ORJİNAL MAKALE / *ORIGINAL PAPER*

EFFECTS OF SOYBEAN PROTEIN SUBSTITUTION WITH PEA IN DIET ON BROILER CHICK SERUM ANTIOXIDANT VITAMIN AND MINERAL LEVELS

Soya Proteini Yerine Bezelye Verilmesinin Broiler Civcivlerinin Serum Antioksidan Vitamin ve Mineral Düzeylerine Etkisi

Semiha DEDE[1], Nuriye Tuğba BİNGÖL[2], Dide KILIÇALP KILINÇ[3], Yeter DEĞER[1], İbrahim Hakkı YÖRÜK[4], Mehmet akif KARSLI[5]

[1] *Veteriner Fakültesi, Biyokimya Anabilim Dalı, Van Yüzüncü Yıl Üniversitesi, Van, Türkiye.*
[2] *Veteriner Fakültesi, Hayvan Besleme Anabilim Dalı, Van Yüzüncü Yıl Üniversitesi, Van, Türkiye.*
[3] *Sağlık Bilimleri Fakültesi, Adnan Menderes Üniversitesi, Aydin, Türkiye.*
[4] *Fen Fakültesi, Kimya Bölümü, Van, Türkiye.*
[5] *Veterinerlik Fakültesi, Hayvan Besleme Anabilim Dal, Kırıkkale Üniversitesi, Kırıkkale, Türkiye.*

Geliş Tarihi: 04.09.2019, Kabul Tarihi: 17.10.2019

ÖZET

Bu çalışma, soya proteini yerine farklı düzeylerde bezelye verilmesinin broiler civcivlerinin serum antioksidan vitamin ve mineral düzeylerine etkisinin belirlenmesi için planlandı. Bu amaçla, bir bazal (kontrol) diyeti hazırlandı ve soya fasulyesi unu proteininin bazal diyetteki bezelye ile ikame edilmesiyle üç deneysel diyet belirlendi. Çalışma grupları, %20 (%20 Bezelye) ve%40 (%40 Bezelye) olarak, soya küspesi ham proteini (kontrol) yerine bazal diyetteki bezelye proteini ile ikame edilerek oluşturuldu. Civcivler, yukarıda belirtilen diyetlerle, her bir alt gruptaki 5 civciv grubunda (her deneme grubu için toplam 16 civciv) beslendi ve deneyler, 42 gün sürdü. Kan örnekleri her gruptaki 12 piliçten toplandı. Serum Ca, Fe, Mg, Mn, Zn, retinol, tokoferol ve D vitamini düzeyleri belirlendi. Serum α-tokoferol, Fe, Mn ve Mg düzeylerinin değişmediği, ancak retinol ve D vitamini düzeylerinin P20 ve P40 gruplarında arttığı tespit edildi. P20 grubunda Zn düzeyi azalırken, Ca düzeyleri anlamlı olarak arttığı görüldü. Soya fasulyesi diyetindeki bezelye seviyelerinin serum mineral ve vitamin düzeylerini çeşitli şekillerde etkileyebileceği sonucuna varılabilir.

Anahtar kelimeler: Piliç, bezelye, vitamin, mineral, serum

ABSTRACT

The aim of the present study was to determine the effects of soybean meal protein substitution with pea at different levels in broiler diets on serum antioxidant vitamin and mineral levels. A basal (control) diet was prepared and three experimental diets were determined by the substitution of soybean meal protein with pea in basal diet; 20% (20% Pea) and 40% (40% Pea) of soybean meal crude protein (control) were substituted with pea protein in the basal diet to obtain the treatment groups. Chicks were fed with the above-mentioned diets in groups of 5 chicks in each subgroup (a total of 16 chicks for each treatment group) and the experiments lasted for 42 days. The blood samples were collected from 12 chicks in each group. Serum Ca, Fe, Mg, Mn, Zn retinol, tocopherol and vitamin D levels were determined. It was determined that serum α-tocopherol, Fe, Mn and Mg levels did not change, however retinol and vitamin D levels increased in P20 and P40groups. Ca levels significantly increased while Zn levels decreased in the P20 group. It can be concluded that pea levels in soy bean based diet may affect serum mineral and vitamin levels in various ways.

Key words: Broiler, minerals, pea, vitamins,

Sorumlu Yazar: Semiha DEDE, Veteriner Fakültesi, Biyokimya Anabilim Dalı, Van Yüzüncü Yıl Üniversitesi, Van, Türkiye, E-mail: sdede@yyu.edu.tr.

INTRODUCTION

It was advised to utilize different protein sources in broiler diets due to high protein requirement of broiler chicks. Increases in feed cost and limitations in the use of conventional protein sources in broiler diet forces researchers to assess the use of local and cheaper protein sources such as pulses in broiler diets. Pea (*Pisum sativum L.*), a plant commonly cultivated in the Mediterranean region, is rich in protein and can be utilized as a source of protein in broiler diet as, an alternative protein source to soybean meal. Even though peas have been used as natural plant protein for human and animal nutrition and are considerably rich in starch when compared to several grains, the use of pea in poultry diet is still very limited (Longstaff and McNab, 1987; Ravindran and Blair, 1992; Mariotti et al., 2001; Laudadio and Tufarelli, 2010; