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EFFECT OF PROBIOTICS AND HERBALS ON HEALTH AND SHEDDING OF RESISTANT *ESCHERICHIA COLI* IN PIGLETS

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Abstract
The purpose of this study was to evaluate the effect of probiotics, herbals and buckwheat bran (*Fagopyrum esculentum* L.*)* on growth, profile of blood, gut microbiota, profile of fatty acid in meat and shedding of resistant *Escherichia coli (E. coli)* in piglets. A total of 44 piglets *(Sus scrofa domesticus)* from age of day 14 to 56 were divided into 4 groups. Control received basal diet (group C), basal diet + probiotics (group P), basal diet + 3% buckwheat bran (group PB) and basal diet + 1.5% herbals (group H). No effect was observed in growth in all groups. The count of *Lactobacillus* spp. increased ($p < 0.05$) in jejunum in group P. In the faeces, *Enterobacteriaceae* decreased in the group P ($p < 0.05$) of 35 days old piglets, but *Enterobacteriaceae* and *E. coli* decreased in the group H ($p < 0.05$) of 56 days old piglets. The prevalence of resistance to at least one antibiotic class was 66.7% before and 50% after the experiment in all groups. Multidrug resistance of *E. coli* was not observed in 14 days old piglets, but was observed in 50% and more in all of study groups of 56 days old piglets. The fatty acid composition of *Longissimus thoracis* muscle had higher levels of α-linolenic acid and palmitoleic acid ($p < 0.05$), but lower level of stearic acid ($p < 0.05$) in group P. In conclusion, probiotics and herbals improved gut microbiota, fatty acid profile and affected shedding of resistant *E. coli*, but not growth performance.
Key words: buckwheat bran, gut microbiota, fatty acids, growth performance, blood profile.

Introduction

Nowadays typical is an intensive pig *(Sus scrofa domesticus)* farming. Pigs are commonly housed at high densities that can promote the spread of diseases. Antibiotics are used for treatment and prevention of diseases, and promoting of growth. The heightened use or misuse of antibiotics is contributing to the alarming increase of antibiotic resistance in bacteria, therefore it is an increasingly serious threat to global public health (WHO, 2014). In the European Union the use of antibiotics as growth promoters have been banned since 2006. Following feed supplementation with heavy metals has increased in pig breeding, but it might promote the spread of antimicrobial resistance too (Liedtke & Vahjen, 2012). There is an increasing interest in finding alternative means to improve the health of pigs and reduce the use of antibiotics. Probiotics have been studied and used as an in-feed supplement. Probiotics promote development of healthy microbiota, reduce enteric pathogens, luminal pH and improve mucosal immunity in piglets (de Lange *et al*., 2010). Buckwheat (*Fagopyrum esculentum* L.*)* seed is nutritionally important and flour of it is used as the prebiotic (Coman *et al*., 2013). Herbs have a number of beneficial effects, of which antimicrobial and antioxidant activity is the most important (Windisch *et al*., 2007). It is not enough to focus on alternative means to influence the spread of resistance. The aim of our study was to find out the impact of probiotics, buckwheat bran and composition of herbals on growth performance, blood and fatty acid characteristics, gut microbiota and shedding of resistant *E. coli* in piglets.

Materials and Methods

Feed additives

As feed additives were used buckwheat bran, herbal powder, and commercially available probiotics 'ProbioHelp'. Buckwheat (*Fagopyrum esculentum* L.*)* bran was chosen from an organic farm. Composition of herbals was made by authors: the aerial parts of St. John's wort (*Hypericum perforatum*) and leaves of greater plantain (*Plantago major*) and nettle (*Urtica dioica*) were collected in the Livani and Dobele districts of Latvia in 2015 and authenticated by the Institute of Horticulture of the LLU.

