier	7	13.1
9	Female	Siberian Husky	10	18.8
10	Male	Mixed breed (Cane Corso / East Siberian Laika)	2	37.4
11	Female	Labrador	11	23.9

3.3. Behavioural tests

3.3.1. Trial design

In order to investigate satiety, all dogs were subjected to a total of three days of behavioural testing. One day at the end of each treatment period. On a testing day, two behavioural tests, i.e. a runway and an attention bias test, were performed. A test day started with the handler and dog arriving at the test area in the morning where the dog got its morning meal at a previously set time between 07:00 – 09:30 h. Should the dog have refused to eat or did not eat the majority (approximately 90 %) of the morning meal, the dog was not tested that day. The time for consumption of the morning meal was recorded. After which the dog was subjected to a series of tests, consisting of a runway and an attention bias test. The dogs were subjected to the tests at *15 minutes*, *3 hours* and *6 hours* after consumption of their morning meal. At ***15 minutes*** post-meal consumption, the dogs were subjected to both the runway and attention bias test. At ***3 hours*** post-meal consumption, the dogs were subjected to only the runway test. At ***6 hours*** post-meal consumption, the dogs were subjected to both the runway and attention bias test. The order in which the dogs are subjected to the runway and attention bias tests at *15 minutes* and *6 hours* post-meal consumption was randomized. The attention bias test was only performed two out of three times during a day of testing to lower the risk of the dogs learning that the food bowl was empty and therefore would not approach it.

3.3.2. Runway test

In order to test the strength of the feeding motivation for the different diets, dogs were assessed in a runway test in which the running speed towards a food bowl placed at the end was measured. In order to control for any effect of food preference for one of the diets, two bowls were placed at the end of the runway: one containing the experimental feed and the other containing the control (wheat) feed. Therefore, there were three combinations of food, Wheat/Wheat, Wheat/Oats and Wheat/Rye, tested on three separate occasions.

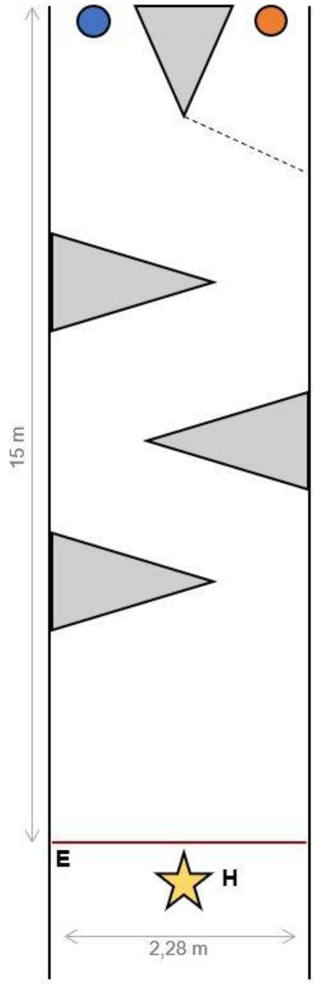

Figure 1. *Runway set-up. Red line = starting line, Grey, triangle areas = unmovable barriers, Dotted line = movable barrier, Blue circle (top left) = Treatment feed, Orange circle (top right) = Control feed, Star = Dog, E = Experimenter, H = Handler.*

All dogs (n=11) had to run a straight fixed track (15 m) to obtain a food reward at the end of the runway (Figure 1). The food reward consisted of either the experimental feed the dog was currently being fed (treatment feed) or the control feed (wheat diet) which was the same through the whole experiment. A total of 4% of each dogs' daily food amount was divided over the runs, resulting in food rewards of 1.0 – 2.5 g (i.e. 2 – 5 kibbles) at the end of each run. Each dog ran a total of six runs in each runway test, three consecutive runs for each feed at the end of the runway. The different food rewards were placed in different bowls separated from each other with barriers. One movable part of the barrier was moved to hinder the dog from approaching the wrong bowl. The treatment feed was always placed to the left and the control feed was always placed to the right in the runway, but the first bowl (left or right) to be tested was randomly assigned to each dog but balanced for treatment.

The runway test started with a guided run in which the handler, normally the owner or otherwise a stand-in for the owner, guided the dog by the leash from the start of the runway up to where the bowl with the food reward (either the treatment or the control feed) were stationed. The dog then had the opportunity to see and smell the food in the bowl but was not allowed to eat it, so that the dog could learn that there was a bowl of food at the end of the runway. Once the dog had smelled the food it was taken back to the start of the runway. The first guided run was then followed by two unguided runs. At the start of the unguided runs, the dogs' leash was removed and given a free command at which point the dog would start moving towards the bowl at the end of the runway. The time it took from the moment the dog crossed the starting line (two paws over the line) until it had placed its snout in the bowl at the end was recorded by the experimenter with a stopwatch. After each run, the experimenter refilled the bowl, and any remaining food was replaced with the new. The procedure was then repeated with one guided run and two unguided runs to the second bowl containing either the treatment or control feed. If the dog did not put its snout in the bowl within one minute of crossing the starting line, or if the dog did not cross the starting line within one minute, it was scored with the maximum time of 60 seconds. If the dog did not eat the food reward after putting its' snout in the bowl, it was noted in the protocol and the run was excluded from the analysis. To not affect their dogs behaviour the handlers were instructed to be stationary and ignore the dog once the dog was released and until it had reached the bowl or one minute had passed.

The reason for having two different locations for the different food rewards was so that the dogs would know what to expect at the end of the runway, and also to clarify the difference in case dogs had trouble detecting palatability or scent differences. The barriers in the middle of the track were used to slow the dogs' approach enough so that a measurable difference in latency would be created, this instead of a u-shaped track that previously have been validated in pigs (Souza da Silva, 2012).

3.3.3. Attention bias test

An attention bias test, based on a previously validated method to assess emotional state in monkeys (Boggiani *et al.*, 2018), was modified for the purpose of this study. All dogs (n=11) were presented with two different stimuli (Figure 2); an empty bowl and a toy. The owners were asked to bring the dogs' favourite toy and their own food bowl for this purpose. The reason for this was to have objects that the dog already associated with food and play, instead of having anonymous bowls and toys that might look and smell unfamiliar and might not trigger the same reaction as already familiar objects would. Before the start of the test the handler held the dog by the leash 3.0 m from where the bowl and the toy would later be placed. The bowl and toy were placed 2.0 m apart by the

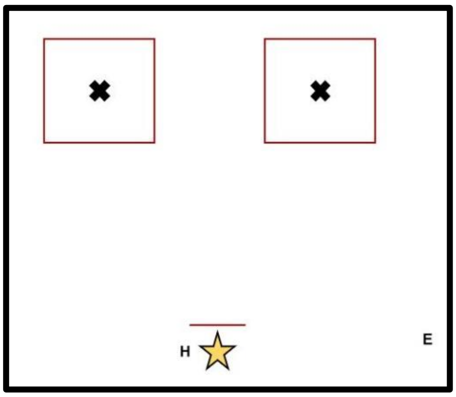

Figure 2. *Attention bias test set-up. Star = Dog, E = Experimenter, H = Handler, X = placement of the items at the start of the test. The squares (1x1m) was useful in measuring the dogs' proximity to the items.*

experimenter, one at a time, at a set distance from the dog, and the order in which items were placed in front of the dog was randomized. Once the items were in place, the dog was set free for one minute, during which the handler and experimenter remained stationary and ignored the dog. The test was video recorded for later analysis. The ethogram for this study and test is specified in Table 4.

