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Effect of dietary fibre type on physical activity and behaviour in kennelled dogs

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ABSTRACT

Dog diets may differ in their effectiveness of maintaining satiety after a meal. Consequently, sensations of hunger, feeding motivation, physical activity, and sensitivity to environmental stressors may be increased. Dietary fibre may be effective in prolonging postprandial satiety depending on type and inclusion level. This study evaluated the effect of fibre fermentability on behaviour in dogs. Sixteen healthy adult dogs were housed individually and fed a low-fermentable fibre (LFF) diet containing 8.5% cellulose or a high-fermentable fibre (HFF) diet containing 8.5% sugar beet pulp and 2% inulin. Dogs were fed two equal portions at 8:30 and 18:30 according to energy requirements. Behaviour of dogs in their home-cage was recorded and analyzed by instantaneous scan sampling (2×24 h with 15 min intervals) and focal sampling continuous recordings (10 min per animal per hour, from 9:00 until 18:00). Dogs were subjected to a behaviour test composed of the subtests open-field, sudden-silence, novel-object, and acoustic-startle. The behavioural responses of each dog were recorded. Scores for the scan and focal samples were expressed per clock hour and DIET $\times$ TIME effects were tested statistically using Residual Maximum Likelihood (REML). Data from the tests were examined using principal component analysis resulting in the compilation of two components. Data were tested statistically for DIET and DIET $\times$ SUBTEST effects using REML. Variables specific for the open-field and novel-object test were analyzed using analysis of variance. For the scans, a significant DIET $\times$ TIME effect was found for resting. At night and in the morning, HFF dogs rested more compared to LFF dogs, but they rested less between 14:00 and 17:00. For the continuous recordings, the main findings were a tendency for DIET $\times$ TIME effect for time spent resting with a pattern consistent with that for the scans. The interaction was significant for inactive-alert (lie with head up or sitting) with HFF-fed dogs having lower values around 10:00–11:00 and higher values hereafter. Finally, time spent tail wagging was significantly higher for LFF-fed dogs just before the evening meal that may indicate higher level of arousal. For the behaviour tests, no significant DIET or DIET $\times$ SUBTEST effects were detected. It is concluded that compared to the LFF diet, the HFF diet increased inactivity in kennelled beagle dogs likely through the prolongation of postprandial satiety. This effect did not change the reaction to stressful events in kennelled laboratory dogs. Enhanced susceptibility to environmental stressors at times of hunger in sensitive companion dogs may occur but requires further study.

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1. Introduction

The behaviour of privately owned dogs may be influenced by dietary effects on satiety. Typically, pet dogs are provided with sufficient nutrients and energy in their diets to meet their requirements, but they may be hungry during part of the day. Hunger and high feed motivation may have an undesirable effect on behaviour such as the facilitation of begging and scavenging behaviour (Weber et al., 2007). These behaviours encourage owners to feed their pet above their energy requirement (Jewell and Toll, 1996) result in overweight and obesity, i.e. the main nutritional disorder in companion animals nowadays (German, 2006). In rats, hunger results in increased levels of anxiety (Asakawa et al., 2001; Carlini et al., 2002). If the same is true for pet dogs, hunger may contribute to the expression of anxiety-related problem behaviours, though this remains speculative as relatively little is known with regard to the relationship between satiety level and behaviour in dogs (Bosch et al., 2007).

Plant-derived dietary fibres may be useful for the development of strategies to influence satiety as these can increase satiety and its duration in dogs (Jewell and Toll, 1996; Bosch et al., 2009). The effectiveness of fibrous ingredients to stimulate and prolong satiety depends on its properties and inclusion level as they are resistant to digestion and absorption in the small intestine, but can be degraded by the microbial population in the large intestine of dogs. The fermentation of dietary fibre yields, apart from several gases, short-chain fatty acids (SCFA, mainly acetate, propionate, and butyrate) (Bergman, 1990). In evolutionary terms, dogs are predominantly carnivore in nature (Bradshaw, 2006) and are not designed to consume plant material containing fermentable dietary fibres that yield these SCFA. This legacy does not mean that the production of SCFA in the large intestine is unnatural and should be prevented. Banta et al. (1979) reported that dogs fed a fortified all meat-based diet had slightly higher concentrations of SCFA in the large intestine than dogs fed a commercial cereal-based diet. Authors suggested that the protein-polysaccharides of the connective tissue would be a source of fermentable substance for the large intestinal microbes. Based on this, it seems reasonable to assume that in evolution wolves (and dogs) are adapted to large intestinal fermentation of animal-based fibre yielding SCFA. These SCFA may play a role in the maintenance of satiety as they stimulate the production and secretion of several satiety-related hormones. One of these is peptide tyrosine tyrosine (PYY), a satiety-promoting hormone released by the enteroendocrine L-cells present in the distal part of the gastrointestinal tract in response to contact with SCFA (Anini et al., 1999). Release of glucagon-like peptide-1 (GLP-1), another satiety-promoting hormone produced by the L-cells (Holst, 2007), is increased in dogs fed fermentable fibre compared to dogs fed low-fermentable fibre (Massimino et al., 1998). In rats, diets supplemented with a fermentable fibre reduce plasma concentrations of ghrelin, a hormone that is associated with feelings of hunger or appetite in humans (Wren et al., 2001), at 8 h after the last meal (Cani et al., 2004). Besides the effects of SCFA on satiety-related hormones, absorbed SCFA (mainly acetate) can also be utilised as an energy source, typically at times when the absorption of nutrients from the digestible fraction of the diet is decreasing or is completed (Bergman, 1990). Thus, different mechanisms have been suggested to make fermentable dietary fibres prolong postprandial satiety in dogs and in this way affect their behaviour, though the latter especially needs to be validated.