Experimental design and collection of samples

The experiment (6 weeks) was conducted in a conventional pig fattening farm in Latvia, in 2015. A total 44 Duroc – Landrace crossbred piglets were used. The age of piglets was 14 days with an average weight of 4.96 ± 0.13 kg. Piglets were divided into 4 treatment groups (11 pigs / pen): group C received basal diet, group P received basal diet + 'ProbioHelp', group PB received basal diet + 'ProbioHelp' + 3% buckwheat bran and group H received basal diet + 1.5% composition of herbals. Buckwheat bran and herbals were mixed into basal diet, but 'ProbioHelp' was added to drinking water, depending on the age of piglets: 14, 28, 35, 42 and 49 days old ones received the concentration of 1%, 0.75%, 0.45%, 0.34%, and 0.32%, respectively. All piglets were given *ad libitum* access to feed and water through local feeder and nipple drinker. The piglets were weighed individually at the beginning of the experiment (on day 14) and on days 35, 49 and 56. Offered and refused feed was weighed daily to calculate the growth parameters -

average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) (Willems, Miller, & Wood, 2013). Blood samples (n = 38) were obtained via jugular vein puncture and sampled into 5 mL EDTAK2 tubes and into 10 mL tubes without anticoagulant at the end of experiment. Blood samples were sent directly to the laboratory of LLU veterinary clinic. The faecal samples (n = 77) were collected from the first defecation after the daily cleaning of the pens on days 14, 35 and 56. On day 56, piglets (four from each treatment group and two from control) were slaughtered. The gastrointestinal tract was removed and digestive contents (n = 14) from jejunum were collected. *Longissimus thoracis* muscles were collected from both sides of the pig carcass for detection of fatty acid profile and transported to the laboratory shortly afterwards.

Haematological and serum biochemical measurements

Count of white blood cells (WBC), red blood cells (RBC), granulocytes (GRAN), lymphocytes (LYM), haematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin, (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured using the blood counter (Exigo eos, Sweden) by photometric method. The levels of serum glucose (GLU), calcium (Ca), phosphorus (P), gammaglutamintransferase (GGT), alkaline phosphatase (ALP) and aspartate aminotransferase (ASAT) were measured using the chemical analyser (Mindray BS-380) by absorbance photometry method.

Microbiological investigation

Faeces were used for enumeration of *Enterobacteriaceae*, *E. coli*, but digestive contents were used for enumeration of *Enterobacteriaceae*, *E. coli* and *Lactobacillus* spp. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination were done according to LVS ISO 6887-1:1999. The count of *Enterobacteriaceae* was carried out based on LVS ISO 21528-2:2007, *E. coli* - on LVS ISO 16649-2:2007, but the count of *Lactobacillus* spp. according to MRS (Biolife) manufacturer's instructions with little modification. Briefly, the faecal, digestive contents (10 g) were diluted with 90 mL of 1% peptone broth (Maximum Recovery Diluent, Biolife) and then homogenized. Counts of bacteria in the faecal samples were conducted by plating serial 10-fold dilutions (in 1% peptone solution) in Violet Red Bile Glucose agar (Biolife), Tryptone Bile X- Gglucuronide Medium - TBX (Oxoid), and MRS Agar with Tween® 80 (Biolife) to isolate the *Enterobacteriaceae*, *E. coli* and *Lactobacillus*, respectively. Colonies were expressed as log10 cfu g^{-1}.

Confirmation of E. coli and antimicrobial susceptibility testing

Two of *E. coli* isolates from TBX agar (from each faecal sample) were streaked with a sterile bacterial loop onto Levine EMB Blue Agar (Biolife) and incubated at 37 °C for 24 h. Isolates were assumed to be *E. coli* if showed typical blue black colonies with green metallic shine and additional basic biochemistry confirmed (oxidase test, citrate utilization and indole production tests).

E. coli isolates were phenotypically tested via agar disk diffusion to 12 antibiotics using BD BBL Antimicrobial Susceptibility Disks (ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), cefazolin (30 µg), cefotaxime (30 µg), imipenem (10 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), trimethoprim (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), enrofloxacin (5 µg) according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2008). Inhibition zones were measured on the Mueller-Hinton agar plates (Biolife) and interpreted according to the manufacturer's recommendations. If *E. coli* isolates were resistant to cefotaxime (30 µg), phenotypic confirmatory test for ESBL producing *E. coli* was used according to CLSI recommendation using both cefotaxime (CTX, 30 µg) and ceftazidime (CAZ, 30 µg) disks alone and in combination with clavulanic acid (CA, 10 µg) (BD BBL).

Analysis of fatty acid profile

The fatty acids (n = 36) were detected in muscle *Longissimus thoracis* samples by gas chromatography method (BIOR-T-012-131-2011) and analyses were performed in the accredited laboratory - BIOR.

