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# Highly viscous guar gum shifts dietary amino acids from metabolic use to fermentation substrate in domestic cats

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## Abstract

The present study evaluated the potential of affecting amino acid metabolism through intestinal fermentation in domestic cats, using dietary guar gum as a model. Apparent protein digestibility, plasma fermentation metabolites, faecal fermentation end products and fermentation kinetics (exhaled breath hydrogen concentrations) were evaluated. Ten cats were randomly assigned to either guar gum- or cellulose-supplemented diets, that were fed in two periods of 5 weeks in a crossover design. No treatment effect was seen on fermentation kinetics. The apparent protein digestibility ($P=0{\cdot}07$) tended to be lower in guar gum-supplemented cats. As a consequence of impaired small-intestinal protein digestion and amino acid absorption, fermentation of these molecules in the large intestine was stimulated. Amino acid fermentation has been shown to produce high concentrations of acetic and butyric acids. Therefore, no treatment effect on faecal propionic acid or plasma propionylcarnitine was observed in the present study. The ratio of faecal butyric acid:total SCFA tended to be higher in guar gum-supplemented cats ($P=0{\cdot}05$). The majority of large-intestinal butyric acid is absorbed by colonocytes and metabolised to 3-hydroxy-butyrylcoenzyme A, which is then absorbed into the bloodstream. This metabolite was analysed in plasma as 3-hydroxy-butyrylcarnitine, which was higher ($P=0{\cdot}02$) in guar gum-supplemented cats. In all probability, the high viscosity of the guar gum supplement was responsible for the impaired protein digestion and amino acid absorption. Further research is warranted to investigate whether partially hydrolysed guar gum is useful to potentiate the desirable *in vivo* effects of this fibre supplement.

**Key words: Acylcarnitine: Guar gum: Exhaled breath hydrogen: Apparent protein digestibility**

Guar gum is a polysaccharide extracted from the endosperm of guar plant seeds. The guar plant (*Cyamopsis tetragonolobus*) is an annual leguminous plant bearing pods with round light brown seeds[1–3]. Guar gum consists of galactomannans, polysaccharides that are inert for digestive enzymes in the human small intestine[3]. In cats, the *in vivo* effects of guar gum intake have been investigated in blends with other fibre sources[4], but never as the unique source of soluble fermentable fibre. *In vitro* research demonstrated that guar gum generated high concentrations of propionic acid upon fermentation with feline faecal inoculum[4,5]. However, further *in vivo* research on the end product profile upon guar gum fermentation is crucial, as recent work by Verbrugghe *et al.*[6–8] suggested an amino acid-sparing effect of propionic acid in domestic cats fed fructan-supplemented diets. In strict carnivores, amino acids are continuously processed to yield glucose via the gluconeogenic pathway[9–11]. Propionic acid may be used as an alternative gluconeogenic substrate[12], and as a consequence, amino acids might be spared. The major goal of the treatment of patients with hepatic[13] and renal failure[14], for example, is decreasing amino acid decarboxylation. To achieve this decrease, the amino acid-sparing potential of propionic acid might be advantageous. Also, in healthy cats, a more efficient amino acid metabolism might be beneficial, as the maintenance protein requirement for cats is higher compared to herbivorous or omnivorous species[10,15].

The present study used dietary guar gum to evaluate whether intestinal volatile fatty acid production – in particular propionic acid – affects amino acid metabolism in domestic cats. Therefore, an *in vivo* assessment of guar gum fermentation metabolites in plasma, end product profiles in faeces, and kinetics was performed, and the effect of guar

**Abbreviations:** AUC, area under the curve; HMG, 3-OH-3-methylglutarylcarnitine; ppm, part per million.

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**Table 1.** Macronutrient composition of the experimental diet* and the experimental diet supplemented with guar gum† or cellulose‡

| Nutrient | Diet | Diet + guar gum† | Diet + cellulose‡ |
|---|---|---|---|
| DM (% as is) | 94·7 | 93·5 | 93·7 |
| Crude protein (% DM) | 30·1 | 29·0 | 28·2 |
| Crude fat (% DM) | 11·5 | 11·3 | 11·6 |
| Crude ash (% DM) | 10·8 | 10·5 | 10·5 |
| Crude fibre (% DM) | 1·4 | 2·0 | 4·0 |
| NFE (% DM)§ | 46·2 | 47·2 | 45·7 |
| TDF (% DM) | 8·0 | 10·9 | 10·2 |

NFE, nitrogen-free extract; TDF, total dietary fibre.
* Labelled ingredient composition: rice, lamb meal, oils and fats, minerals and natural antioxidants.
† Vidogum GI, Unipektin Ingredients AG.
‡ Arbocel BWW 40, Rettenmaier und Söhne.
§ NFE (% DM) was calculated as 100 − crude protein − crude fat − crude ash − crude fibre, with all components on DM basis.

gum supplementation on apparent protein digestibility was assessed. Fermentation kinetics were studied by measuring hydrogen concentrations in the expired air as this expired gas originates solely from large-intestinal microbial fermentation[16]. In the present paper, the methods of training the cats for sampling, and the procedure for measurement of the exhaled breath hydrogen are also described.

