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The effect of the combined probiotic preparation on growth performance, digestibility, microbial composition of intestine and faeces of weaned piglets

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A combined probiotic preparation of *Lactobacillus plantarum* ATCC 14917 $1x10^{11}$ CFU/kg, *Lactobacillus fermentum* DSM 20016 $1x10^{11}$ CFU/kg and *Enterococcus faecium* ATCC 19434 $1x10^{11}$ CFU/kg was supplemented to a basal ration with 100, 150 and 200 mg/kg feed and the effects on growth performance, apparent nutrient digestibility, digesta pH and the total number of microbes (*Anaerobe bacteria, Lactobacilli* spp, *Enterococci* spp, *Escherichia coli*) of defined intestinal segments on thirty two weaned piglets (28 days) were studied for six weeks experimental period. The supplementation of combined probiotic improved slightly daily weight gain (DWG) and feed conversion ratio (FCR), kg feed/kg weight gain. Fibre digestibility was slightly increased and fat digestibility was slightly decreased. Overall a positive effect of the probiotic on growth performance was observed. The results indicate that the probiotic preparation may be less suppressive to the *Escherichia coli.* The effects of the probiotic preparation on the microbial composition of the chyme showed no dose–depended effects. Failure of a dose-response and not clear effects on pH, digestibility, microbial composition in faeces and chyme were possibly caused by combination of different strains. However we recommend the level of 150 mg/kg feed combined probiotic as the optimal dose.

Key words: combined probiotic, microbial composition, digesta, mucosa, faeces, weaned piglet

INTRODUCTION

Probiotics are viable microbial feed supplements, which are believed to stimulate growth and the health as well as to modify the ecology of the intestine in a beneficial manner for the host Männer and Spieler (1997), Breves et al. (2000), Männer et al. (2002), Simon et al. (2003). Possible modes of actions are the modification of the intestinal microorganisms and the nutrient availability with response to the morphology and histology as well as the transport physiology. Significant positive effects of probiotics on performance, health, vitality, gut ecology as well digestibility are observed in many studies, although the mode of action of probiotics is not still completely explained (Jadamus et al. (2000); Solano-Aguilar et al. (2000); Benno et al. (2001); Jadamus et al. (2001); Brooks et al. (2003)). Efficiency probiotic on a focus of combined preparation have hardly been concluded. Therefore the aim of the study was to examine the effects of a combined probiotic preparation *Lactobacillus plantarum* ATCC 14917 $1x10^{11}$ CFU/kg, *Lactobacillus fermentum* DSM 20016 $1x10^{11}$ CFU/kg and *Enterococcus faecium* ATCC 19434 $1x10^{11}$ CFU/kg (AKRON s.r.l-Milano) on performance parameters, nutrient digestibility, pH of defined intestinal segments and, secondly, to show possible influence on the faecal microbial flora.

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MATERIAL AND METHODS

The animal trials were carried out at the experimental station of the Institute of Animal Nutrition of the Free University of Berlin, Germany. Thirty two piglets (White x Duroc) of three litters were transferred after weaning (28 days) to flatdecks and randomly allocated to 4 groups with 8 animals (4 male and 4 female). The basal diet (see Table 1) was either supplemented with 100, 150 and 200 mg/kg of the probiotic preparation or without supplementation (control).

Table 1. Diet composition and calculated nutrient concentration

Diet composition (g/kg feed)		Nutrient concentration (g/kg feed)	
Maize	620	ME (MJ/kg)	12.82
Soyabean meal	275	Crude protein	197.80
Soya oil	50	Crude fat	34.30
Fish meal	30	Crude fibre	31.40
Limestone	10	Calcium	9.10
Monocalcium phosphate	15	Posphorus	7.68
Vitamin -mineral premix[a]	12	Lysine	11.77
L-Lysine	10	Methionine+Cystine	7.64
Methionine+cystine	10	Threonine	8.04
Threonine	10	Tryptophane	2.37
Tryptophane	3		

[a] Contents in 1 kg: 1,200,000 IE vit. A, 120,000 IE vit. D_3, 4000 mg vit. E, 200 mg vit. B_1, 600 mg Vit. B_2, 2500 mg Niacin, 400 mg Vit. B_6, 4500 µg Vit. B_{12}, 20,000 µg Biotin, 1800 mg Pantothenic acid, 160 g Na, 50 g Mg,10,000 mg Zn, 7500 mg Fe, 7500 mg Mn, 150 mg J, 70 mg Co and 40 mg Se.

