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The Synbiotic Effect of Lactobacilli and Flaxseed on Selected Intestinal Microflora and Organic Acid Levels in Weaned Piglets

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ABSTRACT

The study investigated the influence of the administration of Lactobacillus plantarum – Biocenol™ LP96 and flaxseed (alone and in combination) on the selected intestinal microflora and on organic acid levels in the digestive tract of weaned piglets. On day 7 after weaning, the supplementation of piglets with mixture of Lactobacillus plantarum and flaxseed had the most pronounced beneficial effect on the gut microbiota when compared with Lactobacillus plantarum and flaxseed groups. The supplementation significantly enhanced the population of jejunal and ileal lactic acid bacteria, caecal total anaerobes and short-chain fatty acids which was manifested by a significant decrease in the pH level in the large intestine. Our results indicated a synbiotic effect of L. plantarum and flaxseed combination on the growth and metabolic activity of selected microflora in weaned piglets.

KEYWORDS : probiotic, flaxseed, intestinal microflora, weaned pig

INTRODUCTION

The autochthonous gastrointestinal (GI) microflora plays an important role in the development of resistance against pathogens (Krause et al., 2006). Stimulation of this microflora can be achieved by manipulating the composition of the diet or adding a wide range of compounds including probiotics, prebiotics and other natural components **(**Bomba et al., 2002).

Lactobacilli posses properties that make them a promising candidate for probiotics (Zhou et al., 2012). *Lactobacillus plantarum* is a plant-associated lactic acid bacterium that has also been found in human, murine and porcine gastrointestinal tract. It is are able to ferment a broad spectrum of plants, is tolerant against bile salts and low pH (Brajdes and Vizireanu, 2013) and shows antagonistic properties against potential intestinal pathogens (De Vries et al., 2006). These properties make it a promising probiotic feed additive. Flaxseed (FS) is a rich source of polyunsaturated fatty acids (PUFAs), lignans and mucilaginous water-soluble polysaccharides (fibre), all of which have been found to confer numerous health benefits (Pauletti et al., 2000). Flaxseed mucilage water-soluble polysaccharides, which are neither hydrolyzed by the endogenous digestive enzymes nor absorbed by the host, have been identified as prebiotic agents for the indigenous microflora of the gut (Gibson and Roberfroid, 1995).

Only few studies were conducted on the effects of FS on intestinal microbial activity in weaned pigs as most swine research on FS was primarily focused on changing fatty acid profiles in finishing pigs (Marcinčák et al., 2010).

The aim of this study was to investigate the effect of the administration of *Lactobacillus plantarum* – Biocenol™ LP96 and flaxseed, as a source of α-linolenic acid and fibre (alone and in combination), on lactic acid bacteria (LAB), *coliforms*, total aerobes and anaerobes, *Bifidobacterium* sp., *Enterococcus* sp. and organic acid levels in the digestive tract of weaned piglets.

Materials and methods

Probiotic bacteria

The probiotic strain *Lactobacillus plantarum* – Biocenol™ LP96 was selected from the gut contents of healthy suckling piglets. Cheddar cheese was used as a vehicle for the probiotic strain. The probiotic bacteria were added to the milk during typical Cheddar cheese production (referred to as probiotic cheese). The cheese that was used as a control was a similar Cheddar cheese but without the probiotic strain (referred to as control cheese).

Animals, their housing and diets

The State Veterinary and Food Administration of the Slovak Republic approved the experiment under protocol number 2108/07-221 and the animals were handled and sacrificed in a humane manner in accordance with the guidelines established by the respective commission. A total of 64 clinically healthy piglets - crossbreds Yorkshire x Pietrain- (32 barrows and 32 gilts), 28 ±1 days old, 7.3 ±0.3 kg of BW, were obtained from the University Agricultural Farm in Zemplínska Teplica. Based on the litter origin, gender of animals and body weight at weaning, the piglets were randomly allocated to pens (4 piglets per pen). A completely randomized experimental design was used and piglets were divided into four treatment groups, with four pens per treatment. The treatments included: Control (control cheese + control oil); L group (probiotic cheese + control oil); LFA group (probiotic cheese + whole crushed flaxseed); FA group (control cheese + whole crushed flaxseed). Throughout the study, the animals were fed a meal form of basal diet **(Table 1).** The basal diet was supplemented with whole crushed flaxseed cultivar Flanders (Agrola Kožušice, Czech Republic): 100 g flaxseed (i.e. 45.8 g lipids)/1 kg basal diet for LFA and FA groups and control oil: 45.8 g sunflower oil /1 kg basal diet for control and L groups. **Table 2** gives the fatty acid profile of flaxseed, sunflower oil and the basal diet. During the administration period, 10 g/animal/day of 1-month-ripened Cheddar cheese containing *Lactobacillus plantarum* – Biocenol™ LP96 strain (1.5×10^8 CFU/g of cheese) was provided to the experimental piglets of groups L and LFA. In addition, piglets of the control group and group FA received 10 g/animal/day of control cheese without *Lactobacillus plantarum* – Biocenol™ LP96 culture. The probiotic and control cheese were supplied to piglets once a day (individually in the morning) in the form of a grated cheese deposited on the surface of feed. The animals fell for the cheese and consumed it instantaneously. In the same period the animals had ad libitum access to water and feed supplemented with whole crushed flaxseed or control oil.