## Materials and methods

### Animals

Ten healthy adult domestic short-hair cats, with a mean body weight of 4·5 (SEM 0·5) kg and a mean age of 5·8 (SEM 2·7) years, were included in the present study. Five female and five male cats were used and all cats were castrated, except for one intact male. Before inclusion in the study, the cats underwent a thorough physical examination, and after an overnight fast, blood samples were taken for complete blood count and serum biochemistry analyses.

### Experimental design and diet

The experiment was set up in a crossover design, with two periods of 5 weeks and two fibre supplements (4 % guar gum; Vidogum GI, Unipektin Ingredients AG, cold viscosity: 3600–4500 mPas, hot viscosity: 4500–5300 mPas) or 4 % cellulose (Arbocel BWW 40, Rettenmaier und Söhne) on a DM basis). The ten selected cats were randomly divided into two groups (group 1 and group 2). During the first 17 d of each period, the cats were fed twice a day (at 08.00 and 20.00 hours), while during the last 18 d, they were fed four isoenergetic meals per day (at 06.00, 12.00, 18.00 and 24.00 hours). For 2 d between both periods, the cats were fed the experimental diet without fibre supplementation. All animals were individually fed and group housed with five cats per group in between meals. A commercially available dry cat food (Trovet Hypoallergenic lamb and rice; Netlaa bv) was fed at maintenance energy requirement[17] (418·4 kJ/kg$^{0·67}$ per d, based on ideal body weight). This diet had a moderate protein content (30·1 % DM) and rice was the only source of predominantly insoluble fibre. The analysed chemical composition and the ingredient composition specified by the manufacturer of the diet are shown in Table 1. Powdered supplements were first thoroughly mixed with 1 g of a commercially available canned diet (Hill's Prescription Diet Canine/Feline a/d; Hill's Pet Nutrition, Inc.) to improve the supplement intake. Consecutively, this mixture was blended with the dry food by hand. Cats were weighed weekly and the amounts of feed were adjusted to maintain a stable body weight. Cats had *ad libitum* access to fresh drinking-water. The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2010/153; EC 2011/003) and was in accordance with institutional and national guidelines for the care and use of animals.

### Sampling

Blood was collected via jugular venipuncture at 2 h after the first meal in week 5 of each period (four meals per d). Immediately after collection, blood samples were placed into vacutainer tubes containing lithium heparin. Additionally, in week 5 of each period, a total faecal collection was performed over a 5-d period, and on the last day fresh faecal samples were collected within 30 min of voiding. During faecal collection, cats were housed individually. Exhaled breath samples were collected as depicted in Fig. 1. The cats were trained once daily for this sample collection for a period of 3 months preceding the trial. An anaesthetic mask designed for cats was placed on the nose and mouth of the cat for increasing periods of time. At the end of the training period, the cats were sampled with minimal restraint and discomfort for 30–45 s, as per the

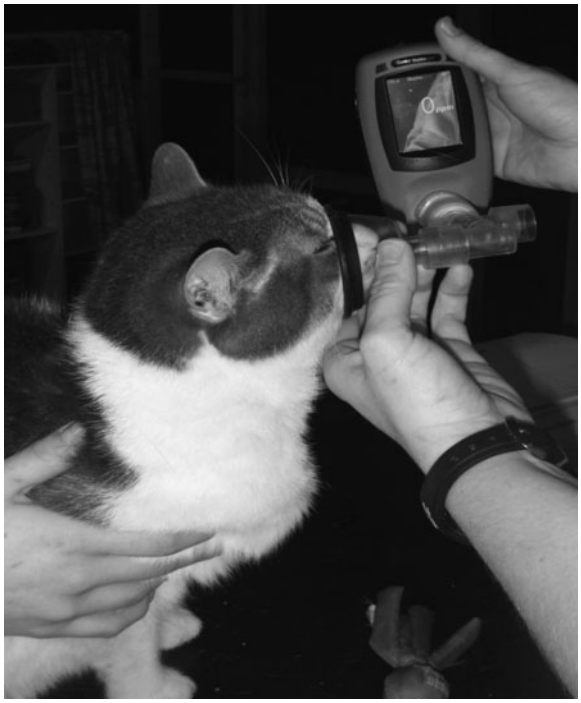

**Fig. 1.** Equipment and sampling technique for the hydrogen breath test used to study fermentation kinetics in nine cats fed a moderate-protein diet supplemented with guar gum (Vidogum GI, Unipektin Ingredients AG) or cellulose (Arbocel BWW 40, Rettenmaier und Söhne) in a 10-week crossover study.

