

## Dietary supplementation of coated butyrate in healthy dogs: effect on apparent digestibility, faecal flora and faecal volatile fatty acids

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**ABSTRACT:** The aim of the present study was to determine the effect of dietary addition of coated butyrate on gut health related faecal traits in dogs. A food with or without coated butyrate was tested in a crossover study with 17 dogs. Faecal samples were collected and frozen until analysis for proximate components. Fresh faecal samples were collected for bacterial culturing and short chain fatty acids (SCFA) analysis. The addition of coated butyrate to a dog diet had neither effect on faecal consistency score, dry matter content and faecal production nor on digestibility coefficients. Absolute and relative faecal contents of SCFA were not different for the two foods. The bacterial nitrogen (N) content of the faeces was significantly lower after butyrate addition compared to the control diet. In conclusion, the addition of butyrate to a dog food had no effect on faecal characteristics but the faecal bacterial protein content was significantly reduced by butyrate addition.

**Keywords:** butyric acid; faecal flora; digestibility; dog

Vegetable ingredients are frequently included in dry foods for dogs for economical reasons. Because of the use of vegetable ingredients, the fibre contents of these foods are higher especially if compared to standard canned food. Fibre inclusion in dog foods may promote and regulate normal bowel function by increasing bulk and water content of the chyme and modulation of the intestinal transit. In addition, end products of proper microbial fermentation maintain a healthy colon (Diez and Istasse, 1997; Gross et al., 2000). Fibre rich diets are also advised in certain disease states like obesity, diabetes mellitus and colitis to decrease the energy density of the food, to increase satiety, to regulate glucose metabolism and to regulate intestinal transit (Jewell and Toll, 1996; Diez and Istasse, 1997; Nelson et al., 1998).

Fermentation of soluble fibre is accompanied by the production of short chain fatty acids (SCFA) such as acetic, propionic and butyric acid. SCFA are quickly absorbed and can also deliver energy

to the animal. In contrast to other animals like pigs where SCFA may supply up to 76% of the basal metabolic rate, Herschel et al. (1981) found that SCFA only provided approximately 7% of the basal energy requirement in dogs. SCFA may also play a role in regulation of gastrointestinal cell metabolism, division and differentiation (Roberfroid and Delzenne, 1998). Other possible effects of SCFA are enhanced mesenteric blood flow, decreased intestinal permeability and stimulated intestinal mucosal expression of the enteroglucagon gene. Butyrate is the preferred metabolic fuel for colonocytes and stimulates colonocyte proliferation and differentiation both *in vitro* and *in vivo* (Ziegler et al., 2003). In contrast to the trophic effect on normal colonocytes, butyrate decreases proliferation and differentiation of tumour cell lines, causes apoptosis (Ziegler et al., 2003) and inhibits proliferation in *in vitro* hyperproliferation states (Bartram et al., 1994). The *in vitro* study in dogs of Drackley et al. (1998) suggested that butyrate also would be

a major fuel for colonocytes *in vivo* but the *in vitro* data also showed species differences in intestinal fuel metabolism between dogs and rats.

The positive effects of butyrate can be achieved by adding fermentable fibre to the diet. However fibre may also interact with nutrient digestibility and availability (Gross et al., 2000) and too large amounts of fermentable fibre can induce negative faecal characteristics such as loose stools (Diez et al., 1997). Therefore, direct addition of butyrate to dog food might be a valuable alternative to dietary fibre. Another advantage is that the addition of one specific SCFA can be tested.

The aim of the present study was to determine the effect of dietary addition of coated butyrate on gut health related faecal traits in dogs.