This study is a part of an experiment evaluating the use of fermentable fibre in diets for dogs to increase satiety. Here it is investigated if dietary inclusion of fermentable fibre, by increasing satiety, influences behaviour in dogs and is a candidate strategy to combat unwanted behaviour. Dietary effects on digestibility, satiety-related hormones, and voluntary food intake is presented elsewhere (Bosch et al., 2009). It was expected that dogs fed fermentable fibre were less active in their home-kennel and less responsive in challenging situations.

2. Materials and methods

2.1. Animals and housing

Nine intact male and seven intact female healthy adult beagle dogs of 2–6 years-of-age with body weights (BWs) between 7.2 and 11.4 kg were housed individually in kennels within one room at the Laboratory of Animal Nutrition of Ghent University (Merelbeke, Belgium). Twelve kennels were 1.5 m × 1.5 m and four kennels measured 1.2 m × 1.6 m. Metal plate partitions between kennels prevented visual and physical contact, i.e. direct behavioural interactions between dogs. Dogs had toys in their kennel during the entire study. Food was provided twice daily in two equal portions at 8:30 and 18:30 and fresh water was available *ad libitum*. Dogs were submitted to weekly health checks and weighed every 2 weeks. Animal housing and experimental procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine of the Ghent University (Belgium, EC 2007/40).

2.2. Treatments

The diets differed in the fermentability of fibrous ingredients used. The low-fermentable fibre (LFF) diet contained cellulose as a fibre source whereas the high-fermentable fibre (HFF) diet contained a combination of sugar beet pulp and inulin. Differences in fermentability of fibres were confirmed *in vitro* (Sunvold et al., 1995; Bosch et al., 2008) and *in vivo* (Bosch et al., 2009). Diets were formulated to be iso-nitrogenous, iso-energetic on a digestible energy basis, and iso-fibrous on a total dietary fibre basis. Ingredient and nutrient compositions of both diets are shown in Table 1. Each dog was individually fed to meet its daily energy requirement, which was estimated at 415 kJ of metabolisable energy/kg $BW^{0.75}$. Diets were in mash form and fed after mixing with an equal amount of lukewarm water to increase palatability. Food intake was recorded during each meal throughout the entire experimental period.

Table 1
Composition of the low-fermentable fibre (LFF) and high-fermentable fibre (HFF) diets.

	LFF	HFF
Ingredient composition (g/kg as is)[a]		
Wheat starch (pre-gelatinised)	468.75	463.00
Poultry meat meal (low-ash)	285.00	275.00
Poultry fat	135.00	135.00
Cellulose	85.00	–
Sugar beet pulp (molassed)	–	85.00
Inulin	–	20.00
Premix[b]	10.00	10.00
Digest	10.00	10.00
Molasses	4.25	–
Titanium(IV) oxide	2.00	2.00
Nutrient composition (g/kg DM)[c]		
Ash	37.5	42.0
Starch	372.4	367.5
Sugar	13.6	41.6
Crude protein	274.1	262.2
Crude fat	191.4	191.2
TDF	123.7	93.9
IDF	110.9	74.7
SDF	12.8	19.2
NSP	111.0	95.1
Energy content (kJ/100 g DM)		
Gross energy	2294	2300

[a] Wheat starch, Pregel Wheat Alpha (Meneba, Weert, The Netherlands); poultry meat meal, Meat Meal 63 (Sonac, Lingen, Germany); poultry fat (Sonac, Lingen, Germany); sugar beet pulp, molasses (Research Diet Services, Wijk bij Duurstede, The Netherlands); inulin, Beneo IPS (Orafti, Tienen, Belgium); cellulose, Arbocel BWW40 (J. Rettenmaier Benelux, Zutphen, The Netherlands); premix (Twilmij B.V., Stroe, The Netherlands); digest, Luxus Digest N8008 (AFB International, Nuland, The Netherlands); titanium(IV) oxide (Sigma–Aldrich Chemie B.V., Zwijndrecht, The Netherlands).

[b] The premix provided per kilogram of diet: Ca, 0.41 g; P, 0.07 g; Mg, 0.05 g; K, 0.1 g; Na, 0.01 g; Cl, 0.09 g; linoleic acid, 0.15 g; PUFA, 0.17 g; lysine, 0.05 g; methionine, 0.02 g; methionine + cysteine, 0.04; threonine, 0.04 g; tryptophan, 0.02 g; vitamin A, 17,500 IU; vitamin D_3, 2000 IU; vitamin E, 100 mg; vitamin K_3, 2 mg; vitamin B_1, 10 mg; vitamin B_2, 10 mg; niacin, 50 mg; pantothenic acid, 25 mg; vitamin B_6, 7.5 mg; vitamin B_{12}, 50 μg; biotin, 300 μg; choline chloride, 475 mg; folic acid, 1.25 mg; vitamin C, 100 mg; Fe, 75 mg; Mn, 35 mg; Cu, 5 mg; Zn, 75 mg; I, 1.75 mg; Co, 2 mg; and Se, 0.2 mg.

[c] TDF, total dietary fibre; IDF, insoluble dietary fibre; SDF, soluble dietary fibre derived by subtracting the IDF content from the TDF content; NSP, non-starch polysaccharides, derived by subtracting the crude protein, crude fat, starch, and sugar content from the organic matter content (de Leeuw et al., 2004). Inulin is not recovered in the TDF fraction (Prosky and Hoebregs, 1999) resulting in an underestimation of the TDF content in the HFF diet of approximately 18 g/kg DM (20 g/kg included in the diet with 90% pure inulin). Similarly, the NSP content of the HFF diet is underestimated with approximately 18 g/kg DM as inulin was included in the analyzed sugar content.

Dogs were randomly assigned to one of the two dietary treatments (LFF or HFF diet), with dietary groups being balanced for BW and gender (blocking factors). Dogs in LFF group ($n = 8$) included four males and had a mean BW ± SEM of 9.7 ± 0.5 kg. The HFF group ($n = 8$) included 5 males and dogs weighed on average 9.8 ± 0.5 kg.

