Dietary supplementation of propionylated starch to domestic cats provides propionic acid as gluconeogenic substrate potentially sparing the amino acid valine

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Abstract
In strict carnivorous domestic cats, a metabolic competition arises between the need to use amino acids for gluconeogenesis and for protein synthesis both in health and disease. The present study investigated the amino acid-sparing potential of propionic acid in cats using dietary propionylated starch (HAMSP) supplementation. A total of thirty cats were fed a homemade diet, supplemented with either HAMSP, acetylated starch (HAMSA) or celite (Control) for three adaptation weeks. Propionylated starch was hypothesised to provide propionic acid as an alternative gluconeogenic substrate to amino acids, whereas acetic acid from HAMSA would not provide any gluconeogenic benefit. Post-adaptation, a 5-d total faecal collection was carried out to calculate apparent protein digestibility coefficients. Fresh faecal and blood samples were collected to analyse fermentation endproducts and metabolites. The apparent protein digestibility coefficients did not differ between supplements ($P=0.372$) and were not affected by the protein intake level ($P=0.808$). Faecal propionic acid concentrations were higher in HAMSP than in HAMSA ($P=0.018$) and Control ($P=0.003$) groups, whereas concentrations of ammonia ($P=0.007$) were higher in HAMSA than in HAMSP cats. Tendencies for or higher propionylcarnitine concentrations were observed in HAMSP compared with HAMSA ($P=0.090$) and Control ($P=0.037$) groups, and for tiglyl + 3-methylcrotonylcarnitine concentrations in HAMSP as compared with Control ($P=0.028$) cats. Methylmalonylcarnitine concentrations did not differ between groups ($P=0.740$), but were negatively correlated with the protein intake level ($r=-0.459$, $P=0.016$). These results suggest that HAMSP cats showed more saccharolytic fermentation patterns than those supplemented with HAMSA, as well as signs of sparing of valine in cats with a sufficient protein intake.

Key words: Acylated starch; Domestic cats; Fermentation; Gluconeogenesis; Propionic acid

Intestinal microbial fermentation and the consequent production of metabolites, such as SCFA, are considered to be beneficial for most animals, even in a strict carnivorous species like the domestic cat. In particular, the amino acid-sparing potential of fermentation-derived propionic acid, hypothesised by Verbrugghe et al., may be advantageous for cats in both health and disease conditions. Propionic acid, produced upon intestinal microbial fermentation of

Abbreviations: DS, degree of substitution; HAMSA, acetylated high-amylose maize starch; HAMSP, propionylated high-amylose maize starch.

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carbohydrates, can be used as an alternative gluconeogenic substrate in domestic cats\(^{(6)}\), potentially reducing the demand on amino acids for gluconeogenesis. Guar gum fermentation has been shown to produce high concentrations of propionic acid upon incubation with faecal inoculum from cats fed two diets, contrasting in both protein and fibre concentrations and sources\(^{(7)}\). The high viscosity or the small-intestinal fermentation of this soluble fibre supplement, however, appeared to have impaired the assessment of the amino acid-sparing potential of propionic acid\(^{(9)}\). Therefore, another approach for supplying the liver with fermentation-derived propionic acid was searched for.

Acylated starches are comprised of either low- or high-amylose maize starch that has been esterified with acetic, propionic or butyric acid to a high degree of substitution (DS) (DS between 0·2 and 0·3\(^{(9)}\), where DS is defined as the number of hydroxyl groups on each D-glucopyranosyl unit derivatised by substituent groups\(^{(10,11)}\). These modified starches are classified as resistant starch type 4, as they are only partially digestible in the small intestine of rats\(^{(12-14)}\) and humans\(^{(16)}\). In the large intestine, however, the ester bond can be cleaved by bacterial enzymes, releasing the coupled SCFA. The residual starch carrier is then available for fermentation by the intestinal microbiota as well, leading to further production of SCFA\(^{(9,16)}\). While acylated starches have never been used in the intestinal microbiota as well, leading to further production of SCFA, residual starch carrier is then available for fermentation by the intestinal microbiota as well, leading to further production of SCFA\(^{(9,16)}\).

