ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LXI 205 Number 6, 2013

http://dx.doi.org/10.11118/actaun201361061839

EFFECT OF PROBIOTICS IN THE PIG NUTRITION ON THE PATHOGENIC BACTERIA COUNTS IN THE GUT

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Received: June 26, 2013

Abstract

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The objective of the present study was to evaluate the efficacy of a probiotic culture in the digestive tract of sows. Two groups of healthy sows with 40 animals each were fed a standard feed three weeks after weaning. From the beginning of the fourth week (day 0), one group received the same diet enriched with the probiotic monoculture of *Enterococcus faecium* SF 68, the second (control) group was given the same diet without probiotics. The samples of faeces were collected (to the sterile containers) at days 0, 15, 30 (end of the probiotic consumption) and 40 (end of the wash-out period), respectively. In the probiotic group, significant decrease ($P < 0.05$) of numbers of *E. coli* and *Clostridium* spp. in faeces was found. The results indicate a positive effect of probiotics consumption on the digestive tract of sows and it can be used for a decrease of the incidence of the diarrhoeic diseases that are frequent in the pig husbandry.

probiotics, intestinal microflora, pigs, *Enterococcus faecium*

Lactobacilli, bifidobacteria, streptococci, *Clostridium perfringens* and *Escherichia coli* are the main microorganisms of the pig digestive tract (Metzler, Mosenthin, 2008). Beneficial bacterial cultures ingested within feed are able to proliferate and colonize the gut. These probiotic cultures adhere to the gut mucosa and compete for the binding sites with pathogenic bacteria, such as *Escherichia coli* or *Clostridium perfringens* A. However, probiotic microorganisms colonize the gut only transiently. Therefore, regarding the maintenance of the stable level in the intestine, probiotics should be a part of every feeding ration (Václavková, Lustyková, 2011).

Probiotics are live microorganisms with beneficial effects on the host's health. The stability of the probiotic preparations is their key characteristic and therefore viability of probiotics can not be compromised after oral intake (Kauer *et al.*, 2002; Isolauri *et al.*, 2004).

Genus *Enterococcus* belongs to the lactic acid bacteria and it is a regular constituent of the digestive tract. Some species of this genus, namely *E. faecium* and E. *faecalis*, are used as probiotics both in humans and in animals due to their ability to adhere on the gut mucosa and their resistance to the broad spectrum of antibiotics. These species are often used also in the form of the pharmaceutical preparations. In humans, probiotics are administered for treatment of diarrhea, irritable bowel syndrome, decreasing plasma cholesterol level and boosting the host's immune system (Lojanica *et al.*, 2010).

In animals, enterococcal probiotics are used mainly for prevention and treatment of diarrhea, immune stimulation and improvement of growth. Probiotics can be advantageously used in farm animals for reduction of the zoonotic pathogenic agents occurring in the digestive tract (Franz *et al.*, 2011; Gaggia *et al.*, 2010). It is evident that probiotics have a positive effect on the animal performance and consequently on an economic profit of a breeder (Václavková, Lustyková, 2011).

Results of the many recent experiments (Lojanica *et al.*, 2010; Maré, 2009; Simon, 2005; Steiner, 2009) confirm favourable effect of probiotics on growth of sows after weaning and feed intake and conversion. Application of probiotics in the fattening pigs improves daily weight gains and nutrients conversion (Václavková, Lustyková, 2011).

The objective of the present experiment was to evaluate an effect of the probiotic culture of *Enterococcus faecium* SF 68 on the optimal feed utilization and intestinal colonization in sows.

MATERIALS AND METHODS

The research was focused on evaluation of an effect of the probiotic strain *Enterococcus faecium* SF 68 on counts of *Escherichia coli, Escherichia coli* O157:H7 and *Clostridium* spp. including *Clostridium perfringens* A isolated from the digestive tract of the weaned sows.

Sows of the hybrid Czech Improved White x Landrase x Durock in the mean initial live weight of 27 kg at the age of 9 weeks (at the start of the experiment), originating from the private South Moravian farm were used in the experiment. Sows were kept in the pens with the area of 24 m^2 with 20 animals each. The average temperature and relative humidity in the stable was 18 °C and 70% respectively. Sows were divided in two groups with 40 animals each: experimental probiotic group and a control group without probiotics. Both groups were fed the same standard feed mixtures A1 and A3 for the first three weeks of fattening. From the fourth week, for the time interval of 30 days, the daily ration of the experimental group was enriched with probiotic preparation of *Enterococcus faecium* SF 68 (NCIMB10415); the content in the ration was $1x10^9$ CFU/g. One metric ton of the feed mixture was supplemented with probiotic *Enterococcus faecium* SF 68 in the amount of 35 g/day.

