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Effects of the Replacement of Soybean Meal with Pea as Dietary Protein Source on the Serum Protein Fractions of Broilers

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ABSTRACT

The aim of this study was to determine the effects of the replacement of different levels of protein derived from soybean meal with that from peas in broiler diets on serum protein fractions. A corn-soybean meal basal diet was formulated as the control diet (Control=C) (NRC, 1994), and then pea was added to the control diet to replace 20% (P20) or 40% (P40) of the crude protein of the control diet. The diets were randomly fed to 12 pens per treatment, each housing five birds, for 42 days. Blood samples were collected from 36 birds (3 birds x 4 pens x3 treatments) and the serum protein fractions were separated. Gamma-globulin percentage was higher in group P20 compared with C and P40 groups. Total protein, beta-globulin, and gamma-globulin concentrations were significantly higher in group P20 compared with those of both control and P40 group (p<0.05).

INTRODUCTION

After the ban of the use of animal products as protein sources in the livestock diets (European Commission Decisions No. 98/272/CE and 2000/374/CE), producers were forced to utilize soybeans (*Glycinemax* L. Merr.) in poultry diets due to its particularly high protein content. Nevertheless, consumer resistance has developed against the inclusion of soybeans in many places, because most of it derives from genetically modified crops and cannot be used in for organic farming (Vicenti *et al*., 2009). The cost of soybeans also dramatically increased after the ban, increasing feed costs. The increase in feed costs and the restrictions on the supply of conventional animal protein sources to livestock has stimulated researchers to evaluate the use of alternative local legume protein sources that are not commonly utilized in poultry diets (Laudadio & Tufarelli, 2010).

Under these circumstances, researchers have started to search for non-genetically modified feedstuffs that could meet the protein requirements of poultry. One protein source alternative to soybean may be peas (*Pisum sativum* L.), which is widely produced in the Mediterranean region and has high nutritional values (Ravindran & Blair, 1992; Castell *et al*., 1996). Although pea seeds are significantly high in starch compared with many other cereal grains, their use is still very limited in poultry diets. It is well-known that starch is the greatest single dietary source of energy in animal diets (Longstaff & McNab, 1987).

Peas have been used for a natural source of plant protein in human and animal nutrition (Mariotti *et al*., 2001; Musa *et al*., 2012). The aqueous extract of green peas contains three fractions (albumin, legumin, and vicilin) (Malley *et al*., 1975).

The two main protein groups in blood serum are albumin and globulin. Serum proteins can be further separated by electrophoresis into five protein fractions (albumin, and α1-, α2-, β-, and γ-globulins). Serum protein electrophoresis (SPEP) measures the levels of specific proteins in the blood to help identifying various conditions, such as nutritional deficiencies, some diseases, liver disorders, acute inflammatory and proliferative diseases, immune status, tissue damage like trauma, and many physiological disorders (Manojlović *et al*., 1993; Erstad, 1996; Karagul *et al.*, 2000; Mehmetoglu, 2002; Murray, 2003; SPEP).

The objective of this study was to investigate the effects of the replacement of soybean meal with peas at different levels as dietary protein source on the serum protein fractions of broilers.

MATERIAL AND METHODS

Birds and housing

A total of 60 one-day-old Ross 308 broilers, obtained from a commercial hatchery, were allotted according to initial body weight into the 12 floor pens (five birds/pen) on wood-shavings litter in an environmentally-controlled room. The temperature was maintained at 35°C during the first week and then gradually reduced to 22°C at 35 days of age. The birds received 23 h of fluorescent illumination per day and were allowed free access to the diets and clean water. The experimental procedures were approved by Yuzuncu Yil University Animal Ethics Committee.

Treatments

The starter diet contained 24% crude protein (CP) and 13.2 MJ metabolizable energy/kg and was fed from weeks 1 to 3, and the finisher diet (21% CP and 13.5 MJ/kg) was fed from weeks 4 to 6 weeks. The chemical composition of the feedstuffs used in the diets was analyzed before feed formulation. A corn-soybean meal basal diet was formulated as the control diet (Control=C) (NRC, 1994), and then pea was added to the control diet to replace 20% (P20) or 40% (P40) of the crude protein of the control diet. The composition and chemical analyses of the starter and finisher diets are presented in Tables 1 and 2. The diets were randomly fed to 12 pens per treatment, each housing five birds, for 42 days.

