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The content of short chain fatty acids in the jejunal digesta, caecal digesta and faeces of growing pigs

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ABSTRACT

Short chain fatty acids (SCFAs) are intermediates from microbial fermentation of proteins or saccharides. Little is known about their production in different parts of intestine and therefore the aim of the present study was to examine, using 7 double-cannulated gilts (initial BW: 25 ± 0.5 kg), the production of the SCFAs in a jejunal digesta, caecal digesta and faeces. After a 25-d recovery period, a 70-d experimental period followed. On days 28; 42; 56 and 70, samples of jejunal, caecal digesta and faeces were collected. In the samples of jejunal and caecal digesta and in faeces, the content of dry matter (DM), crude protein (CP) and SCFAs was determined. The highest content of DM ($P < 0.001$) was found in faeces (29.2%), then in caecal digesta (10.5%) and in jejunal digesta (8.8%). The content of CP in jejunal digesta was lower ($P < 0.001$) when compared with that in the caecal digesta and faeces. The content of lactate was greater ($P < 0.001$) in jejunal (69.1 mmol/L) than in caecal digesta (36.3 mmol/L) or faeces (2.5 mmol/L). However, the content of other SCFAs was higher ($P < 0.001$) in caecal digesta and faeces when compared with that in jejunal digesta. Concentrations of acetate, butyrate and propionate were higher ($P < 0.001$) in caecal digesta and faeces when compared to jejunal digesta, however in the faeces were lower ($P < 0.001$) than in caecal digesta. The contents of isobutyrate, isovalerate and caproate were greater ($P < 0.001$) in the faeces (2.2; 4.1; 0.28 mmol/L) than in caecal digesta (0.3; 0.6; 0.11 mmol/L). In caecal digesta, the content of acetate, propionate and butyrate increased with decreasing the content of lactate ($P < 0.05$). On d 28 and 70 the concentration of lactate in faeces was higher (2.9; 2.6 mmol/L) when compared with d 42 and 56 (1.8; 0.8 mmol/L; $P < 0.001$). The content of acetate in jejunal digesta was higher ($P < 0.05$) on d 56 (17.8 mmol/L) in comparison with d 28, 42 and 70 (11.5; 10.0 and 12.1 mmol/L), and isobutyrate was the lowest on d 28 ($P < 0.05$). The content of SCFAs, with the exception of lactate, was greater in caecal digesta and faeces in comparison with that in jejunal digesta, and the content of acetate propionate and butyrate in caecal digesta increased with decreasing content of lactate.

1. Introduction

Digestive processes in an organism are characterized by a decomposition of nutrients into simple substances which are absorbed through a wall of the small intestine or pass through the upper parts of the gastrointestinal tract (GIT), where there are the substrates for the microbiota (Cummings and Macfarlane, 1991). As a result of the microbial activity, depending on a substrate source and bacterial species, different compounds are produced. For example, the fermentation of carbohydrates by saccharolytic bacteria provides the short chain fatty acids (SCFAs), H_2 and CO_2 (Macfarlane and Macfarlane, 2003), while the fermentation of proteins and amino acids by proteolytic bacteria results in the production of the short branched-chain fatty acids, phenols, amines and CO_2 (Roberforoid, 2005). The level of the fermentation is different in individual parts of a bowel. In the caecum and colon the fermentation is greater than in the small intestine, due to of a larger and more variable microbial population (Gaskins, 2001). Moreover, the intense fermentation in colon and caecum is accompanied by a low pH (Cummings et al., 1987; Macfarlane et al., 1992a; Fallingborg, 1999).

Also, the content of particular SCFAs varies in the different parts of the GIT. The content of lactic acid is greater in the small intestine, whilst the content of acetic, propionic and butyric acids is greater in the caecum (Franklin et al., 2002; Meimandipour et al., 2011). This is the result of the bacterial activity in the caecum, where the lactic acid is converted into the acetic, propionic and butyric acids (Belenguer et al., 2007).

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Studies have been conducted dealing with the production of SCFAs in the GIT of humans (Cummings et al., 1987; Fernandes et al., 2014), chickens (Meimandipour et al., 2011) or piglets (Franklin et al., 2002). However, due to the fact that it is too difficult to have an insight into the GIT of the living subjects, most of these works used data obtained from the subjects *post mortem* (Cummings et al., 1987; Meimandipour et al., 2011) or brought information about the SCFAs in the faeces (Fernandes et al., 2014). Franklin et al. (2002) studied the production of the SCFAs in different parts of intestine in pigs. The pigs, used in their study, were fitted with the cannulas in the jejunum, ileum or caecum, individually, with the one cannula for each pig. However, there is no information about the production of SCFAs from the different parts of GIT, only from the one subject. Moreover, information about the time course production of SCFAs in the GIT is limited. Meimandipour et al. (2011) studied the production of the SCFAs in different parts of a bowel in the chickens, and Franklin et al. (2002) - in weaning piglets. Therefore, the aim of the present study was to observe the production of the SCFAs in the jejunum, caecum and colon of the double-cannulated growing pigs, each fitted with two T-cannulas, one in the jejunum and other one in the caecum.

2. Material and methods

2.1. Animals and experimental design

The study was conducted in Laboratory of Pig Nutrition at Research Institute of Animal Production Nitra. All of the experimental procedures were reviewed and approved by the Animal Care Committee of the Research Institute of Animal Production Nitra in accordance with the appropriate EU regulations (No. 745/09–221).