Faeces were taken in both groups of sows at day 0 (end of the third week of fattening by the standard feed mixture), day 15 (middle of the supplementation of the experimental group with probiotics), day 30 (end of the supplementation of the experimental group with probiotics) and day 40 (10 days after discontinuation of probiotics intake in the experimental group). Faeces were taken to the sterile containers (Vitrum, Czech Republic).

Ten grams of the aseptically taken excrement samples were homogenized in the propagation medium Nutrient Broth Peptone (Himedia; India) and it was used in microbiological analysis. Spread plate method was used for enumeration of the following groups of microorganisms: species *Enterococcus faecium* on the HICrome medium *E. faecium* (Himedia, Italy) with the addition of the *E. faecium* supplement (Himedia, Italy) at the temperature of 37 °C for 48 h aerobically; genus *Clostridium* on the medium Anaerobic Agar (Himedia, Italy) at the temperature of 37 °C for 48 h anaerobically; species *Escherichia coli* on the TBX Agar (Noack, France); species *Escherichia coli* O157:H7 a *Clostridium perfringens* were determined in microbiology laboratory Mendel University in Brno.

After cultivation was finished, typical colonies grown on the Petri dishes were counted and the results were expressed as colony-forming units (cfu)/g. PCR procedures described by Dutka-Malen *et al.* (1995) and Hu *et al.* (1999) were used for the genus and species identification.

Microbiological traits were measured in two repetitions in each faecal sample. The means of these two measurements were used for statistical evaluation. Software Statistica 8 (StatSoft Inc., Tulsa, OK, USA) was used for calculation of the basic statistical characteristics, regressions (including significance testing of the linear and quadratic term) and differences between groups regarding microbial counts (one-way classification of the variance-ratio test, including post-hoc Tukey's HSD test).

RESULTS AND DISCUSSION

Verification of the declared counts of the probiotic strain in the feed mixture

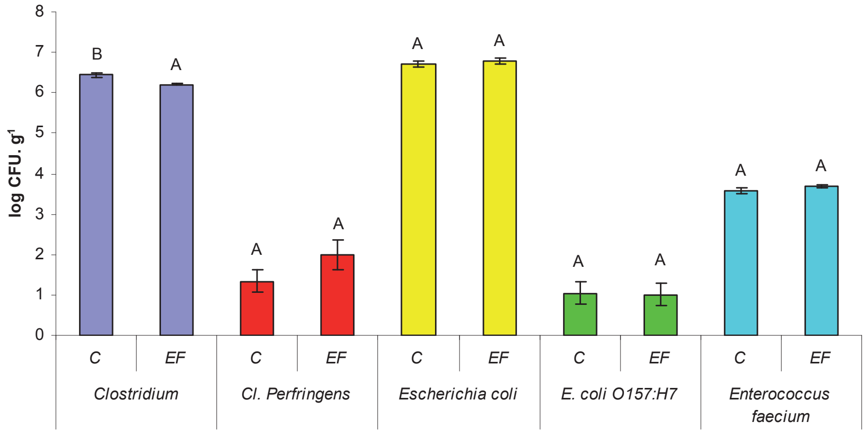
Counts of the probiotic strain *Enterococcus faecium* SF 68 in the experimental feeding mixture were determined by the same method that it was used for analysis of the sow faeces. The counts was found at the level of 1.5×10^9 cfu/g and it was in accordance with counts declared by the producer.