Table 1 – Composition of the starter diets (Weeks 1-3)

	Diets		
	C	P20	P40
Ingredients [%]			
Corn	48.02	44.87	41.70
Soybean meal	41.16	32.94	24.71
Pea	-	11.32	22.64
Sunflower Oil	5.68	5.68	5.68
Fish Meal	2	2	2
Limestone	2.05	1.5	1.5
Di calcium phosphate	0.20	0.85	0.85
Vit+Min premix	0.30	0.30	0.30
DL- Methionine	0.15	0.20	0.29
NaCl	0.44	0.34	0.33
Analysis [%]			
Dry Matter	93.20	93.61	93.83
Crude protein	23.66	23.88	23.78
Ash	7.64	5.02	6.75
Ether Extract	6.69	7.22	6.86
Crude Fiber	4.74	5.26	4.97
*ME (MJ/kg)	13.21	13.26	13.25

Vitamin-Mineral premix (IU or mg kg^{-1} diet): Vitamin A: 12000 IU; Vitamin D_3: 1500 IU; Vitamin E: 30 mg; Vitamin K_3: 5 mg; Vitamin B_1: 3 mg; Vitamin B_2: 6 mg; Vitamin B_6: 5 mg; Vitamin B_{12}: 0.03 mg; Nicotine amid: 40 mg; Calcium-D-pantothenate: 10 mg; Folic acid: 0.075 mg; Choline chloride: 375 mg; Antioxidant: 10 mg; Manganese: 80 mg; Iron: 80 mg; Zinc: 60 mg; Copper: 8 mg; Iodine: 0.5 mg; Cobalt: 0.2 mg; Selenium: 0.15 mg.

*Metabolizable energy calculated according to Titus and Fritz (1971).

Table 2 – Composition of the finisher diets (Weeks 4-6)

	Diets		
	C	P20	P40
Ingredients [%]			
Corn	56.35	53.85	51.32
Soybean meal	33.03	26.44	19.85
Pea	-	9.06	18.12
Sunflower Oil	5.88	5.88	5.88
Fish Meal	2	2	2
Limestone	2	2	2
Di calcium phosphate	0.11	-	-
Vit+Min premix	0.30	0.30	0.30
DL- Methionine	0.06	0.09	0.16
NaCl	0.27	0.38	0.37
Analysis [%]			
Dry Matter	93.59	93.66	93.25
Crude protein	21.18	21.45	21.38
Ash	6	5.24	5.08
Ether Extract	7.24	7.73	6.70
Crude Fiber	5.28	4.79	4.35
*ME (MJ/kg)	13.50	13.59	13.60

Vitamin-Mineral premix (IU or mg kg^{-1} diet): Vitamin A: 12000 IU; Vitamin D_3: 1500 IU; Vitamin E: 30 mg; Vitamin K_3: 5 mg; Vitamin B_1: 3 mg; Vitamin B_2: 6 mg; Vitamin B_6: 5 mg; Vitamin B_{12}: 0.03 mg; Nicotine amid: 40 mg; Calcium-D-pantothenate: 10 mg; Folic acid: 0.075 mg; Choline chloride: 375 mg; Antioxidant: 10 mg; Manganese: 80 mg; Iron: 80 mg; Zinc: 60 mg; Copper: 8 mg; Iodine: 0.5 mg; Cobalt: 0.2 mg; Selenium: 0.15 mg.

*Metabolizable energy calculated according to Titus and Fritz (1971).

Collection of blood samples

On day, blood samples were collected from the wing vein from three birds per replicate, totaling 36 birds (3 birds x 4 pens x3 treatments). Serum samples was separated by centrifugation at 500 g for 10 min for biochemical analyses.

Biochemical analyses

Total protein blood levels were determined by the biuret method. Serum protein fractions were separated using the Helena Lab-Titan III® Serum Protein Electrophoresis device (Cat No. 3023), Helena Lab - Titan III Cellulose acetate cards, and Electra HR Buffer (Cat No. 5805), and buffer solutions (Helena, Bioscience Europe, UK). The obtained bands were evaluated for serum protein fractions using the software Platinum 3.0.