## MATERIAL AND METHODS

Seventeen adult beagle dogs between 1.7 and 8 years old were used in a crossover study with two treatments: a control food (Premium Regular, Bento Kronen, Belgium) and the control food with the coated butyrate (Adimix 30 coated, Nutri Ad, Kasterlee, Belgium). Adimix, as a source of butyrate, was included in the diet at 0.5% on as-fed basis and contained 30% of sodium butyrate. Butyrate content of the supplemented diet was 0.117% on as-fed basis. The coating was of vegetable origin and contained 54% of palmitic acid (C16:0) and 43% of stearic acid (C18:0). The nutrient analysis of both foods is given in Table 1. Every period consisted of an adaptation period of 6 days and a faecal collection period of four days. The dogs were fed to maintain body weight: each dog received 180 g of

food per day, though in some dogs the food amount was increased to 210 g. An external marker (Celite, VWR, Belgium) was added to the food at 1% on as-fed basis. During collection periods, individual food and water intakes were noted daily. In the morning, faeces of each dog were scored for their consistency (score of 1–5; 1: watery, 2: lower consistency but still formed faeces, 3: ideal, 4: hard, 5: constipation), weighed, collected and frozen until proximate analysis. Apparent nutrient digestibility coefficients were calculated by the external marker method with acid-insoluble ash (Kotb and Luckey, 1972):

$$\text{Nutrient digestibility (\%)} = 100 (1 - (\text{Cnf} \times \text{Cmv}) / (\text{Cnv} \times \text{Cmf}))$$

where:

Cnf = faecal nutrient concentration

Cmv = concentration of marker in the food

Cnv = nutrient concentration in the food

Cmf = concentration of marker in the faeces

Faecal bacterial N was determined according to the method of Mason (1969) adapted by Hesta et al. (2003). In principle, bacterial protein is estimated through determining the difference in nitrogen content in the neutral detergent fibre fraction with or without sodium dodecyl sulphate (SDS) treatment. During the day, fresh faecal samples were collected and placed in an anaerobic jar at 5°C. The jar was made anaerobic by the use of Anaerogen (Oxoid, United Kingdom). The fresh faecal sample was used for culture of total anaerobes, sulfide reducing anaerobes, *Salmonella*, *Lactobacilli* and *E. coli* and were analysed for SCFA concentration

Table 1. Nutrient analysis of the control feed and the butyrate supplemented feed

Nutrient (%) (as fed basis)	Butyrate	Control
Dry matter	88.5	89.0
Crude protein	24.2	24.9
Crude fat	11.3	11.6
Crude ash	6.73	6.77
Crude fibre	2.69	2.64
Starch	34.9	35.0
Insoluble ash	1.18	1.18
Metabolisable energy (MJ/kg)*	–	16.91

\*information from the company

Table 2. Effect of butyrate supplementation on feed and water intake, faecal characteristics and apparent nutrient digestibility (mean value  $\pm$  SD)

	Butyrate	Control	<i>P</i>
Feed intake (g/day)	185 $\pm$ 14	186 $\pm$ 12	0.407
Water intake (g/day)	535 $\pm$ 115	549 $\pm$ 128	0.694
Faecal consistency score*	2.9 $\pm$ 0.26	2.9 $\pm$ 0.28	0.782
Faecal dry matter (%)	30 $\pm$ 1.6	30 $\pm$ 3.0	0.908
Faecal production (g/day)	123 $\pm$ 18	124 $\pm$ 22	0.769
ADC of dry matter (%)	78.1 $\pm$ 1.3	78.1 $\pm$ 1.3	0.998
ADC of crude protein(%) %	78.0 $\pm$ 1.7	78.1 $\pm$ 1.7	0.831
ADC of crude fat (%)	94.3 $\pm$ 1.3	94.4 $\pm$ 1.2	0.928
ADC of crude ash (%)	21.4 $\pm$ 4.4	21.8 $\pm$ 3.4	0.755
ADC of crude fibre (%)	32.8 $\pm$ 13.3	27.5 $\pm$ 13.5	0.343
ADC of N-free extract (%)	85.4 $\pm$ 1.5	85.6 $\pm$ 1.9	0.730

ADC = apparent digestibility

\*faecal consistency score (1: watery, 2: lower consistency but still formed faeces, 3: ideal, 4: hard, 5: constipation)

by gas chromatography (Van Nevel and De Meyer, 1977).

The normally distributed values were statistically analysed by a paired *t*-test. The faecal consistency code was analysed by the Friedman test. All statistical analyses were performed using SPSS (SPSS 12.0 Chicago USA).

