AgroLife Scientific Journal - Volume 8, Number 1, 2019
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

EFFECTS OF DIETARY PEA SEEDS (*Pisum sativum* L. cv. Tudor) ON PERFORMANCE, CARCASS TRAITS, PLASMA BIOCHEMISTRY AND INTESTINAL MICROFLORA IN BROILER CHICKS

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Abstract

The effects of dietary partial replacement of soybean meal (SBM) with peas (cv. Tudor), on performance, carcass traits, plasma biochemistry and intestinal microflora in broiler chicks, was assessed. One-day-old broilers (n=400) divided in 2 groups were fed 2 isocaloric and isonitrogenous diets: control diet (C) based on corn-sorghum-SBM and experimental diet (PT) that contained 200 g/kg peas (cv. Tudor). Results shown that PT addition significantly affect performance, BWG of broilers was higher in starter (13.23%; P<0.0001), finisher (3.21%; P=0.014) and overall period (4.28%; P<0.0001); FI was increased with 7.54% (P=0.005) in starter, 0.89% (P=0.001) in finisher, respectively 1.19% (P=0.043) in overall period vs. C diet. The FCR was decreased in finisher (P=0.024) and total period (P<0.0001) in PT group. Feeding PT did not affect the carcass yields but increased breast yield (P=0.038), and a tendency to increase the legs yield and to decrease the abdominal fat was observed. Plasma total protein content was higher in PT group (P=0.045). Digestive organs weight, length of the intestinal tract and intestinal pH of broilers were not affected by feeding PT diet. No differences were found in the ileal population of Enterobacteriaceae and E. coli among treatments, except the counts of Lactobacillus spp. that increased (5.83%; P<0.0001) in PT group. In conclusion, dietary peas (cv. Tudor), in the amount of 200 g/kg, as partial replacement for SBM, improve performance and carcass traits, has a positive effect on plasma total protein and alter ileal microflora by increasing Lactobacillus spp.

Key words*: broiler performance, blood biochemistry, carcass traits, peas, ileal microflora.*

INTRODUCTION

The limited supply and the increased price of soybean meal (SBM), as conventional source of vegetable proteins for poultry feed, have stimulated researchers to evaluate the use of alternative local legumes protein sources that are not commonly utilized in poultry diets (Khattab et al., 2009; Laudadio et al., 2011). Peas (*Pisum sativum* L.) could be used as an alternative protein source to soybean due to its high nutritive value comparable to soybean (Fru-Nji et al., 2007; Tufarelli et al., 2012). The use of legumes is limited due to the content of some antinutritional factors (ANFs; phytic acid, condensed tannins, lectins, protease and α-amylase inhibitors), compounds that decline the nutritional value of pea (Alonso et al., 1998; Collins et al., 2006) and may affect gastrointestinal tract (GIT) physiology (Salgado et al., 2002). In poultry, the microorganisms that colonize the GIT during the early posthatch period form a synergistic relationship with their host. Gastrointestinal microorganisms have a major role in the absorption of energy and other nutrients, and on the response of poultry to ANFs (Choct et al., 2006). However, the previous study that evaluated the effects of dietary pea seeds inclusion in broilers have not been consistent. Several reports have shown that the inclusion of high amount of raw peas in diets have a detrimental effect on the performance of broilers and laying hens (Igbasan and Guenter, 1997; Diaz et al., 2006). Other study reported that dietary pea inclusion achieves similar performance and carcass traits (Dotas et al., 2014), or improve the performance and meat quality of broiler chickens (Laudadio and Tufarelli, 2010) and guinea fowl (Laudadio et

al., 2012). Although diet composition is considered one of the major factors that may influence the microbial activity in the GIT of birds, little literature data are available about the broiler chicks response of dietary peaseeds on GIT development and health. Moreover, the appreciable progress done in plant breeding to improve the cultivars required more research to evaluate the nutritive value of new pea seeds variety and its effects on broilers diets, as alternative protein source.Therefore, the aim of this study was to determine the effects of the partial replacement of SBM with pea seeds (*Pisum sativum* L. cv. Tudor) on performance, carcass traits, plasma biochemistry, gastrointestinal development and ileal microflora of broiler chicks.

MATERIALS AND METHODS

Broilers and experimental design

All procedures used in the trial were approved by the Ethical Committee of the National Research Development Institute for Animal Biology and Nutrition (Baloteşti, Romania) according to the European legislation for animals protection (OJEU, 2010). Four hundred 1-d-old unsexed broilers (Cobb 500; 44.56 ± 0.95 g), purchased from a local hatchery, were weighed and randomly allotted in 8 floor pens (50 birds/pen) on wood shavings litter into 2 treatments. Each dietary treatment had 4 replicate pens. Broilers were fed two diets: a control diet (C) based on corn-sorghum-SBM and an experimental diet (PT) that contained 200 g/kg peas (cv. Tudor), as partial replacement of SBM. The chemical composition of peas cv. Tudor, obtained from locally crop by SCDA Secuieni, was 89.53% dry matter, 20.68% CP, 0.81% crude fat, 7.04% crude fiber, 2815 kcal/kg ME, 1.80% lysine, 0.98% met + cys. The isocaloric and isonitrogenous 3-phases diets (Table 1) were formulated, according to the age of broilers (starter 1 to 10 d, grower 11 to 22 d and finisher 23 to 35 d), to supply the nutritional requirements of Cobb 500 hybrid (Cobb-Vantress, 2015). Throughout the feeding trial, fresh water and feed (mash form) were supplied *ad libitum*. Birds were raised in environmentally controlled conditions, provided a 23 h L: 1 h D lighting program, and were immunized according to the routine protocol. Determined performance parameters were body weight (BW) and feed intake (FI) for grower phases and overall period, in order to calculate body weight gain (BWG) and feed conversion ratio (FCR). Mortality was registered daily in order to correct the FCR.

Table 1. Ingredients and chemical composition of diets for grower phases

Item	Starter (1-10 d)		Grower (11-22 d)		Finisher (23-35 d)	
	C[1]	PT[2]	C	PT	C	PT
Ingredients, g/kg						
Corn	275.4	217.0	307.0	240.0	332.7	263.0
Sorghum	275.4	217.0	307.0	240.0	332.7	263.0
Soybean meal	300.0	210.0	250.0	174.0	210.0	144.0
Peas (cv. Tudor)	-	200.0	-	200.0	-	200.0
Corn gluten meal	59.3	63.0	50.0	55.7	35.0	36.0
Sunflower oil	37.8	41.2	36.0	40.0	43.0	47.0
Monocalcium phosphate	17.9	17.9	16.2	16.8	14.6	15.4
Calcium carbonate	14.8	14.8	14.0	14.1	12.8	12.7
Salt	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin-mineral premix[3, 4]	10.0[3]	10.0[3]	10.0[3]	10.0[3]	10.0[4]	10.0[4]
DL-methionine	1.5	1.7	2.3	2.6	2.1	2.5
L-lysine HCl	4.3	3.8	3.9	3.2	3.5	2.4
Choline HCl	0.6	0.6	0.6	0.6	0.6	0.6
Chemical analysis, %						
Dry matter	89.10	89.08	88.67	88.77	88.59	88.53
Crude protein	22.09	22.05	20.03	20.01	18.10	18.20
Crude fiber	3.57	3.92	3.63	3.77	4.34	4.07
Crude fat	5.45	5.66	5.18	5.73	6.23	6.39
Lysine, total	1.323	1.322	1.193	1.191	1.055	1.053
Methionine+cysteine, total	0.985	0.983	0.892	0.891	0.822	0.820
Calcium	0.93	0.93	0.83	0.84	0.77	0.78
Phosphorus total	0.85	0.85	0.80	0.80	0.76	0.76
Ash	6.33	6.18	5.72	5.60	5.14	5.07
Calculated analysis						
ME (MJ/kg)[5]	12.62	12.59	12.98	12.99	13.34	13.38

[1]Control diet; [2]Peas (cv. Tudor) diet
[3]Provided per kg diet: vitamin A, 4.47 mg; vitamin D_3, 0.12 mg; vitamin E, 80 mg; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 9 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.020 mg; vitamin B_5, 15 mg;vitamin B_3, 60 mg; vitamin B_9, 2 mg; Mn, 100 mg; Zn, 100 mg; Fe, 40 mg; Cu, 15 mg; I, 1.0 mg; Se, 0.30 mg; Co, 0.25 mg, lasalocid sodium, 60 mg.
[4]Provided per kg diet: vitamin A, 2.90 mg; vitamin D_3, 0.12 mg; vitamin E, 50 mg; vitamin K_3, 3 mg; vitamin B_1, 2 mg; vitamin B_2, 8 mg; vitamin B_6, 3 mg; vitamin B_{12}, 0.015 mg; vitamin B_5, 12 mg; vitamin B_3, 50 mg; vitamin B_9, 1.5 mg; Mn, 100 mg; Zn, 100 mg; Fe, 40 mg; Cu, 15 mg; I, 1.0 mg; Se, 0.30 mg; Co, 0.25 mg.
[5]calculated using regression equations (NRC 1994).