2.3. Behavioural observations

2.3.1. Twenty-four hour activity patterns

During week 5 of the experiment, on two non-consecutive days, the dogs' behaviour was continuously recorded on video for a period of 24 h and activity patterns were analyzed by instantaneous scan sampling using intervals of 15 min. Dogs were scanned for the locomotion states resting (lying with head rested), lying (lying with head up), sitting, standing, standing-up and walking. Video analysis was conducted by one person who was ignorant of the dogs' experimental treatment.

2.3.2. Interprandial behaviour

The behaviour of the dogs between meals was evaluated in more detail by focal sampling continuous recording, using again the video recordings collected during the two non-consecutive days. Per hour, bouts of 10 min were analyzed starting at 30 min after the morning meal (approximately 9:00) until 30 min before the evening meal (approximately 18:00). The total observation time per dog was 200 min. In addition to the behaviours described in Section 2.3.1, the following were recorded for the duration of occurrences: posture (high, neutral, and low), autogrooming, nosing, manipulations of the environment, and tail wagging. Behaviours scored for the frequency of occurrence were: body shaking, stretching, yawning, drinking, coprophagia, defecating, and urinating. The number of changes of locomotion states (CLS) was calculated as the sum of the frequency of occurrence of each locomotion state minus 1 (starting locomotion state). For a description of the behaviours, see Beerda et al. (1998). As the positions of the ears and legs were difficult to assess from video recordings, only the position of the tail was used as an indicator of posture. Behavioural observations were performed by one person who was ignorant of the dogs' experimental treatment and recorded using the Observer 5.0 software package (Noldus Information Technology B.V., Wageningen, The Netherlands).

2.3.3. Behavioural responses

Seven weeks after the start of the study, the dogs were studied for anxiety levels as assessed in an open-field test situation. The behaviour test was composed of the subtests 'open-field' (OF), 'sudden-silence' (SS), 'novel-object' (NO) and 'acoustic-startle' (AS). Subtests (for a description, see below) were performed conjointly in the described order. Dogs were tested between 13:30 and 15:20. Prior to testing, the dogs were habituated to procedures. This involved the experimenter walking the dog during each of the 5 days preceding the test to the test location, which was approximately 50 m from the kennels. The dogs did not enter the test arena; thus, they were habituated to procedures prior to testing (i.e. being taken out of the cage, walking to the test location), but the novelty of the test arena was maintained. One day before the first behavioural tests, urine collected from multiple dog kennels was mixed with water and dispersed over the floor and walls of the test arena after which the arena was cleaned again with water. In this way the smell of urine was always present and possible influences of odour on behaviour were assumed to be similar for all dogs. After each test, the arena was cleaned again with water before the next test started.

Dogs were introduced into a test arena measuring 3 m × 3 m, with floor markings indicating 16 sections measuring 75 cm × 75 cm squares each. Dogs were left

undisturbed for 5 min and observed for their behaviour (OF) with background white noise on at an intensity of 60 dB. The background noise was switched off leaving the dog in a silent test arena for 1 min (SS). Next, a standard size plastic shopping bag filled with paper was lowered from the ceiling in the centre of the test room almost touching the floor and the dogs' responses were recorded for 5 min (NO). Finally a short sound blast (~1 s, ~96 dB) was produced using a fog horn (Motip Dupli B.V., Wolvega, The Netherlands) whereafter behavioural responses were recorded for 1 min (AS).

Live observations, done by one observer, were performed behind a one-way screen to prevent the dogs reacting on the observer. A digital lightweight videocamera was mounted above the test arena enabling clear view of the dog's behaviour during the test. Live observations were computer aided as described above.

The dogs' behavioural responses during the test were measured by continuous recording of the behaviours described previously (see Sections 2.3.1 and 2.3.2) as well as the following behaviours: vocalisations (barking; growling, howling, whining, and yelping), floor licking, panting, oral behaviours, paw lifting, trembling, crouching, and freezing. For a description of howling, see Lund and Jørgensen (1999) and for the other behaviours, see Beerda et al. (1998). For the OF, the number of line crossings and the total time spent in the outer sections (i.e. the 75 cm × 75 cm squares next to the walls) were recorded. The latency period for a dog to make contact to the novel-object and the time spent within 1 m of it were recorded for the NO test.

2.4. Data processing

2.4.1. Twenty-four hour activity patterns

Four scan samples per hour were summed resulting in 24 observations per dog per day with behavioural parameter scores ranging from 0 to 4. The general activity levels of the dogs were of most interest and the locomotion states lying and sitting were pooled and labelled 'inactive-alert'. Standing, standing-up, and walking were pooled and labelled as 'active'. Resting was maintained as a separate category.

2.4.2. Interprandial behaviour

Behaviours scored for duration of occurrence were expressed as percentage of the observation time (i.e. 10 min) resulting in 10 observations per dog per day with behavioural parameter scores ranging from 0 to 100%. Behaviours scored as frequencies were expressed as times per 10 min. For the purpose of data reduction the scores for body shaking, stretching, and yawning were summed and analyzed as a single parameter. The posture of the dogs was recorded only when they were standing, standing-up or walking and scores were expressed as percentage of the summed time standing, standing-up and walking. Tail wagging was treated in the same way. The behaviours drinking, coprophagia, defecating, and urinating were observed less than 15 times across dogs throughout the experiment and were, therefore, excluded from further analysis.

2.4.3. Behavioural responses

Again behaviours scored for duration of occurrence were expressed as percentage of the observation time (i.e. 1 or 5 min) and those scored as frequencies were expressed as time per 1 or 5 min. The locomotion states lying and sitting as well as standing and standing-up were summed and labelled 'inactive-alert' and 'standing', respectively. The number of behavioural parameters was reduced by pooling the scores for autogrooming and floor licking (hereafter referred to as 'licking'), whining and yelping ('vocalising'), urinating and defecating ('eliminating'). The behaviours resting, manipulations of the environment, high posture, body shaking, yawning, barking, growling, howling, and trembling were observed less than 15 times during the behaviour tests across dogs and excluded from further analysis. As dogs showed either a neutral or low posture during the tests, only percentage of observation time in low posture was analyzed.