manufacturer's recommendation. The mask was connected to a hydrogen monitor (*cfr. infra*). In week 3 of the first period (days 15–16), a pilot study was performed to establish the ideal measuring time points. All cats were sampled once prior to the morning meal and at every 30 min for 11·5 h postprandially to determine individual hydrogen concentrations over time. The resulting curves revealed that hourly sampling during 6 h was appropriate and this schedule was used in the main study during week 4 of each period. At each time point, two consecutive measurements were done 5 min apart and the means of both values were calculated. All measurements were repeated on two consecutive days.

### Chemical analyses

The experimental diet was analysed for DM by drying to a constant weight at 103°C and for crude ash by combustion at 550°C. Crude protein was calculated from Kjeldahl nitrogen (6·25 × N, ISO 5983-1, 2005), crude fat was analysed by the Soxhlet method (ISO 1443, 1973) and crude fibre by acid-alkali digestion (ISO 5498, 1981). Nitrogen-free extract was calculated by subtracting crude ash, crude protein, crude fat and crude fibre on a DM basis from 100. Total dietary fibre was analysed as described by Prosky *et al.*[18]. Faecal pH was measured using a portable pH meter (Hanna Instruments Belgium). Additionally, faecal consistency was scored as described by Hesta *et al.*[19]. The SCFA were analysed using GC after extraction with diethyl ether[20]. The ammonia $(NH)_3$ was analysed by steam distillation and titration, as described by Bremner & Keeney[21]. Indole, phenol and *p*-cresol were extracted using hexane and analysed using GC–MS/MS, as previously described[7]. Faecal samples of total collections were lyophilised and pooled per cat per period. Pooled faeces were sieved through a 1 mm mesh for hair removal, ground up in a grinding mill (1 mm mesh, Brabender Rotary Mill; Brabender GmbH & Company KG) and proximate analyses were performed as described above. Bacterial nitrogen was analysed by the method of Mason[22], with adaptations previously described by Hesta *et al.*[23]. The hydrogen concentration in the exhaled air samples was measured using a hydrogen monitor (Gastrolyzer; Bedfont Scientific). The electrochemical sensor sensitivity was 1 part per million (ppm) and the measurable concentration range was between 0 and 500 ppm. Before sampling, the hydrogen monitor was calibrated according to the manufacturer's instructions. Different calibration conditions (location, temperature, zeroing) were tested during the training period and pilot study, and the best calibration protocol was used during the test periods (zeroing and calibration in experimental room at 19°C). Plasma acylcarnitine (free carnitine, acetyl-, propionyl-, butyryl- + isobutyryl-, isovaleryl- +2-methylbutyryl-, 3-hydroxy(OH)-isovaleryl-, 3-OH-butyryl-, tiglyl- + 3-methylcrotonyl-, methylmalonyl- and 3-OH-3-methylglutarylcarnitine (HMG)) and amino acid profiles (valine, leucine, methionine, phenylalanine, tyrosine, glycine, alanine, ornithine and citrulline) were analysed using LC–MS/MS, as previously described[24]. Furthermore, plasma 1- and 3-methylhistidine were analysed, according to Spackman *et al.*[25].

### Calculations

The apparent protein digestibility coefficients were calculated using the following formula[26]:

$$\text{Apparent protein digestibility} = ((\text{nutrient intake} - \text{nutrient excretion})/\text{nutrient intake}) \times 100\,\%.$$

### Statistical analysis

For all data, normality of distribution was examined using the Kolmogorov–Smirnov test prior to further analyses. In the pilot study, the mean hydrogen concentration of all cats on the same supplement was calculated at each time point and these normally distributed data were further analysed using repeated measures ANOVA (time as within-subject factor, treatment as between-subject factor). Furthermore, area under the curve (AUC) was calculated for each cat and treatment effects were evaluated using the Student's independent samples *t* test. All data from the main study were analysed in a univariate general linear model ANOVA for crossover designs to test the effects of period, treatment, group and cat nested in group. Day-to-day variability of the hydrogen measurements was tested using data from the main study, analysed in the same cats on consecutive days. The AUC was calculated for each cat for both days. Student's independent-samples *t* tests were performed on the calculated AUC in both periods. For all analyses, Superior Performing Software Systems version 19 (SPSS, Inc.) was used. Statistical significance was set at $P<0{\cdot}05$.

