Dietary fibre fermentability but not viscosity elicited the ‘second-meal effect’ in healthy adult dogs

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Abstract
The present study evaluated the effects of fibre fermentability and viscosity in a morning meal on glucose, insulin and glucagon-like peptide-1 (GLP-1) responses to a glucose challenge later in the day in six healthy female dogs. For this purpose, two Latin square design experiments were performed. In Expt 1, dogs were fed a low-fibre (LF; 1% Solka-Floc (International Fiber Corporation) and 1% soya hulls) diet, a low-fermentable fibre (LFF; 5% Solka-Floc (International Fiber Corporation) and 3% soya hulls) diet or a high-fermentable fibre (HFF; 5% pectin and 3% short-chain fructo-oligosaccharides) diet. In Expt 2, dogs were fed a low-viscosity fibre (5% Solka-Floc (International Fiber Corporation) and 3% soya hulls) diet, a moderate-viscosity fibre (MVF; 2% Solka-Floc (International Fiber Corporation), 2% soya hulls, 2% psyllium and 2% pectin) diet or a high-viscosity fibre (HVF; 4% psyllium and 4% pectin) diet. Dogs were fed at 08.00, 12.00 and 16.00 hours on days 1–6 of each period. On day 7, dogs were fed at 08.00 hours and then dosed with maltodextrin at 12.00 hours. Data were analysed to identify baseline and incremental AUC (IAUC) changes among the treatments. In Expt 1, glucose IAUC0–180 min was lower (P<0.05) in dogs fed the HFF v. LF and LFF diets. Insulin and GLP-1 IAUC0–180 min were not affected. In Expt 2, baseline GLP-1 was greater (P<0.005) and baseline insulin was lower (P<0.05) in dogs fed the HVF v. MVF diet, but glucose, insulin and GLP-1 IAUC0–180 min were not affected. In summary, HFF in a morning meal has the potential to decrease blood glucose response in a consequent meal.

Key words: Dietary fibres: Dogs: Second-meal effect: Glucagon-like peptide-1

Obesity is one of the most common diseases and is a risk factor for many co-morbidities in humans and companion animals, including diabetes mellitus. In dogs, obesity leads to altered blood lipids, glucose intolerance and insulin resistance(1,2), which is similar to humans. Many human and canine studies have highlighted the benefits of dietary fibre, especially soluble fibre, which results in decreased postprandial hyperglycaemia(3–5), greater insulin sensitivity(6,7) and altered gastrointestinal peptide release(8,9). The efficacy of dietary fibre, however, differs according to the source, physical properties and fermentation capacity in the lower part of the gut(10). For example, when dogs were fed a diet containing high-fermentable fibre (HFF; 6% sugarbeet pulp, 2% gum arabic and 1.5% fructo-oligosaccharides) compared with that containing low-fermentable fibre (LFF; 7% cellulose), intestinal glucagon-like peptide-1 (GLP-1) secretion, which acts as an incretin hormone by stimulating insulin secretion, inhibiting glucagon secretion and delaying gastric emptying(11), was increased and improved glucose homeostasis(3). In addition, the supplementation of short-chain fructo-oligosaccharides (scFOS) has been demonstrated to improve insulin sensitivity and modulate the transcription of genes involved in fatty acid or glucose metabolism in the adipose tissue of obese dogs(7).

In addition to the immediate postprandial effect that may occur, certain dietary fibres have also been shown to alter glycaemic response several hours later in a subsequent meal. The ability of an ingredient to affect a subsequent meal has been referred to as the ‘second-meal effect’. In dogs, obesity causes insulin resistance(12), which gives rise to hyperinsulinaemia and glucose intolerance(13). Thus, fibres eliciting the second-meal effect may be used to improve postprandial hyperglycaemia in obese or diabetic dogs. Recent human studies have shown that evening meals rich in non-digestible carbohydrates decrease postprandial glucose to a high-glycaemic index breakfast(14–17). The hypothesis is...
purported to be due to the SCFA (acetate, propionate and butyrate) produced by the fermentation of dietary fibre by the colonic microbiota. In general, SCFA may act directly on cells or receptors, through the action of gut hormones, or via other unknown mechanisms. There are several hypotheses of how SCFA may modulate glucose metabolism: (1) SCFA may increase insulin sensitivity by influencing adipocyte metabolism and decreasing lipolysis, resulting in decreased NEFA concentrations (180); (2) butyrate and butyrate-associated factors play a possible role in moderating glucose-associated inflammation by modulating NF-κB activation (189) and activating PPAR-γ (20); (3) propionate effects on hepatic carbohydrate metabolism to increase glucose use and decrease glucose production (21); (4) SCFA may up-regulate gene expression of the proglucagon gene to increase the secretion of GLP-1 and related peptides (22,23). Because GLP-1 is known to delay gastric emptying (24) and stimulate insulin secretion (25,26), it may contribute to the second-meal effect of fermentable dietary fibre consumption (27). Due to decreased gastric emptying rates and delayed macronutrient digestion and absorption in the gastrointestinal tract (28), diets rich in viscous dietary fibres may alter glucose and insulin responses immediately following a meal, but its effects on a subsequent meal are not known. The potential to elicit a second-meal effect probably depends on dietary fibre type and dosage. To our knowledge, there has not been previous research comparing the effects of dietary fibre fermentability and viscosity on the second-meal effect in dogs.