All videos were analysed using the Mangold INTERACT software version 16.1.0 (Mangold International GmbH, Arnstorf, Germany). For each behaviour from the attention bias ethogram the duration was measured, except 'Choice', which was defined as the first object the dog chose to approach (bowl or toy). Proximity to the items and gaze direction were always recorded even if the dog displayed other behaviours at the same time. E.g. if the dog were playing while in proximity of the owner, or if the dog was holding the toy but were clearly gazing at the bowl. To not affect their dogs behaviour the handlers were instructed to be stationary and ignore the dog once the test had started.

Table 4. *Ethogram for the attention bias test*

BEHAVIOUR	**DEFINITION**
Choice & Latency	
Choice	The choice made to approach (i.e. touch) either the Bowl or Toy first
Latency to approach	Time (s) from start of the test for the dog to touch the Bowl or Toy for the first time
Item directed behaviour	
Attention	The sum of gazing towards, touching, proximity to item (bowl & toy) and individual play (toy)
Gaze direction	Dog turning its head towards the bowl, toy or other direction
Proximity to item	Within a dogs' length of bowl/toy
Touch	Dog sniffing (within 3 cm of object) or touching the object (bowl/toy) without picking it up
Individual play	Any motor activity directed towards the Toy/Bowl, including chewing/biting/shaking from side to side/holding it in its mouth/scratching/batting with paw/tossing/chasing toy in movement WITHOUT any interactions with a human[1]
Human directed behaviour	
Attention	The sum of gazing towards, being in proximity of a human (handler or experimenter) and/or the dog is engaged in human directed play (Invitation to play and Social play)
Gaze direction	Dog turning its head towards the owner or experimenter
Proximity to owner	Within a dogs' length of owner
Proximity to experimenter	Within a dogs' length of experimenter
Invitation to play	Standing in front of human placing toy on the ground between dog and human, looking from toy to human, or human to toy, and backing away from the toy
Social play	Any motor activity directed towards a toy, with physical contact (chewing/biting/shaking from side to side/holding it in its mouth/scratching/batting with paw), when interacting with a human[1]

[1] These definitions were adapted from the ethogram by Hall *et al.* (2017)

3.4. Test environment

The testing was performed in an indoor area, the runway test in a corridor and the attention bias test in a room next to the corridor. The local were provided by Akademiska Hus (Akademiska Hus AB, Uppsala).

3.5. Statistical analysis

All data were analysed using R (The R Project for Statistical Computing) software version 3.6.2. All data was checked for normality and heteroscedasticity by visual inspection, and if the assumptions of the model were not met then the data was $\log_{10}$ or square root transformed. A non-parametric Kruskall-Wallis rank sum test was used if the data still did not meet assumptions of normality after data transformation. All normally distributed variables were analysed fitting Linear Mixed Models (LMMs) or Generalized Linear Mixed Models (GLMMs) using the 'lme4' package. Dog ID was included as a random effect in all models. Post-hoc contrasts were calculated using 'emmeans' package in R where appropriate. One dog was not tested on *Day 2* because she did not eat the morning meal. One dog fell ill prior to his last day of tests (*Day 3*) and was therefore not tested for that day.

The log-transformed latency to approach the bowl in the runway test was analysed with treatment (wheat diet, oat diet, rye diet), day (*Day 1*, *Day 2*, *Day 3*), time post-meal consumption (*15 minutes*, *3 hours*, *6 hours*), feed order (Wheat-Oats-Rye, Oats-Rye-Wheat, Rye-Oats-Wheat), run (1-6) and food reward (control or treatment feed) as fixed effects and dog ID as random effect. Three-way interaction between fixed effects were also tested. Nine runs were excluded prior to analysis because the dogs did not consume any of the food reward at the end of the runway on those runs.

The attention bias data was analysed with treatment (wheat diet, oat diet, rye diet), day (*Day 1*, *Day 2*, *Day 3*), time post-meal consumption (*15 minutes*, *6 hours*) and feed order (Wheat-Oats-Rye, Oats-Rye-Wheat, Rye-Oats-Wheat) as fixed effects and dog ID as random effect. Three-way interaction between fixed effects were also tested. The probability of the dogs choosing the bowl first was evaluated using a generalized mixed-effects model with a logistic link function with treatment and time as fixed effects and dog ID as a random effect.

The latency to approach the toy did not meet assumptions of normal distribution and therefore a non-parametric test (Kruskall-Wallis rank sum test) was used instead with treatment and time post-meal consumption as fixed effects. The invitation to play did not either meet the assumptions of normality and the same non-parametric test where therefore used with treatment, day and time post-meal consumption as fixed effects. The approach bowl data was $\log_{10}$-transformed while gaze bowl, gaze toy, gaze owner, gaze experimenter, touch bowl, touch toy, social play, bowl proximity, toy proximity, owner proximity, experimenter proximity data were square root transformed. If required, any outliers with residuals larger than 2.5 were excluded from analysis to meet assumptions of normality.

4. Results

4.1. Runway test

Treatment had an effect on latency to reach the bowl ($F(2,528.83) = 5.74$, $p<0.01$), and post-hoc analysis showed that dogs on the wheat diet were significantly quicker than dogs on either the oat diet ($p<0.05$) or rye diet ($p<0.01$). There was no significant difference between the oat diet and rye diet (Figure 3).

Effect of time post-meal consumption on the latency to reach the bowl was significant ($F(2,527.07) = 29.44$, $p<0.001$). Post-hoc analysis showed that the dogs were slowest at *15 minutes* (*15 min* vs *3 hours*, $p<0.0001$; *15 min* vs *6 hours*, $p<0.0001$) and dogs were also significantly slower at *3 hours* compared to *6 hours* ($p<0.05$) post-meal consumption (Table 5).

There was a tendency for a treatment by time interaction ($p<0.1$), where dogs on the wheat diet tended to be faster than dogs on the oat diet and rye diet at *3 hours* post-meal consumption while this difference was less obvious in either the morning (*15 minutes*) or in the afternoon (*6 hours*, Figure 3).

Feed order also significantly affected the latency ($F(2,8.03) = 20.15$, $p<0.001$, Figure 3). Further analysis with post-hoc revealed that dogs with the feed order Oats-Rye-Wheat were significantly slower compared to the Wheat-Oats-Rye ($p<0.001$) and Rye-Wheat-Oats ($p<0.01$) feed order. No significant difference was found between the Wheat-Oats-Rye and Rye-Wheat-Oats feed order.

Effect of day on the latency was significant ($F(2,528.67) = 6.36$, $p<0.01$). Post-hoc analysis shows that dogs were significantly slower on *Day 1* compared to *Day 3* ($p<0.01$, Table 7).

The type of food reward (control wheat diet or experimental) at the end of the runway showed no effect on the latency to reach the bowl at the end of the runway ($F(1,527.07) = 1.11$, $p=0.293$).