Biological material and analysis

The pigs from all groups were sacrificed with 1 ml/kg BW T61® (Intervet International B.V. Boxmeer, Netherlands) intracardially on days 3 and 7 post-weaning. Digesta samples were taken from the jejunum, ileum, colon and caecum. Culture-based microbial enumeration was con-

ducted using the samples of intestinal contents diluted with a sterile anaerobic diluent (0.4 g $NaHCO_3$, 0.05 g L-cysteine-HCl, 1 ml resazurin (0.1%), 7.5 ml mineral solution I (0.6% K_2HPO_4), 7.5 ml mineral solution II (1.2% NaCl, 1.2% $(NH_4)_2SO_4$, 0.6% KH_2PO_4, 0.12% $CaCl_2$, 0.25% $MgSO_4$) and 84 ml distilled water (pH 6.8) and stomached for 2 min under a CO_2 atmosphere. The following selective agars were employed: Trypticase soy blood agar (Oxoid Unipath, Ltd., Basingstoke, UK) with 10% sheep blood for total aerobes and Schaedler agar (BBL Microbiology systems, Cockeysville, USA) with 1% vitamin K_1 - hemin solution for total anaerobes, MRS agar (Merck) for lactic acid bacteria, MacConkey agar (Oxoid Unipath) for *coliforms,* M-Enterococcus agar for enterococci (Becton and Dickinson) and modified Wilkin-Chalgreen agar (Rada et al., 1999) for *Bifidobacterium* sp. For anaerobes, the plates containing the inoculated media were kept in anaerobic jars for 24 hours before analysis. Incubation of the inoculated media for anaerobic bacteria was carried out at 37 °C for 3 days under anaerobic conditions (Gas Pak Plus, BBL). Plates for the enumeration of aerobic bacteria were incubated for 24 hours at 37 °C. Numbers of colony-forming units (CFU) were expressed as log CFU per gram of the wet intestinal content. The pH level of the intestinal contents was immediately measured in undiluted samples. The concentration of organic acids in the intestinal content was determined by capillary isotachophoresis.

All data are presented as means ± SEM. The data were evaluated with GraphPadPrism version 3.00 by one-way ANOVA followed by a Tukey's multiple comparison test.

Results

Microbiological analysis of intestinal samples taken from piglets on day 3 after weaning (**Table 3)** showed a significantly higher numbers of LAB ($p<0.001$) in the jejunum, ileum and caecum and significantly lower counts of *coliforms* in the ileum ($p<0.05$) and colon ($p< 0.001$) in L group and significantly higher numbers of LAB ($p<0.001$) in the jejunum, ileum and caecum and significantly lower numbers of *coliforms* in the colon ($p<0.01$) of animals from the LFA group in comparison with the control. Similarly, significantly higher counts of LAB in the jejunum ($p<0.05$) and ileum ($p<0.001$) were found in animals from the FA group compared to control animals. On the other hand, lower counts of LAB were observed in the jejunum, ileum, colon and caecum in comparison with the LFA and/or L groups. The experimental diets had no significant effect on the numbers of total aerobes, total anaerobes, *Bifidobacterium* sp. and enterococci in the caecum. The supplementation of pigs with the diet containing probiotic cheese (L group) as well as mixture of probiotic cheese and flaxseed (LFA group) resulted in a significant increase in the ratio of LAB and *coliforms* plate counts in the contents of all intestinal segments compared to the control group.