## Results

### Energy intake and body weight

The mean energy intake was compared for the last 2 weeks of each period, as sampling occured during these weeks. No treatment effect was found ($P=0{\cdot}25$). One cat had to be excluded from the study due to continuous food refusal and weight loss. All cats that were supplemented with guar gum lost weight, while only one group lost weight when the cellulose-supplemented diet was fed. As a result, treatment ($P<0{\cdot}01$) and group ($P<0{\cdot}01$) effects were observed in the mean weight difference between the start and the end of each period (guar gum −0·37 kg; cellulose: −0·01 kg).

### Faecal parameters

Faecal parameters are shown in Table 2. The faecal pH was lower in guar gum-supplemented cats compared to the cellulose-fed cats ($P<0{\cdot}01$). No treatment effect was seen on the faecal consistency score, despite the numerically lower score (softer faeces) for guar gum-supplemented cats. Total faecal production over the 5-d collection period and DM of the pooled faecal samples did not differ between both supplements ($P=0{\cdot}15$ and $P=0{\cdot}60$, respectively). Cellulose-supplemented cats tended to show higher faecal concentrations of acetic acid ($P=0{\cdot}07$), and with calculation of the ratio of faecal acetic acid:total SCFA, this treatment effect

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**Table 2.** Faecal characteristics and fermentation end products from nine cats fed a moderate protein diet supplemented with guar gum* or cellulose† in a 10-week crossover study

| | Supplement | | | | *P* | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | GG | SEM | Cell | SEM | Treat. | Period | Group | Cat × group |
| Faecal pH | 5·26 | 0·08 | 6·55 | 0·32 | <0·01 | 0·75 | 0·53 | 0·39 |
| Faecal consistency score‡ | 2·12 | 0·39 | 2·36 | 0·38 | 0·16 | 0·42 | 0·01 | 0·07 |
| Total faecal production (g/5 d) | 378·33 | 76·46 | 305·22 | 41·87 | 0·15 | 0·16 | 0·01 | 0·06 |
| Faecal DM (%) | 30·82 | 2·67 | 31·68 | 2·55 | 0·60 | 0·36 | 0·02 | 0·93 |
| Acetic acid (mg/l) | 547·57 | 55·78 | 794·56 | 112·70 | 0·07 | 0·85 | 0·54 | 0·27 |
| Propionic acid (mg/l) | 395·35 | 58·87 | 406·31 | 61·55 | 0·90 | 0·96 | 0·18 | 0·35 |
| Butyric acid (mg/l) | 633·61 | 131·46 | 609·81 | 156·23 | 0·69 | 0·71 | 0·50 | <0·01 |
| Isobutyric acid (mg/l) | 32·70 | 7·69 | 20·15 | 7·64 | 0·10 | 0·51 | 0·02 | 0·16 |
| Isovaleric acid (mg/l) | 89·83 | 17·11 | 58·09 | 13·84 | 0·04 | 0·37 | 0·07 | 0·06 |
| Valeric acid (mg/l) | 399·26 | 84·26 | 241·61 | 54·67 | 0·03 | 0·30 | 0·13 | 0·05 |
| Total SCFA (mg/l)§ | 2098·33 | 248·95 | 2130·54 | 331·79 | 0·95 | 0·61 | 0·20 | 0·04 |
| Faecal $NH_3$ (mg/l) | 390·83 | 45·41 | 226·65 | 37·86 | 0·01 | 0·15 | 0·81 | 0·20 |
| Indole (mg/l) | 0·36 | 0·13 | 0·06 | 0·02 | 0·02 | 0·22 | 0·14 | 0·39 |
| *p*-Cresol (mg/l) | 1·57 | 0·29 | 0·50 | 0·12 | 0·01 | 0·08 | 0·12 | 0·26 |
| Phenol (mg/l) | ND | | ND | | | | | |

GG, guar gum; Cell, cellulose; Treat., treatment; ND, parameter could not be determined (below detection limit).
* Vidogum GI, Unipektin Ingredients AG.
† Arbocel BWW 40, Rettenmaier und Söhne.
‡ Faecal consistency was scored on a five-point scale, as described by Hesta *et al.*[19].
§ Total SCFA = acetic + propionic + butyric + isobutyric + isovaleric + valeric acids.

reached statistical significance ($P=0·01$, data not shown). Faecal propionic acid ($P=0·90$), butyric acid ($P=0·69$) and total SCFA ($P=0·95$) concentrations did not differ between both fibre supplements. The faecal butyric acid:total SCFA ratio, however, tended to be higher for guar gum-supplemented cats ($P=0·05$). Faecal isovaleric acid ($P=0·04$), valeric acid ($P=0·03$), indole ($P=0·03$), *p*-cresol ($P=0·01$) and $NH_3$ ($P=0·01$) concentrations were higher in guar gum-supplemented cats. In addition, the percentage of faecal bacterial nitrogen tended to be higher ($P=0·08$) in cats fed the guar gum-supplemented diet (data not shown).