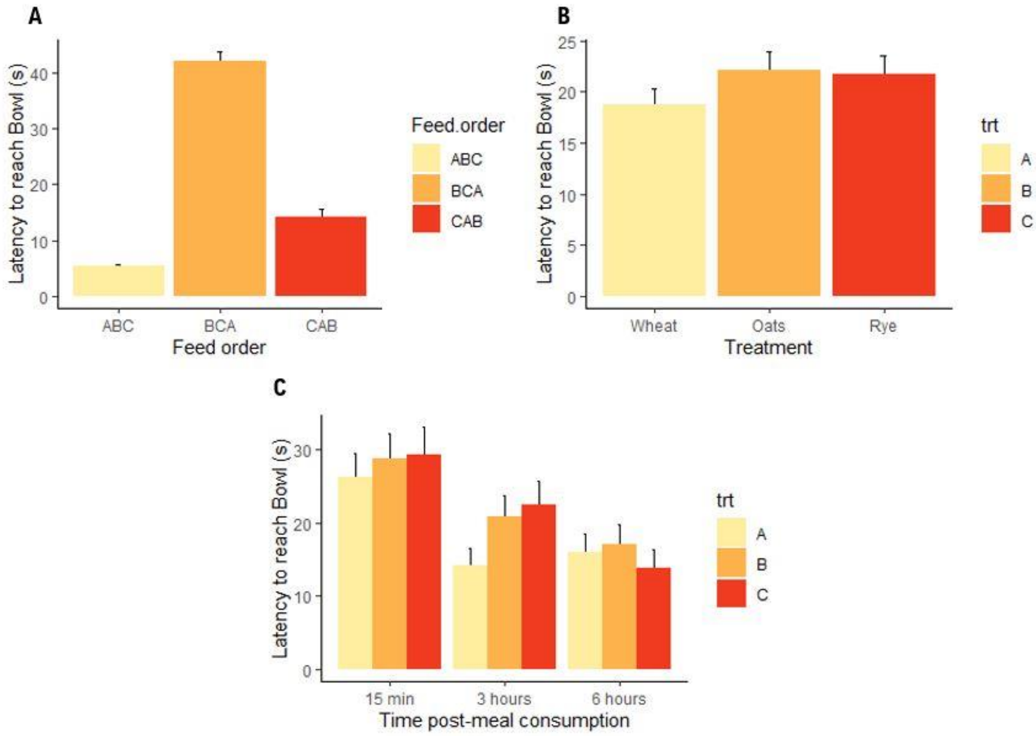

Figure 3. *(**A**) Effect of feed order, (**B**) diet (**C**), diet and time post-meal on the mean ± SEM latency to reach the bowl in the runway test (trt = treatment, A = wheat, B = oats, C = rye, ABC = Wheat-Oats-Rye, BCA = Oats-Rye-Wheat, CAB = Rye-Wheat-Oats).*

Table 5. *Effect of diet, time post-meal and day on the mean ± SEM latency to reach the bowl in the runway test.*

	Day									**Effects**				
	Day 1			Day 2			Day 3							
	Rye	Oats	Wheat	Rye	Oats	Wheat	Rye	Oats	Wheat	TR	T	D	FO	TR*T
Average over the three time points														
Latency to reach bowl (s)	15.3 ± 2.6	46.2 ± 2.7	5.7 ± 0.1	44.1 ± 3.2	5.4 ± 0.1	12.1 ± 2.3	5.3 ± 0.2	15.2 ± 2.8	36.8 ± 3.0	**	***	**	***	#
15 minutes post-meal														
Latency to reach bowl (s)	24.5 ± 6.8	59.3 ± 0.7	5.6 ± 0.2	57.2 ± 2.8	5.1 ± 0.2	23.9 ± 6.2	5.3 ± 0.3	24.7 ± 6.1	48.5 ± 4.3	-	-	-	-	-
3 hours post-meal														
Latency to reach bowl (s)	15.9 ± 4.1	42.5 ± 4.8	5.7 ± 0.1	46.1 ± 5.5	5.3 ± 0.1	6.2 ± 0.5	5.3 ± 0.3	15.2 ± 4.9	28.7 ± 5.2	-	-	-	-	-
6 hours post-meal														
Latency to reach bowl (s)	6.9 ± 0.5	37.7 ± 5.2	5.8 ± 0.1	29.2 ± 6.0	5.8 ± 0.2	6.3 ± 0.5	5.4 ± 0.3	5.8 ± 0.5	33.2 ± 5.4	-	-	-	-	-

p<0.1, * p<0.05, ** p<0.01, *** p<0.001 and ns = non-significant, TR = Treatment, T = Time post-meal consumption, D = Day, FO = Food order, TR*T = treatment by time interaction

4.2. Attention bias test

4.2.1. Choice and Latencies to approach

The probability of the dogs choosing the bowl first was numerically higher at *6 hours* (μ = 58.1 ± 9.0 %) compared to *15 minutes* (μ = 35.5 ± 8.7 %) post-meal consumption for all treatments, although this difference was not statistically significant, no treatment effects were found on the probability to choose the bowl first. Time post-meal consumption had a significant impact on the latency to approach the bowl ($F(1,37.04) = 8.37$, $p<0.01$), and post-hoc analysis revealed that the dogs were significantly faster to approach the bowl at *6 hours* compared to *15 minutes* post-meal consumption (Table 6). Latency to approach the bowl was also affected by day ($F(2,37.03) = 4.39$, $p<0.05$), post-hoc analysis showing that on *Day 1* dogs were significantly slower to approach the bowl compared to *Day 2* ($p<0.05$) and tended to be faster than on *Day 3* ($p<0.1$, Table 7).

No significant effect of treatment, time post-meal consumption, day or feed order on the latency to approach the toy were found (Table 6; Table 7).

4.2.2. Item directed behaviour and attention

Day had a significant effect on total duration dogs were gazing at the bowl ($F(2,36.57) = 8.17$, $p<0.01$). Dogs spent significantly less time gazing at the bowl on *Day 1* compared to *Day 2* ($p<0.001$) and tended to gaze at the bowl less on *Day 1* compared to *Day 3* ($p<0.1$), with no difference between *Day 2* and *Day 3* ($p=0.278$, Table 7).

The total duration of the dogs touching the bowl was also significantly affected by day ($F(2,36.40) = 4.51$, $p<0.05$) in the way that dogs were touching the bowl more during *Day 1* compared to *Day 2* ($p<0.05$, Table 7).

The total duration the dogs spent in proximity of the bowl was significantly affected by a treatment by day interaction ($F(4,13.23) = 4.77$, $p<0.05$), with a tendency for a main effect of day ($F(2,34.32) = 2.54$, $p<0.1$). Post-hoc analysis revealed that, on *Day 1*, dogs on the wheat diet spent significantly less time in proximity of the bowl compared to dogs on the oat diet ($p<0.05$) and tended to spend less time in proximity compared to dogs on the rye diet ($p<0.1$, Table 7).

The total duration of attention the dogs directed towards the bowl was affected by a treatment by day interaction ($F(4,13.22) = 4.42$, $p<0.05$, Figure 4), in the way that dogs on *Day 2* on the wheat diet were directing significantly less attention towards the bowl compared to dogs on the oat diet ($p<0.05$). There was also a significant main effect of day ($F(2,33.51) = 10.92$, $p<0.001$). Post-hoc analysis revealed that dogs directed more attention towards the bowl on *Day 1* compared to *Day 2* ($p<0.001$) and *Day 3* ($p<0.05$, Table 7).

The total duration of gazing at the toy was significantly affected by day ($F(2,33.13) = 5.21$, $p<0.05$), with dogs gazing at the toy significantly longer during *Day 1* compared to *Day 2* ($p<0.01$) and a tendency for an effect of treatment was also found ($F(2,33.12) = 3.22$, $p<0.1$), with dogs on the wheat diet gazing slightly longer at the toy than dogs on the oat diet (Table 7).

Dogs touching the toy was significantly affected by treatment ($F(2,32.63) = 4.37$, $p<0.05$) and day ($F(2,32.62) = 3.39$, $p<0.05$), post-hoc test showing that dogs on the wheat diet spent less time touching the toy compared to dogs on the oat diet ($p<0.05$) and dogs also spent significantly less time touching the bowl on *Day 2* compared to *Day 3* ($p<0.05$, Table 7).

Duration spent in proximity of the toy tended to be affected by a treatment by day interaction ($F(4,16.43) = 2.78$, $p<0.1$). Dogs on the wheat diet spent more time in proximity of the toy during *Day 1* compared to dogs on the oat diet. Dogs on the rye diet spent more time in proximity of the toy during *Day 3* compared to the dogs on wheat and oat diets (Table 7).