Data statistical analysis

Data were analyzed using R version 3.3.2. All results were presented as the mean ± SEM (standard error of the mean). Duncan`s multiple range test was used to compare the means at level of significance of $p < 0.05$.

Results and Discussion

There is a wide interest in the improvement of feeding strategies using different feed additives that stimulate growth performance and minimize the use of antibiotics. In our study no differences were observed in average daily feed intake (ADFI), average daily gain (ADG) or feed conversion ratio (FCR) in all treatment groups. However, the weight gain of group PB was significantly higher than in other groups ($p < 0.05$) (Table 1). The initial weight of group PB was

Table 1

Effects of treatment on performance of 14 - 56 days old piglets

Traits	Group C	Group P	Group PB	Group H
Initial weight (kg)	4.500 ± 0.141^{b}	4.673 ± 0.208^{b}	5.836 ± 0.255^{a}	4.827 ± 0.236^{b}
Final weight (kg)	19.982 ± 1.063^{b}	19.482 ± 0.614^{b}	23.255 ± 0.465^{a}	20.273 ± 0.864^{b}
Weight gain (kg)	15.482 ± 1.138^{b}	14.809 ± 0.630^{b}	17.418 ± 0.501^{a}	15.445 ± 0.681^{b}
ADG (g d^{-1})	0.369 ± 0.027^{a}	0.353 ± 0.015^{a}	0.415 ± 0.012^{a}	0.368 ± 0.016^{a}
ADFI (g d^{-1})	0.580 ± 0.082^{a}	0.620 ± 0.088^{a}	0.658 ± 0.087^{a}	0.577 ± 0.082^{a}
FCR	1.681 ± 0.148^{a}	1.795 ± 0.085^{a}	1.599 ± 0.044^{a}	1.606 ± 0.083^{a}

ADG – average daily gain; ADFI – average daily feed intake; FCR – feed conversion ratio; a and b – means in the same row with different letters are significantly different ($p < 0.05$).

significantly higher compared to the other groups. Effect of probiotic bacteria has been studied before. Some researchers observed that probiotic bacteria in piglet diet produced a positive effect on growth (Liu *et al*., 2015). Whereas, other researchers have shown that probiotics have no positive effect of growth performance on piglets (Speiser *et al*., 2015), similar to our results. The factors which could affect ADG are different: species of probiotics, method of administration, age of pigs, components of basal diet and others (Zimmermann *et al*., 2016). A beneficial effect of probiotics on ADG was observed when the basal diet contained maize (*Zea mays* L.) as the principal feed ingredient, but not barley (*Hordeum vulgare* L.) or wheat (*Triticum aestivum* L.) (Zimmermann *et al*., 2016), which were principal feed ingredients in basal diet in our experiment. The lowest ADFI was observed in group H, but it was not significant. That might be attributed to the special organoleptic properties of herbs which may reduce diet palatability (Yan, Meng, & Kim, 2012).

All serum biochemical and haematological parameters after feeding trial were within the reference intervals (Klem *et al*., 2010), except ALP, which was slightly higher compared to the results in groups P, H and C, respectively, - higher by 1.0%, 1.5%, 11.9%. A higher level of ALP (without ASAT) is always an indicator of intense bone formation in the growth phase, particularly, if the level of phosphorus in diet is lower (Luiz *et al*., 2012). The RBC was lower ($p <$