### *Apparent protein digestibility coefficients*

The mean apparent protein digestibility coefficients in guar gum- and cellulose-supplemented cats were 71·8 (SEM 3·6) and 79·7 (SEM 1·0) %, respectively, and tended to be lower in the guar gum-supplemented cats ($P=0·07$).

### *Hydrogen concentrations*

*Pilot study*. The hydrogen concentration over time curves revealed a clear maximal hydrogen concentration in only one cat. The variability between cats was very high, as seen in Fig. 2. Numerically, the guar gum-supplemented cats showed a higher mean hydrogen concentration at all time points. However, no significant time ($P=0·20$) or treatment ($P=0·20$) effect could be observed. In one cat, the sampling could only be performed hourly and this cat was therefore excluded from the statistical analysis of the pilot study data (guar gum *n* 5; cellulose *n* 4). No treatment effect on the AUC of the hydrogen concentration over time curves was seen ($P=0·20$).

*Main study*. The mean hydrogen concentration per supplementation group for the six measured time points is shown in Fig. 3. The mean hydrogen concentration of guar gum-supplemented cats over all measured time points (3·20 (SEM 1·05) ppm) was numerically higher than for cats supplemented with cellulose (2·11 (SEM 0·47) ppm). This difference, however, was not statistically significant ($P=0·325$) and the variation between cats was very high. In both periods, no significant differences were found between AUC of all cats for two consecutive days ($P=0·18$ for period 1 and $P=0·72$ for period 2; data not shown).

### *Plasma metabolites*

Plasma metabolites are shown in Table 3. No treatment effects were found for 1- and 3-methylhistidine, free carnitine, acetyl-, propionyl-, butyryl- + isobutyryl-, methylmalonyl-, 3-OH-3-methylglutaryl-, tiglyl- + 3-methylcrotonyl- and 3-OH-isovalerylcarnitine. However, guar gum-supplemented cats showed higher 3-OH-butyrylcarnitine concentrations in plasma compared to cellulose-supplemented cats ($P=0·02$). For isovaleryl- + 2-methylbutyrylcarnitine, a trend towards higher plasma concentrations was observed when cats were supplemented with guar gum ($P=0·09$). Plasma leucine concentrations were lower ($P=0·02$), and plasma valine ($P=0·08$) and phenylalanine ($P=0·06$) concentrations tended to be lower in guar gum-supplemented cats. On the contrary, guar gum-supplemented cats showed higher plasma alanine concentrations ($P=0·02$). For ornithine, citrulline, methionine, tyrosine and glycine, as well as the ratios methylmalonylcarnitine:valine, propionylcarnitine:methionine and HMG:leucine, no treatment effects were observed (data not shown).

## Discussion

The present study was set up to investigate large-intestinal guar gum fermentation in domestic cats, with the emphasis on the amino acid-sparing potential of propionic acid. This mechanism was proposed by Verbrugghe *et al.*[6–8]

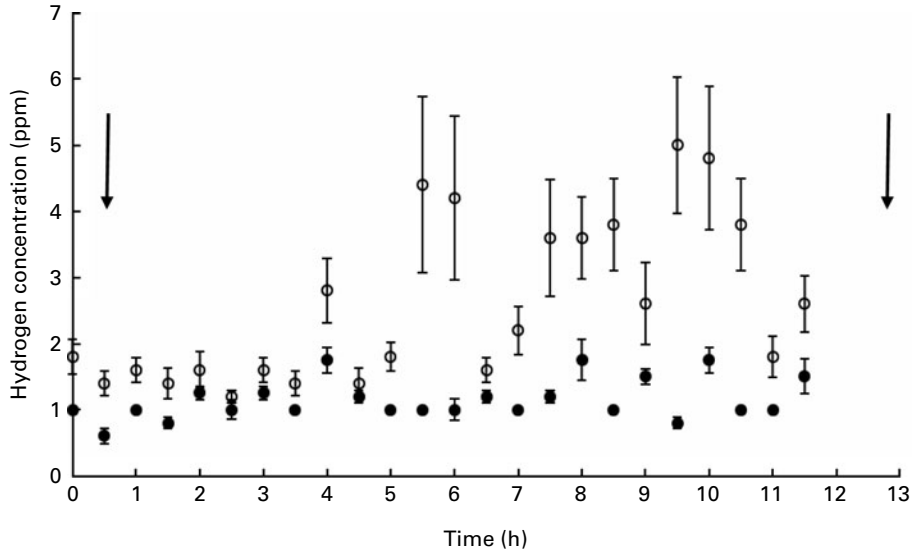


**Fig. 2.** Mean hydrogen concentration with their standard errors over time for nine cats fed a moderate-protein diet supplemented with guar gum (Vidogum GI, Unipektin Ingredients AG; ○, *n* 5) or cellulose (Vidogum GI, Unipektin Ingredients AG; ●, *n* 4) in the pilot experiment. The arrows indicate times of meal consumption. ppm, Parts per million.