Sample collection

On d 35 of the trial, after 12 h fasting period, from sixteen birds per treatment blood samples (4 mL) were collected from wing vein in heparinized tubes. Plasma samples was obtained by centrifugation at 2700 rpm for 10 min (Multifuge 3L-R, Heraeus, Hanau, Germany) and stored in Eppendorf tubes at -20ºC until biochemical analyses.

After blood samples collection, the birds were killed by cervical dislocation. Carcasses were eviscerated manually and the GIT was excised. The digestive organ size (gizzard, liver, pancreas, spleen) and the length and weight of intestinal segments (duodenum, jejunum, ileum, and cecum) were evaluated.

The pH of fresh digesta were measured at the terminal end of each intestinal segment. Samples of ileal digesta were aseptically collected in plastic tubes and preserved at - 20°C until bacteriological analyses.

Chemical analyses

The samples of feed ingredients and diets were analysed in duplicate for basal chemical composition (dry matter, crude protein, ether extract, crude fiber and crude ash) using standardized methods (OJEU, 2009).

Plasma biochemistry protein profile (total protein, albumin, total bilirubin, creatinine, urea), energetic profile (glucose, cholesterol, triglycerides) and enzymatic profile [alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gammaglutamyltransferase (GGT)] were assayed using commercial kits Accent-200 MG (Cormay, Wiosenna, Poland) on BS-130 chemistry analyzer (Bio-Medical Electronics Co., China).

A portable pH meter (WTW ProfiLine pH 3310, Germany) were used to determine the intestinal digesta pH by inserting the pH meter electrode (WTW Sentix 41, Germany) into the distal sections of each intestinal segment (duodenum, jejunum, ileum and caecum).

Bacterial populations from ileum digesta samples were determined on selected media using a colony counter (Scan 300, Interscience France). In brief, *Enterobacteriaceae* and *E. coli* were counted using a classical isolation medium Levine agar, *Lactobacillus* strains was enumerated on MRS agar, after incubation of decimal diluted subsamples at 37°C for 48 h. The *Salmonella* spp. was determined using Rambach-*Salmonella* agar, after incubation of diluted subsamples at 37°C for 24 h. The results were expressed as a logarithm (base 10) of colony-forming units per gram of sample.

Statistical analysis

All data were analysed using the GLM procedure of the software SPSS (SPSS, 2011). One-way analysis of variance (ANOVA) with the Tukey's comparison test was used to evaluate statistical significance of differences between dietary treatments. Replication was considered as the experimental unit for performance parameters and bird as the experimental unit for biochemistry and intestinal parameters. The results are expressed as means and standard error of the mean (SEM); statistical differences were significant at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Performance

The effects of dietary treatments on body weight gain, feed intake and feed conversion ratio of broiler chicks are given in Table 2. There was significant difference ($P<0.05$) among treatments for BWG and FI during the starter, finisher and overall periods. Thus, in starter, finisher and overall period the BWG of broilers fed PT diets was higher with 13.23% ($P<0.0001$), 3.21% ($P=0.014$), respectively 4.28% ($P<0.0001$) and FI was increased with 7.54% ($P=0.005$) in starter, 0.89% ($P=0.001$) in finisher, respectively 1.19% ($P=0.043$) in overall period compared to C diets. The FCR in finisher and total period decreased in PT group with 3.35% ($P=0.001$), respectively 3.93% ($P<0.0001$) *vs.* C group. The overall mortality rate was low in the PT group (1.40 *vs.* 1.50%; $P>0.05$) and was not related with dietary treatments.

Table 2. Growth performance of broilers fed dietary peas[1]

Item	Control	Peas (cv. Tudor)	SEM	*P-value*
Body weight gain, g				
Starter (1 to 10 d)	210.77[b]	238.65[a]	5.00	*<0.0001*
Grower (11 to 22 d)	684.35	708.89	7.50	*0.112*
Finisher (23 to 35 d)	980.58[b]	1019.08[a]	4.49	*0.014*
Overall (1 to 35 d)	1875.70[b]	1966.62[a]	15.65	*<0.0001*
Feed intake, g				
Starter (1 to 10 d)	260.96[b]	280.64[a]	2.75	*0.005*
Grower (11 to 22 d)	1029.42	1031.27	2.59	*0.997*
Finisher (23 to 35 d)	2047.05[b]	2065.21[a]	5.77	*0.001*
Overall (1 to 35 d)	3337.43[b]	3377.12[a]	4.80	*0.043*
Feed conversion ratio, g feed:g gain				
Starter (1 to 10 d)	1.24	1.18	0.02	*0.108*
Grower (11 to 22 d)	1.50	1.45	0.02	*0.137*
Finisher (23 to 35 d)	2.09[a]	2.02[b]	0.01	*0.024*
Overall (1 to 35 d)	1.78[a]	1.71[b]	0.02	*<0.0001*