Dogs share many similarities with humans in terms of gastrointestinal anatomy and physiology. For example, dogs and humans have a strikingly similar stomach morphology, gastric emptying characteristics and postprandial hormone response (29,30). Therefore, dogs are commonly used as a suitable human model for obesity and satiety studies and for testing the effects of diet on postprandial hormone and metabolite concentrations (31-33). The dog model allows for experiments whereby diet, food intake, feeding time, food preference, environment and exercise can be strictly controlled, and allows for repeated blood collections. The objective of the present study was to evaluate the effects of dietary fibre fermentability and viscosity fed in a morning meal on glucose, insulin and GLP-1 responses to a glucose challenge later in the day in healthy dogs. We hypothesised that the inclusion of fermentable (Expt 1) and viscous (Expt 2) fibres would elicit a second-meal effect, namely decreasing glycaemic response and increasing insulin and GLP-1 secretion, in response to an oral glucose load 4 h after their consumption.

Materials and methods

Animals and diets

A total of six healthy adult intact female hounds (age 5·0 (SEM 0·1) years; body weight 23·0 (SEM 2·0) kg; body condition score on a nine-point scale 4·5–5·5) were used in these experiments. Dogs were individually housed in pens (2·3 m × 1·1 m) in the animal facility of the Edward R. Madigan Laboratory at the University of Illinois. The room was environmentally controlled (20°C) with a 16 h light–8 h dark cycle. Dogs were allowed access to various toys for behavioural enrichment and to exercise outside of their cages and socialised with each other and humans for approximately 1 h at least 5 d/week except for collection days.

The dietary ingredient and chemical composition of six experimental diets are shown in Table 1. Based on previous in vitro fermentation experiments (34,35) and digesta viscosity experiments (36), dietary fibres were selected on their level of fermentability and viscosity. In Expt 1, dogs were randomly assigned to one of three treatments: a low-fibre (LF) diet containing 1% Solka-Floc (International Fiber Corporation) and 1% soya hulls; a LFF diet containing 5% Solka-Floc (International Fiber Corporation) and 3% soya hulls; a HFF diet containing 5% pectin (HM (high-methoxyl) Pectin; TIC Gums) and 3% scFOS (SynergyC; BENE0-Group). In Expt 2, dogs were randomly assigned to one of three treatments: a low-viscosity fibre (LVF) diet containing 5% Solka-Floc (International Fiber Corporation) and 3% soya hulls; a moderate-viscosity fibre (MVF) diet containing 2% Solka-Floc (International Fiber Corporation), 2% soya hulls, 2% psyllium (KV Vet Supply) and 2% pectin; a high-viscosity fibre (HVF) diet containing 4% psyllium and 4% pectin. All the diets were formulated to meet all nutrient recommendations provided by the Association of American Feed Control Officials (2009) and were manufactured at Kansas State University’s Bioprocessing and Industrial Value-Added Program facility (Manhattan, KS, USA) under the supervision of Pet Food & Ingredient Technology, Inc. (Topeka, KS, USA). Dogs were fed to maintain body weight. Initial food intake was determined by calculating the maintenance energy requirement (37) and by using previous feeding records. Dogs were weighed weekly and food intake was adjusted to maintain body weight and body condition score throughout the study. Water was available ad libitum throughout the experiment. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before experimentation.