upon supplementation of fructans in a commercially available canned cat food. To test this hypothesis, guar gum was supplemented to the experimental diet, as *in vitro* fermentation of this fibre source demonstrated high propionic acid concentrations[4,5]. After 24 h of *in vitro* incubation with faecal inocula from donor cats fed different diets, guar gum yielded approximately 1 mmol extra propionate per g of incubated organic matter compared to fructan fermentations[5]. *In vitro* data should be extrapolated very carefully to *in vivo* situations. Guar gum is completely indigestible by small-intestinal enzymes[3]. In healthy adult cats, small-intestinal transit time is on average 2 to 3 h[27], whereas total colon transit time is between 22 and 25 h[28], depending on the measurement technique and diet of the cats. Therefore, a theoretical extra propionic acid production of 1 mmol/g of organic matter of guar gum might also be expected *in vivo*, within the large intestine. As concentrations of propionic acid in portal blood are assumed to be 1/1000th of the concentrations present in the colon and 80 % of the portal blood concentration of propionic acid is metabolised in the liver[29], every gram of organic matter of guar gum reaching the large intestine might provide $8 \times 10^{-4}$ mmol of propionic acid to the gluconeogenic process. The present experiment aimed to confirm the amino acid-sparing hypothesis; however, further research for exact quantification of the amino acid-sparing potential of guar gum and propionic acid is still warranted.

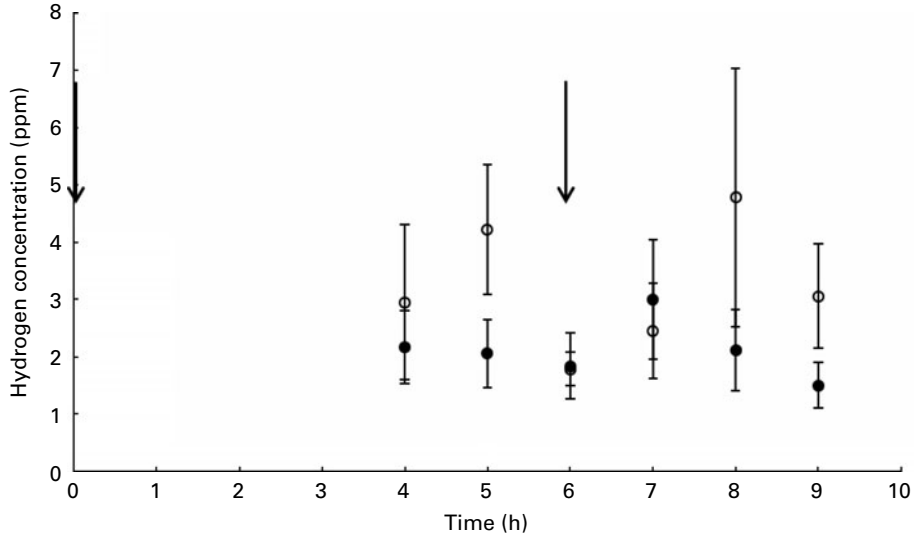


**Fig. 3.** Mean hydrogen concentrations with their standard errors at six time points measured in cats fed a moderate-protein diet supplemented with guar gum (Vidogum GI, Unipektin Ingredients AG; ○, *n* 9) or cellulose (Arbocel BWW 40, Rettenmaier und Söhne; ●, *n* 9) in a 10-week crossover study. The arrows indicate two out of four meal consumption times. ppm, Parts per million.

**Table 3.** Fermentation end product metabolites in plasma from nine cats fed a moderate protein diet supplemented with guar gum* or cellulose† in a 10-week crossover study