2.5. Statistical analyses

Behavioural parameters were analyzed with linear mixed models (LMMs) using Residual Maximum Likelihood (REML) in the statistical package ASReml (Gilmour et al., 2006). For the non-normal distributed binary or count data, LMM takes the actual distribution into account and implements REML-type analyses (Pryce et al., 1999).

The following statistical model was used for parameters of the 24-h activity patterns:

$$Y_{ijklm} = +\mathrm{GENDER}_i + \mathrm{DIET}_j + f(\mathrm{TIME}_{jk}) + \mathrm{DAY}_l + \mathrm{DOG}_m + \mathrm{error}_{ijklm}$$

where Y_{ijklm} is a measurement recorded for dog m with gender i, which was fed diet j, on day l at time point k. DAY (1 or 2) and DOG (1–16) make up the random component of the model, with GENDER (male, female), DIET (LFF or HFF) and TIME (0:00, 1:00,..., 23:00), here fitted as a spline per diet group to describe the occurrence of the behaviour in time, representing the fixed component. For interprandial behaviour parameters the statistical model was similar except for the time frame: 9:00, 10:00,..., 18:00. The validity of the statistical model used is in part dependent on the normal distribution of the data or even better the residuals produced by it. This was checked by means of making histograms of the frequencies of residuals and no major deviations were detected. Violation of homogeneity of variance was monitored by plotting fitted values against residuals and there were no indications that variances changed with the level of measurement. Variance parameter values were stable and convergence failures did not occur, meaning that the REML log-likelihood changes between iterations were less than 0.002 × iteration number (for details, see Gilmour et al., 2006). Data reduction was established by means of principal components analysis (PCA, Jolliffe, 1986) on behavioural response parameters, following similar procedures as described by van Reenen et al. (2004). Principal components represent linear combinations of the behavioural scores and reflect the underlying correlation matrix, but correlations (represented by loadings) may become inflated due to effects of, for example, gender or

dietary treatments. This was controlled for by first conducting a PCA on residuals (64 records) calculated by REML (GenStat version 10.2 Lawes Agricultural Trust, 2007), using a LMM with GENDER (male, female), SUBTEST (OF, SS, NO, AS) and DIET (LFF, HFF) in the fixed component and DOG ($n = 16$) in the random component. A total of 15 parameters (inactive-alert, standing, walking, low posture, nosing, tail wagging, freezing, panting, licking, eliminating, vocalising, oral behaviours, crouching, paw lifting, and CLS) were fed into the PCA analysis on residuals. For the calculation of principal component scores, which were derived from the scores for the multiple behavioural parameters corrected for their respective loadings on a given principal component, a second PCA was performed on the raw scores for only the behavioural parameters with loadings above 0.50 or below −0.50 on principal components in the first PCA (i.e. on residuals). The scores for PCA components and individual behavioural response parameters (those not included in one of the PCA components), were analyzed with LMMs using Residual Maximum Likelihood (REML) in the statistical package ASReml (Gilmour et al., 2006). The following statistical model was used:

$$Y_{ijkl} = +\text{GENDER}_i + \text{DIET}_j + \text{SUBTEST}_k + \text{DOG}_l + \text{error}_{ijkl}$$

where Y_{ijkl} is a measurement recorded for dog l with gender i, which was fed diet j, at subtest k. DOG (1–16) makes up the random component of the model, with GENDER, DIET, and SUBTEST representing the fixed components. Variables specific for the OF subtest, i.e. time spent in the outside sections and number of line crossings, and the NO subtest, i.e. latency to contact and time spent within 1 m of the NO, were analyzed using ANOVA in GenStat version 10.2 (Lawes Agricultural Trust, 2007). The statistical model used was:

$$Y_{ij} = +\text{GENDER}_i + \text{DIET}_j + \text{error}_{ij}$$

where Y_{ij} is a measurement recorded for a dog with gender i, which was fed diet j. Differences were considered to be significant at $P \leq 0.05$.

3. Results

3.1. Animals

Dogs consumed all the food provided readily and remained healthy throughout the study. Both groups of dogs lost an average of 5% of their initial BW with no significant difference between dietary treatment groups (data not shown).

3.2. Behavioural observations

3.2.1. Twenty-four hour activity patterns

The amount of time that dogs were active (stood/walked), inactive-alert (lay down/sat) or resting changed during the day ($P < 0.001$ for each state, Fig. 1). Only, for resting this diurnal rhythm differed between the dietary treatment groups ($P = 0.045$ for DIET × TIME effect). Dogs were active during a 2–4 h period around the morning meal at 8:30 and during a 3–4 h period before the evening meal at 18:30. Typically, they rested during the periods from 1:00 to 6:00 and from 10:00 to 11:00. The resting pattern of the HFF dogs differed from that of the LFF dogs in that they rested more between 0:00 and 7:00 (see least square means in Fig. 1). Less clear-cut were the increased levels of resting in the HFF dogs as compared to the LFF dogs during the first 4 h after the morning meal, and the decreased levels around 14:00–17:00. During the latter period the HFF dogs were relatively inactive, and together the results indicate that dogs in the HFF group showed relatively low activity levels, especially during the night. General activity levels were unaffected by gender.

