EFFECTS OF SIMULTANEOUS ADMINISTRATION PROBIOTIC AND ANTIBIOTIC ON DIGESTIBILITY IN PIGLETS

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Abstract

In our 13-day experiment, piglets weaned at the age of 28 days and divided into two groups of six piglets each were used. The piglets were fed *ad libitum* with a commercial mix for early weaning of piglets, and had unrestricted access to water. For the experimental group (E), a feed mix with *Bacillus toyoi* spores in the final concentration 1.10^3 CFU per 1 g of feed mixture was used. A total of 8g Colistin sulphas per 100 kg live weight were administered in 2 daily doses by adding them to the feed in the trough. Piglets in the control group (C) received feed mix with no supplements. During the experiment, weight of piglets was monitored individually and the amount of feed consumed was monitored per group. Digestibility was determined by an analysis of faeces collected in boxes on days 5, 6 and 7 of the experiment, and adjusted according to the content of sand in the faeces and the feed. During the experiment, the incidence and intensity of diarrhoeas and changes in health were monitored in individual piglets. At weaning and on subsequent days, rectal swabs were taken to monitor the incidence of selected bacterial agents of infections and of the probiotic strain *B.toyoi*. Bacteriological examination was made by selective cultivation.
The comparison of parameters monitored in the two groups of piglets clearly shows that experimental piglets received more feed (505g) compared with the control group (346 g), which is a difference of 46.5 %. While the mean weight gain of piglets in the experimental group was 209.2 g/day, it was only 205.4 g/day in the control group. All the parameters of feed mix digestibility determined at the end of the first week of experiment were higher in the controls. The admixture of tyocerin and colistin had the greatest effect on the digestibility of crude fat (C= 56.32 %, E= 47.85 %) and crude fibre (C= 52.58 %, E= 48.24 %), but the differences found are not statistically significant. No case of diarrhoea was reported in any of the piglets from either group during the experiment.
It is no surprise that no weight increases in piglets receiving toyocerin and colistin simultaneously with their feed were found in the feeding experiment and that substantially higher feed consumption per kg of weight gain was found instead.

Introduction

In response to the increase in the prevalence of *E. coli*-related diarrhoeas among weaned piglets in the Czech Republic in 2000 and 2001, colistin administered in water or feeds began to be used with increasing frequency instead of aminoglycosides. On many swine farms, colistin became the drug of choice in the prevention of enteritis caused by pathogenic *E.coli* strains. Furthermore *in vitro* test have demonstrated that colistin (polymyxin E) is also highly active against some other species of Gram- negative bacteria present in swine intestine, such as *Salmonella spp., Campylobacter jejuni, Klebsiella pneumonie, Pseudomonas aeruginosa* and *Fusobacterium spp*., but not against *Proteus spp., Providentia spp.* (PRESCOTT, 2000) and all Gram - positive bacteria including (*B.cereus*), which are colistin resistant. (Colistin administered per orally to piglets passes practically unabsorbed through their gastrointestinal tract, with over 90% of it being excreted in faeces where it is bound to bacterial phospholipid membranes).
When analysing reasons for the poor effectiveness of the therapeutic use of colistin on some pig farms, we repeatedly found that also toyocerin (probiotic spores of *Bacillus cereus var. toyoi*) was administered in parallel as a feed supplement to piglets during pot-weaning period.

Toyocerin was supplemented to feed mixtures as a preventive measure aimed at suppressing clinical symptoms of colibacilosis and reducing post-weaning gain losses (SIMON et al., 2001). Experience of farmers and veterinary practitioners on the farms, however, showed that the desired effect was not obtained, and doubts about the usefulness of this combination prevailed. The aim of this 13-day experiment was to compare a group of weaned piglets receiving toyocerin and colistin in the feed with a control group receiving no additives or antibiotics in their feed.

Material and methods

Piglets from two litters weaned at 28 days of age and divided into two groups of 6 each were used in the experiment. Piglets were fed *ad libitum* with a commercial feed mixture for early piglet weaning (Table 1) with free access to drinking water. The feed mixture for the experimental group contained 0.1 kg toyocerin per 1 tonne of mixture, i.e. the final concentration was 1.10^3 CFU per 1 g of feed mixture. Added to the feed in the trough, colistin sulphate (polymyxin E - bactericidal antibiotic produced by *Bacillus colistinus*) was administered 2x daily at a total daily ration of 8g per 100 kg live weight.