| | Supplement | | | | *P* | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | GG | SEM | Cell | SEM | Treat. | Period | Group | Cat × group |
| Plasma amino acid profile (μmol/l) | | | | | | | | |
| Val | 24·96 | 1·80 | 27·83 | 3·02 | 0·08 | 0·39 | 0·28 | 0·05 |
| Leu | 328·45 | 21·87 | 389·29 | 27·34 | 0·02 | 0·29 | 0·15 | 0·12 |
| Met | 54·74 | 5·76 | 64·20 | 5·84 | 0·11 | 0·41 | 0·48 | 0·03 |
| Phe | 61·55 | 3·54 | 71·14 | 2·51 | 0·06 | 0·41 | 0·62 | 0·28 |
| Tyr | 44·29 | 3·55 | 45·27 | 2·45 | 0·82 | 0·57 | 0·05 | 0·23 |
| Orn | 49·40 | 7·53 | 46·33 | 3·45 | 0·67 | 0·98 | 0·67 | 0·14 |
| Cit | 34·44 | 4·61 | 40·91 | 3·41 | 0·23 | 0·89 | 0·12 | 0·31 |
| Gly | 421·34 | 35·24 | 433·85 | 26·56 | 0·71 | 0·97 | 0·72 | 0·07 |
| Ala | 840·67 | 74·04 | 642·38 | 64·55 | 0·02 | 0·73 | 0·63 | 0·05 |
| Plasma acylcarnitine profile (μmol/l) | | | | | | | | |
| Free carnitine | 23·07 | 1·58 | 18·84 | 2·06 | 0·14 | 0·04 | 0·20 | 0·48 |
| Acetylcarnitine | 4·02 | 0·23 | 4·07 | 0·19 | 0·78 | 0·20 | 0·98 | 0·97 |
| Propionylcarnitine | 0·44 | 0·08 | 0·26 | 0·04 | 0·19 | 0·53 | 0·51 | 0·98 |
| Butyryl+isobutyrylcarnitine | 0·37 | 0·04 | 0·37 | 0·03 | 0·11 | 0·97 | 0·80 | 0·21 |
| Methylmalonylcarnitine | 0·09 | 0·01 | 0·08 | 0·01 | 0·63 | 0·19 | 0·21 | 0·68 |
| 3-OH-3-methylglutarylcarnitine | 0·01 | 0·00 | 0·02 | 0·00 | 0·21 | 0·59 | 0·78 | 0·70 |
| Isovaleryl + 2-methylbutyrylcarnitine | 0·32 | 0·06 | 0·21 | 0·05 | 0·09 | 0·18 | 0·80 | 0·11 |
| 3-OH-isovalerylcarnitine | 0·15 | 0·02 | 0·12 | 0·02 | 0·11 | 0·53 | 0·17 | 0·26 |
| 3-OH-butyrylcarnitine | 0·07 | 0·01 | 0·04 | 0·01 | 0·02 | 0·67 | 0·68 | 0·69 |
| Tiglyl + 3-methyl-crotonylcarnitine | 0·10 | 0·02 | 0·10 | 0·01 | 0·59 | 0·08 | 0·25 | 0·31 |
| 3-Methylhistidine | 11·44 | 1·29 | 10·26 | 1·21 | 0·20 | 0·17 | 0·58 | <0·01 |
| 1-Methylhistidine | 13·06 | 1·55 | 12·43 | 1·73 | 0·27 | 0·84 | 0·44 | <0·01 |

GG, guar gum; Cell, cellulose; Treat., treatment; +, components cannot be separated.
* Vidogum GI, Unipektin Ingredients AG.
† Arbocel BWW 40, Rettenmaier und Söhne.

Due to the low propionic acid concentrations in peripheral blood, analysing this end product is only possible in portal blood. Likewise, measuring butyric acid in peripheral blood is impossible due to its nearly complete absorption and metabolism in colonocytes[30]. To circumvent difficult and invasive sampling techniques for measuring these SCFA, their respective carnitine esters can be analysed in plasma from the peripheral blood. Plasma acylcarnitine concentrations are representative of the mitochondrial acyl-CoA pool, and as a consequence, these parameters reflect metabolites available for the citric acid cycle[7,31]. Additionally, some of the analysed plasma acylcarnitines represent metabolites of the catabolic pathway of, especially branched-chain, amino acids[32]. Large-intestinal and hepatic carbohydrate and amino acid metabolism can thus be assessed by studying such metabolites.

A potential problem arising with the use of guar gum *in vivo* is its high viscosity[3]. In previous *in vitro* work[5] and in the study described here, highly viscous guar gum was used. As the apparent protein digestibility tended to be lower in guar gum-supplemented cats, the gelling properties of the guar gum supplement have impaired protein digestion and absorption in the small intestine. Although plasma amino acid concentrations are not a direct measure of amino acid absorption, a significantly lower concentration of leucine and a trend towards lower valine and phenylalanine concentrations in plasma of guar gum-supplemented cats suggest decreased protein digestion and amino acid absorption from the small intestine. A higher concentration of L-alanine in plasma of guar gum-supplemented cats might indicate a higher amino acid turnover, as alanine is an important transport metabolite for amino groups in animals[32].