For supplying the liver with fermentation-derived propionic acid, they were weighed weekly to enable adjustments of the food amounts until amounts needed to maintain stable body weight were achieved. At all times, cats had ad libitum access to tap drinking water provided by automatic drinking fountains and refreshed daily. The cats were group-housed between meals with a maximum of ten cats per group (randomised for housing, not housed per treatment group). The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2012/06), and was in accordance with institutional and national guidelines for the care and use of laboratory animals.

A homemade diet was formulated, and the ingredient composition and the analysed nutrient content are depicted in Table 1. The homemade diet consisted of cooked (boiled in water for 10 min) chicken breast meat (without skin, bones and visible fat) as the protein source and white rice (steam cooked separately for 10 min) as the main carbohydrate source. After cooking, the chicken and rice were thoroughly ground and mixed. Then, the mixture was divided into approximate daily portions pooled for all cats, and frozen at \(-20^\circ\)C. Every day one pooled portion was transferred to 4°C to be defrosted gradually over a 2-d period. The day before feeding, the pooled portion was accurately weighed and subdivided into individual portions. In total, diets were kept 3 d at 4°C until being fed to the animals. Before feeding, the portions were allowed to warm to room temperature and blended with rapeseed oil (8·1 % of total food amount (TFA); Vandemoortele koolzaadolie; Vandemoortele Lipids). They were weighed weekly to enable adjustments of the food amounts until amounts needed to maintain stable body weight were achieved. At all times, cats had ad libitum access to tap drinking water provided by automatic drinking fountains and refreshed daily. The cats were group-housed between meals with a maximum of ten cats per group (randomised for housing, not housed per treatment group). The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2012/06), and was in accordance with institutional and national guidelines for the care and use of laboratory animals.

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### Materials and methods

#### Animals

A total of thirty healthy adult domestic shorthair cats (fifteen female and fifteen male), with a mean body weight and age of 4·0 (sd = 0·9) kg and 5·6 (sd = 3·0) years, respectively, were included in the present study. All cats were castrated, except for five females that remained intact. Before inclusion in the study, the cats were declared healthy based on a thorough physical examination and complete blood count and serum biochemistry analyses. The cats were divided into three groups (two treatment groups and one control group), consisting of ten cats each (n 10), considering equal distribution of age, body weight, body condition score\(^{(16)}\), BMI\(^{(17)}\) and neuter state.

#### Experimental design and diet

All cats were fed on the same homemade diet (see below) to fulfill maintenance energy requirements (418·4 kJ/kg\(^{-0.67}\); National Research Council\(^{(18)}\)) during a 3-week adaptation period with two isenergetic meals per d in individual housing. They were weighed weekly to enable adjustments of the food amounts until amounts needed to maintain stable body weight were achieved. At all times, cats had ad libitum access to tap drinking water provided by automatic drinking fountains and refreshed daily. The cats were group-housed between meals with a maximum of ten cats per group (randomised for housing, not housed per treatment group). The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2012/06), and was in accordance with institutional and national guidelines for the care and use of laboratory animals.

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faecal SCFA and ammonia (NH₃) were performed as previously described(13–30) or acetylated (DS 0–23) Hylon VII high-amylose maize starch (HAMSP and HAMSA, respectively), both prepared by the National Starch & Chemical Company. Since in general acetic acid is not a gluconeogenic substrate(19), the HAMSA supplement was used as a negative control for gluconeogenic amino acid sparing, when compared with HAMSP, which is hypothesised to provide the liver with additional gluconeogenic propionic acid as compared with the baseline propionic acid yield from fermentation of the residual starch carrier in both HAMSP and HAMSA. The control supplement was celite (Celite 545; VWR International), which consists of non-digestible and non-fermentable ash(20).

