Effect of pea and probiotic and/or acidifier supplementation on growth performance and composition of caecal microbiota of broiler chicken[1]

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Growth performance and composition of caecal microflora were measured in broiler chickens fed diets formulated with/without white pea and supplemented with dietary probiotic and/or encapsulated acidifier. One-day-old broiler females allotted into 8 groups of 12 birds and maintained individually were fed isoprotein and isoenergetic wheat and soybean meal-based diets with/without white pea (150 g/kg), unsupplemented or supplemented with probiotic (1g/kg) and/or acidifier (1 g/kg). Performance was measured for 5 weeks, birds were killed and samples of caecal digesta prepared. The caecal microbiota was characterized by fluorescent *in situ* hybridization (FISH) and terminal-restriction fragment length polymorphism (T-RFLP).
Inclusion of pea into diet increased feed intake, but negatively affected FCR (P<0.05), while neither probiotic nor acidifier supplementation affected performance. Total bacterial numbers in caecal contents were higher in birds fed pea diets (P<0.01), but were not affected by any dietary supplement. Neither pea inclusion nor probiotic supplementation affected the *Lactobacillus/Enterococcus* and *Streptococcus/Lactococcus* counts in caecal contents. But, the composition of the *Lactobacillus/Enterococcus* population was altered by inclusion of pea, and acidifier supplementation increased the *Lactobacillus/Enterococcus* counts. In conclusion, dietary inclusion of pea and encapsulated acidifiers may change the bacterial community structure in the distal parts of the chicken gastrointestinal tract.

Keywords: acidifier; probiotic; pea; caecal microflora; broiler chickens

Introduction

In recent years there is an increasing demand in European countries for a domestic protein alternatives to imported soybeans, the most often used are peas (*Pisum sativum* L). Pea seeds contain about 20-25% crude protein, 47% starch, 1.2% crude fat and 5% α-galactosides. Daveby at al. (1998) found in chickens the following apparent ileal digestibility of crushed pea components: protein 79%, starch 77%, fat 80%, total dietary fibre polysaccharide residues 8%. Weurding et al. (2001) reported, that only 71% of pea starch digested in the whole gastrointestinal tract is digested before the ileum and 91% before the posterior ileum, while for cereal grains the respective values are much higher (90 and 98%). Incomplete digestion of pea nutrients in the small intestine supplies substrates for bacterial fermentation in the posterior ileum and caeca. Choct et al. (1996) reported that increased small intestinal

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fermentation is partly responsible for the anti-nutritive activity of wheat non-starch polysaccharides in chickens. It is not clear how undigested pea nutrients can affect the bacterial community structure in distal parts of chickens gastrointestinal tract, particularly when diets without antibiotic growth promoters are supplemented with different feed additives.

The objective of the study was to determine whether the inclusion of pea into diet and supplementation with acidifier and probiotic would affect the composition of the caecal microflora in broiler chickens.

Materials and methods

The experimental design consisted of 2 x 2 x 2 factorial arrangement of the treatments. Two isoprotein and isoenergetic basal diets were formulated: S - wheat and soybean meal-based diet and P - the wheat and soybean meal-based diet containing 150 g white pea in 1 kg (Table 1). Both diets were prepared without or with supplemental probiotic (Lab YuccProbio, Mifarmex, containing *Lactobacillus casei/paracasei, L. brevis, L. plantarum*, *Saccharomyces cerevisiae* and *Yucca Schidigeri* extract), without or with supplemental acidifier (Galliacid, Vetagro, a mixture of fumaric acid, formic Ca, propionic Ca and sorbic K encapsulated with saturated plant oils). All diets were without growth-promoting antibiotic but contained an ionophore coccidiostat (salinomycin, 70 ppm) and were cold pelleted.

Table 1. Composition of diets, g/kg air-dry matter

Component	S diets				P diets			
	S	SPro	SAc	SProAc	P	PPro	PAc	PProAc
White pea (21% CP)	-	-	-	-	150	150	150	150
Wheat (11% CP)	354.5	354.5	354.5	354.5	249.4	249.4	249.4	249.4
Soybean meal (44.6% CP)	359.0	359.0	359.0	359.0	314.5	314.5	314.5	314.5
Maize (9.9% CP)	200	200	200	200	200	200	200	200
Limestone	12	12	12	12	12.1	12.1	12.1	12.1
Monocalcium phosphate	13	13	13	13	13.3	13.3	13.3	13.3
NaCl	3	3	3	3	3	3	3	3
Rapeseed oil	50	50	50	50	50	50	50	50
Mineral-vitamin premix*	3	3	3	3	3	3	3	3
L-Lys (78%)	1	1	1	1	-	-	-	-
DL-Met (98%)	1.5	1.5	1.5	1.5	1.7	1.7	1.7	1.7
Avizyme 1300	1	1	1	1	1	1	1	1
Wheat starch	2	1	1	-	2	1	1	-
Probiotic	-	1	-	1	-	1	-	1
Acidifier	-	-	1	1	-	-	1	1
Crude protein	220	220	220	220	220	220	220	220
ME/ MJ/kg	12.31	12.31	12.31	12.31	12.44	12.44	12.44	12.44