Thus, a larger load of undigested protein and unabsorbed amino acids could have reached the large intestine in guar gum-supplemented cats, as a result of which more protein fermentation has occurred. This hypothesis was supported by higher concentrations of isovaleric and valeric acids, $NH_3$, indole and *p*-cresol in faecal samples of guar gum-supplemented cats. In addition, guar gum might have stimulated microbial protein synthesis and turnover, as a tendency towards a higher percentage of bacterial nitrogen was observed in cats fed the guar gum-supplemented diet. In addition, in the plasma of these cats, a trend towards higher concentrations of isovaleryl- + 2-methyl-butyrylcarnitine was noted, which in turn suggests a higher microbial leucine and isoleucine degradation in the large intestine[32]. Besides isovaleric, isobutyric and valeric acids, $NH_3$ and phenolic compounds, bacterial degradation of amino acids generally produces acetic and butyric acids, in proportions depending on the bacterial species composing the microbiota[33]. The extensive increase in protein fermentation in the large intestine could explain the absence of treatment effects on plasma propionylcarnitine and faecal propionic acid concentrations. These findings again highlight the difficulties of extrapolating *in vitro* data to *in vivo* situations. As plasma propionylcarnitine concentrations showed no treatment effect, the absorbed propionic acid concentrations were assumed to be similar for guar gum- and cellulose-supplemented cats. Therefore, no

differences in hepatic amino acid and carbohydrate metabolism between treatments were expected. The conclusion was supported by the absence of treatment differences for plasma 1- and 3-methylhistidine, as both parameters were analysed in the present study as markers of endogenous protein catabolism[34,35].

An unexpected finding in the present experiment was a significantly higher concentration of 3-OH-butyrylcarnitine in plasma of guar gum-supplemented cats. In colonocytes, butyric acid can be converted to the ketone body 3-OH-butyryl-CoA[36], which can be measured in plasma as the carnitine ester 3-OH-butyrylcarnitine. As the ratio of faecal butyric acid: total SCFA was higher in guar gum-supplemented cats, a higher butyric acid production upon microbial fermentation of guar gum is suspected, explaining the treatment difference on 3-OH-butyrylcarnitine. This metabolite is partly converted to acetyl-CoA through the process of β-oxidation[36] , and partially absorbed into the bloodstream[37]. Likewise, acetyl-CoA can be absorbed from colonocytes into the bloodstream[37]. Acetyl-CoA is involved in many other metabolic pathways and is challenging to trace within the body. A concomitant increase in HMG was expected, as its CoA is a metabolite in the β-oxidation pathway[36]. Nonetheless, no differences in plasma HMG concentrations due to treatment could be observed, which might be due to the absorption of 3-OH-butyryl-CoA into the bloodstream and the conversion to acetoacetyl-CoA in the liver. Acetoacetyl-CoA can be converted in the liver to acetoacetate through pathways that do not involve the production of HMG[37].

Besides studying the end product profile, fermentation kinetics of the fibre supplements were addressed using measurements of hydrogen concentrations in the exhaled breath. Reproducibility of the measurements was considered to be sufficient as no significant differences were found between the AUC calculated from measurements on two consecutive days for all cats, as has been shown in dogs[38].

The main aim of the pilot experiment was to establish the time point at which maximal fermentation occurred in each cat, which was defined as the maximal expired hydrogen concentration. An estimation of the orocaecal transit time of the feed on both supplements could then also be made. However, this maximum concentration could only be clearly determined for one cat in the measurement period of 11·5 h. The experimental protocol was changed following the pilot study, and the cats were fed four isoenergetic meals per d in the actual study starting from day 18 of each period. As such, a more consistent delivery of the fibre supplements to the gut microbiota was intended, and a constant fermentation of the guar gum supplement during the day was expected. However, data of the actual experiment did not reveal a significant treatment effect. Different hypotheses can be proposed for the absence of the expected postprandial rise of exhaled hydrogen concentrations in guar gum-supplemented cats in the pilot study and the absence of treatment effects in the actual experiment. One possible explanation is the increase in protein fermentation, as these processes are reported not to produce hydrogen[39]. Secondly, the extreme hydrogen-binding activity of guar gum, because of a large amount of hydroxyl groups in its chemical structure, might also explain the absence of a treatment effect[1]. Furthermore, guar gum slows down small-intestinal transit due to its viscous nature in pigs[40], humans[41] and dogs[42]. In these experiments, the delay in small-intestinal transit time did not consist of several hours, so it cannot explain the absence of a hydrogen concentration increase during 11·5 h postprandially.

## Conclusions

Guar gum fermentation did not evoke an increase in faecal propionic acid and plasma propionylcarnitine concentrations, suggesting a similar large-intestinal propionic acid production and absorption in guar gum- and cellulose-supplemented cats. The physicochemical properties of the gum have impaired the protein digestion and amino acid absorption within the small intestine, hence amino acid utilisation in cats' metabolism. Therefore, viscosity counteracts the amino acid-sparing potential of dietary fibre sources. Further experiments with partially hydrolysed guar gum of lower viscosity may be useful to potentiate the desirable *in vivo* side effects of this fibre supplement.

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