**Sampling**

After an overnight fast (12 h), preprandial blood samples were aseptically drawn from the jugular vein before the study (general blood work for inclusion of the cats in the study, see above) and on the first day of the collection period (week 4). Immediately after collection, blood samples were placed into Vacutainer® tubes containing lithium heparin or serum clot activator, and serum and plasma were separated –sonically vibrated, rotated(4), then centrifuged for 10 min at 13 300 x g, a subsample of 60 µl was combined with 140 µl of water. A quantity of 10 µl of this dilution was injected on a liquid chromatography system consisting of a Thermo Fisher Scientific Accela U-HPLC pumping device, coupled with an Accela Autosampler and Degasser. Chromatographic separation was achieved on an HSS-C18 column (1.8 µm; 50 mm × 2.1 mm) (Waters), kept at 40°C. The mobile phase, consisting of 50 mM-ammonium acetate and acetonitrile, was pumped isocratic (i.e. the same proportions of solvents are used throughout the entire run instead of using a gradient) at a flow rate of 0.3 ml/min for 10 min. Detection was performed on a photodiode array (PDA) detector (Thermo Fisher Scientific) at 270 nm. Remaining 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 and Company KG), and proximate analyses as well as analyses of bacterial N were performed as previously described(8). Plasma acylcarnitine and amino acid profiles, 1- and 3-methylhistidine were analysed as previously described(8). Serum urea, creatinine, total protein and plasma creatine kinase were analysed spectrophotometrically (ARCHITECT c Systems and the AEROSET System; Abbott Products sa/nv) using commercial kits (Urea N, Creatinine, Total protein, and Creatine kinase kit; Abbott Products sa/nv). The DS of the acylated starches was determined by using 13C-NMR spectroscopy (DRX-500 spectrometer; Bruker), by using the resolution of the six glucose carbons as assigned by Dais & Perlin(22).

**Calculations**

Apparent protein digestibility coefficients were calculated based on dietary nutrient intake and faecal nutrient excretion based on total faecal collection(8). Energy, crude protein, crude fat and amino acid intake were calculated per cat per kg metabolic weight per d. In the plasma acylcarnitine profile, the ratio of the concentrations of methylmalonyl- and propionylcarnitine was calculated.

**Statistical analysis**

For all statistical analyses, SPSS version 21 (IBM) was used. Statistical significance was set at $P<0.05$. Before further analyses of all data, normality was examined using the Kolmogorov–Smirnov test on standardised residuals ($P>0.01$). Homogeneity of variances was tested by means of the Levene’s test for equality of error variances. If the significance of the latter test was below 0.05, a logarithmic transformation of the data was done, which resolved the variance heterogeneity in most cases (faecal propionic acid and acid excretions and concentrations, methylmalonylcarnitine:propionylcarnitine ratio, plasma creatine kinase). If this transformation did not restore the homogeneity of variance, the data were analysed non-parametrically (see below; apparent protein digestibility, 3-hydroxy (OH) 3-methylglutaryl carnitine, 3-OH isovaleryl- + 2-methyl-3-OH butyrylcarnitine). Outliers in the normally distributed data were detected if the standardised values (Z scores) exceeded a value of $(n – 1)/\sqrt{n}$(23).
All normally distributed data were analysed using a univariate ANOVA to test the effects of supplement (HAMSP, HAMSA, Control) with the protein intake level as a covariate. This covariate was included in the model to correct for the fact that more than half of the cats had a protein intake below the recommended minimal requirements (3.97 g/kg\(^{0.67}\); see National Research Council\(^{(18)}\)), which might bias the results. The differences between treatments were unravelled using Fisher’s least significant difference (LSD) post hoc test. If the covariate protein intake level was significant for a specific parameter, Pearson product moment correlation coefficients were calculated between the protein intake level and the respective parameter. Data that were not normally distributed (faecal pH) were analysed non-parametrically by means of Kruskal–Wallis tests for independent samples, again with supplement as a factor. As far as protein intake level, non-normally distributed data were divided into two categories: protein intake below (category 1 = PIB) and above (category 2 = PLA) the recommended minimal requirement (3.97 g/kg\(^{0.67}\)/d; see National Research Council\(^{(18)}\)). This grouping was done irrespective of treatment and resulted in five out of ten cats with PIB for HAMSP, six out of eight for HAMSA and six out of nine for Control (exclusion of three cats; see Results section). This categorical variable was also used as a factor in Kruskal–Wallis tests for independent samples. Dunn’s post hoc tests were performed to determine treatment differences. A single-ordered contingency table was used with faecal scores as the ordered columns, while supplement or protein intake levels were the unordered rows. The differences between supplements and the effects of protein intake levels were detected using an exact Kruskal–Wallis test with a \(\chi^2\) test of association.