A total of 7 gilts (*Landrace* x *Slovak White*; initial BW 25 ± 0.5 kg) were used in the study. The pigs, fitted with the T-cannulas in a jejunum and caecum, were individually housed in balance cages with feeder and nipple drinker. The cages were made of stainless steel construction with artificial slatted floor. After a 25-d recovery postoperative period, a 70-d experimental period followed. The samples of jejunal, caecal digesta and faeces were collected on days 28, 42, 56 and 70. After opening the T-cannulas in the jejunum and caecum, the polyethylene bags were fitted to the cannulas and the jejunal and caecal digesta were collected at the same time for 3 h, started at 11:00 h. After this time the cannulas were closed and the collection was finished. The digesta was accumulated into the bags and was continuously moved into plastic containers and stored in a refrigerator at 4 °C for subsequent chemical analysis. When the plastic bags were fitted to the cannulas, faeces were collected directly from a rectum and were stored in a refrigerator at 4 °C for chemical analysis. The samples of digesta and faeces were analyzed immediately when the collection was finished.

In the study a standard commercial pig grower diet was used (Table 1). The diet was administered twice daily at 7:00 and 16:00 h, in two equal meals at the daily rate of 90 g/kg$^{-0.75}$. Water was offered *ad libitum*.

2.2. Chemical analyses

The content of nutrients in the basal experimental diet was analyzed in accordance with AOAC (1990) procedures. The samples of digesta and faeces were analyzed for the content of dry matter (DM), crude protein (CP) (AOAC, 1990) and SCFAs. The content of SCFAs was analyzed by a gas chromatography using a gas chromatograph GC 8000 Top (CE Instruments, Hindley Green, Wigan, UK). The lactic acid was determined after the oxidation as acetaldehyde. Before the analyzes of SCFAs, the samples of faeces in an amount of 15 g diluted in 15 ml of distilled water and samples of digesta in an amount of 15 ml were centrifuged at 4000 g and 18 °C for 5 min. The 5 ml of supernatant was preserved with 5% HCl in ratio 1:1 and stored at 7 °C. Prior to injection into the gas chromatograph, supernatant was centrifuged at 14000 g and 17 °C for 10 min. The column (length 1.8 m) with stationary phase SP 1200 (Supelco) contained 10% SP 1200, 1% H_3PO_4, and acid-washed 80/100 Chromosorb W. Flame ionization detector was used for detection. Nitrogen was used as a carrier gas.

Table 1
Ingredient and nutritional composition of diet (as-fed basis).

Item	
Ingredient (%)	
Maize	58.7
Soybean meal	22.4
Barley	11.6
Alfalfa meal	4.0
Monocalcium phosphate	2.0
Salt	0.5
Vitamin-mineral premix[a]	0.5
Limestone	0.3
Analyzed content of nutrients (g/kg)	
Dry matter	900.8
Starch	410.1
Crude protein	145.6
Crude fiber	34.0
Ash	29.5
Total sugars	26.3
Reducing sugars	3.2

[a] Supplied per kilogram of diet: vitamin A, 9000 IU; vitamin D_3, 1500 IU; α– tocopherol, 35 mg; vitamin B_1, 1.7 mg, vitamin B_2, 6 mg; vitamin B_6, 2.5 mg; Ca-pantothenate, 15 mg; niacin, 38 mg; vitamin K_3, 2 mg; biotin, 0.12 mg; cyanocobalamin, 0.03 mg; choline, 156 mg; Fe, 103 mg; Zn, 116.5 mg; Mn, 49.0 mg; Cu, 40 mg; I, 1.2 mg; Co, 0.4 mg; Se, 0.3 mg; lysine, 3.82 g; methionine, 0.8 g; and threonine, 1.72 g.

2.3. Statistical analyzes

The experimental data were subjected to One-Way ANOVA using Statgraphics Plus 3.1 software (Statistical Graphics Corp., Rockville, MD). When the treatment effects were significant ($P < 0.05$) or tended to be different ($P < 0.10$), the means were separated using a Fisher's LSD procedure. Each animal was considered as an experimental unit. The data shown are means of the values obtained in the experiment using separate pools of samples from 7 animals. A regression analysis was used for the determination of the relationship between the lactate, acetate, propionate and butyrate in the caecal digesta. The relationships were evaluated using the General Linear Model of Statgraphics Plus for windows version 3.1 software (Statistical Graphics Corp., Rockville, MD).

3. Results

The pigs remained in good health throughout the whole study, and they consumed all the feed offered. The content of DM was greater ($P < 0.001$) in the faeces than in the caecal digesta, and the lowest one was in the jejunal digesta (Table 2). The content of CP in the jejunal digesta was 8% and 10% lower ($P = 0.003$) in comparison with that in the caecal digesta and faeces, respectively.

The concentration of lactate was lower in the caecal digesta and faeces when compared with that in the jejunal digesta ($P < 0.001$), and the lowest concentration was in the faeces. It was 93% lower in faeces than the caecal digesta (Table 2).

In the caecal digesta, the content of the lactate decreased linearly ($P < 0.05$) with increasing the content of acetate (Fig. 1a), propionate (Fig. 1b) and butyrate (Fig. 1c).

The content of acetate, propionate and butyrate in the faeces decreased ($P < 0.001$) by 7%, 46% and 27% in comparison with that in caecal digesta, however, the content of isobutyrate, isovalerate and caproate was 737%, 634% and 147% greater in the faeces than in the