* supplied per kg diet: vit. A. 12500 IU; vit. D_3 2750 IU; (mg) vit. E 35; vit B_1 2; vit B_2 6; biotine 0.2; vit B_6 4.5; vit B_{12} 0.015; vit K_3 3; niacine 30; folic acid 2; Ca panthotenate 15; choline 600; Mn 60; Zn 55; Se 0.2; Co 0.2; Cu 15; Fe 30; J 1, salinomycin 70 and Ca 470

Ninety six one-day-old Ross broiler females were divided into 8 groups, 12 birds in a group and kept for the first week of life in a battery cages. At 8th day of life birds were weighed and placed in the individual cages. Chickens from the first day of life were provided with the experimental diets (1-3 d mash, from 4th day pellets) and water *ad libitum.* Feed intake and body weight were measured at weekly intervals after 4 h starving. At 36h and 37th day of age all chickens were weighed and killed by cervical dislocation, the abdomen was opened, the gastrointestinal tract was excised, and the luminal content of caeca was collected.

Caecal contents were mixed with paraformaldehyde fixative in proportion 1:3, kept for 3h at 4°C and pelleted by centrifugation (2 min, 5000 g). Pellets were washed two times with PBS buffer (130 mM sodium chloride, 10 mM sodium phosphate, pH 7.2), resuspended in 50% (vol/vol) ethanol/PBS and stored at -20°C. Total bacterial numbers in caecal contents were determined after staining with acridine orange. *Lactobacillus* and *Enterococcus* numbers as well as *Streptococcus* and *Lactococcus* numbers in caecal contents were determined with the aid of fluorescent *in situ* hybridization (FISH) by using the Lab158 (Harmsen et al. 1999) and Strc493 (Franks et al. 1998) oligonucleotide probes. The composition of the caecal microbiota was characterized by terminal-restriction fragment length polymorphism (T-RFLP) by digestion restriction endonucleases HhaI, RsaI, MseI, MspI and BfaI.

Data were analyzed as a 2x2x2 factorial arrangement using Statgraphics Plus ver. 5.1 program.

Results and discussion

The dietary inclusion of pea increased the feed intake, but negatively affected feed conversion ratio (P<0.05), whereas neither probiotic nor acidifier supplementation affected performance. Total bacterial numbers in caecal contents were higher in birds fed pea diets (P<0.01), which could indicate an increased ceacal fermentation of undigested nutrients. Total bacterial numbers were not affected however by any of the dietary supplements. Neither pea inclusion nor probiotic supplementation affected the *Lactobacillus/Enterococcus* and *Streptococcus/Lactococcus* counts in caecal contents, but inclusion of acidifier increased the *Lactobacillus/Enterococcus* counts. The composition of the caecal *Lactobacillus/Enterococcus* population as determined by the T-RFLP method was different in birds fed the pea and the soy diets. In conclusion, dietary inclusion of pea and encapsulated acidifiers may change the bacterial community structure in the distal part of the chicken gastrointestinal tract. Further, the inclusion of pea had a negative influence on bird performance in terms of feed conversion ratio (Table 2).

Table 2. Effect of soybean meal (S) and pea (P) diets, probiotic (Pro) and acidifier (Ac) supplementation on performance and bacterial counts in the caecal digesta of broiler chickens

Item		S diets		P diets			Probability		
	Probiotic	- Ac	+ Ac	- Ac	+ Ac	RMSE	Pro	Ac	S vs P
Performance 1-35 day of life									
Body weight gain[1], kg	- Pro	1.97	2.07	2.00	2.01	0.020	0.184	0.406	0.753
	+ Pro	2.04	2.04	2.07	2.06				
Feed intake[2], kg	- Pro	2.85	3.07	3.13	3.08	0.049	0.619	0.415	0.024
	+ Pro	3.03	3.02	3.10	3.09				
Feed conversion ratio (FCR)[3], kg	- Pro	1.45	1.49	1.57	1.53	0.006	0.288	0.991	0.005
feed/kg BWG	+ Pro	1.49	1.48	1.50	1.51				
Bacterial counts in caecal contents									
Total number of bacteria[4]	- Pro	10.4	10.3	10.5	10.5	0.025	0.620	0.805	0.007
log AFU/g	+ Pro	10.4	10.4	10.4	10.5				
Lactobacillus/Enterococcus[5]	- Pro	8.92	9.09	8.81	9.11	0.162	0.354	0.048	0.461
log AFU/g	+ Pro	8.64	8.98	9.03	8.94				
Streptococcus/Lactococcus[6]	- Pro	9.54	9.78	9.82	9.87	0.125	0.839	0.675	0.870
log AFU/g	+ Pro	9.82	9.78	9.67	9.63				

RMSE - residual mean square error; the following interactions were significant at * P<0.05; ** P<0.01: [3]SP x Pro*;[4] SP x Ac*

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