**Results**

Of the study animals, one cat from the HAMSA group was excluded during the study due to medical problems unrelated to the experiment. Data obtained from two other cats (one from the HAMSA group, one from the Control group) were excluded from the statistical analyses due to post-experimental death of the cats unrelated to the experiment. Multiple outliers were noticed in the datasets of the latter cats, which may be explained by the underlying medical problems.

**Nutrient intake, body weight and apparent protein digestibility coefficients**

Data are presented in Table 2. Daily energy, crude protein and crude fat intakes were below the offered amounts for most of the cats, but did not differ between supplements (\(P = 0.744, 0.148\) and \(0.805\), respectively). Logically, energy and crude fat intakes were positively correlated with protein intake (\(r = 0.900\) and \(0.800\), respectively; \(P < 0.001\) for both parameters). All cats, with the exception of two neutered males, lost weight during the experiment (overall mean of body-weight change over 4-week experiment: \(0.28\) (SEM 0.04) kg; range \(-0.1\) to \(0.75\) kg), but no effect of supplement was seen (\(P = 0.754\)). On the contrary, this body-weight difference was negatively correlated with protein intake (\(r = -0.842\); \(P = 0.000\)). The apparent protein digestibility coefficients were high in all cats, did not differ between supplements (\(P = 0.372\)) and were not affected by protein intake level (\(P = 0.808\)).

**Faecal parameters**

Faecal parameters are shown in Table 3. Faecal pH differed among supplements (\(P = 0.031\)), with post hoc tests revealing significantly lower values for HAMSP as compared with HAMSA (\(P = 0.043\)) and independent of protein intake level (\(P = 0.228\)). Overall, faecal consistency scores indicated rather wet and soft faeces, combined with a low total faecal DM % for all three supplements. For the faecal consistency score, again, no differences between supplements (\(P = 0.122\)) were observed, even when corrected for protein intake level (\(P = 0.473\)). However, when faecal consistency scores were grouped into three categories (too hard, normal, too soft; data not shown), HAMSP demonstrated a higher incidence of too-soft faeces as compared with HAMSA (\(P = 0.031\)), independent of protein intake level (\(P = 0.473\)). Faecal DM % correlated negatively with protein intake (\(r = -0.464\); \(P = 0.015\)), whereas total faecal production over 5 d correlated positively with protein intake (\(r = 0.591\); \(P = 0.001\)).

**Table 2. Nutrient intake, body weight loss and apparent protein digestibility coefficients from a feline study on the amino acid-sparing potential of propionic acid**

(Mean values, pooled standard errors, statistical significance and Pearson correlations with protein intake level (PI))

| Parameter                        | Supplement       | P<sup>||</sup> | Pearson correlation |
|----------------------------------|------------------|---------------|---------------------|
|                                  | HAMSP*           | HAMSA†        | Control‡            | Pooled SEM§        |                      |                     |
| Energy intake (kJ/kg<sup>0.67</sup>/d) | 319.2            | 251.5         | 270.9               | 14.7               | 0.744               | < 0.001             | 0.900               | < 0.001             |
| Crude protein intake (g/kg<sup>0.67</sup>/d) | 5.0              | 3.9           | 4.4                 | 0.2                | 0.148               | < 0.001             | 1.000               | < 0.001             |
| Crude fat intake (g/kg<sup>0.67</sup>/d)   | 3.6              | 2.8           | 3.1                 | 0.2                | 0.805               | < 0.001             | 0.800               | < 0.001             |
| Body weight loss (kg/4 weeks)     | 0.2              | 0.4           | 0.3                 | 0.0                | 0.754               | < 0.001             | -0.842              | < 0.001             |
| Apparent protein digestibility (%) | 87.1             | 91.3          | 89.3                | 1.1                | 0.372               | 0.808               | NP                  | NP                  |

HAMSP, propionylated high-amylose maize starch; HAMSA, acetylated high-amylose maize starch; NP, analysed non-parametrically, hence no correlation was calculated.