[a,b]Means with different superscript within a row differ significantly ($P<0.05$); [1]n=50 broilers per replicate.

Previous studies have shown that the use of peas in poultry diets did not influence the performance of broiler chickens (Igbasan and Guenter, 1996; Perez-Maldonado et al., 1999; Crepon, 2006; Laudadio and Tufarelli, 2010; Dotas et al., 2014), turkey broilers (Castell et al., 1996) or guinea fowl broilers (Laudadio et al., 2012). Similarly to our findings,

Czerwiński et al. (2010) found that the use of dietary peas in broilers increased feed intake and decreased feed conversion ratio. Therefore, the growth performance of broiler chickens obtained in current study suggest that the peas (cv. Tudor) used did not seem to contain deleterious levels of ANFs.

Contrary to these studies, McNeill et al. (2004) reported inferior performance (growth rate and feed efficiency) in broilers fed diets with 20% peas meal, but the diets used in their study were not been isocaloric and balanced for digestible amino acids.

Diaz et al. (2006) also, observed reduced body weight and decreased feed conversion efficiency when fed broilers with extruded pea seeds. For this reason, possible explanations for the causes of inconsistencies in poultry performance could be attributed to variations in the formulation of diets, the level of inclusion or the form of use (seeds, micronized and dehulled seeds, extruded, meal).

Carcass traits and digestive organ size

Table 3 present the results of carcass traits and relative digestive organ weight of broilers at slaughter. No differences between treatments was observed for carcass weight and carcass yield (P>0.05). However, the yields of breast significantly increase in birds fed peas diet with 9.10% (P=0.038) compared to C. The optimum performance of broiler chicks fed dietary peas (cv. Tudor) could be partially attributed to a well-balanced amino acid profile associated with the supplementation of essential amino acids (lysine and methionine). It is well known that the dietary imbalance ofamino acids could diminishedthe percentage of breast muscles in carcasses of poultry (Nasr, 2011). Our results partially agrees with the previous studies (Masoero et al., 2005; Laudadio and Tufarelli, 2010; Laudadio et al., 2011) that shown no effect of dietary alternative protein source on dressing percentage, breast and drumstick percentages in broilers.

Also, feeding dietary peas tend to increase the thigh and drumstick yields (P=0.062) and to decrease the abdominal fat percentage (P=0.055). Similar results were reported by Laudadio et al. (2012) who found that feedingdehulled-micronized peas in guinea fowl broilers tend to increase the legs yields and decrease significantly the abdominal fat. There were no differences due to dietary treatments in the relative weight of the gizzard, liver, pancreas and spleen (P>0.05).

Table 3. Carcass characteristics and relative digestive organs weight of broilers fed dietary peas[1]

Parameter	Control	Peas (cv. Tudor)	SEM	*P-value*
Carcass traits				
Carcass weight, g	1488.50	1512.30	16.29	*0.198*
Carcass yield, %[2]	70.40	70.78	0.26	*0.504*
Breast, %[2]	24.39[b]	26.61[a]	0.56	*0.038*
Thigh+drumstick, %[2]	19.11	19.88	0.21	*0.062[T]*
Abdominal fat, %[2]	1.27	1.06	0.05	*0.055[T]*
Digestive organ size (g/100 g BW)				
Gizzard	1.55	1.67	0.06	*0.363*
Liver	2.10	1.89	0.06	*0.105*
Pancreas	0.22	0.20	0.007	*0.189*
Spleen	0.08	0.09	0.004	*0.643*

[a,b]Means with different superscript within a row differ significantly (P<0.05); SEM, standard error of means; T, tendency to be influenced by treatment (P<0.10).
[1]n=16 samples for each dietary treatment; [2]% of BW at slaughter.