Counts of the tested microorganisms in the sow faeces

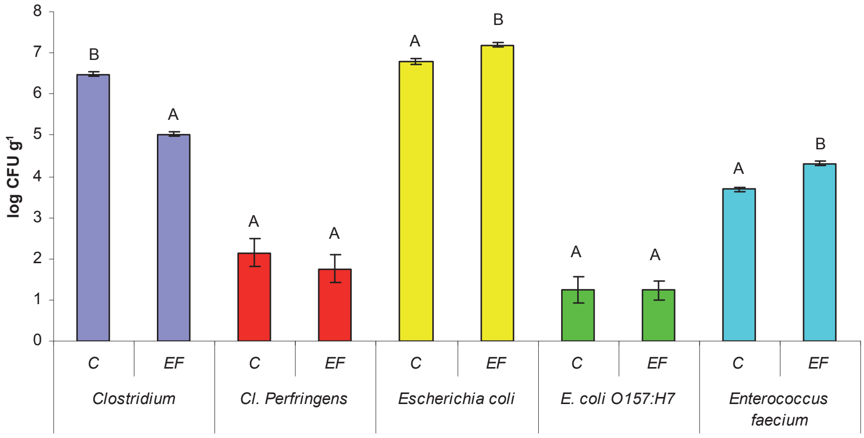
E. coli O157:H7 and *Clostridium perfringens* A were detected in both groups of sows already at the beginning of the experiment (day 0, Fig. 1), which is very undesirable in the pig breeding due to the high pathogenicity of the aforementioned species. Significant differences ($P<0.05$) between experimental and control group of sows were established only in the case of the genus *Clostridium*.

At the day 15, a positive effect of the probiotic mixture was already found in experimental group in comparison to control, namely in the genus *Clostridium* and the species of *E. coli* (Fig. 2); significant increase ($P < 0.05$) of the counts of the species of *Enterococcus faecium* was also established at this phase of the experiment. Therefore, a positive effect of an application of the probiotic feeding mixture on the occurrence of the pathogenic microorganisms was confirmed. It is very important that the pathogenic microorganisms play an important role in an incidence of the diarrhoeic diseases in the weaned sows (Franz *et al.*, 2011).

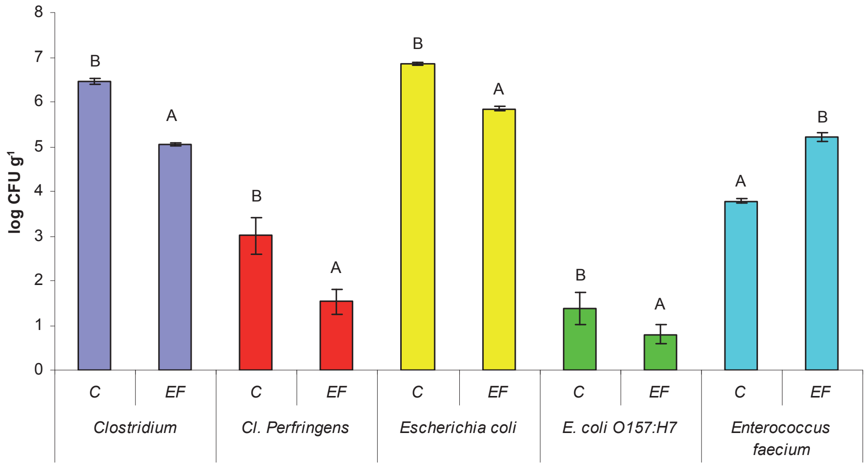
It is evident from Fig. 3 that counts of the *Clostridium* spp., *Clostridium perfringens*, *E. coli* and *E. coli* O157:H7 decreased ($P < 0.05$) in faeces of the experimental group as compared to the control



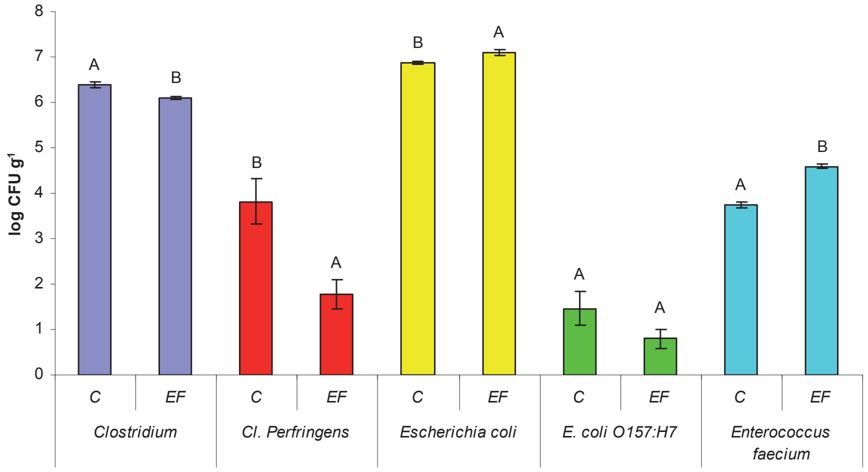
1: *Counts of the tested microorganisms (log cfu/g) in faeces of the control (C) and experimental (EF) group of sows at the beginning of the experiment (day 0)*
Data are mean and indicate significant differences (A: P < 0.01,B: P < 0.05). Comparisons were made between the experimental group and control group.