Experimental design

Dogs were fed the LF diet three times daily for 2 weeks before experimentation to allow for adaptation to the feeding pattern. The research was then conducted as two studies using a replicated 3 × 3 Latin square design. Each period in both experiments included a 6 d adaptation phase, followed by blood collection on day 7. Dogs were fed the experimental diets three times daily at 08.00, 12.00 and 16.00 hours on days 1–6. On day 7, dogs consumed their 08.00-hour meal as usual. At 12.00 hours, a baseline blood sample (0 min) was collected via jugular venepuncture. Following the baseline sample, 25 g maltodextrin in 120 ml of water were administered using a 60 ml syringe without a needle in place of their regular second meal. A maltodextrin solution, which is commonly used to test glycaemic and insulininaemic responses in humans and dogs, was given by dripping the solution from the syringe into the mouth of the dogs to ensure consumption within 10 min. This method of dosing was done slowly to minimise stress...
and avoid aspiration. Blood samples were then collected at 10, 20, 30, 45, 60, 90, 120 and 180 min after dosing.

**Chemical analyses**

Diet subsamples were collected and ground using a Wiley mill (Model 4; Thomas Scientific) through a 2 mm screen and dry ice in preparation for chemical analyses. Diet samples were analysed for DM and organic matter according to the Association of Official Analytical Chemists (38). Crude protein was measured using a Leco Nitrogen/Protein Determinator (Model FP-2000, Leco Corporation) according to the Association of Official Analytical Chemists (38). Fat concentrations were determined by acid hydrolysis according to the American Association of Cereal Chemists (39) followed by diethyl ether extraction (40). Total dietary fibre was determined according to Prosky et al. (41). Gross energy was measured using a bomb calorimeter (Model 1261; Parr Instrument Company).

**Blood collection and analysis**

The same blood collection and handling procedures for the measurement of serum glucose, serum insulin and plasma active GLP-1 were used in both experiments. A total of 6 ml of blood were collected at each time point via jugular venepuncture using 10 ml syringes. Then, one drop of blood from syringes was immediately used to measure glucose concentration using the glucose oxidase method (AlphaTRAK Blood Glucose Monitoring System; Abbott Laboratories). Another 2 ml of blood were then immediately transferred into a precooled Vacutainer tube (no. 367835; Becton, Dickinson and Company) containing EDTA and 20 μl dipeptidyl peptidase IV inhibitor (10 μl/ml blood; Millipore), and centrifuged at 1000 g at 4 °C for 10 min for the measurement of plasma active GLP-1. The remaining blood was transferred to a serum separator tube (no. 367985; Becton, Dickinson and Company) to allow clotting to occur, and then centrifuged at 1300 g at room temperature for 15 min for the measurement of serum insulin. After centrifugation, the supernatant was collected into its respective cryovial and stored at −20 °C (for serum) or −80 °C (for plasma) until further analysis.

Serum insulin was determined using a Rat Insulin Enzyme Immunoassay kit (Cayman Chemical). Plasma active GLP-1 concentration was analysed using a Glucagon-Like Peptide 1 (Active) ELISA kit (Millipore). Both methods have been validated for use in dogs previously (30, 42).