3.2.2. Interprandial behaviour

Patterns in resting, inactive-alert, high posture, and tail wagging between meals are presented in Fig. 2. The amount of time dogs rested during the day tended to vary differently for dogs in the LFF and HFF groups ($P = 0.095$ for DIET × TIME effect), with results replicating those reported for 24 h activity patterns. Typically, dogs rested from 10:00 to 13:00. Compared to the LFF dogs, the HFF dogs rested more during the period from 10:00 to 11:00 and less during the period from 12:00 to 13:00 and around 16:00. In a reversed way, such DIET × TIME effect ($P = 0.028$) was found for inactive-alert (Fig. 2). The time dogs were inactive-alert decreased up to 3–4 h after the morning meal followed by an increase until 16:00 and decrease hereafter. The HFF dogs were less inactive-alert around 10:00–11:00 than the LFF dogs but more thereafter. The postures of the dogs changed during the day, with differences between dietary groups ($P = 0.036$ for DIET × TIME effect on high posture). High postures were observed most during the times dogs were active, i.e. after the morning meal until 11:00 and after 14:00. Differences between dietary groups were most pronounced at 16:00 and 17:00, with higher postures in HFF dogs than LFF dogs. Tail wagging occurred especially around 1 h before the evening meal with less tail wagging in HFF dogs than in LFF dogs ($P < 0.001$ for DIET × TIME effect). Other behaviours did not show DIET × TIME or DIET effects and are presented in Table 2. TIME effects occurred in that dogs decreased their activity (including CLS and nosing) after the morning meal until 11:00. From 12:00 to 18:00 dogs became increasingly active. Dogs tended to groom themselves just after the morning meal (9:00–10:00) and in the afternoon (13:00–15:00). Gender did not significantly affect any of the behaviours measured between meals.

3.2.3. Behavioural responses

For the purpose of data reduction, behavioural parameters recorded during the behaviour tests were investigated for interrelationships using PCA. Two PCA components explained a substantial part of the variation (20 and 14%) in a dataset of 15 parameters. The first component grouped behaviours indicative of 'restlessness-exploration', namely walking (loading of 0.91), nosing (0.91), CLS (0.87) and, reversely related to these, inactive-alert (−0.85). The second component grouped oral behaviours (0.81), paw lifting (0.56) and, reversely related to these, standing (−0.60), and was interpreted as 'anxiety'.

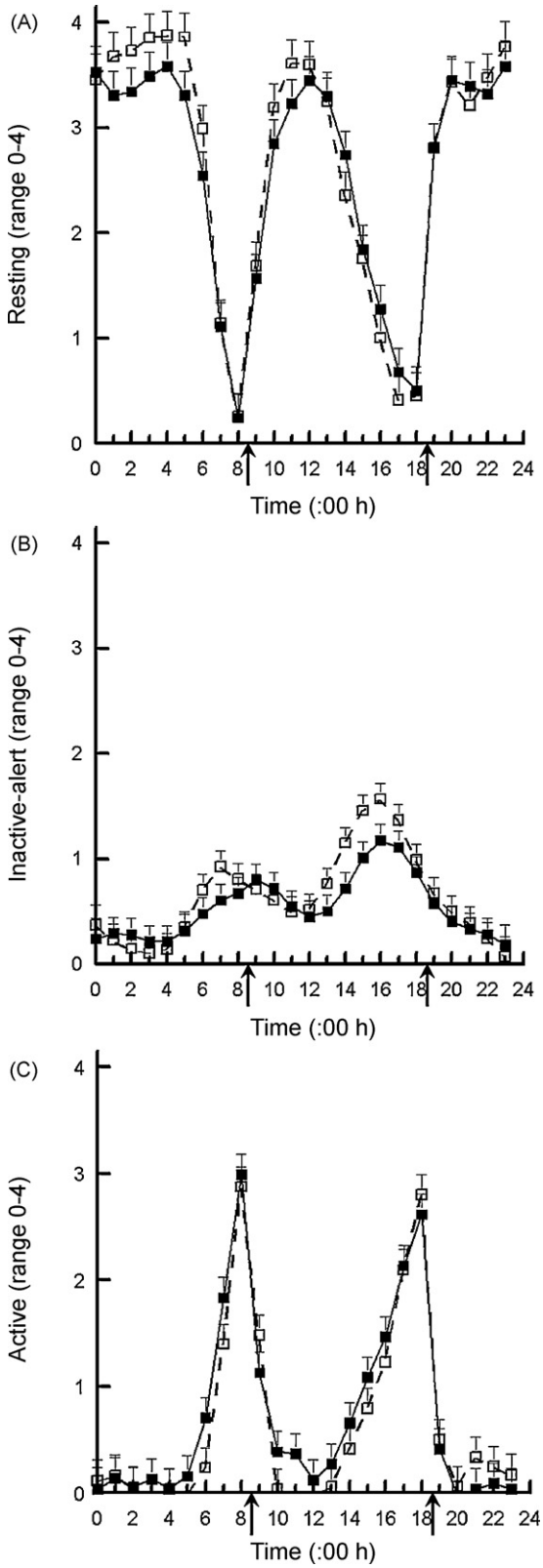

Fig. 1. Diurnal rhythms in resting (lie with head down, panel A), inactive-alert (lie with head up or sitting, panel B) and active (stand or walk, panel C). Least square means and standard errors by the statistical model represent the times a behaviour was observed per hour per dog, given four scans per hour. Results are presented separately for the dogs fed the low-fermentable fibre ((■) $n = 8$) and the high-fermentable fibre diet ((□) $n = 8$). The arrows represent feeding times (8:30 and 18:30).

Component scores were calculated from the dogs' scores for the different behavioural parameters, weighing behavioural parameters relative to their loadings.

Least squares means for component scores (i.e. restlessness-exploration and anxiety) and behavioural parameters that did not fit these components are presented in Table 3. Effects of DIET or DIET × SUBTEST were in none of the cases significant. Also, SUBTEST specific parameters like distance to the novel-object or latency to contact it were not significantly different between the groups.