The diets were offered ad libitum and animals had free access to water. The probiotic preparation included the following strains: *Lactobacillus plantarum* ATCC 14917 $1x10^{11}$ CFU/kg, *Lactobacillus fermentum* DSM 20016 $1x10^{11}$ CFU/kg and *Enterococcus faecium* ATCC 19434 $1x10^{11}$ CFU/kg. During the six weeks period body weight (BW), daily weight gain (DWG) and feed conversion ratio (FCR), kg feed/kg body weight gain were measured weekly. Three piglets from each trial group were euthanised one week after probiotic administration by intracardial injection of T61 (Fa. Hoechst) after sedation with Stresnil*. Immediately after death, the abdomen was opened and ligatures were applied to collect digesta samples for pH measurement in defined segments of the duodenum, jejunum, ileum, caecum and colon. This operation was finished between 12-14 hours after death.

For determination of intestinal bacteria, the "Selective Media" method was used (CATC-agar (Citrat Acid Tween Carbonate - agar base) for *Enterococci* spp, MRS-agar (*Lactobacillus* agar acc to Man Rogosa and Sharp) for *Lactobacilli* spp and Mac Conkey for *Enterobacteria*). The colony of *aerobe and anaerobe* microorganisms by visual numbering were measured on agar plate.

The apparent nutrient digestibility was determined by the indicator method during the last week of the experiment using chromium (III) oxide (0.5%). Data are presented as arithmetic means with standard deviations (Mean ± SD). One-way analysis of variance and Student's t-test ($P< 0.05$) were performed to test the differences between levels of the probiotic in the diet.

* Approved by competent authority according to Council Directive 86/609/EEC of 24 November1986 on the approximation of laws, regulations and administrative provisions of the Member States, regarding the protection of animals used for experimental and other scientific purposes.

RESULTS AND DISCUSSION

The results of the growth parameters are presented in Table 2.

The body weight gain was improved with graded levels of the probiotic preparation from 4.9 up to 31.7%. Caused by the high coefficient of variation the differences were not significant. The FCR (kg feed/kg weight gain) was improved with graded levels by 0.6 up to 7.3%. The differences were not significant. Because of the low dose-response between 150 and 200 mg/kg feed, the level of 150 mg/kg feed seems to be the optimal dose.

The same results showed Lessard and Brisson (1987) on the experiments with weaned piglets, used LFP- *Lactobacillus-Fermentation-Product.* This probiotic contents *Lactobacillus bulgaricus, Lactobacillus casei, Streptococcus thermophilus*, produced in Quebec, Canada. The basal diet was supplemented with 100 mg LFP/kg feed.

The feed intake and the daily weight gain (DWG) were increased respectivly 11.8% and 10.4%, compared with the control group. The feed conversion ratio (FCR) was in the same level.

Table 2. Effects of probiotic preparation on performance parameters (Mean ± SD)

Parameters		Probiotic Dose (mg/kg feed)			
		Control	100	150	200
Production	n[1]				
Initial BW, kg	8	5.6 ± 1.1	5.5 ± 1.1	5.6 ± 1.2	5.6 ± 1.0
BW 6th week[2]	5	19.5 ± 5.1	19.8 ± 5.8	23.1 ± 3.2	22.3 ± 7.0
Feed intake, kg		24.5 ± 7.5	25.4 ± 6.4	29.8 ± 5.4	30.4 ± 7.5
DWG, g[3]		325 ± 153	341 ± 128	427 ± 71	436 ± 123
FCR[4]		1.79 ± 0.48	1.78 ± 0.31	1.65 ± 0.05	1.66 ± 0.15

[1] Number of animals, (8 piglets/ every group, at the beginning of the experiment)

[2] Number of animals, (5 piglets/every group, one week after probiotic supplementation). n = 4 at treatment 150 mg/kg in 6th week.

[3] DWG for whole experimental period.

[4] FCR for whole experimental period.