**Table 1. Ingredient and chemical composition of the experimental diets fed to dogs**

<table>
<thead>
<tr>
<th>Items</th>
<th>LF</th>
<th>LFF</th>
<th>HFF</th>
<th>LVF</th>
<th>MVF</th>
<th>HVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewer’s rice (%)</td>
<td>35.00</td>
<td>29.00</td>
<td>29.00</td>
<td>29.00</td>
<td>29.00</td>
<td>29.00</td>
</tr>
<tr>
<td>Poultry by-product meal (%)</td>
<td>34.30</td>
<td>33.25</td>
<td>33.25</td>
<td>33.25</td>
<td>33.25</td>
<td>33.25</td>
</tr>
<tr>
<td>Maize, yellow, ground (%)</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Poultry fat (%)</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Maize gluten meal (%)</td>
<td>4.54</td>
<td>5.59</td>
<td>5.59</td>
<td>5.59</td>
<td>5.59</td>
<td>5.59</td>
</tr>
<tr>
<td>Egg product, dried (%)</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Solka-Floc (%)</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Soy hulls, raw (%)</td>
<td>1.00</td>
<td>3.00</td>
<td>3.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pectin, HM rapid (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pea starch (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.00</td>
<td>–</td>
</tr>
<tr>
<td>Maize gluten meal (%)</td>
<td>4.54</td>
<td>5.59</td>
<td>5.59</td>
<td>5.59</td>
<td>5.59</td>
<td>5.59</td>
</tr>
<tr>
<td>Egg product, dried (%)</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Polysaccharides (%)</td>
<td>1.00</td>
<td>5.00</td>
<td>–</td>
<td>5.00</td>
<td>2.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Chemical composition (DM basis) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM (%)</td>
<td>93.55</td>
<td>93.38</td>
<td>92.91</td>
<td>93.38</td>
<td>93.25</td>
<td>92.55</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>34.61</td>
<td>33.88</td>
<td>33.81</td>
<td>33.88</td>
<td>32.09</td>
<td>32.39</td>
</tr>
<tr>
<td>Acid-hydrolysed fat (%)</td>
<td>17.83</td>
<td>18.28</td>
<td>17.99</td>
<td>18.28</td>
<td>17.43</td>
<td>16.08</td>
</tr>
<tr>
<td>TDF (%)</td>
<td>10.48</td>
<td>9.64</td>
<td>9.64</td>
<td>9.64</td>
<td>9.64</td>
<td>9.64</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.05</td>
<td>5.85</td>
<td>5.78</td>
<td>5.88</td>
<td>5.88</td>
<td>5.94</td>
</tr>
<tr>
<td>Gross energy (kcal/g)</td>
<td>5.32</td>
<td>5.35</td>
<td>5.29</td>
<td>5.35</td>
<td>5.28</td>
<td>5.19</td>
</tr>
<tr>
<td>Gross energy (kJ/g)</td>
<td>22.26</td>
<td>22.38</td>
<td>22.13</td>
<td>22.38</td>
<td>22.09</td>
<td>21.71</td>
</tr>
</tbody>
</table>

LF, low fibre; LFF, low-fermentable fibre; HFF, high-fermentable fibre; LVF, low-viscosity fibre; MVF, moderate-viscosity fibre; HVF, high-viscosity fibre; HM, high-methoxyl; scFOS, short-chain fructo-oligosaccharides; TDF, total dietary fibre.

* International Fiber Corporation.
† Provided per kg of diet: vitamin A palmitate, 36 mg; vitamin D3, 27 mg; vitamin E acetate, 288 mg; vitamin K, 2.16 mg; thiamin, 30.6 mg; riboflavin, 30.6 mg; pantothenic acid, 50.4 mg; nicotinic acid, 124.2 mg; pyridoxine, 30.6 mg; biotin, 10.08 mg; folic acid, 1.08 mg; vitamin B12, 115 μg.
‡ Provided per kg of diet: Mn (as MnCO3), 18.0 mg; Fe (as C6H8O7·xFe), 135.0 mg; Cu (as Cu2(OH)2CO3), 18.0 mg; Zn (as ZnCO3), 180.0 mg; I (as KIO3), 1.8 mg; Se (as Na2SeO3), 396.0 μg; Co (as CoSO4), 3.8 μg.
§ Because the TDF assay cannot quantify scFOS, this value was determined by adding the amount of scFOS present in the diet to the uncorrected TDF value.
Statistical analyses

For the baseline samples, data were analysed using the MIXED procedure of SAS 9.2 (SAS Institute, Inc.) for testing the main effect of experimental diet and including random effects of dog and period. For post-second-meal samples, all data were expressed as the incremental change from baseline (baseline subtracted) to minimise the differences in baseline concentrations among dogs, and then analysed using the MIXED procedure of SAS 9.2 (SAS Institute, Inc.) as repeated measures. The main effects of diet and time were tested and the diet x time interaction was evaluated if significant. Random effects of dog and period were included in the model. Means were separated for diets using the PDIFF statement in the MIXED procedure for individual time points after detecting a significant diet effect using SLICE/time. Differences in the all positive peak areas using GraphPad Prism version 5.00 were considered as significant and £ time interaction was evaluated if significant.