Table 2

Effects of treatment on blood parameters in 56 days old piglets

Parameter	Group C	Group P	Group PB	Group H
WBC 10^{9} L^{-1}	23.780 ± 0.530^{a}	24.373 ± 1.175^{a}	21.336 ± 1.360^{a}	22.600 ± 1.274^{a}
GRAN 10^{9} L^{-1}	9.740 ± 0.464^{a}	11.027 ± 0.949^{a}	8.664 ± 0.703^{a}	9.664 ± 0.625^{a}
LYM 10^{9} L^{-1}	11.900 ± 0.539^{a}	11.055 ± 0.447^{a}	10.936 ± 0.720^{a}	11.064 ± 0.666^{a}
RBC 10^{12} L^{-1}	6.712 ± 0.159^{a}	6.670 ± 0.107^{a}	6.995 ± 0.095^{a}	6.288 ± 0.120^{b}
HCT %	34.900 ± 0.460^{a}	35.418 ± 0.641^{a}	35.145 ± 0.757^{a}	33.818 ± 0.627^{a}
HGB g dL^{-1}	10.960 ± 0.147^{a}	10.882 ± 0.133^{a}	10.927 ± 0.201^{a}	10.418 ± 0.195^{a}
MCV fl	$52.040 \pm 0.830^{a,b}$	53.112 ± 0.772^{a}	50.218 ± 0.201^{b}	53.827 ± 0.754^{a}
MCH pg	16.320 ± 0.252^{a}	16.327 ± 0.141^{a}	15.628 ± 0.220^{b}	16.618 ± 0.192^{a}
MCHC g dL^{-1}	31.400 ± 0.145^{a}	30.818 ± 0.270^{a}	31.145 ± 0.142^{a}	30.864 ± 0.192^{a}
ASAT U L^{-1}	75.240 ± 8.367^{a}	48.009 ± 4.606^{b}	$60.018 \pm 3.306^{a,b}$	68.373 ± 6.707^{a}
ALP U L^{-1}	$\uparrow 335.560 \pm 36.074^{a}$	$\uparrow 304.381 \pm 1.029^{a}$	260.327 ± 7.018^{b}	$\uparrow 303.082 \pm 10.044^{a}$
Ca mmol L^{-1}	2.928 ± 0.068^{a}	2.918 ± 0.036^{a}	2.728 ± 0.031^{b}	2.756 ± 0.053^{b}
P mmol L^{-1}	2.890 ± 0.059^{a}	2.810 ± 0.064^{a}	2.975 ± 0.028^{a}	2.871 ± 0.044^{a}
GGT U L^{-1}	45.200 ± 2.172^{a}	49.345 ± 8.201^{a}	37.682 ± 2.670^{a}	46.009 ± 2.487^{a}
GLU mmol L^{-1}	4.560 ± 0.093^{a}	4.832 ± 0.241^{a}	4.665 ± 0.119^{a}	4.715 ± 0.139^{a}

WBC – white blood cells; GRAN – granulocytes; LYM – lymphocytes; RBC – red blood cells; HCT – haematocrit; HGB – haemoglobin; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; ASAT – aspartate aminotransferase; ALP – alkaline phosphatase; Ca – calcium; P – phosphorus; GGT – gammaglutamintransferase; GLU – glucose; a and b means in the same row with different letters are significantly different ($p < 0.05$). ↑ the level is higher compared to the reference interval (Klem *et al*., 2010).

Table 3

Effects of treatment on digestive microbial populations of 35 and 56 days old piglets

Item (log_{10} cfu g^{-1})	Group C	Group P	Group PB	Group H
faecal 35 d:				
Enterobacteriaceae	3.37 ± 0.47[a]	1.73 ± 0.38[b]	2.78 ± 0.43[a,b]	2.95 ± 0.47[a,b]
E. coli	2.91 ± 0.29[a]	1.66 ± 0.38[a]	2.05 ± 0.35[a]	2.92 ± 0.44[a]
faecal 56 d:				
Enterobacteriaceae	5.19 ± 0.70[a,b]	5.41 ± 0.23[a]	3.93 ± 0.52[b,c]	3.18 ± 0.37[c]
E. coli	4.94 ± 0.60[a]	5.02 ± 0.22[a]	3.85 ± 0.49[a,b]	3.16 ± 0.33[b]
jejunum 56 d:				
Enterobacteriaceae	5.29 ± 2.69[a]	3.92 ± 0.56[a]	3.04 ± 0.85[a]	2.99 ± 0.67[a]
E. coli	4.74 ± 2.21[a]	3.54 ± 0.46[a]	2.96 ± 0.83[a]	2.98 ± 0.67[a]
Lactobacillus spp.	6.27 ± 0.82[b]	7.75 ± 0.24[a]	6.62 ± 0.14[a,b]	5.98 ± 0.51[b]

a, b and c – means in the same row with different letters are significantly different ($p < 0.05$).

0.05) in group H, compared with group C. ASAT was lower in group P ($p < 0.05$) and group PB ($p > 0.05$), compared with group C. The level of phosphorus was higher in group BP (Table 2).