On day 7 after weaning (**Table 4**), the numbers of LAB ($p<0.001$) were significantly higher and counts of *coliforms* ($p<0.001$) were significantly lower only in the jejunum of animals from the L group when compared to the control animals. On the other hand, animals from the LFA group showed significantly higher numbers of LAB ($p<0.001$) and significantly lower counts of *coliforms* ($p<0.001$) in contents of all intestinal segments compared to the control and L group. In addition, in the caecum of LFA animals a significant increase in total anaerobes and *Bifidobacterium* sp. ($p<0.01$) was recorded in comparison with the control and L group. Significantly lower counts of total aerobes in LFA animals were observed when compared with the control group ($p<0.001$) and L group ($p<0.01$). A similar response to the diet containing flaxseed (FA group) was observed in the colon and caecum. Significantly higher counts of LAB ($p<0.001$) and *Bifidobacterium* sp. ($p<0.01$) and lower counts of *coliforms* and total aerobes ($p<0.01$) were recorded in comparison with the control and L group. The counts of total anaerobes tended to be higher compared to the control and L group, however, the difference was insignificant. There were no significant differences in the jejunum and ileum between the FA group and the control. On the other hand, significantly lower counts of LAB ($p<0.001$) and significantly higher counts of *coliforms* ($p<0.001$) in comparison with the LFA group were recorded. In the contents of all intestinal segments, the ratio between LAB and *coliforms* plate counts was the greatest for the diet containing probiotic cheese and flaxseed (LFA group).

Table 5 shows the concentration of organic acids in the content of the jejunum, ileum and caecum of weaned piglets. The concentration of lactic acid in the jejunum and ileum of the L group was significantly higher ($p<0.01$) on day 3 post-weaning and in the jejunum ($p<0.05$) on day 7 after weaning in comparison with the control. No significant differences were seen between the two groups in the concentration of jejunal and ileal acetic acid. Similarly, changes in the concentrations of caecal short chain fatty acids (SCFAs) and lactate were insignificant. On the other hand, in animals from the LFA group, significantly higher levels of jejunal ($p<0.05$) and ileal ($p<0.01$) acetic acid were recorded in comparison with the control group on day 3 post-weaning. On day 7 after weaning the piglets fed probiotic cheese and flaxseed (LFA group) had significantly increased concentration of acetic acid in the jejunum ($p<0.001$) and ileum ($p<0.01$) in comparison with the control group and L group. Also the ileal concentration of lactic acid was increased in these piglets ($p<0.05$) when compared with the control group. The administration flaxseed with/whitout probiotic cheese (LFA and FA group) had no effect on caecal SCFAs and lactate on day 3 post-weaning. However, on day 7 post-weaning animals from the LFA group showed significantly higher levels of caecal acetic and propionic acids ($p<0.001$) as well as butyric acid ($p<0.01$; $p<0.05$) in comparison with the control and the L group. Piglets fed the flaxseed (FA group) had increased caecal concentrations of acetic, butyric and propionic acids compared to the control or L group (propionic acid) but decreased levels of acetic and propionic acid when compared with the LFA group. The differences in concentration of lactic and acetic acid in the content of the jejunum and ileum between control animals and the FA group were insignificant and in some cases, their levels were lower when compared with the L and LFA groups.

On day 7 post-weaning, a decreased level of pH was recorded in the digestive tract of LFA piglets with significant differences in the colon ($p<0.01$) compared to the control group and a significantly decreased pH in the caecum ($p<0.01$) in comparison with the control and L group. Similarly, low pH levels were recorded also in the FA group with significantly lower levels ($p<0.01$) in the colon compared to the control group and in the caecum compared to the control and L group.

Discussion

The present study compared the effect of the administration of *Lactobacillus plantarum*– Biocenol™ LP96 and flaxseed (alone and in combination) on lactic acid bacteria, *coliforms*, total aerobes and anaerobes, *Bifidobacterium* sp., *Enterococcus* sp. and organic acid levels in the digestive tract of weaned piglets.