## RESULTS

Food and water intake were not influenced by the treatment (Table 1). The addition of coated butyrate to the control food neither affected faecal consistency score, dry matter content and faecal production nor

apparent digestibility coefficients (dry matter, crude protein, crude fat, ash, crude fibre and nitrogen-free extract) (Table 2). Absolute and relative faecal contents of acetic, propionic and butyric acid were not different between the two foods (Table 3).

The total amount of anaerobic bacteria, sulfide (S) reducing anaerobes, *Lactobacilli* and *E. coli* did not show any differences (Table 4). *Salmonella* was not found in any faecal sample after enrichment. The estimated faecal bacterial protein content expressed on a dry matter basis as well as expressed as a percentage of faecal protein content or as a percentage of dietary protein intake was significantly lower after butyrate addition compared to the control diet (Table 5).

Table 3. Effect of butyrate supplementation on faecal amount of SCFA (mean value  $\pm$  SD)

	Butyrate	Control	<i>P</i>
Acetic acid (%)*	57.7 $\pm$ 3.5	56.5 $\pm$ 3.8	0.313
Propionic acid (%)*	31.8 $\pm$ 2.3	32.9 $\pm$ 3.5	0.232
Butyric acid (%)*	10.4 $\pm$ 2.1	10.5 $\pm$ 1.8	0.869
Acetic acid (mg/kg)	9081 $\pm$ 1776	8668 $\pm$ 2203	0.552
Propionic acid (mg/kg)	4974 $\pm$ 807	4945 $\pm$ 855	0.933
Butyric acid (mg/kg)	1611 $\pm$ 277	1577 $\pm$ 336	0.715

\*as a % of sum of acetic, propionic and butyric acid excretion

Table 4. Effect of butyrate supplementation on faecal bacterial concentration (mean value  $\pm$  SD)

	Butyrate	Control	<i>P</i>
Total anaerobes*	7.03 $\pm$ 1.41	7.56 $\pm$ 1.33	0.113
Sulphite reducing anaerobes*	8.32 $\pm$ 0.47	8.42 $\pm$ 0.64	0.477
<i>Lactobacilli</i> *	8.07 $\pm$ 0.64	7.94 $\pm$ 0.85	0.126
<i>E. coli</i> *	5.22 $\pm$ 1.55	5.66 $\pm$ 1.24	0.958

\*in Log colony forming units per g faeces

Table 5. Effect of butyrate supplementation on estimated faecal bacterial protein (mean value  $\pm$  SD)

	Butyrate	Control	<i>P</i>
Bacterial protein (% AF)	0.91 $\pm$ 0.31	1.24 $\pm$ 0.31	0.006
Bacterial protein (% DM)	3.08 $\pm$ 1.08	4.19 $\pm$ 0.89	0.004
Bacterial protein (g/100 g faecal protein)	11.26 $\pm$ 4.03	14.91 $\pm$ 3.01	0.008
Bacterial protein (g/100 g protein intake)	2.46 $\pm$ 0.85	3.31 $\pm$ 0.8	0.005

AF = fed basis; DM = dry matter

## DISCUSSION

Overdosing of butyrate might induce an osmotic effect resulting in increased faecal moisture content and worse faecal consistency (NRC, 2006), but no changes were observed in the present trial. In the present study, physiological doses of butyric acid (BA) were used since food and faecal BA concentrations were comparable.

The absolute and relative faecal concentrations of SCFA were not altered after butyrate supplementation. This however does not exclude an effect of the added butyrate in the large intestine since 95–99% of the produced SCFA are rapidly absorbed in the large intestine (NRC, 2006). Since it was not feasible to fistulate 17 dogs we could not measure SCFA in the colon nor in the vena porta; consequently faecal measurement was chosen. The absence of a difference in freshly collected faecal butyrate content of both groups shows that the coating of the butyrate was released in the gastrointestinal tract. Due to the nature of the coating (saturated fatty acids), most probably the coating was lost in the more distal part of the small intestine. However, from the present study design no statements can be made with regard to place where the coating was lost and further research is needed to clarify this. Yet, depending on the desired effect, the place where the coating