Dogs behaved differently during the different phases of the behaviour test (OF, SS, NO, AS) as indicated by significant SUBTEST effects. In short, tail wagging typically occurred during the OF and SS tests whilst dogs tended to crouch in response to NO and AS and to freeze in response to SS and AS tests. Minor differences between sexes existed in that males showed higher frequencies of urination and defecation compared to females ($P < 0.001$) and they spent relatively more time within 1 m from the novel-object ($P = 0.017$).

4. Discussion

The HFF diet was formulated to prolong satiety, which was indicated by the reduced ($P = 0.058$) food consumption of a freely available standard food 6 h after the morning meal in dogs fed HFF (mean uptake ± SEM of 404 ± 70 g) as compared to those fed low-fermentable fibre (LFF, 250 ± 46 g) (Bosch et al., 2009). Beforehand, we assumed that prolonged satiety in dogs fed the HFF diet would lower spontaneous physical activity during parts of the diurnal cycle. Indeed, dogs fed the HFF diet rested more during the night and just before the morning feeding and were relatively inactive (more inactive-alert) in the afternoon compared to the LFF-fed dogs. The a priori assumption that the dogs fed the HFF diet would be less anxious during the behaviour tests, was not confirmed in the present study.

In this study multiple parameters were measured, which increased the risk of a Type 1 error. This was controlled in part by data reduction following PCA. In addition, we interpreted statistically significant effects on behavioural parameters in the context of other findings. However, by adapting a relatively liberal approach the study is somewhat explorative in nature (see Pocock, 1997 for a review).

The present findings associate reduced satiety with increased spontaneous activity, which is in line with earlier reports on energy restriction induced increases in spontaneous activity in dogs (Crowell-Davis et al., 1995), pigs (Beattie and O'Connell, 2002), and rats (Martin et al., 2007). In rats, increased spontaneous activity has been demonstrated to occur after intracerebroventricular injection of the hunger-hormone ghrelin (Jászberényi et al., 2006). Regarding the feeding of fermentable fibre, studies in sows and pigs have shown calming effects of diets containing fermentable fibre sources on physical activity

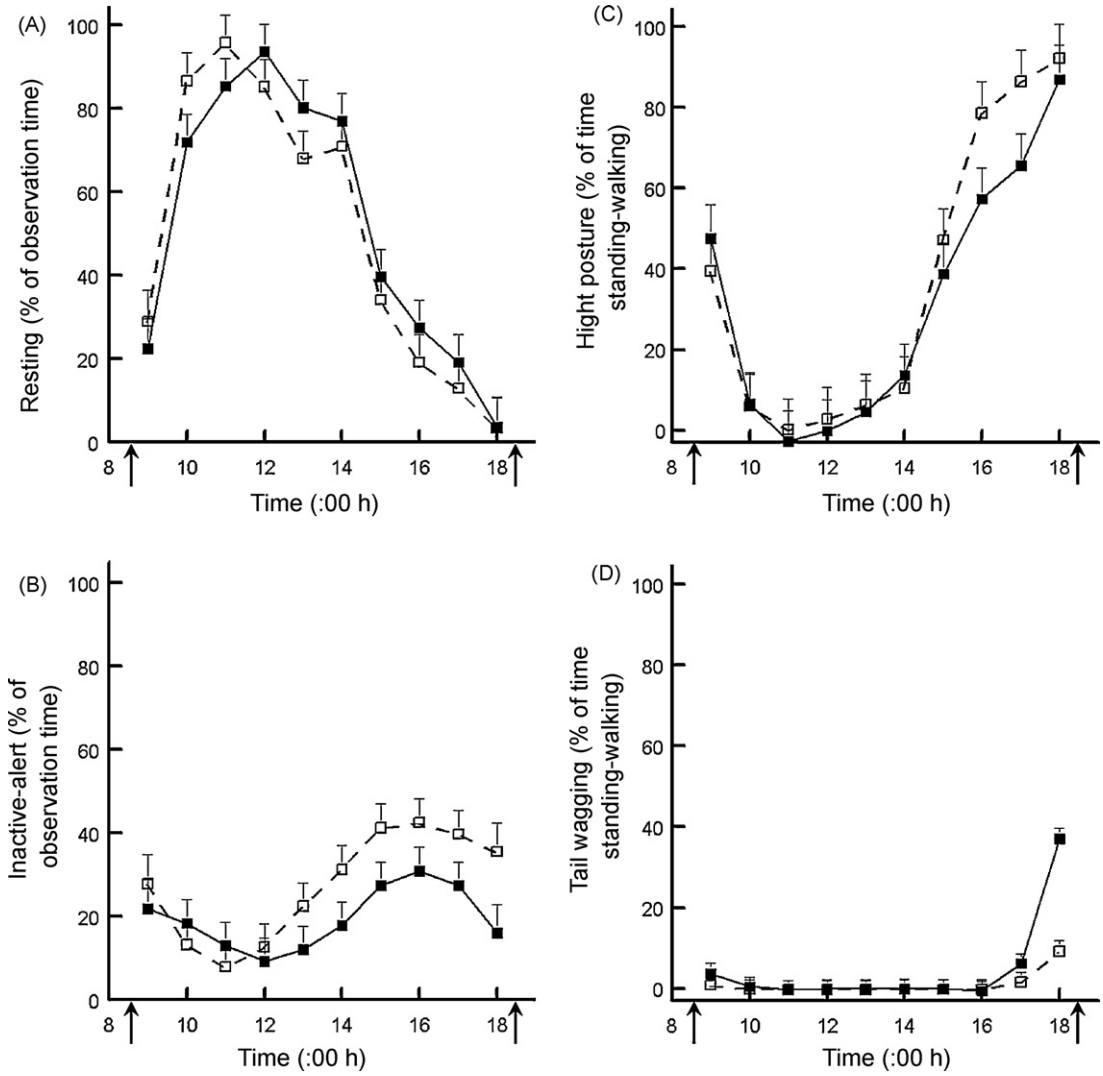

Fig. 2. Interprandial patterns in resting (lie with head down, panel A), inactive-alert (lie with head up or sitting, panel B), high posture (panel C), and tail wagging (panel D). Least square means and standard errors by the statistical model represent the time spent on a behaviour as percentage of observation time per hour per dog. Results are presented separately for dogs fed the low-fermentable fibre ((■) $n = 8$) and the high-fermentable fibre ((□) $n = 8$) diet. The arrows represent feeding times (8:30 and 18:30).