As indicated by the results of our experiment, the administration of *Lactobacillus plantarum*– Biocenol™ LP96 affected positively the population of LAB in the jejunum, ileum and caecum of pigs on day 3 post-weaning and in the jejunum on day 7 post- weaning. This was manifested by the increased ratio of LAB: *coliforms* plate count. This could be an explanation of increased concentration of jejunal and ileal lactic acid, the principal fermentation end product of LAB, which was significantly higher when compared to the control group. Lactic acid is the essential microbial metabolite present in the stomach and small intestine of pigs, with lactic acid bacteria such as *Lactobacillus* spp. and *Streptococcus* spp. being the most important producing microbes (Ohashi and Ushida, 2009). Although no significant differences were observed in intestinal pH in the jejunum or ileum, the increased post-weaning production of lactic acid could positively affect intestinal health and prevent disease. These findings are important relative to the management of weaned piglets, because lactate has been shown to have antibacterial effects on *E. coli* and *Salmonella* species (Nout et al., 1989).

The lower abundance of *coliforms* in the jejunum, ileum and colon of *Lactobacillus plantarum* group pigs, compared with the control pigs, supports the view that probiotics inhibit the growth of potential pathogens. The lactobacilli themselves can also colonize the gut mucosa forming a biological barrier to pathogenic microbes. Therefore, the lactobacilli preparations can enhance pig resistance to *E. coli* infection by regulating the balance of microflora. Van Winsen et al. (2001) showed a reduction in faecal *Enterobacteriaceae* by supplementing the diet of pigs with *L. plantarum*. Feeding a spray-dried metabolite of *L. plantarum* decreased *Enterobacteriaceae* in the faeces of post weaning rats (Loh et al., 2009). Flaxseed is very rich in α-linolenic acid, and contains high level of mucilaginous water-soluble polysaccharides. Previously, some studies reported effect n-3 PUFA and prebiotic effect of the mucilage fraction of flaxseed on the intestinal microbiota (Hekmatdoost et al., 2008; Alzueta et al., 2003; Smith et al., 2004).

Despite significantly higher counts of LAB in the jejunum and ileum in animals fed the flaxseed (FA group) compared to control animals (but lower than in the L group) on day 3 post-weaning, more remarkable changes were recorded in the colon and caecum on day 7 post-weaning. These changes were demonstrated by increased lactic acid bacteria and bifidobacteria counts as well as by decreased counts of *coliforms* and total aerobes in comparison with the control and L group. Similarly, Kiarie et al. (2007) reported that inclusion of flaxseed in the diet of piglets shifted microbial activity from the ileum to the hindgut, which resulted in lower faecal pH. Polysaccharides of flaxseed may stimulate the growth and metabolic activity of beneficial bacteria, resulting in production of organic acids or short chain fatty acids, which in turn results in reduced concentration of pathogens or potential pathogens. Higher production of caecal acetic, propionic and butyric acids which resulted in lower pH in the colon and caecum were confirmed by our observations. The increased concentration of acetic acid may be associated with higher counts of caecal bifidobacteria. *Bifidobacterium* spp. and *Bacteroides* spp. are able to effectively utilize oligosaccharides, such as arabinoxylans (Van Laere et al., 2000). The arabinoxylan breakdown has been shown to increase propionate production during *in vitro* studies involving human intestinal microbiota (Hopkins et al., 2003). Moreover, Mori et al. (1997) reported that *Propionibacterium* and some other intestinal bacteria produced growth-promoting factors for bifidobacteria, of which one substance was identified as quinone.

The acquisition of data on the efficacy of synbiotic products (a mixture of probiotics and prebiotics) as feed additives in pigs needs further investigation. However, results of *in vivo* trials are promising, showing a synergistic effect coupling probiotics and prebiotics in the reduction of food-borne pathogenic bacterial populations (Nemcová et al., 2007). Our data indicated that synbiotic supplementation mixture of *Lactobacillus plantarum* – Biocenol™ LP96 and flaxseed on day 7 post-weaning had the most pronounced beneficial effect on the gut microbiota. It might be the combination of probiotics and prebiotics that improves the survival rate of probiotics during their passage through the digestive tract, thus contributing to enhancement of the probiotic effect. Recently, we investigated the influence of administration of flaxseed oil on interaction of *Lactobacillus plantarum*– Biocenol™ LP96 and *E. coli* O8:K88ab:H9 in the gut of germ-free piglets. The administration of flaxseed oil positively influenced the counts of *L. plantarum* in the jejunum and ileum of gnotobiotic piglets, indicating that the intake of these fatty acids may influence the intestinal levels of this bacterial strain (Nemcova et al., 2012). A synbiotic relationship between *Lactobacillus plantarum* – Biocenol™ LP96 and flaxseed could indicate a synergism. The increase in the concentration of SCFAs compared to other groups may indicate indirect fermentation of the end products produced by lactobacilli or direct fermentation of flaxseed by other bacteria. Lactate is normally metabolized to acetate or propionate by lactate-utilizing bacteria and to butyrate by *Megasphaera elsdenii*, some *Clostridium* spp. and *Eubacterium hallii* (Belenguer et al., 2006). An increased butyrate production may be associated with significantly higher numbers of total anaerobes observed in our study. This might be of specific interest since butyrate is used as the primary energy source by colonocytes (Wong and Jenkins, 2007). An increase in the number of lactic acid bacteria and caecal total anaerobes with the accompanying increase in the production of organic acids during the post-weaning period is valuable for the development of protective flora because piglets are vulnerable to pathogenic infections during this period.