Results

Expt 1

Baseline concentrations of glucose, insulin and active GLP-1 in Expt 1 were not affected (P>0.05) by the dietary treatments (Table 2). Fig. 1 presents the incremental changes in glucose, insulin and active GLP-1 concentrations for 3h after dosing with maltodextrin. In general, blood glucose concentration (Fig. 1(a)) peaked 20–30 min after dosing and then gradually decreased over time until approaching baseline at approximately 180 min. In dogs fed the LF and LFF diets, the first peak was followed by another peak after 60–90 min. In contrast to dogs fed the HFF diet that had a sharp decrease in glucose concentration from 60 to 90 min, dogs fed the LF and LFF diets maintained high glucose levels throughout the 90 min postprandial period. Blood glucose concentration was lower (P<0.05) during the HFF treatment compared with the LFF or LF treatment at 90 min. Blood glucose during the LFF and LF treatments approached, but did not return to baseline by 180 min. Incremental changes in blood insulin concentrations had similar two-peak patterns among the dietary treatments (Fig. 1(b)). Insulin concentrations in dogs fed the HFF, LF and LFF diets returned to baseline at approximately 90, 120 and 180 min, respectively. Active GLP-1 concentrations were highly variable throughout the 180 min postprandial period (Fig. 1(c)). Dogs fed the HFF and LF diets had GLP-1 peaks at 20 min that quickly dropped to baseline at approximately 90 min. Dogs fed the LFF diet, however, had a GLP-1 peak at 45 min, which gradually decreased to baseline at 120 min.

Because AUC values consider the entire postprandial curve, they are a better indicator of treatment response than individual time points. Dogs fed the HFF diet had lower (P=0.049) postprandial glucose IAUC0–180 min when compared with dogs fed the LF and LFF diets. IAUC0–180 min values for insulin and active GLP-1 were not different among the dietary treatments. Fasting basal insulin:glucose ratio and AUCinsulin:AUCglucose did not differ among the test diets (Table 2).

Expt 2

Baseline glucose concentrations did not differ (P>0.05) among dogs fed the LVF, MVF and HVF diets (Table 3). Baseline GLP-1 concentrations were greater (P=0.005) in dogs fed the HVF diet, while baseline insulin concentrations in dogs fed the HVF diet tended to be lower (P=0.10) than dogs fed the MVF diet.

Table 2. Serum glucose, insulin and plasma active glucagon-like peptide-1 (GLP-1) concentrations in dogs fed a low-fibre (LF), low-fermentable fibre (LFF) or high-fermentable fibre (HFF) diet

<table>
<thead>
<tr>
<th>Items</th>
<th>LF</th>
<th>LFF</th>
<th>HFF</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/l)</td>
<td>1042</td>
<td>1028</td>
<td>1130</td>
<td>41</td>
<td>0.106</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>13-5</td>
<td>10-8</td>
<td>161-2</td>
<td>28.9</td>
<td>0.169</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>7-8</td>
<td>6-8</td>
<td>9-1</td>
<td>1.7</td>
<td>0.610</td>
</tr>
<tr>
<td>Insulin:glucose*</td>
<td>0-13</td>
<td>0-10</td>
<td>0-14</td>
<td>0-02</td>
<td>0-150</td>
</tr>
<tr>
<td>IAUC0–180 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/l)</td>
<td>40 700</td>
<td>45 620</td>
<td>30 050</td>
<td>5870</td>
<td>0.049</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>10 027</td>
<td>15 110</td>
<td>12 033</td>
<td>2737</td>
<td>0.453</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>401</td>
<td>506</td>
<td>319</td>
<td>124</td>
<td>0.501</td>
</tr>
<tr>
<td>IAUCinsulin:AUCglucose†</td>
<td>0-23</td>
<td>0-35</td>
<td>0-41</td>
<td>0-08</td>
<td>0-358</td>
</tr>
</tbody>
</table>

LF, low-fibre; LFF, low-fermentable fibre; HVF, high-fermentable fibre. IAUC, incremental AUC.

* Mean values within a row with unlike superscript letters were significantly different (P<0.05).

† Ratio of area under the insulin:glucose curve.
GLP-1 concentrations were highly variable throughout the 180 min postprandial period (Fig. 2(c)). GLP-1 concentration in the MVF treatment had a sharp peak at 20 min ($P=0.006$) that quickly dropped near the baseline at 45 min and did not change over time after 45 min. GLP-1 incremental change in the LVF treatment peaked at approximately 30 min, and then remained at a lower level after 60 min. Dogs fed the LVF diet had a GLP-1 peak at 10 min, but thereafter neither changed over time nor returned to baseline. No dietary treatment effects were observed in the postprandial IAUCAUC~180 min of glucose, insulin, and active GLP-1. Fasting basal insulin: glucose ratio and AUC_{insulin}:AUC_{glucose} did not differ among the test diets (Table 3).