* HAMSP (n:10): Propionylated Hylon VII high-amylose maize starch (National Starch & Chemical Company).
† HAMSA (n:8): Acetylated Hylon VII high-amylose maize starch (National Starch & Chemical Company).
‡ Control (n:9): Celite 545 (VWR International).
§ Standard error of the mean of data grouped over all supplements.
|| Statistical significance is set at \(P < 0.05\).
the latter positive correlation, the faecal fermentation endproducts were expressed as absolute excretions over 5 d, instead of concentrations in the fresh faecal samples. Faecal acetic, propionic, butyric, valeric, isobutyric and isovaleric acid, and ammonia excretions were positively correlated with protein intake level (for P values, see Table 3). No effects of supplement (P = 0.690) or protein intake (P = 0.176) were seen on faecal bacterial N excretion when expressed in percentage of the total N excretion or of supplement when expressed in concentrations (data not shown; P = 0.214).

**Serum and plasma parameters**

Data are shown in Table 4. Among plasma amino acid profiles, no significant differences between supplements were seen even when corrected for protein intake level (for r and P values, see Table 4). Among plasma acylcarnitine profiles, tendencies for higher propionylcarnitine concentrations (overall P = 0.086) were observed in HAMSP as compared with HAMSA (P = 0.090) and Control (P = 0.037), while trends for a lower methylmalonylpropionylcarnitine ratio (overall P = 0.089) were found for HAMSP as compared with HAMSA (P = 0.058) and Control (P = 0.054). Additionally, trends for higher tiglyl-3-methylcrotonylcarnitine concentrations (overall P = 0.067) were seen in the plasma of HAMSP (P = 0.028) as compared with Control cats. All above-mentioned observations were independent of protein intake (for r and P values, see Table 4).
### Table 4. Plasma and serum parameters of a feline study on the amino acid-sparing potential of propionic acid
(Mean values, pooled standard errors, statistical significance and Pearson correlations with protein intake level (PI))