2: *Counts of the tested microorganisms (log cfu/g) in faeces of the control (C) and experimental (EF) group of sows in the middle of the probiotic administration (day 15)*
Data are mean and indicate significant differences (A: P < 0.01,B: P < 0.05). Comparisons were made between the experimental group and control group.



3: *Counts of the tested microorganisms (log cfu/g) in faeces of the control (C) and experimental (EF) group of sows at the end of the probiotic administration (day 30)*
Data are mean and indicate significant differences (A: P < 0.01,B: P < 0.05). Comparisons were made between the experimental group and control group.



4: *Counts of the tested microorganisms (log cfu/g) in faeces of the control (C) and experimental (EF) group of sows ten days after discontinuation of the probiotic administration (day 40)*
Data are mean and indicate significant differences (A: $P < 0.01$, B: $P < 0.05$). Comparisons were made between the experimental group and control group.

group. Regarding species *E. faecium*, significant increase of its counts established at the day 15 still persisted ($P < 0.05$) at the end of the administration period (day 30; Fig. 3).

The effect of the probiotic culture of *Enterococcus faecium* SF 68 on the decrease of the deleterious bacteria still persisted ten days after the administration. It was demonstrated by significant differences ($P < 0.05$) between C- and EF-group (Fig. 4). Only one exception was *E. coli* O157:H7, where the significant differences between groups of sows were not established.

The final effect of probiotics on the animal performance and consequently on an economic profit of a breeder is an important measure for their evaluation in the pig nutrition. The present study found a positive effect of the probiotic culture of *Enterococcus faecium* SF 68 added to the feed mixture on the growth performance of sows, daily live weight gains and nutrients conversion, respectively. However, significant differences ($P < 0.05$) between C- and EF-groups were established only at the end of the experiment (day 40). The same results reported also Veizaj-Delia *et al.* (2010). According to Jorgensen and Hansen (2006), probiotics administration to sows in the period from two weeks *ante partum* till weaning affected positively the losses of sows until weaning.

Number of studies (e.g. Franz *et al.*, 2011) demonstrated a favourable effect of probiotics on the composition of the gut microflora, namely an increase of the microorganisms favourably influencing host's health and a decrease of the potentially deleterious microorganisms, which was confirmed also in the present study.

Administration of probiotics can play an important role in constipation, diarrhoea, intestinal inflammations and boosting the resistance of an organism (Viet *et al.*, 2009). The present study (Figs. 2–4) demonstrated not only the increase of counts (cfu/g) of the probiotic strain *E. faecium* SF 68 in the course of the whole experiment, but also the growth reduction of the tested potentially pathogenic bacteria *E. coli* and *Clostridium* spp. These bacteria can cause various infections and diarrhoeic diseases that are very undesirable in the pig farming from the economic viewpoint (De Angelis *et al.*, 2007).

Despite the positive effects of the probiotic enterococci on the pathogenic bacteria (*E. coli*, *Clostridium* spp.) in the farm animals, the studies dealing with this topic are very exceptional in the available literature (Szabó *et al.*, 2009). Taras *et al.* (2007) and Apás *et al.* (2010) reported an increase of numbers of enterococci, where a positive effect in mice and goats was observed.

SUMMARY

Results of the present study indicate a positive effect of the addition of the probitic strain *Enterococcus faecalis* SF 68 on the composition of the intestinal microflora in pigs. A favourable increase of numbers of *E. faecium* was observed in the course of the present experiment with the concomitant decrease of the counts of the tested pathogenic microorganisms (*E. coli*, *Clostridium* spp.) that are the agents of both the diarrhoeic and other diseases of pigs. Moreover, a positive effect of the tested probiotic strain was demonstrated regarding the growth performance of sows, daily weight gain and nutrients conversion rate, which are the important markers of the economics in the pig fattening.

Acknowledgement
This research was supported by grant IGA MENDELU TP5/2012.

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