Plasma profile

Generally, plasma biochemical parameters at 35 d (Table 4), were not affected by the dietary peas (cv. Tudor) inclusion, except the concentration of total protein that was significantly increase (13.2%; P=0.045) compared with control. The other components of plasma protein profile (albumin, total bilirubin, creatinine and urea), considered as a relevant marker of protein metabolism and synthesis in poultry organism, were lower, but not differ significantly between treatments (P>0.05).

The results of present study are in line with Bingol et al. (2016), who reported that the substitution of 20% soybean protein with pea protein in broilers significantly increased blood total protein and the beta and gamma-globulins concentrations, and indicated also that peas could stimulate the proteins synthesis related with immunity. In our study, it was observed that the concentrations of glucose, cholesterol and triglycerides were decreased in PT group compared with C, with no significantly difference between treatments (P>0.05). Previous studies with rats have shown that dietary pea proteins reduce blood cholesterol and triglyceride levels (Rigamonti et al., 2010; Sirtori et al., 2012) and have decreased the blood lipid profile (Spielmann et al., 2008).

Dai et al. (2013) reported that pea protein could have hypocholesterolemic effect due to the low

methionine and high arginine content. Dietary peas inclusion favorised cholesterol conversion into bile acid in hamsters fed a high fat diet, exerting hypolipidemic activity, that was assigned to the fact that peas contained high amounts of unsaturated fatty acid and phytosterol. It is known that occurrence in blood of certain intercellular origin enzymes in abnormal amounts indicate organs or tissue injure (Wilson, 2008). Thus, the liver injury could abnormally increase the values of its specific enzymes (ALT, AST, LDH and GGT) in the blood (Kaplan et al., 2003). In this study, plasma enzymes activity (AP, ALT, AST and GGT) was not influenced by dietary treatments (P>0.05), and was noticed that dietary peas (cv. Tudor) addition did not affect the function of liver and kidney, as important health status parameters.

Table 4. Plasma metabolic profile of broilers fed dietary peas[1]

Plasma parameter	Control	Peas (cv. Tudor)	SEM	*P-value*
Total protein (g/dL)	2.50[b]	2.81[a]	0.09	*0.045*
Albumin (mg/dL)	1.53	1.50	0.10	*1.000*
Total bilirubin (mg/dL)	0.47	0.44	0.07	*0.893*
Creatinine (mg/dL)	0.95	0.50	0.08	*0.173*
Urea (mg/dL)	8.18	6.52	0.32	*0.211*
Glucose (mg/dL)	199.84	192.87	3.55	*0.909*
Cholesterol (mg/dL)	95.98	90.00	2.42	*0.992*
Triglycerides (mg/dL)	52.17	50.33	1.46	*0.973*
AP (U/L)	43.84	44.15	1.79	*1.000*
AST (U/L)	59.36	52.67	1.41	*0.249*
ALT (U/L)	46.98	55.85	1.31	*0.169*
GGT (U/L)	34.68	47.33	2.42	*0.247*

[a,b]Means with different superscript within a row differ significantly (P<0.05); SEM, standard error of means; [1]n=16 samples for each dietary treatment.
AP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gammaglutamyltransferase.

Intestinal parameters and ileal microflora

The effect of diets on length of the intestinal tract and intestinal digesta pH of broilers at 35 d of age are shown in Table 5. No significant differences were observed in relative weights and lengths of the digestive compartments or in pH values of intestinal digesta (P>0.05) between birds fed the different dietary treatments. In our study the values of intestinal pH, considered as important parameters that influence the protein digestion and solubility (Recoules et al., 2017), range between normal limits (5.5 - 6.5) reported for monogastric animals on different segments of intestinal tract (Ao et al., 2008). Moreover, the absence of an effect of dietary peas inclusion on intestinal development and pH was confirmed by growth performance results, which were superior than control group.

Table 5. Relative intestinal weight and length, intestinal digesta pH of broilers fed dietary peas[1]

Item	Control	Peas (cv. Tudor)	SEM	*P-value*
Weight (g/100 g BW)				
Duodenum	0.66	0.59	0.02	*0.122*
Jejunum	1.53	1.43	0.03	*0.164*
Ileum	1.01	1.14	0.05	*0.232*
Caecum	0.59	0.54	0.02	*0.237*
Length (cm/100 g BW)				
Duodenum	1.44	1.37	0.02	*0.164*
Jejunum	3.69	3.66	0.07	*0.875*
Ileum	2.67	2.73	0.12	*0.819*
Caecum	0.84	0.81	0.02	*0.437*
pH				
Duodenum	5.59	5.72	0.06	*0.285*
Jejunum	5.91	6.04	0.04	*0.786*
Ileum	6.17	6.12	0.10	*0.937*
Caecum	6.56	6.61	0.03	*0.670*

[1]n=16 samples for each dietary treatment; SEM, standard error of means.

Analysis of intestinal microflora is important in evaluate intestinal health, and also to support the productive performance obtained in this study (Table 6). Changes in the intestinal microbial population of broiler chickens couldbe related with the efficient feed utilization (Zulkifli et al., 2009).

Table 6. Ileal microbial populations (log10 cfu/g) of broilers fed dietary peas[1]

Item	Control	Peas (cv. Tudor)	SEM	*P-value*
Enterobacteriaceae	7.156	7.112	0.02	*0.139*
E. coli	5.543	5.450	0.12	*0.843*
Lactobacillus spp.	6.031[B]	6.383[A]	0.07	*<0.0001*
Lactobacillus:E. coli ratio	1.088	1.171	0.03	*0.200*

[AB]Means with different superscript within a row differ significantly (P<0.01); [1]n=16 samples for each dietary treatment; SEM, standard error of means; *Salmonella* spp. not detected.

The results of our study shown that the bacteriological composition of ileal content was not affected by the inclusion of peas (cv. Tudor) in chicken diets (P>0.05), except the counts of *Lactobacillus* spp. that significantly increased with 5.83% (P<0.0001), compared with C diet.

No differences were found in the ileal population of *Enterobacteriaceae* and *E. coli* among dietary treatments at 35 d of age. The *Lactobacillus: E. coli* ratio increased in the ileal content of broilers fed peas diet, but the

difference was not significantly (P>0.05). *Salmonella* spp. was not detected in the samples of ileal content. Probably, in present study, the improvement in performance of broilers fed peas cv. Tudor may be due to the presence of beneficial bacterial species such as *Lactobacillus* spp. and/or absence of detrimental bacterial species.

It is stated that bacterial populations from gastrointestinal tract play a crucial role in broiler growth and health status, modulating the development of the digestive tract, affecting nutrient digestion and absorption and stimulating gut immune functions (Choct, 2009; Lee et al., 2010; Torok et al., 2011). Moreover, Han et al. (2016) reported the small intestine has the highest concentration of bacterial cells, principally *Lactobacillus, Enterococcus*, and various *Clostridiaceae* and the dominant genus was *Lactobacillus* accounting for almost 70% of the total. To our knowledge little publisheddata are available about the influence of dietary pea seeds on ileal microflora composition in broilers. Czerwiński et al. (2010), evaluated the effect of pea and probiotic and/or acidifier supplementation on growth performance and composition of caecal microbiota of broiler chicken.These authors noticed that dietary inclusion of pea (150 g/kg) and encapsulated acidifiers may change the bacterial community structure in the distal part of the chicken gastrointestinal tract. Recently, Tusnio et al. (2017) reported that partial replacement of soybean meal with pea seeds in pigs diets did not considerably change microbial activity and intestinal morphology, indicating that they were a good alternative protein source to soybean meal.

CONCLUSIONS

The use of peas (*Pisum sativum* L. cv. Tudor) in the amount of 200 g/kg in broiler diets as a partial replacementof SBM, improve growth performance and breast yields, has a positive effect on plasma total protein and alter ileal microflora by increasing *Lactobacillus* spp.

In addition, it is widely accepted that the use of alternative local legumes protein sources in poultry diets is an important tool to increase the economic efficiency.

ACKNOWLEDGEMENTS

"This work was supported by Romanian Ministry of Research and Innovation, Program 1. Development National Research-Development, Subprogram 1.2. Institutional Performance - Projects funding excellence in R&D, Contract No.17 PFE and Ministry of Agriculture and Rural Development, Project ADER 6.1.1."

REFERENCES

Ao T., Cantor A.H, Pescatore A.J., Pierce J.L., 2008. *In vitro* evaluation of feed-grade enzyme activity at pH levels simulating various parts of the avian digestive tract. Animal Feed Science and Technology, 140: p. 462-468.

Alonso R., Orue E., Marzo F., 1998. Effects of extrusion and convention al processing methods on protein and antinutritional factor contents in pea seeds. Food Chemistry, 63 (4): p. 505-512.

Bingol N.T., Dede S., Karsli M.A., Deger Y., Kiliçalp D. Kılınç, Çetin S., 2016. Effects of the replacement of soybean meal with pea as dietary protein source on the serum protein fractions of broilers. Brazilian Journal of Poultry Science, 18 (4): p. 639-644.