| Parameter                              | Supplement | Control | P | Pearson correlation |
|----------------------------------------|------------|---------|---|---------------------|
|                                        | HAMSP*     | HAMSA†  |   |                     |
|                                        |            |         |   |                     |
| Valine (μmol/l)                         | 133.44     | 133.03  | 138.93 | 4.65 | 0.793 | 0.260 | NS     | NS     |
| Leucine (μmol/l)                        | 134.16     | 146.11  | 139.66 | 4.64 | 0.913 | 0.160 | NS     | NS     |
| Methionine (μmol/l)                     | 42.60      | 41.42   | 44.21  | 1.31 | 0.725 | 0.885 | NS     | NS     |
| Phenylalanine (μmol/l)                  | 54.85      | 52.91   | 51.96  | 1.48 | 0.390 | 0.083 | NS     | –0.275 0.165 |
| Tyrosine (μmol/l)                       | 37.59      | 41.79   | 40.27  | 1.66 | 0.937 | 0.177 | NS     | NS     |
| Ornithine (μmol/l)                      | 24.60      | 24.05   | 22.61  | 0.97 | 0.683 | 0.764 | NS     | NS     |
| Citrulline (μmol/l)                     | 33.40      | 37.14   | 29.06  | 1.64 | 0.164 | 0.147 | NS     | NS     |
| Glycine (μmol/l)                        | 345.31     | 355.20  | 349.28 | 9.67 | 0.977 | 0.149 | NS     | NS     |
| Alanine (μmol/l)                        | 699.37     | 630.26  | 652.30 | 21.45 | 0.372 | 0.594 | NS     | NS     |
| Plasma free carnitine (μmol/l)          | 27.92      | 29.44   | 28.43  | 2.47 | 0.984 | 0.327 | NS     | NS     |
| Acetylcarnitine (μmol/l)                | 7.96       | 7.36    | 7.15   | 0.58 | 0.432 | 0.048 | NS     | –0.329 0.093 |
| Propionylcarnitine (μmol/l)             | 0.27       | 0.20    | 0.17   | 0.02 | 0.086 | 0.268 | NS     | NS     |
| Butyryl-+isobutyrylcarnitine (μmol/l)   | 0.22       | 0.24    | 0.22   | 0.02 | 0.955 | 0.539 | NS     | NS     |
| Methylmalonylcarnitine (μmol/l)         | 0.06       | 0.06    | 0.05   | 0.00 | 0.740 | 0.028 | NS     | –0.459 0.016 |
| Methylmalonylpropionylcarnitine (μmol/l)| 0.24       | 0.40    | 0.36   | 0.03 | 0.089 | 0.749 | NS     | NS     |
| 3-OH 3-Ch3 glutaryl carnitine (μmol/l)   | 0.01       | 0.01    | 0.01   | 0.00 | 0.817 | 0.306 | NP     | NP     |
| Isovaleryl-+2-Ch3 butyrylcarnitine (μmol/l)| 0.23     | 0.19    | 0.23   | 0.07 | 0.479 | 0.149 | NS     | NS     |
| 3-OH isovaleryl-+2-Ch3:3-OH butyrylcarnitine (μmol/l)| 0.10   | 0.11    | 0.08   | 0.05 | 0.738 | 0.103 | NP     | NP     |
| 3-OH butyrylcarnitine (μmol/l)          | 0.06       | 0.06    | 0.08   | 0.02 | 0.546 | 0.010 | NP     | –0.502 0.008 |
| Tiglyl-+3-Ch3 crotonylcarnitine (μmol/l)| 0.05       | 0.05    | 0.03   | 0.00 | 0.067 | 0.130 | NS     | NS     |
| Serum creatine kinase (U/l)             | 314.70     | 189.75  | 330.00 | 47.06 | 0.666 | 0.506 | NS     | NS     |
| Plasma 3-methylhistidine (μmol/l)       | 27.34      | 29.85   | 29.54  | 1.82 | 0.947 | 0.517 | NS     | NS     |
| Serum creatinine (mg/l)                 | 25.71      | 24.76   | 27.75  | 1.52 | 0.743 | 0.507 | NS     | NS     |
| Serum creatinine (mg/l)                 | 18.1       | 18.3    | 17.9   | 0.7  | 0.963 | 0.747 | NS     | NS     |
| Serum urea (mg/l)                       | 390.0      | 452.5   | 414.4  | 13.2 | 0.459 | 0.085 | NS     | –0.442 0.021 |
| Serum total protein (g/l)               | 77.5       | 76.0    | 77.2   | 1.5  | 0.828 | 0.020 | 0.453 | 0.018 |

HAMS, propionylated high-amyllose maize starch; HAMSA, acetylated high-amyllose maize starch; NS, P value for protein intake > 0.1; OH, hydroxyl; CH3, methyl; NP, analysed non-parametrically, hence no correlation was calculated.

* Mean values within a row with unlike superscript letters were significantly different (P < 0.05).
† HAMSP (n = 10): Propionylated Hylan VII high-amyllose maize starch (National Starch & Chemical Company).
‡ HAMSA (n = 8): Acetylated Hylan VII high-amyllose maize starch (National Starch & Chemical Company).
§ Control (n = 9): Celite 545 (VWR International).
‖ Standard error of the mean of data grouped over all supplements.
‖‖ Statistical significance is set at P < 0.05.
¶ Data subjected to log transformation to resolve heterogeneity of variance.

Discussion
The present study is the first to investigate the fermentation metabolite and endproduct profiles of acylated starches in domestic cats. High-amyllose maize starches esterified with propionic (HAMSP) or acetic (HAMSA) acid were used in this experiment. The aim of feeding these supplements was to compare the metabolic effects of acetic and propionic acids after de-esterification of the latter molecules from the starch residue in the large intestine and absorption into the blood. In general, acetic acid is known not to be a gluconeogenic substrate (19), whereas propionic acid is considered to be a gluconeogenic substrate post-absorption (6). Propionic acid, therefore, has the potential of sparing the dietary and endogenous amino acids from participation in gluconeogenesis (3,4). Amino acids are channelled at a high rate to gluconeogenesis in domestic cats (2,4).