Group H received a diet supplemented with composition of herbals, wherein one of the three components was nettle. In traditional medicine, nettle is used for treatment of anemia. The beneficial effect of nettle on the erythropoiesis was similar to that of iron-containing preparations (Upton, 2013). The mean of MCV and MCH was the highest in group H, therefore reduced count of RBC cannot be associated with iron deficiency. In our study probiotics decreased the level of ASAT; it coincided with other studies about the effect of probiotics on layer chicks (*Gallus gallus domesticus* L.) (Hatab, Elsayed, & Ibrahim, 2016). At the same time, there are other authors who have not observed this tendency (Liu *et al*., 2015). Group PB had the highest level of phosphorus, it could be explained with the fact that group PB received basal diet supplemented with buckwheat bran, which is an additional source of phosphorus, and therefore resulted in a lower level of ALP in group PB ($p < 0.05$).

Compared with the group C, diet supplementation with probiotics increased the count of *Lactobacillus* spp. in jejunum in both group P ($p < 0.05$) and group PB ($p > 0.05$). In the faeces of 35 days old piglets, the count of *Enterobacteriaceae* decreased in the group P ($p < 0.05$) compared to the group C. In the faeces of 56 days old piglets, the count of *Enterobacteriaceae* and *E. coli* decreased in the group H ($p < 0.05$) compared to the group C, but the total count of *Enterobacteriacea* in group PB compared to the group P ($p < 0.05$) and group C was decreased ($p > 0.05$) (Table 3).

We observed that diet supplemented with probiotics increased the count of *Lactobacillus* spp. and decreased count of *Enterobacteriaceae* and *E. coli*, which coincides with other authors (Liu *et al*., 2015)**.** Probiotic bacteria increased the production of short chain fatty acids in an *in vitro* study and these help to reduce digest pH and growth of pathogenic bacteria (Gibson, 1999). We observed beneficial effect of herbal supplementation on shedding of faecal microbial, particularly, at the age of 56 days. Similar to other authors (Yan, Meng, & Kim, 2012), in our study, herbals decreased the count of *Enterobacteriaiaceae* and *E. coli* in faeces of piglets. Some herbals contain essential oils with strong antimicrobial activity, particularly, phenolic structures, which damage cell membrane of bacteria (Lambert *et al*., 2001), but with little inhibition towards *Lactobacillus* spp. (Si *et al.,* 2006), that can be seen in our study, too. Buckwheat flour has demonstrated the potential of prebiotic. It has also been reported that extracts of methanol from buckwheat inhibited *E. coli* (Coman *et al*., 2013). Buckweat bran did not increase count of *Lactobacillus* spp. in jejunum, but reduce count of *Enterobacteriaceae* and *E. coli* in our study. After weaning, piglets pass through acute and adaptive phases. Primarily the effect of acute phase is on the reduced feed intake. It could take seven days, when weaned piglets learn to eat dry feed and the intake of dry matter content reaches volume as it was before weaning (Pluske, Hampson, & Williams, 1997). Considering this fact, we assumed that piglets did not ingest enough feed that was supplemented with herbals and therefore we did not observe the beneficial effects of herbals to faecal microflora. The effect of probiotics in acute phase is higher if consumed with water.

During the study, a total of 80 *E. coli* were isolated from faeces of 14 and 56 days old piglets. Before experiment, higher prevalence of resistant *E. coli* was observed to ampicillin (42%) and trimethoprim (33%). After experiment, higher resistance was observed to tetracycline in groups P (50%), PB (50%)

Table 4

Antibiotic resistant *E. coli* isolates from 14 and 56 days old piglets

Antibiotic	14 days old piglet (n = 12)	56 days old piglet			
		Group C (n = 16)	Group P (n = 20)	Group PB (n = 16)	Group H (n = 16)
Ampicillin	5 (42%)	4 (25%)	5 (25%)	6 (38%)	5 (31%)
Amoxicillin-clavulanic acid	0	2 (13%)	1 (5%)	0	0
Cefazolin	0	1 (6%)	1 (5%)	3 (19%)	4 (25%)
Cefotaxime	0	1 (6%)	0	0	0
Imipenem	0	0	0	0	0
Gentamicin	0	0	0	0	0
Tetracycline	2 (17%)	5 (31%)	10 (50%)	8 (50%)	7 (44%)
Ciprofloxacin	0	0	0	0	0
Trimethoprim	4 (33%)	4 (25%)	10 (50%)	7 (44%)	6 (38%)
Trimethoprim-sulfamethoxazole	1 (8%)	3 (19%)	9 (45%)	6 (38%)	5 (31%)
Chloramphenicol	0	6 (38%)	0	0	0
Enrofloxacin	0	0	0	0	0

and H (44%), and to trimethoprim – in groups P (50%) and PB (44%). Resistance to chloramphenicol and cefotaxime was observed only in group C (38% and 6%, respectively) (Table 4).