Castell A.G., Guenter W., Igbasan F.A., 1996. Nutritive value of peas for non-ruminant diets. Animal Feed Science Technology, 60: p. 209-227.

Choct M., 2009. Managing gut health through nutrition. British Poultry Science, 50: p. 9-15.

Cobb-Vantress, 2015. Coob 500 Broiler Performance and Nutrition Supplement. Available from: http://www.cobbvantress.com.

Collins C.L., Eason P.J., Dunshea F.R., Higgins T.J.V., King R.H., 2006. Starch but not protein digestibility is altered in pigs fed transgenic peas containing α-amylase inhibitor. Journal of Science of Food and Agriculture, 86: p. 1894-1899.

Crepon K., 2006. Nutritional value of legumes (pea and faba bean) and economics of their use. In Recent Advances in Animal Nutrition. P.C. Garnsworthy and J. Wiseman, ed. Nottingham University Press, Nottingham, UK, p. 332-366.

Czerwicski J., Hřjberg O., Smulikowska S., Engberg R.M., Mieczkowska A., 2010. Influence of dietary peas and organic acids and probiotic supplementation on performance and caecal microbial ecology of broiler chickens. British Poultry Science, 51 (2): p. 258-269.

Dai F.J., Hsu W.H., Huang J.J., Wu S.C., 2013. Effect of pigeon pea (*Cajanus cajan* L.) on high-fat diet-induced hypercholesterolemia in hamsters. Food and Chemical Toxicology, 53: p. 384-391.

Diaz D., Morlacchini M., Masoero F., Moschini M., Fusconi G., Piva G., 2006. Pea seeds (*Pisum sativum*), faba beans (*Vicia faba* var. Minor) and lupin seeds (*Lupinus albus* var. Multitalia) as protein

sources in broiler diets: Effect of extrusion on growth performance. Italian J. of Animal Sci., 5: p. 43-53.

Dotas V., Bampidis V.A., Sinapis E., Hatzipanagiotou A., Papanikolaou K., 2014. Effect of dietary field pea (*Pisum sativum* L.) supplementation on growth performance, and carcass and meat quality of broiler chickens. Livestock Science, 164: p. 135-143.

Fru-Nji F., Niess E., Pfeffer E., 2007. Effect of graded replacement of soybean meal by faba beans (*Vicia faba* L.) or field peas (*Pisum sativum* L.) in rations for laying hens on egg production and quality. Japan Poultry Science, 44: p. 34-41.

Han G.G., Kim E.B., Lee J., Lee J.-Y., Jin G., Park J., Huh C.-S., Kwon I.-K., Kil D.Y., Choi Y.-J., Kong C., 2016. Relationship between the microbiota in different sections of the gastrointestinal tract and the body weight of broiler chickens. Springerplus, 5:911.

Igbasan F.A., Guenter W., 1996. The enhancement of the nutritive value of peas for broiler chickens: An evaluation of micronization and dehulling processes. Poultry Science, 75: p. 1243-1252.

Igbasan F.A., Guenter W., 1997. The influence of micronization, dehulling and enzyme supplementation on the nutritional value of peas for laying hens. Poultry Science, 76: p. 331-337.

Kaplan L.A., Pesce A.J., Kazmierczak S.C., 2003. Liver Function. In: Clinical Chemistry. Ed: Sherwin, J.E., 4th ed. Mosby. An affiliate of Elsevier Science, St. Lauis, Toronto.

Khattab R.Y., Arntfield S.D., Nyachoti C.M., 2009. Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation. LWT-Food Science and Technology, 42: p. 1107-1112.

Laudadio V., Tufarelli V., 2010. Growth performance and carcass meat quality of broiler chickens fed diets containing micronized-dehulled peas (*Pisum sativum* cv. Spirale) as a substitute of soybean meal. Poultry Science, 89: p. 1537-1543.

Laudadio V., Tufarelli V., 2011. Pea (*Pisum sativum* L.) seeds as an alternative dietary protein source for broilers: Influence on fatty acid composition, lipid and protein oxidation of dark and white meats. J. of American Oil Chemical Society, 88: p. 967-973.

Laudadio V., Tufarelli V., 2012. Effect of treated field pea (*Pisum sativum* L. cv. Spirale) as substitute for soybean extracted meal in a wheat middlings-based diet on egg production and quality of early laying brown hens. Archiv Fur Geflugelkunde, 76: p. 1-5.