Piglets in the control group were fed the same mixture with no supplements. In the experiment, the weight of individual piglets and the amount of feed consumed by each of the two groups were monitored. Digestibility was determined by an analysis of faeces collected in boxes on days 5, 6 and 7 of the experiment by a conversion to the HCl-insoluble ash portion in the faeces and the feed. During the experiment, the incidence and intensity of diarrhoeas and changes in health were monitored in individual piglets. At weaning and on subsequent days (2, 5, 7, 9, 12 and 13), rectal swabs were collected from individual piglets to monitor the incidence of normal and pathogenic *E.coli* strains and other selected bacterial causative agents of piglet diarrhoea. Selective cultivations were used monitor the shedding of the probiotic *B.cereus var. toyoi* strain. Rectal swab specimens were transported in commercial transport medium Amies (Copan Italy). For direct isolations of bacteria from swab specimens, blood agar made by adding ovine blood to the base (Columbia blood agar base CM331, Oxoid) was used. The Mac Conkey agar No.3 (CM115, Oxoid) and the selective agar Bacillus cereus selective medium (Oxoid) were also used. The number of the bacteria isolated was determined semi quantitatively on the basis of typical morphology of the colonies. *E.coli* isolates with haemolytic activity on blood agar were identified biochemically using the commercial set ENTEROtest24 (PLIVA-Lachema Diagnostika), and further typed for the presence of fimbrias K88 using the specific antiserum (ALEXA et al., 1992). *B.cereus* isolates were also identified biochemically. The probiotic strain was identified by the PCR method for the gene assay (GRANUM et al., 1999).

Furthermore, to prove the identity of the probiotic strain *B.cereus var. toyoi* in rectal swabs, an antibiotic phenotype was used whereby every isolate was examined by the disk diffusion test for susceptibility to 12 selected antibacterial substances according to the NCCLS specific antibiotic phenotype confirmation protocol.

Digestibility was determined by an analysis of faeces collected in boxes on days 5, 6 and 7 of the experiment. The digestibility was determined by calculating the content of nutrients in the feed and faeces concerning the content of the marker (insoluble portion of ash). The content of dry matter, crude protein, crude fat, crude fibre, ash and organic matter were determined. To determine the dry matter content, specimens were dried at 105°C for 4 hours and weighed. To calculate he crude protein content in samples, nitrogen content was first determined by Coulometric titration and then used in the formula: crude protein = N x 6.25. The fat content was determined by weighing the remains of extraction by diethyl ether. The fibre content was determined as the solid matter remaining after acid and alkaline hydrolysis by sulphuric acid and sodium hydroxide solution, respectively, and after weighing the ash content. The ash content was calculated by weighing the remaining part after complete combustion of organic substances at 550°C. The content of insoluble portion of ash in HCl was determined by weighing the ash remaining after dissolving it in diluted hydrochloric acid. At the same time, a laboratory analysis of the feeding mixture for early piglet weaning used in the experiment was performed (Table 2).

Tab. 1.: Composition of feeding mixture for early piglet weaning

Wheat	%	16.1
Barley	%	30.0
Corn	%	25.0
Soybean meal	%	20.0
Fish Flour	%	4.0
Vegetable Fat	%	1.3
Mineral and vitamin supplement	%	3.6
Calculated content of amino acids		
Lysine	g/kg	13.1
Methionine	g/kg	3.7
Sulphur amino acids	g/kg	7.0
Threonine	g/kg	8.6
Tryptophan	g/kg	2.5

Tab. 2.: Content of nutrients in the feeding mixture

Dry matter	g/kg	888.13
Crude protein (N * 6.25)	g/kg	202.09
Crude fat	g/kg	50.22
Crude fibre	g/kg	37.17
Ash	g/kg	48.49
ME_P	MJ/kg	14.41

Results

A comparison of parameters monitored for 13 days in piglets of the two groups (Table 3) showed that the quantity of feed consumed by piglets in the experimental group was greater by 46.5% than the quantity of feed consumed by piglets in the control group (505 g and 346 g, respectively). Checks of individual piglets' weight during the experiment showed that all piglets of the control group gained weight. In the experimental group, only 5 piglets showed weight gains. The mean daily weight gain of piglets in the control group over the experimental period was 205.4 g/day compared with 209.2 g/day in piglets from the experimental group.

Tested at the end of the first week of the experiment, all indices of feed mixture nutrients digestibility were higher in the control group piglets (Table 4). The parameters most affected by the addition of toyocerin and colistin were digestibility of fat (C= 56.32 %, E= 47.85 %) and of fibre (C= 52.58 %, E= 48.24 %), but the differences found were not statistically significant.

No diarrhoea symptoms were recorded among the piglets of the two groups at any time of the experiment.

Sporadic findings of a haemolytic *E.coli* strain were made by cultivation of repeatedly collected rectal swabs in the experimental group on days 2 (1 piglet) and 12 (1 piglet) of the experiment. In the control group, haemolytic isolates of *E.coli* were demonstrated in a total of nine cases. The findings were made repeatedly, at least twice in three piglets of the 6 tested. In one piglet, a haemolytic *E.coli* strain was found repeatedly on five occasions, and in the last three collections (days 9, 12 and 13) the haemolytic strain incidence was dominant compare to remain bacteria. Antigen K88 production was not demonstrated in any of the haemolytic *E.coli* isolates in either of the groups of piglets. Using selective isolation designed to assay the probiotic *B.cereus var. toyoi* strain, we were able to demonstrate the microorganism in rectal swab collected from all piglets of the experimental group from day 2 to day 13 of the experiment. The quantities found in rectal swab specimens were in the range 10^1 to 10^2 CFU per rectal swab. At this stage, the excretion in faeces from piglets in the experimental group was continuous without significant variations. In the rectal swabs of control group piglets, on the other hand, no *B.cereus var.toyoi* was demonstrated.