The first prerequisite to enable the study of the supplements’ metabolic effects is the de-esterification of acylated starch within the large intestine into SCFA and resistant starch residues. In the rat, the ester bond is degraded by colonic bacterial enzymes. Consequently, acylated starches supply the large intestine with significant quantities of de-esterified SCFA in addition to those derived from the fermentation of resistant starch residues (5). The present study provided......
evidence that the HAMSP ester bond is degraded in the feline large intestine, since faecal propionic acid concentrations were the highest in this group. Faecal pH was the lowest in HAMSP, indicative of an extensive de-esterification of propionylated starch as well. Remarkably, faecal acetic acid concentrations were not significantly higher in the HAMSA cats as compared with the other supplements and the faecal concentrations of branched-chain fatty acids and ammonia were highest in HAMSA-supplemented cats.

A possible explanation for the difference in large-intestinal fermentation pattern between HAMSA (proteolytic) and HAMSP (saccharolytic) might lie in the structural difference between the two supplements. Due to the longer chain length of the esterified SCFA in HAMSP in comparison with HAMSA, the molecular structure of HAMSP is more compact than that of HAMSA(11). Therefore, the ester bond in HAMSA may be more accessible for bacterial enzymes and HAMSA might have been de-esterified already in the feline small intestine before reaching the large intestine, especially since higher numbers of bacteria have been shown to reside in the feline small intestine as compared with other species(25–27). The small-intestinal microbial de-esterification of HAMSA and the potential fermentation of resistant starch residues within the small intestine might have lowered the pH in this region of the gastrointestinal tract. A decreased small-intestinal luminal pH would impair the optimal functioning of endogenous digestive enzymes(28), impairing small-intestinal protein digestion and stimulating large-intestinal protein fermentation, reflected in the high levels of ammonia, branched-chain fatty acids, phenol and p-cresol in the faeces of cats consuming the HAMSA supplement. It has to be noted that the present study is the first at our laboratory that detected phenol in feline samples due to optimisation in terms of sensitivity of the analysis protocol compared with previous studies(4,5,7,8). The apparent protein digestibility coefficients did not differ between groups, but these parameters reflect total-tract rather than small-intestinal digestibility.

The second prerequisite is the absorption of SCFA from the intestine into the blood, which can be estimated based on plasma acylcarnitine profiles. In the plasma of HAMSP-supplemented cats, concentrations of propionylcarnitine were higher (in comparison with Control) or tended to be higher (in comparison with HAMSA) compared with other supplements, consistent with a (tendency towards a) higher propionic acid absorption from the large intestine. Hence, a higher availability of propionic acid for hepatic metabolism may be assumed. This expected result shows that a sufficient intake level of the dietary supplements had been achieved. Higher propionylcarnitine concentrations were not accompanied by a rise in plasma concentrations of methylmalonylcarnitine in HAMSP-supplemented cats. Methylmalonyl-CoA, measured in plasma as methylmalonylcarnitine, can be produced from fermentation-derived propionic acid or upon degradation of valine and isoleucine(29). A concomitant increase in propionylcarnitine through fermentation and a lack of increase of methylmalonylcarnitine might thus be due to a sparing of valine and isoleucine(3,4). In contrast, the higher plasma tiglyl- + 3-methylcrotonylcarnitine concentrations in HAMSP, as compared with the Control group, are consistent with a higher endogenous leucine and isoleucine breakdown in the former group(29). The sparing of isoleucine by fermentation-derived propionic acid is, therefore, questionable in this experiment.

It has to be noted that the mean energy intake of both treatment groups and the Control group was considerably lower than the calculated maintenance energy requirements (418.4 kJ/kg<sup>0.67</sup>; see National Research Council(18)). This energy intake was not sufficient to maintain body weight in all cats, and for more than half of the cats (seventeen out of twenty-seven; five in HAMSP, six in HAMSA, six in Control), crude protein intake was below the recommended minimal requirements. As a consequence, mean intakes of most amino acids were below the adequate or minimum intake requirements as well(19). Since no significant differences in energy, protein, fat or amino acid intakes were observed between groups, the comparison between supplements as described above are valid, but only applicable for situations of relative protein shortage and energy intakes below maintenance energy requirements. The protein intake level was, therefore, included as a covariate in the statistical analyses, and the data from the cats with a low protein intake might serve as a model for diseased cats in clinical circumstances with a low food and protein intake. Especially under these circumstances, the amino acid-sparing potential of propionic acid would be advantageous. Of course, the simple extrapolation of data from healthy cats with a low energy and protein intake to disease-affected cats may be confounded by the various metabolic differences existing between healthy and diseased cats. Follow-up studies, quantifying the amino acid-sparing effects of propionate in healthy cats with a range of dietary protein intakes and in cats in various disease states, will be important.