There was a treatment by day interaction that significantly affected total duration of individual play ($F(4,12.47) = 3.89$, $p<0.05$) for which post-hoc test revealed no significances or tendencies. There also was a tendency for a main effect of treatment ($F(2,35.11) = 2.80$, $p<0.1$) and a tendency for a treatment by day by time interaction ($F(4,34.99) = 2.47$, $p<0.1$) to effect total duration of individual play, with dogs on the wheat diet spending less time playing individually compared to dogs on the oat diet (Table 6; Table 7).

The total duration of attention that was directed towards the toy was not significantly affected by any of the factors included (Figure 4.).

4.2.3. Human directed behaviour and attention

The total gaze owner duration was significantly affected by treatment ($F(2, 36.41) = 4.06$, $p<0.05$), with dogs on the wheat diet gazing at the owner significantly more than dogs on the oat diet ($p<0.05$). There was a tendency for a treatment by time interaction effect on the gaze owner duration ($F(2,36.01) = 2.76$, $p<0.1$), with dogs on the wheat diet spending slightly more time gazing at the owner at *6 hours* post-meal consumption than dogs on either the oat or rye diet (Table 6.).

The total duration the dogs spent in proximity of their owner was significantly affected by treatment ($F(2,33.68) = 4.09$, $p<0.05$), and post-hoc test showed that dogs on the wheat diet spent significantly less time in proximity of the owner compared to dogs on the rye diet ($p<0.05$). A day by time interaction also significantly affected total duration dogs spent in proximity of the owner ($F(2,33.37) = 5.54$, $p<0.01$), and a tendency for an effect of a treatment by day by time interaction ($F(4,33.34) = 2.56$, $p<0.1$). Dogs spent significantly more time in owner proximity during *Day 3* at *6 hours* post-meal consumption compared to the same time in *Day 1* ($p<0.05$). Dogs on the rye diet seemed to spend more time in owner proximity during *Day 1* at *15 minutes* post-meal consumption compared to dogs on either the wheat or oat diet (Table 7).

Time spent gazing at the experimenter tended to be affected by a treatment by day interaction ($F(4,12.03) = 3.18$, $p<0.1$, Table 7).

A treatment by day interaction ($F(4,13.25) = 5.24$, $p<0.01$), with a main effect of treatment ($F(2,31.81) = 3.41$, $p<0.05$), had significant effects on the total duration dogs spent in proximity of the experimenter (Table 7). Post-hoc test revealed that on *Day 3,* dogs on the rye diet spent significantly more time in proximity of the experimenter than dogs on either the wheat ($p<0.001$) or oat diets ($p<0.01$). There was also a tendency for a treatment by day by time interaction ($F(4,31.39) = 2.17$, $p<0.1$) effect on time spent in proximity of experimenter (Table 6).

There was also a tendency of effect on experimenter proximity due to a treatment by day by time interaction ($F(4,28.41) = 2.40$, $p<0.1$).

The total duration of attention toward humans was significantly affected by an interaction between treatment, day and time post-meal consumption ($F(4, 34.543) = 4.15$, $p<0.01$, Figure 4). Post-hoc analysis showed that on *Day 1* at *15 minutes* post-meal consumption, dogs on the rye diet directed significantly more attention toward humans compared to dogs on the oat diet ($p<0.05$). On *Day 2* at *6 hours* post-meal consumption dogs on the wheat diet directed significantly more attention toward humans compared to dogs on the oat diet ($p<0.01$) and dogs on the rye diet ($p<0.001$). On *Day 3* at both test times dogs on the rye diet directed significantly more attention toward humans than both dogs on the wheat diet (*15 min*: $p<0.01$; *6 hours*: $p<0.001$) and dogs on the oat diet (*15 min*: $p<0.05$; *6 hours*: $p<0.05$).

The variables 'invitational play' and 'social play' showed no significance.

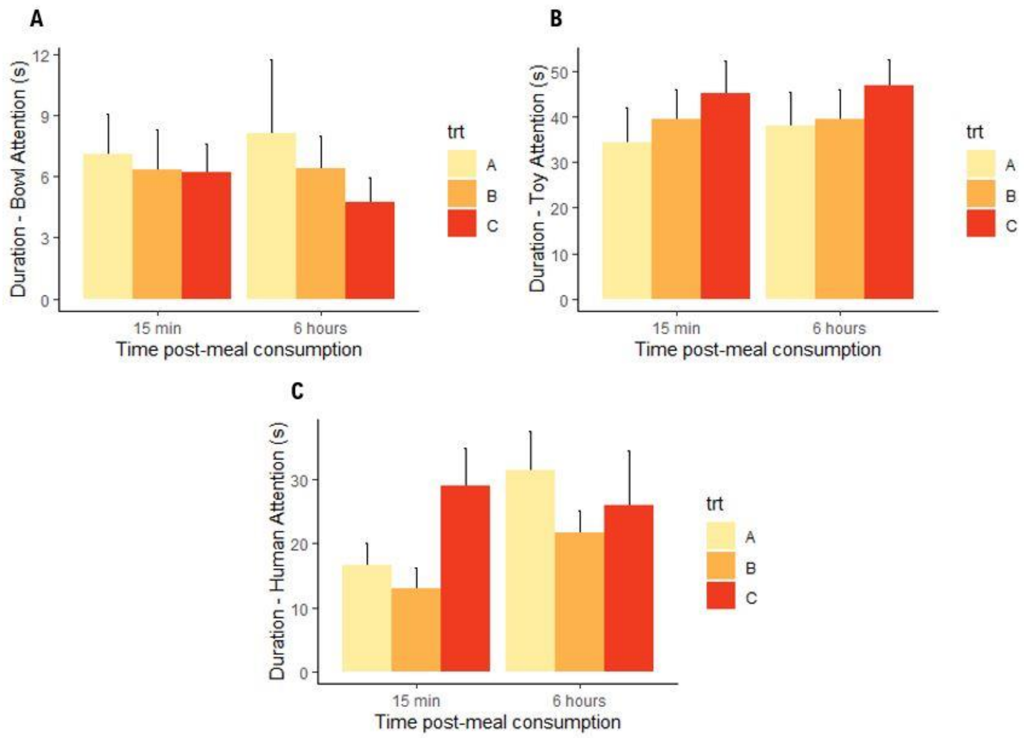

Figure 4. *Effect of diet and time post-meal on the mean ± SEM duration of attention toward (**A**) bowl (**B**), toy and (**C**) humans in the attention bias test (trt = treatment, A = wheat, B = oats, C = rye).*

Table 6. *Effect of time post-meal and treatment on mean ± SEM duration of behaviours in the attention bias test* [1].