Statistical Analysis

The data were subjected to one-way analysis of variance. Duncan test was applied for multiple comparisons of the means. Differences were considered significant at $p<0.05$ using the SPSS 22.0 statistical software.

RESULTS

The serum protein fraction bands obtained by electrophoresis were assessed using the software Platinum 3.0, and the serum levels of albumin; alpha 1, alpha 2, beta, and gamma globulins and their percentages relative to total protein levels were determined, andalbumin to globulin ratio was calculated.

Total protein concentration was significantly higher in the group P20 compared with that of both control and P40 groups ($p<0.05$). Albumin, and alpha 1 and 2 globulin concentrations were not different among treatments. Beta-globulin concentration significantly higher in group P20 compared with that of control and group P40 ($p<0.05$). The percentage of gamma globulin was higher in group P20 compared with that of group P40 ($p<0.05$), but similar to that of the control group. The percentages of the other blood protein fractions and A/G ratio were not affected by dietary treatment. Gama-globulin concentrations were significantly higher in group P20 compared with both the control and P40 groups ($p<0.05$).

DISCUSSION

Some pea cultivars show considerable potential as plant protein sources for broilers (Cowieson *et al*., 2003). Dietary pea inclusion was shown to improve the performance and meat quality of broiler chickens (Laudadio & Tufarelli, 2010). Evaluating four white-flowering pea cultivars (Avia, Laser, Madonna and Miami) Kluth *et al*. (2005) observed that the cultivar with the lowest AA digestibility caused the lowest energy digestibility both at 150 and 300 g/kg diet. The inclusion of peas in broiler diets increased feed intake and decreased gain:feed ratio (Czerwi□ski *et al*., 2010). Thacker *et al*. (2013) stated that increasing the availability of phosphorus in peas meant that less inorganic phosphorus would be required to meet the nutritional requirements of broilers.

The effect of different levels of raw grass pea caused a remarkable decrease in gross energy and lipid apparent digestibility (Riasi *et al*., 2015). Spielmann

Table 3 – Relative levels of serum proteins of 42-d-old broilers fed the experimental diets (C: control, P20: 20% of the dietary protein derived from peas, P40: 40% of the dietary protein derived from peas).

Diets	Albumin	Alpha1 globulin	Alpha2 globulin	Beta globulin	Gamma globulin	Total Protein	A/G ratio
C	32.17±1.42	12.23±1.53	16.71±1.54	14.58±0.96	24.11±1.78^{ab}	2.39±0.09	0.48±0.03
P20	28.37±2.87	14.18±2.07	14.17±1.33	17.21±1.20	26.97±1.13^{b}	2.96±0.17	0.44±0.06
P40	34.93±1.95	15.33±1.08	15.63±2.11	16.07±1.59	21.53±1.01^{a}	2.46±0.07	0.49±0.02

$^{a, b}$Means with different superscripts within a column are significantly different ($p<0.05$).

Table 4 – Serum protein fractions (g/L) of 42-d-old broilers fed the experimental diets (C: control, P20: 20% of the dietary protein derived from peas, P40: 40% of the dietary protein derived from peas).

Diets	Albumin	Alpha1	Alfa2	Beta	Gama
C	0.817±0.053	0.295±0.044	0.372±0.048	0.330±0.0259^{b}	0.575±0.046
P20	0.826±0.059	0.440±0.083	0.414±0.031	0.542±0.067^{a}	0.800±0.056
P40	0.895±0.077	0.400±0.034	0.458±0.065	0.376±0.037^{b}	0.535±0.035

$^{a, b}$ Means with different superscripts within a column are significantly different ($p<0.05$).

et al. (2008) observed that pea protein has a low methionine content and a high arginine content. The addition of pea meal as a source of protein into broiler diets can improve carcass quality and may result in favorable lipid profile, without adversely affecting growth performance traits (Igbasan & Guenter, 1996; Pilarski *et al*., 2005; Laudadio *et al*., 2012).

The broiler serum proteins and fractions can be affected by the application of different feedstaff (Bingol et al., 2016). In this study, total protein concentrations in group P20 were significantly higher than in group P40 ($p<0.05$). It may be assumed that pea amino acid composition influenced total protein concentration, and that resulted from the differences among globulin fractions.