Hale and Newton (1979) used the same probiotic LFP (*Lactobacillus-fermentation-product*) on the weaned piglets. Pigs fed a diet with 0.36 ml/kg LFP required nearly 10% less feed per unit of weight gain than the control group. Also the incidence of scouring decreased ($P< 0.05$) in pigs fed with different levels of LFP. Overall improvement occurred up through the addition of 0.36 ml/kg LFP with no additional benefit from greater amounts. Pollman et al. (1980) showed the effects of microbial feed additives on performance of starter and growing-finishing pigs. One of the experimental group with weaned piglets was fed with 750 mg *Lactobacillus acidophilus*/kg feed. The second experimental group was supplemented with 1250 mg *Streptococcus faecium*/kg feed.

The addition of *Lactobacillus acidophilus* to the feed of young pigs improved average daily weight gain by 9.7 % and the feed conversion ratio by 21.4%, whereas the addition of *Streptococcus faecium* decreased average daily weight gain. The addition of acid lactic improved feed conversion, suggesting that lactic acid as a metabolite produced during fermentation might be the reason for the improvement in performance. The probiotics had no effect on growing-finishing pigs.

In a trial with 90 untreated and 90 treated *(Bacillus cereus*-preparation) weaned piglets, the probiotic treated animals gained 7% more live weight during 6 weeks after weaning with a reduced feed conversion ratio of 2.4%. However, both results were not significant. This points towards a high variation in the response of the individual animals to this type of feed additives (Jadamus 2001).

Simon et al. (2003) concluded the majority of the experiments show trends toward positive effects, however, the significance level of ($P\leq 0.05$) was reached only in 5% of the experiments. Today, trends without statistical significance are also considered as positive effect. Due to the complexity of the intestine, individual variations of animals to probiotic inclusion may be the rule and not the exception. Considering this concept, the range between no effect and significant effects seem to be reasonable.

Table 3. Effects of probiotic preparation on apparent nutrient digestibility and digesta pH of defined intestinal segments (Mean ± SD)

Parameters	Probiotic Dose (mg/kg feed)			
	N[1]	100	150	200
Digestibility (in %)[2]	5			
Dry matter		73.20 ± 10.39	67.20 ± 2.22	75.70 ± 9.52
Crude fat		71.20 ± 2.60	69.00 ± 9.11	70.00 ± 3.77
Crude fibre		54.50 ± 7.48	52.30 ± 5.79	56.40 ± 2.31
Digesta pH	3			
Duodenum		5.74 ± 0.68	5.87 ± 0.83	6.51 ± 0.77
Jejunum		6.17 ± 0.66	6.29 ± 0.51	6.56 ± 0.85
Ileum[3]		6.43 ± 0.77[b]	6.41 ± 0.16[b]	5.25 ± 0.12[c]
Caecum		5.65 ± 0.20	5.79 ± 0.39	5.55 ± 0.09
Colon		6.19 ± 0.38	6.27 ± 0.37	6.18 ± 0.43

[1] Number of animals

[2] Crude nutrients were determined by Weende scheme

[3] Significant differences, indicated with different superscripts

Feeding probiotic preparation slightly increased the crude fibre digestibility compared to the control group in the range of 3.4%, 1.2% and 5.4% at supplementations with 100, 150 and 200 mg/kg feed, respectively. With graded levels of the probiotic preparation pH of the chyme of ileum and caecum was slightly decreased, in contrast the pH of duodenum and jejunum was slightly increased. The low effect of pH was agreement with digestibility results. The pH results in the duodenum and jejunum is in contrast to former results reported by Männer and Spieler (1997). This is possibly caused by the combination of different strains used in this study.

Hale and Newton (1979) supplemented the diets of growing pigs with LFP preparation (*Lactobacillus Fermentation Produced*) and observed that a supplementation of 0.72 mg LFP/kg feed increased the crude fibber digestibility with 14.2% compared to the control group ($P< 0.05$).

These authors assumed that the rate of passage of feed through the digestive tract was decreased by feeding LFP, which allowed more time for digestion of crude fibre. Also the urinary nitrogen excretion was greater than faecal excretion but both combined were less then intake, thus resulting in a positive nitrogen balance. In total, the digestibility of dry matter was decreased 0.4% and the digestibility of crude protein did not change, compared to the control.