Tab. 3.: Weight gains and feed conversion ratio

		Control	**Experiment**	**Index (C = 100 %)**
Number of piglets		6	6	
Starting weight kg	x	7.30	7.30	100.00
	SD	0.84	0.89	
Daily weight gain g	x	205.4	209.2	101.85
	SD	148.2	152.3	
Feed conversion ratio kg/kg	x	1.685	2.420	143.62
Feed intake	g/piglet/day	346	506	146.24

Tab. 4.: Digestibility coefficients (%) of control and experimental groups

		Control	**Experiment**	**Index (C = 100 %)**
Dry matter	x	80.07	78.02	97.44
	SD	0.86	0.51	
Crude fat	x	56.32	47.85	84.96
	SD	3.21	1.59	
Crude protein	x	72.31	68.67	94.97
	SD	1.27	2.09	
Crude fibre	x	52.58	48.24	91.75
	SD	3.67	5.48	
Organic matter	x	81.60	79.48	97.40
	SD	0.84	0.62	
Ash	x	53.55	52.65	98.32
	SD	2.18	2.39	

Discussion

It was not surprising to find that no weight gains were recorded in a feeding experiment where piglets were fed toyocerin- and colistin-supplemented feed. It is true that probiotics are used in animal nutrition, compared to humans, as feed supplements that are supposed to rapidly affect certain parameters, particularly to increase weight gains and prevent diarrhoeas-related losses in finishing operations. But reports of statistically significant improvements in weight gains in piglets or in feed conversion are rare. In a recent study, 35-day old piglets from the control group weighed more than piglets from a group fed the probiotic *B.cereus var. toyoi* strain (TARAS et al., 2005). Comparing the experimental and the control groups in our study, the most surprising was the finding of significantly higher feed consumption per kg weight gain in piglets whose feed was supplemented with a combination of an antibiotic and a probiotic. We believe that the increased feed uptake was a manifestation of an effort to compensate for lower feed conversion rates indicated by reduced digestibility of fat and fibre. Positive effects of toyocerin alone on feed conversion have been reported in a number of studies (KRYIAKIS et al., 2003). Positive effects have also been reported in a recent study, but only referred to piglets from the age of six weeks onwards (TARAS et al., 2005). Feed conversion effects of colistin alone, on the other hand, have been described rarely, and predominantly as negative, particularly regarding the decrease in proteosynthesis. In our opinion, none of the studies reported a combined use of toyocerin and colistin that might have provided a selective advantage for the propagation of the probiotic *B.cereus var. toyoi* strain other than the intrinsic bactericidal effects on *E.coli* and some other G –bacteria in the small intestines and the colon. That strains, moreover, has natural resistance against colistin also because the antibiotic is the product of another, related species, *Bacillus colistinus* strain. The selective advantage of the colistin presence for the probiotic strain was probably manifested by an increase in its numbers in the intestine content, and subsequently by its uninterrupted and constant excretion in faeces of all piglets from the experimental group. Because, however, there was no spontaneous outbreak of a diarrhoeal disease among control group piglets attributable to enterotoxigenic strains of *E.coli (K88+)* in our study either, no conclusions can be drawn in this respect.

From the incidence rate of haemolytic *E.coli (K88-)* strains we may only assume that the propagation of this indicator species was suppressed to such an extent that no repeated incidence was recorded. These isolates showed susceptibility against colistin in the disk diffusion test, which is evidence of unstable levels of colistin in intestinal content. Using a very sensitive technique for the assay of *L.intracellularis* in excrements, we can rule out the existence of proliferative enteropathy in piglets of the two groups, or any negative effect of that infection on feed conversion.

In real life situations on pig farms we have also come across colibacilosis in piglets receiving a diet supplemented with the probiotic *B.cereus var.toyoi* strain and treated at the same time with one of aminoglycosides dissolved in water. In those cases, however, the probiotic strain was just as affected with the bactericidal action as the pathogenic *E.coli* strains. If probiotic strains in feed mixture supplements are to be exploited to their full potential, producers of feed mixtures – and of probiotics in particular – should clearly state which antibiotics are contraindicated with regards the propagation of the probiotic strain in the intestinal tract.

Conclusion

A parallel dietary administration of toyocerin and colistin to an experimental group of piglets over a post-weaning 13 day-period caused an increase in feed consumption compared with the control group. While there were practically no differences between mean weight gains in the two groups (0.5%), there was a decrease in feed digestibility in the experimental group. It follows from the results that effects in those indices were not potentiated. The parallel administration of colistin did, however, provide a selective advantage for the propagation of the probiotic *B.cereus var. toyoi* strain, which was then excreted regularly in excrements from day 2 to day 13 of the experiment. The occurrence of haemolytic strains of *E.coli* (K88-) in the experimental group was only sporadic compared with the controls, which indicates variations in colistin levels in the piglets' individual intestinal compartments.

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