**Discussion**

In the early twentieth century, Staub(43) and Traugott(44) first reported the second-meal effect. In these human studies, they demonstrated that an initial glucose load decreased glycaemic response of a second glucose load within 12 h. Recently, due to the prevalence of obesity and diabetes, the benefit of the second-meal effect has drawn much wider attention. However, the mechanisms involved in the second-meal effect are still unclear. In human subjects, consumption of a low-glycaemic index food in an evening meal has been shown to improve postprandial glucose response during breakfast the next morning. Indeed, these low-glycaemic index foods contain a source of dietary fibre. Dietary fibres are broadly classified into soluble and insoluble forms based on their solubility in water. Fibres may also be classified as viscous v, non-viscous fibre and fermentable v, non-fermentable fibre according to their physico-chemical properties and physiological effects on the host. Until now, there has been no research comparing the effects of dietary fibre type or amount on the second-meal effect. The present study aimed to evaluate the effects of dietary fibre type and amount on the second-meal effect in the dog model.

In the present study, all dogs were fed the dry kibble experimental diets at amounts to maintain body weight and body condition score. Feeding three times daily was used to mimic a human eating pattern, yet staying within the practical limits of conducting an in vivo experiment. Data from the present study, therefore, may be applied to both companion animal and human health.

The aim of the first experiment was to evaluate the second-meal effect of diets containing varying amounts of fermentable fibre. We hypothesised that because a morning meal containing fermentable fibre would increase SCFA production in the colon, it would subsequently modulate postprandial blood glucose and insulin peaks and incremental AUC following an oral glucose load provided 4 h later. We observed that when the HFF diet including 5% pectin and 3% scFOS was consumed in the morning, a decreased blood glucose response resulted during the oral glucose load at noon. This occurred without altering the baseline glucose, insulin and GLP-1 concentrations or postprandial insulin or GLP-1 response. The LF and LFF diets did not alter the glucose, insulin or GLP-1 response after the oral glucose load. Although

LVF diet had a sharp decrease in glucose concentration after 90 min, which returned to baseline at approximately 120 min. Incremental changes in blood insulin concentrations had the similar two-peak pattern among the dietary treatments (Fig. 2(b)). Insulin concentrations in dogs fed the HVF, MVF and LVF diets returned to baseline at approximately 180, 90 and 120 min, respectively. Incremental changes in active

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**Fig. 1.** Incremental changes in (a) serum glucose, (b) serum insulin and (c) plasma active glucagon-like peptide-1 (GLP-1) concentrations in dogs fed a low-fibre (•), low-fermentable fibre (■) or high-fermentable fibre (▲) diet in Expt 1. Values are incremental changes from baseline means. (a) SEM = 5.25; diet, $P=0.288$; time, $P<0.0001$; diet × time, $P=0.116$. Mean values were significantly different among the dietary treatments ($P<0.05$). (b) SEM = 40.85; diet, $P=0.356$; time, $P<0.0001$; diet × time, $P=0.424$. (c) SEM = 1.49; diet, $P=0.575$; time, $P<0.0001$; diet × time, $P=0.457$. 

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Second, SCFA may also improve glucose tolerance and insulin sensitivity by influencing carbohydrate metabolism (49,50). Venter et al. (49) reported that propionate supplementation decreased circulating free fatty acids and improved glucose tolerance, accompanied with a lower NEFA concentration and a slower gastric emptying rate at the second-meal time. This delay in gastric emptying could be the effect of SCFA via GLP-1, but contradictory results are present in the literature. Data reported by Priebe et al. (17) do not suggest that the second-meal effect is due to SCFA mediation of gastric emptying. Those researchers observed a higher glucose clearance rate (suggesting increased uptake into peripheral tissues) in the fermentable fibre treatment, but no difference in the rate of exogenous glucose appearance.