Tortuer (1973) showed the influence of *Lactobacillus acidophilus* in broïler chicks on growth, feed conversion and crude fat digestibility. The addition of *Lactobacillus acidophilus* in broïler chicks diet decreased the digestibility of crude fat.

Table 4. The effect of probiotic preparation on the microbial composition of faeces (CFU*10^6/g wet weight) (Mean ± SD)

Parameters		Probiotic Dose (mg/kg feed)			
		Control	100	150	200
Week					
1st	*Lactobacilli* spp.	95	120	150	170
	Enterococci spp.	0.01	0.94	1.12	1.23
	Escherichia coli.	10	10	32	2
6th	*Lactobacilli* spp.	683 ± 584	223 ± 191	345 ± 403	767 ± 306
	Enterococci spp.	0.018 ± 0.031	0.1 ± 0.131	0.011 ± 0.01	0.028 ± 0.02
	Escherichia coli.	2.35 ± 3.60	15 ± 21.8	0.05 ± 0	0.083 ± 0.057

*Four faeces samples/every group were collected/every week, during the experimental period.

The effect of probiotic preparation on the microbial composition of faeces was examined early, one week after supplementation, because the first week after weaning is critical period for tends to shift the balance of the gut microflora away from beneficial bacteria towards pathogenic bacteria. One week after weaning piglets fed with the probiotic preparation showed increased the concentration of *Lactobacilli* spp. and *Enterococci* spp. compared to the control treatment. Feeding 200 mg probiotic preparation/kg feed induced a reduction of *Escherichia coli*. At the end of the experiment piglets fed with 150 and 200 mg probiotic preparation/kg feed had reduced *Escherichia coli* compared to the control. These results indicate that the probiotic preparation may be less suppressive to the *Escherichia coli*.

Morelli (1995) observed the similar microbial changes in the faeces of weaned piglets, fed with the same combined probiotic preparation.

Table 5. The effect of probiotic preparation on the microbial composition of digesta, one week after probiotic supplementation (log CFU/g wet weight) (Mean ± SD; n = 3)

Parameters		Probiotic Dose (mg/kg feed)			
		Control	100	150	200
Jejunum	*Anaerobe bacteria.*	13.92 ±14.15	12.22 ± 12.45	8.75 ± 8.60	12.98 ± 13.07
	Lactobacilli spp.	10.24 ± 10.44	12.58 ± 12.78	8.36 ± 8.38	11.60 ± 11.55
	Enterococci spp.	7.02 ± 6.98	8.03 ± 8.22	7.00 ± 7.19	7.01 ± 6.97
	Escherichia coli.	7.57 ± 7.74	8.60 ± 8.72	6.00 ± 0.00	7.90 ± 8.02
Ileum	*Anaerobe bacteria.*	13.17 ± 13.36	13.21 ± 13.20	13.21 ± 13.20	12.60 ± 12.72
	Lactobacilli spp.	12.87 ± 13.11	12.69 ± 12.73	12.72 ± 12.95	13.68 ± 13.89
	Enterococci spp.	6.00 ± 0.00	8.82 ± 9.06	7.33 ± 7.55	7.02 ± 7.22
	Escherichia coli.	8.17 ± 8.17	11.00 ± 11.23	12.01 ± 12.25	12.05 ± 12.23
Caecum	*Anaerobe bacteria.*	13.90 ± 13.85	12.69 ± 12.84	13.75 ± 13.87	13.98 ±14.12
	Lactobacilli spp.	13.28 ± 13.48	12.60 ± 12.84	13.43 ± 13.65	13.83 ± 14.05
	Enterococci spp.	6.86 ± 7.04	10.00 ± 10.23	7.80 ± 8.03	6.84 ± 6.70
	Escherichia coli.	12.69 ± 12.93	10.00 ± 10.23	10.82 ± 11.06	10.86 ± 11.04
Colon	*Anaerobe bacteria.*	14.72 ± 14.92	13.04 ± 13.06	13.95 ± 14.18	13.93 ± 14.15
	Lactobacilli spp.	12.55 ± 12.49	13.01 ± 13.23	13.84 ± 14.08	13.92 ± 14.10
	Enterococci spp.	8.82 ± 9.06	9.00 ± 9.23	12.01 ± 12.25	9.12 ± 9.36
	Escherichia coli.	13.44 ± 13.68	11.30 ± 11.53	12.69 ± 12.93	12.39 ± 12.59

The effects of the probiotic preparation on the microbial composition of the chyme showed no dose–depended effects. However there was a tendency for increasing of the concentration of *Lactobacilli* spp. and *Enterococci* spp. in the colon compared to the control.