compared to diets with low-fermentable or low amounts of fibre (de Leeuw et al., 2008). Together, earlier and present findings support the idea that high levels of behavioural activity in dogs can in part be due to hunger. The relationship between activity and hunger in dogs is unknown and it remains obscure which degree of hunger was associated with the specific increase in activity observed in the present study. The differences in spontaneous activity levels and the difference in voluntary food intake reported previously (Bosch et al., 2009) do indicate that dogs fed fermentable dietary fibres experience less hunger than dogs fed a diet containing a low-fermentable fibre.

During the 1–2 h period before the evening meal, dogs on a HFF diet showed a higher posture and less tail wagging than those on a LFF diet. Relatively high postures may be interpreted as a sign of feeling well, i.e. when extrapolation from the finding that low postures are associated with acute and chronic stress (Beerda et al., 1998, 1999b). Increased tail wagging in the LFF dogs was most likely due to relatively high levels of excitement/arousal (Beerda et al., 1999b) as related to the anticipation of food. Increased arousal before feeding was also noticed for dogs restricted in their energy intake (Crowell-Davis et al., 1995). The findings on posture and tail wagging provide further support for the assumption that the HFF dogs were more relaxed than the LFF dogs. In line with this, there were indications ($P < 0.10$) that dogs fed the LFF diet spent relatively more time on scratching the wooden resting-plate in their home-cage (recorded as manipulation of the environment). Such behaviour could be characterised as stereotypic as it was shown repetitively, unvarying, and apparently without function. The low occurrence of repetitive behaviour in dogs fed HFF corresponds with

Table 2
Least squares means and SEM for the behavioural parameters observed between morning and evening meals for dogs fed the low-fermentable fibre (LFF, $n = 8$) and the high-fermentable fibre (HFF, $n = 8$) diet.

Behaviours	LFF		HFF		*P*-value		
	Mean	SEM	Mean	SEM	DIET	TIME	DIET × TIME
Locomotion[a]							
Active	7.4	7.4	4.2	7.4	NS	<0.001	NS
CLS	6.1	10.1	7.5	10.1	NS	<0.001	NS
Posture							
Low	3.7	2.0	3.1	2.1	NS	NS	NS
Neutral	19.2	4.8	19.0	4.9	NS	NS	NS
Other							
Autogrooming	5.6	1.9	9.3	2.0	NS	0.050	NS
Nosing	0.4	0.2	0.2	0.2	NS	0.001	NS
Manipulations	2.0	0.6	0.3	0.6	0.099	NS	NS
Body shaking, stretching, yawning	0.5	0.2	0.5	0.2	NS	NS	NS

[a] Active, standing, standing-up, and walking; CLS, changes in locomotion states.

Table 3
Least squares means and standard errors (SEM) for the behavioural parameters recorded during the behaviour tests for dogs fed the low-fermentable fibre (LFF, $n = 8$) and the high-fermentable fibre (HFF, $n = 8$) diet.

Behaviours	LFF		HFF		*P*-value		
	Mean	SEM	Mean	SEM	DIET	SUBTEST	DIET × SUBTEST
Restlessness-exploration	−0.04	0.35	−0.05	0.36	NS	<0.001	NS
Anxiety	0.00	0.27	0.05	0.27	NS	<0.001	NS
Low posture	48.2	10.6	52.2	10.7	NS	<0.001	NS
Tail wagging	13.1	5.7	5.3	5.8	NS	0.042	NS
Vocalising	0.6	0.8	1.9	0.9	NS	NS	NS
Licking	1.1	0.6	1.6	0.6	NS	NS	NS
Panting	1.6	3.1	6.2	3.1	NS	NS	NS
Freezing	2.6	0.8	2.0	0.9	NS	0.071	NS
Crouching	0.3	0.1	0.4	0.1	NS	<0.001	NS
Eliminating	1.7	0.5	1.6	0.5	NS	<0.001	NS
# Line crossings*	141.0	21.4	127.5	21.7	NS	–	–
Outer sections*	87.0	2.3	85.7	2.3	NS	–	–
Inside meter†	13.9	4.6	21.3	4.7	NS	–	–
Latency contact†	168.2	45.1	136.5	45.8	NS	–	–

*,†Applies to the open-field test (*) or novel-object test (†) only and was analyzed with ANOVA instead of REML.

reports on reduced stereotypic behaviour pigs fed fermentable fibres like sugar beet pulp (Whittaker et al., 1998; Danielsen and Verstergaard, 2001).

Anxiety may be influenced by hunger or satiety. For example, the intracerebroventricular injection of ghrelin in mice and rats increases anxiety as measured with an elevated plus-maze (Asakawa et al., 2001; Carlini et al., 2002). Furthermore, pigs fed a diet high in sugar beet pulp (high-satiating diet) seem to be less aroused in an OF test and less motivated to escape from the environment, suggesting reduced anxiety compared to pigs fed a diet high in starch (low-satiating diet) (Gerrits et al., 2003). To evaluate whether stress responses would be affected by differences between treatment groups, dogs were subjected to several behaviour tests. Dogs were tested around 6 h after the morning meal, approximating the time at which the voluntary food intake measurement by Bosch et al. (2009) was conducted. The tests in the current experiment were used to study anxiety and provoke mild stress. The OF test is frequently used to study exploration and anxiety in rodents (Leussis and Bolivar, 2006). Time spent in the outer sections was evaluated as animals that spent the greatest time in these sections are regarded as more anxious than those that prefer the central region (Royce, 1977). The SS test has been used as a model for mild unexpected stress in rodent studies eliciting immediate behavioural arrest with orientational movements (Hagan and Bohus, 1984; Buwalda et al., 1992). The NO and AS test procedures were similar to tests reported by Beerda et al. (1998) which induced acute stress in dogs. It was expected that dogs fed the HFF diet, which appeared to experience less hunger, would show lowered behavioural stress responses during these tests compared to the more hungry LFF-fed dogs. No effects of dietary treatment or the interaction between treatment and test were found for any of the analyzed variables. For some behavioural variables, the different tests were more or less effective in provoking a response from the dogs. It should be noted that the effect of a test may be confounded with habituation to the experimental room and carry-over effects may have occurred from one test to the next. The lack of treatment effect could be attributed to several possibilities. Firstly, hunger and increased feed motivation may not influence the susceptibility to stress and anxiety in dogs. Secondly, the test procedures used were too mild or too severe preventing variation in stress responses to