Although many SCFA-related effects may function through GLP-1, other possible mechanisms are GLP-1 independent. A third potential mechanism by which SCFA may mediate the second-meal effect is by increasing insulin sensitivity by influencing adipocyte metabolism (49,50). Venter et al. (49) reported that propionate supplementation decreased fasting basal insulin:glucose ratio. Although study in human subjects has also reported that cooked barley kernels, which contain fermentable fibre, increased insulin sensitivity and decreased blood glucose response (17). Studies have also confirmed that the colonic fermentation of scFOS specifically can increase insulin sensitivity (47,48). Increased NEFA release increases the body’s ability to take up glucose, allowing a greater storage of glucose as muscle glycogen. SCFA may also improve glucose tolerance and insulin sensitivity by influencing carbohydrate metabolism (49,50). However, suppression of NEFA release increases the body’s ability to take up glucose, allowing a greater storage of glucose as muscle glycogen (49,50). SCFA may also improve glucose tolerance and insulin sensitivity by influencing carbohydrate metabolism (49,50).

Baseline insulin:glucose concentrations and insulin AUC: glucose AUC were not different (P>0.05), these results indicate that HFF diets may have the potential to increase insulin secretory response or insulin sensitivity in dogs. A previous study in human subjects has also reported that cooked barley kernels, which contain fermentable fibre, increased insulin sensitivity and decreased blood glucose response (17). Although many SCFA-related effects may function through GLP-1, other possible mechanisms are GLP-1 independent. A third potential mechanism by which SCFA may mediate the second-meal effect is by increasing insulin sensitivity by influencing adipocyte metabolism (49,50). Venter et al. (49) reported that propionate supplementation decreased fasting basal insulin:glucose ratio. Although study in human subjects has also reported that cooked barley kernels, which contain fermentable fibre, increased insulin sensitivity and decreased blood glucose response (17). Studies have also confirmed that the colonic fermentation of scFOS specifically can increase insulin sensitivity (47,48). Increased NEFA release increases the body’s ability to take up glucose, allowing a greater storage of glucose as muscle glycogen. SCFA may also improve glucose tolerance and insulin sensitivity by influencing carbohydrate metabolism (49,50). However, suppression of NEFA release increases the body’s ability to take up glucose, allowing a greater storage of glucose as muscle glycogen (49,50). SCFA may also improve glucose tolerance and insulin sensitivity by influencing carbohydrate metabolism (49,50). Venter et al. (49) reported that propionate supplementation decreased fasting serum glucose and maximum insulin increments during glucose tolerance tests. These data were in line with that reported by Todesco et al. (30), who demonstrated that dietary propionate supplementation in bread reduced the blood glucose area in comparison with standard propionate-free bread in healthy subjects. SCFA may mediate the second-meal effect by decreasing pro-inflammatory markers. Priebe et al. (17) reported that an evening meal containing cooked barley kernels prevented the postprandial rise in the pro-inflammatory cytokines IL-6 and TNF-α after the glucose load in the morning. Mechanisms by which SCFA may contribute to reduced inflammation include the modulation of

### Table 3. Serum glucose, insulin and plasma active glucagon-like peptide-1 (GLP-1) concentrations in dogs fed a low-viscosity fibre (LVF), moderate-viscosity fibre (MVF) or high-viscosity fibre (HVF) diet