	Time post-meal consumption						**Effects**					
	15 minutes			*6 hours*								
	Rye	Oats	Wheat	Rye	Oats	Wheat	TR	T	D	TR*T	TR*D	TR*D*T
Choice & Latency												
Latency Bowl	28.2 ± 10.0	28.2 ± 10.1	25.7 ± 9.3	26.6 ± 11.8	25.1 ± 9.5	19.5 ± 8.8	ns	**	*	ns	ns	ns
Latency Toy	3.3 ± 1.5	2.8 ± 0.6	13.3 ± 7.0	2.9 ± 0.6	6.8 ± 3.5	18.5 ± 8.1	ns	ns	ns	ns	ns	ns
Item directed behaviour												
Gaze Bowl	3.2 ± 0.9	2.4 ± 0.9	2.0 ± 0.5	1.8 ± 0.4	2.7 ± 0.9	2.3 ± 0.6	ns	ns	**	ns	ns	ns
Gaze Toy	1.3 ± 0.3	1.7 ± 0.4	1.4 ± 0.3	1.2 ± 0.3	1.8 ± 0.4	1.2 ± 0.5	ns	.	*	ns	ns	ns
Proximity Bowl	2.8 ± 0.7	3.6 ± 1.7	4.4 ± 1.3	2.6 ± 0.8	2.8 ± 1.0	5.3 ± 3.2	ns	ns	.	ns	*	ns
Proximity Toy	7.5 ± 3.7	4.3 ± 2.1	3.2 ± 1.5	7.8 ± 3.3	1.2 ± 0.6	8.6 ± 4.1	ns	ns	ns	ns	.	ns
Touch Bowl	0.2 ± 0.1	0.3 ± 0.1	0.7 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	ns	ns	*	ns	ns	ns
Touch Toy	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	*	ns	*	ns	ns	ns
Individual play	34.7 ± 7.3	33.1 ± 7.2	29.7 ± 7.5	37.8 ± 7.3	36.2 ± 7.1	26.4 ± 7.2	.	ns	ns	ns	*	.
Human directed behaviour												
Gaze Owner	1.9 ± 1.4	2.5 ± 1.4	1.8 ± 1.2	1.7 ± 1.1	1.5 ± 0.8	6.3 ± 2.8	ns	*	ns	.	ns	ns
Gaze Experimenter	2.2 ± 1.1	3.7 ± 3.4	2.8 ± 1.7	2.6 ± 1.7	3.5 ± 1.3	4.2 ± 2.4	ns	*	*	ns	*	ns
Proximity Owner	14.6 ± 4.2	6.6 ± 2.2	5.3 ± 2.3	14.2 ± 4.6	12.3 ± 3.3	5.8 ± 3.4	*	ns	ns	ns	ns	.
Proximity Experimenter	8.5 ± 4.5	2.1 ± 0.9	5.8 ± 2.4	7.0 ± 2.4	3.8 ± 1.4	0.9 ± 0.6	*	ns	ns	ns	*	.
Invitation to play	0.5 ± 0.5	0.5 ± 0.5	0.6 ± 0.6	1.0 ± 1.0	1.4 ± 1.1	0.8 ± 0.6	ns	ns	ns	ns	ns	ns
Social play	1.8 ± 0.8	0.5 ± 0.4	0.3 ± 0.2	0.6 ± 0.4	0.6 ± 0.4	0.5 ± 0.3	ns	ns	ns	ns	ns	ns

. = p<0.1, * = p<0.05, ** = p<0.01, *** = p<0.001, ns=non-significant, TR = Treatment, T = Time post-meal consumption, D = Day, TR*D = treatment by day interaction, TR*T = treatment by time interaction, TR*D*T = treatment by day by time interaction

[1] Attention means in Figure 4.

Table 7. *Effect of treatment and day together with treatment on mean ± SEM duration on behaviours in the attention bias test.*

	Treatment			**Day**									**Effects**					
				Day 1			Day 2			Day 3								
	Rye	Oats	Wheat	Rye	Oats	Wheat	Rye	Oats	Wheat	Rye	Oats	Wheat	TR	T	D	TR*T	TR*D	TR*D*T
Choice & Latency																		
Latency Bowl	27.5±7.4	26.6±6.7	22.6±6.3	36.8±14.0	36.3±11.2	2.5±0.4	41.0±12.0	1.5±0.2	45.3±14.7	1.9±0.03	48.2±11.8	31.4±10.8	ns	**	*	ns	ns	ns
Latency Toy	3.1±0.8	4.8±1.8	15.9±5.2	2.0±0.5	7.7±4.8	10.5±7.1	1.9±0.7	4.1±0.6	20.8±12.4	5.5±2.0	1.8±0.8	17.5±9.3	ns	ns	ns	ns	ns	ns
Item directed behaviour																		
Attention Bowl	5.5±0.9	6.4±1.3	7.6±1.9	4.5±1.1	6.5±1.2	14.7±4.9	3.7±1.2	10.2±2.9	1.2±0.7	8.2±1.9	1.8±0.7	6.9±1.2						
Attention Toy	46.0±4.5	39.5±4.5	36.3±5.2	48.2±8.6	35.9±8.4	40.2±6.9	49.3±9.2	32.7±7.6	31.1±11.5	40.6±6.0	53.3±4.1	36.3±9.8						
Gaze Bowl	2.5±0.5	2.5±0.6	2.1±0.4	1.8±0.5	4.2±1.2	2.9±0.5	1.7±0.6	2.4±0.9	0.5±0.3	4.0±1.2	0.4±0.2	2.6±0.8	ns	ns	**	ns	ns	ns
Gaze Toy	1.3±0.2	1.8±0.3	1.3±0.3	0.9±0.2	2.6±0.6	1.9±0.3	1.0±0.3	1.6±0.3	0.4±0.2	1.9±0.3	0.9±0.2	1.3±0.5	ns	.	*	ns	ns	ns
Proximity Bowl	2.7±0.5	3.2±1.0	4.8±1.5	2.5±0.8	1.6±0.5	10.5±4.3	1.8±0.6	6.3±2.3	0.7±0.5	3.7±1.1	1.3±0.5	3.7±0.8	ns	ns	.	ns	*	ns
Proximity Toy	7.6±2.4	2.7±1.1	5.9±2.2	5.6±4.0	1.0±0.5	11.9±5.0	2.8±1.7	4.0±2.2	5.0±3.3	14.5±5.1	3.3±2.8	0.5±0.3	ns	ns	ns	ns	.	ns
Touch Bowl	0.3±0.1	0.3±0.1	0.6±0.2	0.2±0.2	0.6±0.3	0.9±0.3	0.2±0.2	0.3±0.1	0.02±0.02	0.5±0.2	0.02±0.02	0.6±0.3	ns	ns	*	ns	ns	ns
Touch Toy	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.02	0.1±0.03	0.1±0.03	0.1±0.04	0.1±0.02	0.1±0.03	0.1±0.02	0.4±0.2	0.1±0.02	*	ns	*	ns	ns	ns
Individual play	36.3±5.0	34.6±4.9	28.1±5.1	40.6±8.3	32.1±9.1	25.0±7.0	45.0±9.1	26.6±8.6	24.8±10.2	24.1±7.2	48.7±4.3	33.6±10.2	.	ns	ns	ns	*	.
Human directed behaviour																		
Attention Human	28.3±5.3	18.5±2.9	24.5±3.8	25.1±6.1	17.0±4.6	30.1±3.2	8.8±4.9	20.1±6.4	32.6±9.7	51.0±7.0	18.7±4.3	11.1±4.1						
Gaze Owner	1.8±0.8	2.0±0.8	4.1±1.6	0.2±0.2	1.7±0.8	7.7±3.9	0.2±0.2	3.2±2.1	2.2±1.1	4.9±2.0	0.9±0.6	1.8±1.1	ns	*	ns	.	ns	ns
Gaze Experimenter	2.4±1.0	3.6±1.8	3.5±1.4	1.2±1.2	4.0±1.7	6.5±3.7	0.0±0.0	5.7±4.5	1.0±0.8	6.0±2.1	0.4±0.4	2.3±1.1	ns	*	*	ns	*	ns
Proximity Owner	14.4±3.0	9.5±2.0	5.5±1.9	18.8±5.2	8.1±3.5	3.1±1.7	7.0±4.6	7.0±3.3	9.9±6.3	17.5±5.3	14.6±3.4	5.3±3.0	*	ns	ns	ns	ns	.
Proximity Experimenter	7.7±2.5	3.0±0.8	3.6±1.4	3.0±1.5	1.4±1.0	6.0±2.2	1.1±0.7	5.2±1.7	4.4±3.9	19.2±4.4	2.5±1.2	0.1±0.1	*	ns	ns	ns	*	.
Invitation to play	0.7±0.6	0.9±0.6	0.7±0.4	0.0±0.0	0.9±0.7	1.9±1.0	0.0±0.0	1.7±1.5	0.0±0.0	2.2±1.6	0.0±0.0	0.0±0.0	ns	ns	ns	ns	ns	ns
Social play	1.2±0.5	0.6±0.3	0.4±0.2	1.9±1.1	1.0±0.6	0.4±0.3	0.5±0.5	0.3±0.3	0.9±0.6	1.2±0.6	0.4±0.2	0.0±0.0	ns	ns	ns	ns	ns	ns