Lee K., Lillehoj H.S., Siragusa G.R., 2010. Direct-fed microbials and their impact on the intestinal microflora and immune system of chickens. Journal of Poultry Science, 47: p. 106-114.

Masoero F., Pulimeno A.M., Rossi F., 2005. Effect of extrusion, expansion and toasting on the nutritional value of peas, faba beans and lupins. Italian Journal of Animal Science, 4: p. 177-189.

McNeill L., Bernard K., MacLeod M.G., 2004. Food intake, growth rate, food conversion and food choice in broilers fed on diets high in rapeseed meal and pea meal, with observations on sensory evaluation of the resulting poultry meat. British Poultry Science, 45: p. 519-523.

Nasr J., 2011. Effect of different levels of amino acids on carcasscomposition and yield in broilers. Animal Production Science, 51: p. 1123-1126.

Perez-Maldonado R.A., Mannion P.F., Farrell D.J., 1999. Optimum inclusion of field peas, faba beans, chick peas and sweet lupins in poultry diets. I. Chemical composition and layer experiments. British Poultry Science, 40: p. 667-673.

Recoules E., Sabboh-Jourdan H., Narcy A., Lessire M., Harichaux G., Labas V., Duclos M.J., R´ehault-Godbert S., 2017. Exploring the *in vivo* digestion of plant proteins in broiler chickens. Poultry Science, 96: p. 1735-1747.

Rigamonti E., Parolini C., Marchesi M., Diani E., Brambilla S., Sirtori C.R., Chiesa G., 2010. Hypolipidemic effect of dietary pea proteins: Impact on genes regulating hepatic lipid metabolism. Molecular Nutrition & Food Research, 54: p. S24-S30.

Salgado P., Freire J.P.B., Mourato M., Cabral F., Toullec R., Lallès J.P., 2002. Comparative effects of different legume protein sources in weaned piglets: nutrient digestibility, intestinal morphology and digestive enzymes. Livestock Produc. Sci., 74 (2): p.191-202.

Sirtori C.R., Triolo M., Bosisio R., Bondioli A., Calabresi L., De Vergori V., Gomaraschi M., 2012. Hypocholesterolaemic effects of lupin protein and pea protein/fibre combinations in moderately hypercholesterolaemic individuals. British Journal of Nutrition, 107 (8): p. 1176-1183.

Spielmann J., Stangl G.I., Eder K., 2008. Dietary pea protein stimulates bile acid excretion and lowers hepatic cholesterol concentration in rats. J. of Animal Physiology and Animal Nutrition, 92 (6): p. 683-693.

Torok V.A., Hughes R.J., Mikkelsen L.L., Perez-Maldonado R., Balding K., MacAlpine R., Percy N.J., Ophel-Keller K., 2011. Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. Applied and Environmental Microbiology, 77: p. 5868-78.

Tufarelli V., Naz S., Khan R.U., Mazzei D., Laudadio V., 2012. Milk quality, manufacturing properties and blood biochemical profile from dairy cows fed peas (*Pisum sativum* L.) as dietary protein supplement. Archiv fur Tierzucht, 55: p. 132-139.

Tuśnio A., Taciak M., Barszcz M., Święch E., Bachanek I., Skomiał J., 2017. Effect of replacing soybean meal by raw or extruded pea seeds on growth performance and selected physiological parameters of the ileum and distal colon of pigs. PLoS ONE, 12 (1): e0169467.

Wilson D.D., 2008. Manual of laboratory and diagnostic tests. McGraw-Hills 1st Edition.

Zuidhof M.J., Carney V.L., Schneider B.L., Renema, R.A., Robinson F.E., Betti M., 2006. Broiler meat quality and yield dynamics. Poultry Service Industry Workshop, October 3rd-5th, p. 79-88.

Zulkifli I., Iman Rahayu H.S., Alimon A.R., Vidyadaran M.K., Babjee S.A., 2009. Gut micoflora and intestinal morphology of commercial broiler chickens and Red Jungle Fowl fed diets containing palm kernel meal. Archiv Fur Geflugelkunde, 73: p. 49-55.

***NRC, 1994. Nutrient Requirements of Poultry, 9th ed. National Academy Press, Washington DC, USA.
***OJEU (Official Journal of the European Union L 54), 2009. Commission Regulation (EC) No. 152/2009 laying down the methods of sampling and analysis for the official control of feed. Series L 155: p. 1-66.
***OJEU (Official Journal of the European Union), 2010. Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes. Series L 276: p. 33-79.
***SPSS, 2011. Statistics version 20.0. IBM SPSS Inc, USA.