occur between treatment groups. It is unlikely that the tests were too mild as several behavioural typical indicators of stress were exhibited by the dogs during the tests. The first component contained behavioural elements related to activity and paw lifting. High levels of walking, nosing and changing from one state of locomotion or sector to another may be interpreted as restlessness or avoidance behaviour and may be a sign of moderate stress response (Beerda et al., 1998). Furthermore, paw lifting was found to be an indicator of acute stress (Beerda et al., 1998). The second component was composed of oral behaviours, standing and standing-up, and paw lifting. Oral behaviour has also been shown to be expressed during stressful events (Boissy, 1995; Beerda et al., 1998). Standing, and standing-up relate to immobility. Like avoidance behaviour, immobility may be a manifestation of fear in dogs (Diederich and Giffroy, 2006). Furthermore, the only vocalisations recorded were whining and yelping which were suggested to indicate distress related to fear (Lund and Jørgensen, 1999). Low posture and crouching behaviour occurred mainly during the NO and AS tests which is in accordance with findings of Beerda et al. (1998). Some behavioural variables related to stress like yawning, body shaking, and trembling, however, were not observed or observed at a low frequency. Yawning has been associated with psychological tension or mild stress in primates (Deputte, 1994). Body shaking could be interpreted as a sign of tension release or relief rather than stress (Beerda et al., 1998). It is possible that the tests were not sufficient in intensity to provoke dogs in performing these behaviours. Yet, both behaviours could be performed after leaving the test room rather than during the tests in the test arena. Trembling may not have been displayed because dogs did not experience the relatively high levels of stress that are typically associated with trembling. It is not likely that the tests were too severe in inducing stress as the intensity in the display of stress-related behaviours varied among dogs. Based on the observed behavioural variables, the tests appeared to be effective in provoking acute stress in the dogs. Thirdly, as some dogs in both dietary groups lost some BW during the study it is probable that the dogs experienced hunger. Although the HFF-fed dogs appeared less hungry as they consumed less food during the satiety test (Bosch et al., 2009), these dogs may also have experienced feelings of hunger sufficient to increase stress susceptibility to a similar level as for the LFF-fed dogs. Likewise, the contrasts between treatment groups in hunger or satiety could be not large enough to create differences in behavioural stress responses at the time of testing. The contrast in hunger at which differences in stress susceptibility occur remains to be investigated. To further explore the effects of hunger and satiety on stress susceptibility, it would be important to include positive and negative controls in the study. One possibility to do this is to subject dogs to similar tests either shortly after a meal (fully satiated) or after a considerable time of fasting (hungry).

There were few differences observed in responsiveness during the tests between male and female dogs. Females spent less time inside 1 m from the NO (data not shown) but latency to contact was not affected by gender. The former is in line with results in other studies in which the stress response was more pronounced in female than in male dogs (Beerda et al., 1998, 1999a). The frequency of urination and defecation was higher for males than for females. Most urination and defecation occurred during the OF test and gradually decreased in frequency with each subsequent test. It is suggested that urination during the OF test is primarily an index of territorial marking (Royce, 1977). The motivation to mark the novel test room appeared to be stronger in male dogs than in female dogs which is in agreement with other studies (Beerda et al., 1998; Siwak et al., 2002). It is therefore likely that this behaviour was the result of territorial marking rather than the expression of stress. Overall, it appeared that in the current study male and female dogs were equal in their responsiveness to the applied stressors.

This study indicated that satiety may be prolonged depending on the fermentability of dietary fibre sources used. Assuming that feelings of hunger are unpleasant, these findings may aid in the development of dietary strategies that optimally stimulate satiety in dogs. This is practically relevant as it is likely that satiated dogs will show less begging behaviour and scavenging, behaviours that encourage owners to feed their dog above energy requirements (Jewell and Toll, 1996; Weber et al., 2007). However, for some types of dog breeds, a diet containing fermentable fibre may be less effective as these dogs maintain their appetite regardless of the diet composition and frequency of feeding. Also, the begging behaviour may not only be governed by feelings of hunger but may contain a conditioned component that will be left unaffected by a high-satiating diet. The present study did not show an effect on stress susceptibility in dogs. Extrapolation of this finding to field situations should be undertaken with caution. Dogs in the present study were kennelled laboratory beagle dogs. Under field conditions, depending on the dietary composition and feeding regime dogs may experience feelings of hunger more intensive and for longer periods of time, as well as be subjected to more severe stressful events and be more sensitive these events. As a result, diets high in satiating properties may prevent enhancement of sensitivity to stressful events for some companion dogs of the dog population. It is not to be expected that food treatments are the most effective strategies for reducing anxiety and improving welfare, but in this study they had some effect.

5. Conclusion

Compared to the diet containing the low-fermentable fibre, fermentable dietary fibre enhanced inactivity in kennelled beagle dogs likely through its effects on increasing satiety. These differences in hunger or satiety were not associated with changes in the susceptibility to stressful events in kennelled laboratory dogs.

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