. = p<0.1, * = p<0.05, ** = p<0.01, *** = p<0.001, TR = Treatment, T = Time post-meal consumption, D = Day, TR*D = treatment by day interaction, TR*T = treatment by time interaction, TR*D*T = treatment by day by time interaction

5. Discussion

In agreement with our hypothesis, latencies in the runways decreased with time post-meal consumption irrespective of diet. As expected, dogs on the wheat diet had the shortest latencies in the runway, however, no difference in latency between the oats and rye diet was found. This suggests that oats and rye were equally satiating, despite their differences in dietary fibre content.

The objective with the attention bias test in this study was to investigate the dogs first choice, the sum of attention toward a food-related cue and how those would change with time post-meal consumption and if type of grain in their feed would make a difference. We detected that the latency to approach the bowl was affected by time post-meal consumption with dogs being faster to approach the bowl in the afternoon (at *6 hours*) compared to the morning (at *15 minutes*), similar to what was found in the runway test, indicating that satiety was also measured here. However, no statistical effect of time on choice or attention directed toward the bowl was found. Choice did however show a numerical pattern that supported our hypothesis that dogs would be more likely to choose the bowl with more time after a meal. Type of grain did not affect the choice made by the dogs or the latency to approach the items in the attention bias and even though some variables seemed to support our hypothesized effect of diet, there was no clear patterns.

The increase in feeding motivation throughout the day, shown by the decrease in latencies in the runway and attention bias test, tells us that we indeed managed to measure satiety using two behavioural tests.

5.1. Runway test

Dogs' feeding motivation was generally higher when fed the wheat diet. Thus, suggests that a wheat-based diet is less satiating than either a rye- or oats-based diet, which agrees with the hypothesised outcome. A number of studies have found differences in satiety due to inclusion level of fibre (Bosch *et al.*, 2009; Souza da Silva *et al.*, 2012; 2013) and found differences in energy intake between wheat and rye diets (Jackson *et al.*, 1997; Shur *et al.*, 2017). The amount of food intake did not differ in these studies, but the reduced energy intake in the rye diets still supports our finding that rye is more satiating than wheat. In line with our hypothesis that the oat and rye diet did not differ in terms of overall feeding motivation, this despite the reported higher content of total dietary fibres in rye (Frølich *et al.*, 2013). Even if oats contain less total dietary fibres, it has by far the highest content of β-glucans compared to both wheat and rye. Reportedly, β-glucans has effects on satiety due to the viscous and fermentable properties (EFSA, 2010; Slavin, 2013; Tosh and Miller, 2016). Perhaps β-glucans in oats has an equally satiating effect as the soluble arabinoxylans in rye. This would also explain why oats was shown to be more satiating compared to wheat even if oats reportedly have a lower total dietary fibre content than wheat (Frølich *et al.*, 2013).

Had we instead used some other comparison to the experimental feeds than the wheat diet, e.g. treats, it would have been hard to determine what was satiety and what was food preference. Treats are usually highly palatable, i.e. the dogs 'like' them more. 'Liking' and 'wanting' are somewhat separate components of consummatory behaviour (Spruijt *et al.*, 2001; Morrison and Berthoud, 2007). Typically, 'liking' leads to 'wanting' and ultimately consumption of a food, however, 'wanting' does not necessarily mean that the food is liked or disliked but simply needed to maintain energy balance (Spruijt *et al.*, 2001; Morrison and Berthoud, 2007; Verbeek *et al.*, 2012). Thus, using a treat or another palatable dry food as a comparison in a runway would make it difficult to conclude if the dogs ran faster because they want the food because they were hungry or if they want the food because they simply liked it.

In the runway test it was evident that time after a meal had a large impact on the dogs' feeding motivation, since the latency to reach the bowl with the food reward at the end of the runway steadily decreased post-meal consumption. Palatability did not interfere with the results of this study since there was no effect of food reward, i.e. the wheat diet used as the control compared to each of the diets including wheat vs wheat. This increase in feeding motivation with time and lack of preference for a specific diet indicate that we indeed measured satiety in the runway test. These results agree with previous research in pigs (Souza da Silva *et al.*, 2013). For some species, however, latency may be affected by daily variations in activity (Souza da Silva *et al.*, 2012). This did not seem to be the case with dogs in this study since the effect of time post-meal consumption was very clear. However, the clear difference between the dogs' speeds directly following (*15 minutes*) the morning meal compared to *3*- or *6-hours* post-meal consumption was not as clear when comparing the speed at *3*- to speed at *6-hours* post-meal consumption. Suggesting that the level of feeding motivation ceases to increase, or the increase slowed down, after a certain point after a meal. After a very long time post-meal, level of satiety will probably be the same irrespective of diet, but there is not enough evidence to know when this occurs post-meal.

However, during the runway test it was observed that the floor in the test area was rather slippery for the dogs', which could limit the maximum possible speed in the runway and thus could have affected upon the difference in latency between 3 hours and 6 hours post-meal consumption. When dogs were attempting to run faster, they would sometimes slip, and during the subsequent run they were observably more cautious. Even if the dogs were cautious there was no notable decrease in speed because they did not get frightened or hurt but seemed to learn how to go about the runway to avoid slipping. Thus, there might have been a larger difference between time points and maybe even treatments with slip-reduced flooring in the runway test.

Animals commonly show increased physical activity prior to an expected or announced reward (Spruijt *et al.*, 2001; De Leeuw *et al.*, 2008; D'Eath *et al.*, 2009). Spruijt *et al.* (2001) defines this anticipatory behaviour "…*as responses elicited by rewarding stimuli that lead to and facilitate consummatory behaviour*". Assuming the speed in the runway reflected the level of anticipatory behaviour for the food reward presented at the end, the dogs in this study showed an increase in general physical activity (i.e. faster running speeds) with time post-meal consumption. Similar increase in activity following a meal have been observed in pigs (De Leeuw *et al.*, 2008; Souza da Silva *et al.*, 2013). According to De Leeuw *et al.* (2008) however, the decrease in general activity directly following consumption of a meal has not been shown to be a reliable indicator of immediately-postprandial satiety in pigs. On the other hand, the authors definition of immediately-postprandial were 'within 3 hours'. In this study we tested the dogs two times within the first three hours of a meal resulting in a significantly lower general activity (i.e. slower running speeds) shown by the dogs at *15 minutes* than at *3 hours* post-meal consumption. Had we conducted the first round of tests at thirty minutes or one hour post-meal consumption, perhaps any significant difference in running speeds between first and second round of tests would be less clear or even lost. This will need to be verified by further research of course. The data in this study was very sensitive to individual behaviour since there were only 3-4 dogs in each treatment group. This is most visible in the treatment by day interaction because the slowest dogs seem to have all ended up in the Oats-Rye-Wheat feed order group, despite that the feed order was randomized. Therefore, if repeated, a larger number of subjects would result in more even group distribution and reduce the effect of feed order.

The dogs did not get the chance to get acclimatized to the test area before the first day of testing, which might be the reason that an effect of day was picked up in the runway analysis.