Stabilization of microbial community post-weaning is crucial in attending the gut health and reducing the risk of pathogenic infections. Results of our investigations indicated that the combination of lactobacilli and flaxseed may a prospective way of using biological preparations in the prevention of gastrointestinal diseases in weaned piglets.

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Table 1: Ingredient (%) and chemical composition (g/kg DM) of the basal diet

Ingredient %	
Wheat	27.60
Soybean, extr.	22.00
Maize (8.4% CP)	19.70
Barley	17.00
Oat (11.2% CP)	5.00
Powdered whey	5.00
Calcium carbonate	1.10
Mono-calcium phosphate	1.00
Sodium chloride	0.40
Vitamin-mineral premix 0.5% Vitamin/Mineral premix	0.40
L-Lysine HCl	0.35
L-Threonine	0.35
DL-Methionine	0.10
Chemical composition (g/kg DM)	
CP (g)	187.9
ME (MJ)	12.8
Fibre (g)	38.3
Lysine (g)	11.6
Methionine and Cysteine (g)	6.4
Threonine (g)	7.6
Tryptophan (g)	2.3
Choline (mg)	1352
Vitamin A (IU)	11530
Vitamin D_3 (IU)	1500
Vitamin E (mg)	68.7
Vitamin B_2 (mg)	7.1
Vitamin B_{12} (μg)	26.4
Ca (g)	7.5
P (g)	6.2
Na (g)	1.9
Cu (mg)	10.0
Fe (mg)	163.4
Zn (mg)	125.8
Mn (mg)	72.7

DM-dry matter, CP-crude protein, ME-metabolizable energy

Table 2 Fatty acid profile (percentage)

Fatty acid	Flaxseed	Sunflower oil	Basal diet
Lipids(dm basis)	45,8	ND	2,2
Palmitic, C16:0	5,1	6,3	17,4
Stearic, C18:0	3,7	3,2	2,2
Oleic, C18:1	18,4	22,6	24,7
Linoleic, C18:2	16,1	67,9	51,9
Linolenic, C18:3	56,8	0	3,8

ND – not detected

Table 3 Microbiological analysis of intestinal samples of pigs on 3rd day after weaning

Item	control	L group	LFA group	FA group	SEM	*P* value
jejunum						
Lactic acid bacteria	6.68	8.44[a]	8.81[a]	7.35[a,b,c]	0.14	<0.0001
Coliforms	5.57	5.07	5.40	5.49	0.16	0.1603
L:C ratio	1.17	1.72[a]	1.60[a]	1.34[b]	0.04	<0.0001
ileum						
Lactic acid bacteria	6.63	8.85[a]	9.13[a]	7.91[a,b]	0.15	<0.0001
Coliforms	7.66	6.88[a]	7.45	7.29	0.08	0.019
L:C ratio	0.90	1.28[a]	1.20[a]	1.09	0.05	0.001
colon						
Lactic acid bacteria	8.08	8.89	8.74	8.10[b,c]	0.11	0.003
Coliforms	7.92	6.84[a]	7.02[a]	7.85[b]	0.06	<0.0001
L:C ratio	1.01	1.34[a]	1.24[a]	1.04[b]	0.02	<0.0001
caecum						
Lactic acid bacteria	8.15	8.99[a]	9.00[a]	8.32[b,c]	0.08	<0.0001
Coliforms	7.70	7.50	7.77	7.81	0.10	0.2603
Enterococcus sp.	7.49	7.21	7.20	7.30	0.16	0.1905
Bifidobacterium sp.	7.05	7.21	7.43	7.25	0.15	0.1750
Total aerobes	8.47	7.78	8.16	7.83	0.14	0.1003
Total anaerobes	9.61	9.64	9.81	9.28	0.10	0.2360
L:C ratio	1.05	1.23[a]	1.15	1.09[b]	0.03	0.017