The prevalence of resistance to at least one antibiotic class was observed – 66.7% before and 50% after the experiment in all groups. Most of *E. coli* isolates were resistant only against one antibiotic class in 14 days old piglets (62.5%). Multidrug resistance (resistant to three or more antimicrobial classes) of *E. coli* isolates was not observed in 14 days old piglets, but was observed 50% and more in all of study groups of 56 days old piglets. Resistance against 5 antibiotic classes was observed only in group C (12.5%) (Figure 1).

High prevalence of resistant *E. coli* to amoxicillin in 14 days old piglets and to tetracycline and trimethoprim / trimethoprim-sulfamethoxazole in 56 days old piglets could be explained with antibiotic usage habits in this farm. Resistance to chloramphenicol, and it was of high level, was observed only in the group C. Chloramphenicol has been banned in EU since 1994 for livestock treatment. Despite the fact that some specific antibiotics have not been used in animals for a long time, some resistant genes can be maintained due to a link with the other genes coding resistance to other antibiotics, which are allowed for use in food-producing animals (Diarra *et al.*, 2007). Suckling and weaned piglets are highly sensitive to bacterial infections, therefore more often are pressured of therapeutic antibiotics, as a result, a high prevalence of resistant *E.coli* is observed (Akwar *et al.*, 2008). During the experiment, none of treatment groups received antibiotics additionally and this could

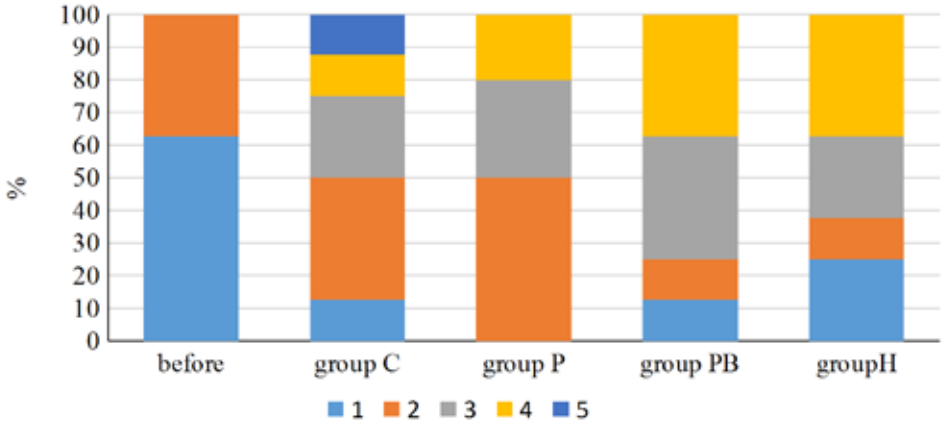

Figure 1. Proportion of resistant *E. coli* before (14 days old piglets) and after experiment (56 days old piglets) against 1, 2, 3, 4 and 5 antibiotic classes.

Table 5

Fatty acid* composition (% of detected fatty acids) of *Longissimus thoracis* muscle in 56 days old piglets