Satiety is a highly subjective, multifaced and complex sensation (Cooper *et al.*, 2015). The satiety effect of rye is well known but as is with all grains, there are still gaps in the knowledge of their effect upon the microbiota (Cooper *et al.*, 2015). In this study mainly one out of three treatments differed from the others enough so that it was detectable using a runway test. Measuring physiological indicators as well as behavioural indicators could produce a more complete picture of the effects of grains on satiety in dogs. As mentioned, this study is part of a larger study that will be published later on, where physiological parameters of satiety will be measured on dogs eating the same feeds as in this study. It will be interesting to combine those results with the ones from this study.

Based on this, using a runway to assess feeding motivation has indeed proven useful and is therefore a recommended method of choice in future studies with a similar research question. If repeated, a larger number of individuals would be preferable in this test to cancel out any unwanted effects of day and feed order.

5.2. Attention bias test

There was no statistically significant difference that supported the hypothesis that dogs would be more likely to choose their bowl at *6 hours* post-meal consumption compared to at *3 hours* post-meal consumption. On the other hand, the numerical difference followed the expected pattern, at least indicating that this study did not suffer such a large discrepancy in expected and actual outcome that has been reported in some studies (Doyle *et al.*, 2010; Burman *et al.*, 2011). While another study concluded that food restricted sheep showed an attentional bias toward a food-related cue through slower disengage from the cue and expression of feeding behaviour (Verbeek *et al.*, 2014). This might mean that the dogs in this study were not hungry enough for it to yield statistical differences, or the food-related cue did not trigger feeding motivation in dogs the way that it was expected to. Dogs were not food restricted in this study in the way that energy intake did not differ between feeding periods but had energy intake been restricted we might have picked up a statistically significant difference. However, restricting energy intake alters the incentive value of foods, which would result in higher feeding motivation because the dogs would ‘want’ the food even more (Morrison and Berthoud, 2007). This might intensify the difference, but there is a chance that the main aim to measure the satiety effect of the grains alone would be lost.

Similar to the results of the runway, the latency to approach the bowl in the attention bias test significantly decreased from *15 minutes* to *6 hours* post-meal consumption indicating that an increase in feeding motivation was measured in the approach like a miniature runway but with no food reward at the end. Demonstrating the same point made in the previous paragraph. Dogs were faster to reach the bowl with every test day, an unexpected outcome with a clear pattern which probably comes from that the dogs learned what to expect and were habituated to the situation.

In this test we used the dogs’ own bowl, because it was already familiar and associated with food. If we instead had used an anonymous bowl in this test might have resulted in a greater effect of day since the only experience the dog would have had with that bowl was that it would have been empty and therefore probably lost interest for it quicker.

The hypothesised increase in attention directed towards the bowl with time post-meal consumption was not seen in the results. The food-related cue was probably not interesting enough for the dogs to generate a longer span of attention than we got. Once the dog had approached the bowl and touched it, they most commonly did not return to investigate it a second time. Some dogs gazed at the bowl from a distance several times, as observed by the experimenter, but usually not for longer than a couple of seconds which is reflected by the results. Dogs attention toward the bowl decreased with every test day, while the attention directed toward the toy was unaffected by day. This day-effect was likely due to dogs learning that there was never any food in their bowl, and they therefore paid less and less attention to it. An improvement of the method could be to add a small amount of food in the bowl that was either accessible or inaccessible to the dogs in order to control for this learning effect. Accessible food is commonly given in small amounts, i.e. minor percentage of their daily intake, when assessing feeding motivation or satiety in animals (Souza da Silva *et al.*, 2012; 2013). The food would thus be consumed quickly if accessible in an attention bias test, and once consumed the dog would likely turn to the toy or the humans. The dogs may be more likely to return to the bowl on more occasions than if the bowl were empty, however there is a strong possibility that the dogs would ignore the bowl once the food reward was consumed and turn their attention elsewhere. Inaccessible food in a preference test has proven to predict consumption of that same food (Thompson *et al.*, 2016), leading us to believe that inaccessible food as a food-related cue in an attention bias test like the one in this study could prove useful. Inaccessible food is especially useful since it requires no food to be consumed in the process, and dogs could therefore be tested on several occasions during a day without the satiating effect of the morning meal being altered. Inaccessible food, however, can be reinforcing in itself (Thompson *et al.*, 2016), which needs to be taken into consideration before including it in any method. If inaccessible food were to be used in the current design of this study it would be difficult to decide whether to use the experimental diets or some other food in the bowl, to avoid any potential effect of food preference and to be sure that satiety is measured.

The wheat and oat diet were the only two diets differing in attention toward the bowl, and this was only during the second day of testing. Going through the separate behavioural indicators included in the attention variables, the effect of treatment does not get any clearer. The lack of treatment significance could depend on the choice of the food related cue and the way that the food related cue was presented in this study. The wheat diet is the diet that stands out the most, because dogs on the wheat diet spent significantly more or less time performing any given behaviour. No other clear pattern was found among the separate behavioural indicators, apart from choice and latency to approach, making it impossible to draw further conclusions based on the results of the attention bias test.

Dogs on the wheat diet gazed more at the owners than either of the other diets. Since the wheat diet was shown to be the least satiating diet it suggests that these dogs were gazing at the owner in an attempt to beg for more food. However, dogs on the wheat diet did not spend more time in proximity of either the owner or the bowl compared to either of the other diets and therefore it is unlikely that increased gazing at the owners was due to begging behaviours.

The least attention to the humans (i.e. owner and experimenter) were generally given by the dogs on the oat diet. One cannot do anything other than speculate as to why this was shown to be the case in this study. Invitational and social play were not often observed in this study, so it was not due to the fact that dogs played more 'with' the humans and the humans were instructed to ignore the dog during the test. There was no significant increase in gazing behaviour at the humans in dogs on the oat diet, which suggests that the dogs did not attempt to beg for more food. Whether this was because of increased satiety or perhaps palatability of the oat diet is impossible to conclude.

Because of the lack of patterns in the results from the attention bias it is hard to speculate whether the behavioural indicators of satiety used in this study were optimal or not. Choice and latency to approach produced some patterns that were of use in this study. Remaining behavioural indicators produced little knowledge when separate, however when put together as indicators of attention some patterns emerged. These patterns were on the other hand also difficult to interpret because of day effects, which are most likely due to the dogs habituating to the stimuli and test situation.

The dogs in this study displayed some exploratory behaviours (e.g. sniffing around the room) during the attention bias test, when not directing their attention towards the bowl, toy or humans (observations by the experimenter – no data available). According to Spruijt *et al.* (2001) exploratory behaviour patterns seem to be a distinctive form of anticipatory behaviour and if anticipatory behaviour facilitates and lead to consummatory behaviour, perhaps it would produce clearer results in future studies investigating satiety through behaviour if such behaviours were included in the ethogram.

Based on the results of this study, when using an attention bias test as the one we used it is important to consider the choice of stimuli. If repeated in the same manner as in this study, some alterations must be made. For instance, one option could be to use food in the test, either accessible or not. A larger number of individuals would be preferable in the attempt to cancel out any unwanted effects of day and feed order. Alterations to the ethogram to include anticipatory behaviour, such as increase in physical activity and exploratory behavioural patterns, in an effort to produce clearer results.