Values are mean ± SEM of log CFU/g of wet intestinal content; n=8

[a] significantly different from control group; [b] significantly different from L group

[c] significantly different from LFA group

L:C ratio - ratio between LAB and *coliforms* plate counts

Table 4 Microbiological analysis of intestinal samples of pigs on 7th day after weaning

Item	control	L group	LFA group	FA group	SEM	*P* value
jejunum						
Lactic acid bacteria	7.03	7.96[a]	8.67[a,b]	7.56[c]	0.12	<0.0001
Coliforms	6.85	5.64[a]	5.02[a,b]	6.37[b,c]	0.14	<0.0001
L:C ratio	1.01	1.50[a]	1.72[a,b]	1.20[b,c]	0.07	0.001
ileum						
Lactic acid bacteria	7.05	7.67	8.94[a,b]	7.80[c]	0.16	<0.0001
Coliforms	7.74	8.12	6.39[a,b]	7.50[b,c]	0.17	<0.0001
L:C ratio	0.91	0.93	1.37[a,b]	1.00[c]	0.08	0.021
colon						
Lactic acid bacteria	7.95	8.36	9.02[a,b]	8.84[a]	0.16	<0.0001
Coliforms	8.70	8.62	7.27[a,b]	7.78[a,b,c]	0.19	<0.0001
L:C ratio	0.91	0.97	1.29[a,b]	1.15[a,b]	0.07	<0.0001
caecum						
Lactic acid bacteria	8.22	8.08	8.90[a,b]	8.88[a,b]	0.11	<0.0001
Coliforms	8.64	8.49	7.01[a,b]	7.13[a,b]	0.21	<0.0001
Enterococcus sp.	6.10	6.43	6.50	6.48	0.31	0.181
Bifidobacterium sp.	7.36	7.41	7.94[a,b]	7.88[a,b]	0.28	0.0044
Total aerobes	9.15	8.83	7.83[a,b]	7.90[a,b]	0.19	<0.0001
Total anaerobes	9.48	9.46	10.11[a,b]	9.86	0.12	0.003
L:C ratio	0.93	0.95	1.30[a,b]	1.24[a,b]	0.07	<0.0001

Values are mean ± SEM of log CFU/g of wet intestinal content; n=8

[a] significantly different from control group; [b] significantly different from L group;

[c] significantly different from LFA group

L:C ratio - ratio between LAB and *coliforms* plate counts

Table 5 The concentration of short chain fatty acids (mmol/L) in the content of the jejunum, ileum and caecum of weaned piglets

Item	control	L group	LFA group	FA group	SEM	*P* value
jejunum						
3rd day after weaning						
Lactic acid	12.75	48.52[a]	27.67	19.47[b]	4.31	0.0016
Acetic acid	14.92	20.57	28.08[a]	14.90	3.23	0.0446
7rd day after weaning						
Lactic acid	20.43	59.89[a]	30.51	33.63	4.79	0.0450
Acetic acid	16.61	14.14	30.02[a,b]	18.36[c]	2.97	<0.0001
ileum						
3rd day after weaning						
Lactic acid	14.29	60.30[a]	25.97	18.19[b]	6.32	0.0012
Acetic acid	10.82	21.07	27.53[a]	12.09[c]	6.70	0.0017
7rd day after weaning						
Lactic acid	23.60	43.59	53.62[a]	41.41	8.55	0.0456
Acetic acid	12.80	16.49	36.94[a,b]	10.50[c]	4.74	0.0038
caecum						
3rd day after weaning						
Lactic acid	3.29	4.70	4.28	3.44	1.78	0.4203
Acetic acid	54.40	72.69	82.83	68.18	6.03	0.1020
Propionic acid	30.41	36.41	30.06	28.22	2.56	0.6647
Butyric acid	9.75	9.61	8.50	9.20	1.87	0.8925
7rd day after weaning						
Lactic acid	3.60	3.90	5.78	5.20	0.90	0.1042
Acetic acid	57.55	77.43	108.10[a,b]	79.08[a,c]	4.80	<0.0001
Propionic acid	24.13	21.77	79.98[a,b]	44.07[a,b,c]	5.50	<0.0001
Butyric acid	6.58	16.23	32.95[a,b]	24.27[a]	4.10	0.0019

[a] significantly different from control group; [b] significantly different from L group;

[c] significantly different from LFA group

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