(Means with their standard errors, n = 6)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LVF</th>
<th>MVF</th>
<th>HVF</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline concentration Glucose (mg/l)</td>
<td>1133</td>
<td>1098</td>
<td>1090</td>
<td>39</td>
<td>0.299</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>138.9</td>
<td>166.4</td>
<td>126.6</td>
<td>40.8</td>
<td>0.102</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>7.3^a</td>
<td>8.3^a</td>
<td>16.6^b</td>
<td>2.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Insulin:glucose*</td>
<td>0.12</td>
<td>0.15</td>
<td>0.12</td>
<td>0.04</td>
<td>0.124</td>
</tr>
<tr>
<td>IAUC_{0–120 min} Glucose (mg/l)</td>
<td>27,910</td>
<td>33,730</td>
<td>35,540</td>
<td>7640</td>
<td>0.332</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>9066</td>
<td>10,271</td>
<td>13,905</td>
<td>4004</td>
<td>0.436</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>586</td>
<td>447</td>
<td>487</td>
<td>144</td>
<td>0.788</td>
</tr>
<tr>
<td>AUC_{insulin-AUC_{glucose}}†</td>
<td>0.29</td>
<td>0.34</td>
<td>0.40</td>
<td>0.10</td>
<td>0.580</td>
</tr>
</tbody>
</table>
Although it often affects glycaemic response in the meal at which it is consumed, it is unknown whether viscous fibres have the ability to affect glycaemic response in a second meal. It is possible and may have contributed in previous studies, but viscosity is difficult to measure and is not always distinguished from non-viscous fibres in such studies. In the second experiment of the present study, the aim was to evaluate the second-meal effect of diets containing highly or lowly viscous fibres by including variable amounts of psyllium, pectin and cellulose in the diets. We hypothesised that due to the delayed gastric emptying, macronutrient digestion and absorption in the gastrointestinal tract\(^{(26)}\), diets causing increased digesta viscosity would also modulate glucose and insulin responses following a glucose load 4 h later. The present data, however, suggest that supplementing 4 or 8% of viscous fibres in the morning meal does not influence postprandial glucose or insulin response (IAUC\(_{0–180\text{ min}}\)) during the oral glucose load at the second-meal time. However, because baseline concentrations were different before the glucose load, any potential second-meal effects may have been masked in this experiment. Given the gastric emptying data that exist for dogs\(^{(51)}\), we expected a considerable proportion of the morning meal to reach the hindgut after 4 h. This time was selected to represent a logical meal sequence for humans or dogs eating multiple meals each day. Because baseline differences were observed, more time after the morning meal may have been needed before the glucose tolerance test. Interestingly, when comparing the two experiments, HFF (5% pectin and 3% scFOS) decreased the postprandial glucose response at the second-meal time, while HVF (4% pectin and 4% psyllium) did not. Because pectin was included in both fermentable and viscous fibre diets, it may not contribute to the second-meal effect independently. In agreement with the present results, Pouteau et al.\(^{(52)}\) recently reported that a chronic 5-week ingestion of apple pectin and acacia gum blend had no effect on glycaemia or lipidaemia, and did not improve the peripheral insulin sensitivity measured by the euglycaemic and hyperinsulinaemic clamp technique in patients with the metabolic syndrome.

Factors other than fermentation or viscosity may also improve postprandial glucose response, although mechanisms are unclear. In healthy women, for example, Weickert et al.\(^{(53)}\) observed an improved postprandial glucose response during lunch in those that ingested bread containing a non-fermentable, insoluble wheat fibre \(\text{v}\). white bread at breakfast. Weickert & Pfeiffer\(^{(54)}\) postulated that other unknown mechanisms contributing to beneficial effects of insoluble dietary fibre consumption could involve increased insulin sensitivity, a shift in the relationship of gut microbiotic communities, as well as direct and indirect influences on yet unknown hormonal and molecular factors in the host.

In the present study, although we did not observe a second-meal difference in GLP-1 IAUC\(_{0–180\text{ min}}\) among the three diets differing in viscosity, baseline GLP-1 concentrations were greater \((P=0.005)\) in dogs fed the HVF diet. Because viscous diets may delay gastric emptying and intestinal transit, the time between the first and second meals may have not been long enough to appropriately test this effect. It has been
reported that viscous dietary fibres may decrease the early postprandial GLP-1 response in healthy human subjects\(^{(9,55)}\).

Karhunen et al.\(^{(39)}\) reported that the postprandial GLP-1 concentration was significantly suppressed after a psyllium fibre- and protein-rich meal, in contrast to the initial increase following the low-fibre/low-protein, high-fibre/low-protein and low-fibre/high-protein meals. The GLP-1 data in that study represented a 2h response after meals\(^{(39)}\). Houda et al.\(^{(56)}\) measured the postprandial GLP-1 response in 8h in pigs when fed diets containing different levels of oat β-glucan and reported that GLP-1 was affected by time. Pigs fed a high-viscous fibre diet had lower GLP-1 concentrations at 90, 120 and 180min postprandial than pigs fed low-viscous fibre diets. At 4h postprandial, however, GLP-1 tended to increase in the high-viscous fibre treatment when compared with the low-viscous fibre treatments\(^{(50)}\).

In conclusion, the present results suggest that highly fermentable fibres have the potential to decrease blood glucose response in a meal fed many hours later, possibly by increasing insulin sensitivity in dogs. Viscous fibres, however, did not appear to contribute to a second-meal effect in dogs, but may have been masked by the timing of our meals. These findings provide valuable information that may be used as a platform for which to design subsequent studies targeting the second-meal effect and its application to obese dog and human nutritional strategies. Further studies in dogs or human subjects may provide new insights into the mechanisms by which dietary fibres contribute to the second-meal effect.

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