Barrow et al. (1980) supplemented the pig diets with a combination of *Lactobacillus fermentum* 14 and *Streptococcus salivarius* 312 for 4 days and observed a significant reduction in the *Escherichia coli* count in both the stomach and duodenum. A significant reduction of *Escherichia coli* number in the stomach was also found, when *Lactobacillus fermentum* was supplemented separate. In cases of diarrhea caused by *Escherichia coli* the treatment as described here was not effective because the count of *Escherichia coli* in the duodenum of culture-fed pigs was still greater than 10^6/g. However, if the antibacterial effect of strain 14 could be increased some effect on scouring due to *Escherichia coli* should follow. This might be accomplished by the feeding of large numbers of organisms or by the administration in a concentrated form of the inhibitory factors produced by *Lactobacillus fermentum* strain 14.

Gedek et al. (1993) showed that the application of 10^8 colony forming units (CFU) of a *Bacillus cereus* preparation/kg feed to piglets reduced counts for *Lactobacilli* spp. *Bifidobacteria*, *Eubacteria* and *Escherichia coli* in the duodenum and jejunum, but increased respective CFU in the ileum, caecum and colon.

Männer and Spieler (1997) showed a significant reduction of *Escherichia coli* CFU in the small intestine of piglets was also noted when an *Enterococcus faecium* preparation was applied. However, at the same time *Lactobacilli* spp. and *Enterococci* spp. counts increased as a trend and statistically significant, respectively (Jadamus et al. 2000).

The results of studies on the ability of probiotic bacteria to reduce the colonization of pathogenic bacteria are ambiguous. Challenge studies with piglets and *Escherichia coli* O141:K85 showed no influence on clinical symptoms, mortality or excretion of hemolytic *Escherichia coli* (De Cupere et al. 1992).

Jadamus et al. (2000) showed that the colonization with mucosa associated *Enterobacteria* spp. was reduced when a probiotic *Bacillus cereus* preparation was supplemented.

The probiotic had no influence on the occurrence of pathogenic *Escherichia coli* as measured with a PCR assay (Goebel et al. 2000). These results point to the fact that hygienic conditions in scientific institutes may sometimes be too favorable to investigate effects of pathogenic bacteria without challenge trials (Simon et al. 2003).

These and the other studies imply that probiotics are able to reduce/enhance specific bacterial groups, but the reduction of total bacterial cell numbers as recorded for antibiotics is probably not a probiotic mode of action. In order to understand the casual relationships which lead to the observed improvements in weight gain and feed conversion or general health of animals, possible interactions between bacteria in the intestine and host animal must be studied. Of special significance are interactions between the metabolism of the host and metabolic activity of intestinal bacterial populations (Simon et al. 2003).

CONCLUSIONS

The supplementation of the combined probiotic preparation induced slightly the performance data. However the differences were not significant. Feeding probiotic preparation slightly increased the crude fibre digestibility in all treated groups. With graded levels of the probiotic preparation pH of the chyme of ileum and caecum was slightly decreased, in contrast the pH of duodenum and jejunum was slightly increased. The probiotic preparation showed increased the concentration of *Lactobacilli* spp. and *Enterococci* spp. compared to the control. The results indicate that the probiotic preparation may be less suppressive to the *Escherichia coli*. The effects of the probiotic preparation on the microbial composition of the chyme showed no dose–depended effects. However there was a tendency for increasing of the concentration of *Lactobacilli* spp. and *Enterococci* spp. in the colon compared to the control. Possibly this was due to the combined probiotic preparation. At the end, we recommend the level of 150 mg/kg feed combined probiotic as the optimal dose.

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