5.3. Present and future considerations

5.3.1. Welfare implications

The knowledge about grains' effect upon satiety and other physiological functions is important for the dogs' overall welfare. The risk of food seeking behaviours turning stereotypic can be lowered if food ingredients that prolong the feeling of satiety are utilized in feed production to a larger extent (Bosch et al., 2007; Morrison and Berthoud, 2007; Bellisari, 2008). Dogs suffering from metabolic diseases typically found in humans are becoming more common, and inclusion of dietary fibres in diets for humans has proven to reduce the risk of these diseases (German, 2006; Frølich et al., 2013; Hur and Lee, 2015). Some of these diseases are symptoms of altered microbiotas in the colon (Hur and Lee, 2015). Dietary fibres have been shown to promote a healthy microbiota, but the knowledge is still scarce (Cooper et al., 2015; Hur and Lee, 2015). To date there is not enough evidence on how consumption of dietary fibres by dogs would lower the risk for stereotyped feeding behaviour or metabolic diseases. Feed producers and researchers need to investigate the use of dietary fibres further so that owners can buy foods that promote good health and welfare in our companion dogs. This study found that wheat was the least satiating grain, which raises the question if the feed producers should alter their dog food recipes and start including more satiating grains like oats and rye. However, addition of satiating fibre in dog feeds is only one way of solving the problems related to satiety. Efforts have been made to increase satiety through the use of different kibble sizes and increased meal volume without increasing energy intake (Serisier et al., 2014; Sagols et al., 2019). Increasing the time it takes to finish a meal may have positive effect on satiety, but this would need to be investigated of course. Satiety related problems, e.g. obesity, are complex and may therefore require application of several strategies in order to be solved

5.3.2. Ethical considerations

We wanted to investigate the feeling of satiety in dogs in this study, and because feelings are subjective to the individual there was no other feasible way of doing this without using dogs. Some results indicate that the number of animals were too low. This might be due to the fact that we used a heterogenous group (i.e. different breeds) of dogs. A heterogenous group of animals require a larger number of individuals in order to balance out their differences and produce significant results. With a group of dogs of the same breed we might not have seen the same effects upon the results as we did. The choice to use privately owned dogs of different breeds was made so that the results could be applied to the general population of dogs in Sweden. Which would not be possible if we used a group of dogs of the same breed. On the other hand, the number of animals used is merely one possible reason for some of our unclear results. There are still potential for improvement of the methods used and should the methods improve there might not be a need for increasing the number of animals used.

Research is an important tool that should be used in future efforts to improve animal welfare. However, the need for using animals for research purposes should continue to be questioned and controlled. Animals cannot speak for themselves and the more we learn about their cognitive abilities and how emotionally complex they really are, it becomes even more evident why they need to be protected from potential suffering. The usage of behavioural tests where the animal has control of their environment to ask scientific questions has the potential to minimize suffering of the animals used. When using behavioural tests to measure metabolic and cognitive responses, however, the results will initially need to be validated together with physiological measures to ensure that the behaviour can be linked to the correct physical responses in the animal. Once that is established, behavioural tests such as the ones in this study can be used as a non-invasive way of measuring metabolic and cognitive responses, such as satiety.

5.3.3. Sustainability and economy

Cereal grains, like wheat, rice, corn, barley and oats, constitute about 30-60% of dog foods (Kempe *et al.*, 2004; Kore *et al.*, 2009). Therefore, it is important to consider how these grains impact not only the health of the dog population but also our environment. Wheat is a grain that is largely used in dog foods (Kore *et al.*, 2009). Based on the results presented here, wheat appear to lack properties to facilitate a sufficient satiety response in dogs on its' own. Using wheat to produce suboptimal dog foods does therefore seem like a poor use of food resources, especially since the demand for human consumption of wheat and other cereals is increasing (Kore *et al.*, 2009).

Rice is another grain that is largely used in dog foods (Kore *et al.*, 2009). Rice requires 2-3 times more water to cultivate than other grains and irrigated rice cultivation are a large source of methane emissions globally (Yao *et al.*, 2017). Every effort counts to limit greenhouse gas emissions and to stop global warming, and we believe that the dog food producers should not be an exception. We did not investigate rice specifically in this study, however, it is worth investigating if oats and rye can be a more sustainable options than rice.

Considering economical aspect for the dog food producers and owners it is important to find solutions that also are financially sustainable. According to some authors, wheat and rice are relatively expensive grains and inclusion of rye has been shown economically beneficial in pig production (Kore *et al.*, 2009; Schwarz *et al.*, 2016). This indicates that inclusion of at least rye could be of economic interest to the dog food producers since it could lower production costs. Which could be an incentive to the producers to investigate rye as a potential resource in their dog foods.

Increasing knowledge about the effect of different grains, or other dietary fibre sources, on overall dog welfare, economy and our planet is needed to develop more sustainable dog foods.

5.4. Conclusion

In conclusion, the results showed that dogs became more feed motivated with time after a meal, suggesting that satiety in dogs was successfully measured. Wheat based diets was shown to possess the least satiating properties compared to both a rye and oat diet and palatability did not seem to interfere with these findings. Statistically, dogs' attention directed toward a food-related cue and the likelihood of dogs choosing the food-related cue did not significantly change over time. Dogs showed a pattern of choosing the bowl more frequent in the afternoon compared to directly following a meal, even though there was no measured statistical difference. The use of a similar control feed as a complement to the treatment feed in the runway to control for palatability proved to be useful because it did not interfere with the effects of the grains in the runway test.

This study can be considered a pilot, taking the first steps to validate the current designs of the attention bias and runway test. There is definitely room for improvement, but the results indicate that these methods could prove useful in this field. A larger number of animal subjects may be needed to avoid unwanted effects of feed order and day when conducting further research. There is still a need of further research into the effect of long-term intake of specific dietary fibres on the microbiota and subsequent satiety and health effects, to promote welfare in our four-legged canine companions.

6. Popular scientific summary

Our companion dogs today are facing welfare problems like obesity and many dogs, obese or not, might be feeling hungry during large parts of the day. Dogs are served energy dense foods that meet all their needs, but these foods need to be served in limited amounts because they are so energy dense to reduce or avoid obesity. Even though this is being done out of care for the dogs wellbeing, it may leave the dogs feeling unsatiated (not full) which may lead to unwanted behaviour like begging and stealing foods from the owners. Including certain dietary fibres, in the form of grains, into dog feeds has the potential to prolong the feeling of satiety after a meal. Different types of grains contain different types and levels of dietary fibres and may therefore be more or less satiating. Wheat is the most common grain used in dog foods, while oat is rarely used, and rye is not used at all. For this reason, the aim of this study was to investigate the satiating effect of three common types of grains (wheat, oats and rye) in dogs. Satiety and the motivation to feed are feelings which are subjective and therefore hard to measure since we, unfortunately, cannot ask the dogs what they are feeling. The good news is that there are ways that these feelings can be measured indirectly. In this study we used two behavioural tests to measure feeding motivation in dogs.

Eleven privately owned dogs received three experimental feeds containing either whole wheat, rolled oats or whole rye. All dogs ate each feed during three different but succeeding three week periods. After each feeding period, the dogs were subjected to two behavioural tests. At 15 minutes, 3 hours and 6 hours after eating a meal the dogs' feeding motivation was measured in a runway test, where dogs ran through a short track to find a small food reward at the end. At 15 minutes and 6 hours after eating a meal the dogs' feeling of satiety was tested in an attention bias test, where the dogs had to make a choice between an empty food bowl and a toy.

The results of these two tests showed us that the dogs feeding motivation increased with time after eating a meal, which confirmed that we indeed managed to measure satiety using these tests. The results from the runway showed that dogs are feeling less satiated on a wheat based diet than on either an oat or a rye based diet. This suggests that dogs might benefit from dog foods containing more satiating grains like oats or rye. However, the results of the attention bias test were not as strong as the runway test, indicating that the test was not sensitive enough or there is room for improvement of the method. This study is a first step towards learning more about how to measure satiety and the welfare benefits of inclusion of different dietary fibres in dog foods. Feed producers and researchers need to investigate the use of dietary fibres further so that owners can buy foods that promote good health and welfare in their dogs.

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