was lost, is probably not that important since rectally administered SCFA also had a trophic effect on distant gut segments (jejunum, ileum) in rats probably through an indirect mechanism (Ichikawa et al., 2002). Similarly, SCFA administered through the stomach also had a trophic effect on the distal colon (Ichikawa et al., 2002). An *in vitro* study in dogs showed that butyrate was not only oxidised by colonocytes but also by mid jejunum enterocytes at a relatively high rate. In contrast to rats, the butyrate oxidation was not reduced by other substances (glucose, glutamine,  $\beta$ -hydroxybutyrate or mixture) (Drackley et al., 1998).

In rats SCFA stimulate large and small intestinal cell proliferation (Ichikawa et al., 2002) and consequently may increase brush border digestion and nutrient absorption through increased villus height. In pigs, other mechanisms for altered nutrient digestibility by organic acids are a lower gastric pH and increased proteolytic activity, decreased proliferation and colonisation of undesirable intestinal bacteria, stimulated pancreatic secretion and intermediate metabolism (Partanen and Mroz, 1999). Indeed in pigs, organic acids increased apparent total tract digestibility of protein, energy and minerals, particularly Ca and P (Partanen and Mroz, 1999). In humans absorption of SCFA increases sodium and water transport since they are

taken up in a concentration-dependent manner by the colonocytes. Moreover, they may increase calcium and magnesium absorption (Ziegler et al., 2003). In this study, butyrate supplementation did not change apparent digestibility coefficients but individual electrolyte and mineral availabilities were not measured.

In piglets, the addition of organic acids (1.8% K-diformate) affected the intestinal flora (Canibe et al., 2001). Similarly, the results of the present study suggest a change in intestinal flora in dogs: the reduction of the bacterial protein suggests a reduction in total bacterial number although, there was no effect on numbers of the cultured bacteria. SCFA, as the major end products of fermentation, may decrease intestinal pH, which may prevent colonisation of pathogenic bacteria and may stimulate health promoting bacteria (Swennen et al., 2006). Although, the pKa value of butyrate is not as high as of lactic acid, addition of butyric acid may also slightly decrease the intestinal pH and influence the bacterial flora. Estimated faecal bacterial protein content decreased after butyrate supplementation but faecal concentrations of total anaerobes, S-reducing anaerobes, *Lactobacilli* and *E. coli* did not change. Since it is not known which bacterial group was decreased, it is difficult to speculate on possible health effects. In pigs, an inhibitory effect of organic acids on coliform population was shown both *in vivo* (Overland et al., 2000) and *in vitro* (Knarreborg et al., 2002). This may be due to inhibition of the bacterial cell metabolism as a result of acidification of the cytoplasm by entrance of the organic acids in the undissociated form followed by dissociation in the more alkaline interior of the bacterial cell (Hunter and Segel, 1973). However, in the present study, coliformes were not decreased by adding BA to the diet of dogs. Apart from the BA coating and dosage, the limited time of BA supplementation (10 days) may also be a factor in explaining the small observed differences in this trial. It may take several weeks before faecal flora changes become apparent. Faecal bacterial profile did change in dogs fed a soluble fibre (oligofructose) for three weeks (Beynen et al., 2002). In the present study, only a few groups of bacteria were cultured and consequently the specific effect might have been missed.

The effect of butyrate could also depend on the quality and composition of the food and on the health status of the animal. In this trial both food and animal health were optimal which can be a

reason for the small observed differences between both treatment groups. In the future, the effect of butyrate in dogs could be tested in a more challenging situation e.g. by feeding high protein, lower quality protein sources. More research is needed to explain the nature of the decreased bacterial protein content in the faeces.

## CONCLUSION

The addition of butyrate to a dog food had no effect on faecal characteristics and nutrient digestibility. The estimated faecal bacterial N content was significantly lower on the butyrate supplemented diet although the faecal concentration of health-improving bacteria such as *Lactobacilli* was not changed.

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