Albumin influences osmotic pressure balance and the transport of substances in the blood. Albumin also prevents blood from leaking out of the vessels (Adkins *et al*., 2002; Metzler & Metzler, 2003; Prinsen *et al*., 2004; Jacobs *et al*., 2005; SPEP). In the present study, no changes in albumin percentage or concentration were observed, suggesting that pea proteins may affect mostly globulin fractions.

Serum globulin concentrations are followed up in liver diseases, chronic infections, acute diffuse glomerulonephritis, sarcoidosis, carcinomas and autoimmune diseases (Karagul *et al*., 2000; Turgut, 2000; Mehmetoglu, 2002; SPEP). The alpha-1 globulin fraction include (α_1-antitrypsin,α_1-acid glycoprotein, α_1-lipoprotein (Apolipoprotein A), α_1-fetoprotein (AFP), transcortine, protein connecting thyroxine, glycoprotein) (Turgut, 2000; Mehmetoglu, 2002; SPEP). Pea proteins markedly reduces blood cholesterol and triglyceride levels in rats (Martins *et al*., 2004; Rigamonti *et al*., 2010; Sirtori *et al*., 2012). Dietary pea has a lowering effect on the blood lipid concentrations of rats (Spielmann *et al*., 2008). Pea protein contains low methionine and high arginine levels, and this is important for the hypocholesterolemic effect. Dietary pea addition promoted cholesterol conversion into bile acid in hamsters fed a high fat diet, thereby exerting hypolipidemic activity, which was attributed to the fact that pigeon pea contained high unsaturated fatty acid levels (Dai *et al*., 2013). Pea protein stimulates the formation and excretion of bile acids, which reducing hepatic cholesterol concentration and a reduced cholesterol secretion via VLDL (Spielmann *et al.,* 2008).

The alpha-2 globulin fraction includes important metabolic and acute-phase proteins, such as α2-macroglobulin and haptoglobin (Mehmetoglu, 2002; Gungor *et al*., 2004; SPEP). In this study, the percentages and concentrations alpha1 and alpha2 globulins were not statistically different among groups. The pea levels added to the diets in the present experiment did not affect alpha-globulin levels.

Beta-globulin proteins help to transport substances, such as iron, through the bloodstream, and help fighting against infections (Karagul *et al*., 2000; SPEP). Beta-globulin concentrations were higher in group P20 compared with both C and P40 groups in the current study.

Gamma globulins are also called antibodies, which help to prevent and fight against infections. Gamma globulins bind to foreign substances, such as bacteria or viruses, causing them to be destroyed by the immune system. Immunoglobulins and the C-reactive protein (CRP) migrate in the γ-globulin area (Erstad 1996; Karagul *et al*., 2000; Mehmetoglu 2002; Murray, 2003). The long-term intake of pea fiber (PF) was shown to improve colonic function via altering colonic barriers, colonic immunity, and metabolism-related protein gene expressions (Che *et al*., 2014). The percentages and concentrations gamma globulin were greater in group P20 compared with group P40 in the current study ($p<0.05$).

Pea proteins increased beta- and gamma-globulin fractions, particularly when added at 20% of dietary CP level. Therefore, both this level and period of inclusion seem to be ideal for broiler chickens. It should be noted that concentration and percentage of gamma-globulin fractions when 20% pea protein was added to the basal diet, indicating that pea may stimulate the synthesis of proteins associated with immunity.

No changes in blood A/G ratio where observed in the pea-fed groups, despite the increase in total globulin concentration.

This study showed that albumin, alpha-1, and alpha-2 globulin concentrations and A/G ratio were not affected by dietary pea addition. However, the replacement of 20% soybean protein with pea protein significantly increased the blood concentrations of beta and gamma-globulins and total protein of broilers. Considering that addition of pea in the basal diet may stimulate the synthesis of proteins of the beta- and gamma-globulin fractions, further studies to determine the optimal level of pea addition to broiler diets are warranted.

CONFLICT OF INTERESTS STATEMENT

The authors have no conflict of interest regarding the publication of this article.

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