The results from the present study indicate that increased propionic acid from dietary supplementation had no impact on amino acid sparing in cats with a low dietary protein intake, since plasma methylmalonylcarnitine concentrations were negatively correlated with protein intake levels. This is in accordance with higher plasma concentrations of this carnitine when protein intake was low. When protein intake was low, a higher catabolism of valine and isoleucine was expected, overriding the potential sparing of these amino acids by propionic acid. The latter cats showed other signs of a higher endogenous protein catabolism to fulfil the metabolic demand for N and energy precursors as well, such as higher concentrations of serum urea and plasma concentrations of all measured free amino acids above the plasma concentrations of kittens fed diets containing each amino acid at minimal requirement(30). However, no significant correlations were found between the protein intake and other carnitines that represent the catabolism of branched-chain amino acids(29), tiglyl- + 3-methylcrotonylcarnitine, isovaleryl- + 2-methylbutyrylcarnitine, 3-OH isovaleryl- + 2-methyl-3-OH butyrylcarnitine from leucine and isoleucine. Likewise, creatinine, creatine kinase and 3-methylhistidine were not affected by protein intake level. However, the latter parameters are not sensitive or affected by other factors, such as stress or restraint of the animal(31–33). In contrast, in cats...
consuming adequate levels of protein, increased dietary propionic acid was associated with signs of sparing of the amino acid valine (see above).

Another remarkable effect of the low protein intake was the higher plasma concentrations of 3-OH butyrylcarnitine combined with a lower faecal butyric acid excretion. This carnitine ester is an estimator of the concentration of 3-OH butyryl-CoA, which is a metabolite in the β-oxidation pathway in colonocytes. In this pathway, acetyl-CoA and ketone bodies are produced from large-intestinal fermentation-derived butyric acid to yield energy for the colonocytes (34). A possible explanation for the effect of protein intake level on this parameter is that faecal butyric acid excretion and, by extrapolation, large-intestinal butyric acid production were lower when protein intake was lower. Large-intestinal butyric acid is known to stimulate the mRNA expression of an important rate-limiting enzyme in the β-oxidation pathway, namely 3-OH 3-methylglutaryl-CoA (HMG) synthase(35,36). A consequence of lower concentrations of butyric acid is a lower activity of HMG synthase and accumulation of metabolites higher in the pathway, including 3-OH butyryl-CoA, which can be absorbed into the blood(37) and was detected via higher plasma concentrations of 3-OH butyrylcarnitine. The lower activity of HMG synthase can also explain why an increase in 3-OH butyryl-CoA is not accompanied by an increase in HMG. These observations contrast with previously published results(8), wherein a higher ratio of faecal butyric acid to total SCFA in guar gum-, as compared with cellulose-, supplemented cats was accompanied by higher concentrations of plasma 3-OH butyrylcarnitine. From this discrepancy it can be concluded that 3-OH butyryl-CoA is a metabolite from β-oxidation in colonocytes, which appears to be rapidly absorbed into the hosts’ blood in cases of high concentrations in colonocytes by a higher production (as described by Rochus et al. (8)) or an accumulation (as in the present study).

Conclusions

The HAMSP supplement produced a different, more saccharolytic, fermentation pattern in the feline large intestine as compared with HAMSA. The HAMSP-supplemented cats appeared to show sparing of valine, whereas HAMSA- and Control-fed cats did not. The energy and protein intake of all cats was below the offered amounts and in cats with a low protein intake the amino acid-sparing potential of propionic acid was not sufficient to compensate for the higher endogenous protein catabolism. Further studies to explore the ideal dose of dietary HAMSP supplementation and to quantify the amino acid-sparing effect in domestic cats are warranted.

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