Fatty acid	Group C	Group P	Group PB	Group H
C18:3 n3 α-linolenic acid	0.90 ± 0.12^{b}	1.80 ± 0.10^{a}	1.60 ± 0.10^{a}	1.50 ± 0.10^{a}
C20:4 n6 arachidonic acid	1.40 ± 0.2^{a}	$0.75 \pm 0.05^{a,b}$	$1.05 \pm 0.15^{a,b}$	0.50 ± 0.10^{b}
C18:2 n6 linoleic acid	10.37 ± 0.70^{a}	11.60 ± 0.40^{a}	11.85 ± 0.75^{a}	9.65 ± 0.75^{a}
C18:1 oleic acid	$41.63 \pm 0.49^{a,b}$	42.55 ± 0.95^{a}	39.60 ± 0.60^{b}	42.55 ± 0.25^{a}
C16:1 palmitoleic acid	3.07 ± 0.26^{b}	4.55 ± 0.25^{a}	$4.25 \pm 0.55^{a,b}$	3.25 ± 0.15^{b}
C16:0 palmitic acid	24.50 ± 0.61^{a}	24.25 ± 0.15^{a}	25.75 ± 0.05^{a}	24.65 ± 0.45^{a}
C18:0 stearic acid	13.13 ± 0.13^{a}	9.40 ± 0.60^{b}	$11.20 \pm 1.30^{a,b}$	13.10 ± 0.70^{a}
SFA	40.22 ± 0.49^{a}	36.70 ± 0.25^{b}	40.08 ± 1.23^{a}	40.53 ± 1.13^{a}
MUFA	$47.15 \pm 0.63^{a,b}$	49.03 ± 1.08^{a}	45.65 ± 0.75^{b}	$48.05 \pm 0.20^{a,b}$
PUFA	13.40 ± 0.85^{a}	14.90 ± 0.60^{a}	15.10 ± 0.80^{a}	12.25 ± 0.95^{a}
PUFA/SFA	0.33 ± 0.02^{a}	0.41 ± 0.01^{a}	0.38 ± 0.03^{a}	0.30 ± 0.03^{a}

* - not all detected fatty acids are showed in table; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.; PUFA: SFA ratio of polyunsaturated fatty acids and saturated fatty acids; a and b means in the same row with different letters are significantly different ($p < 0.05$).

explain the fact that the prevalence of resistant *E. coli* in weaned piglets (56 days old) was lower compared to 14 days old piglets. Large proportion of multi-drug resistant *E. coli* in all treatment groups was explained by the uptake of resistant genes from the environment as well as diet received by piglets, supplemented with zinc oxide. According to previous studies, feed supplementation with heavy metals, particularly zinc, increase proportion of multi-drug resistant *E.coli* in piglets (Bednorz *et al*., 2013). Clay minerals also could have selection effect on uptake of resistant genes (Jahanbakhsh *et al*., 2015), but they do not give any information about the impact of different herbals. Probiotics have demonstrated to leave impact on the transfer of antibiotic resistance genes, but information about the strains with higher effect and mechanism of this effect is not known (Moubareck *et al*., 2007). Differences of proportion of resistant *E. coli* between treatment groups indicate that feed supplementation with natural feed additives (herbals and probiotics) could have selective effect on resistant bacteria or uptake of resistance genes.

The fatty acid composition of *Longissimus thoracis* muscle showed that piglets fed with basal diet supplemented with probiotics had higher levels of α-linolenic acid and palmitoleic acid ($p < 0.05$), but lower level of stearic acid ($p < 0.05$) compared to group C. Only level of α-linolenic acid was higher in groups H and PB compared to group C. SFA was lower ($p < 0.05$) and PUFA/SFA rations were higher ($p > 0.05$) in group P (Table 5).

SFA have a negative effect on cardiovascular system of humans. According to Department of Health (1994), the recommended PUFA/SFA rations to reduce the risk of cardiovascular diseases is greater than 0.4 (Wood *et al*., 2008). Pigs are monogastric animals and some of PUFA (linoleic acid, α-linolenic acid) pass through the stomach unchanged. They are absorbed into the blood flow through small intestine and get into tissues (Wood *et al.,* 2008). As basal diet was the same for all treatments groups, differences of fatty acid composition in group P proved that probiotics can improve the fatty acid profile of pork. Effect of probiotics on reduction of SFA and increased level of MUFA and PUFA in pigs has been reported before (Ross, Nieuwenhove & González, 2012).

Conclusions

Dietary supplementation with probiotics, buckwheat bran or herbals did not affect the growth performance. Probiotics increased *Lactobacillus* spp. in jejunum. Probiotics consumed with water more effectively reduced *Enterobacteriaceae* in acute phase, but herbals were more effective in reduction of *Enterobacteriaceae* and *E. coli* in adaptive phase of post weaning. Buckwheat bran did not exert the effect of prebiotic, but more effectively reduced *Enterobacteriaceae* and *E. coli in vivo.* Differences of proportion of resistant *E. coli* between treatment groups indicated that feed supplementation with probiotics and herbals could have selective effect on resistant bacteria or uptake of resistance genes. These results confirmed that probiotics can improve the fatty acid profile of pork. Probiotics reduced SFA and increased the level of MUFA and PUFA in pigs.

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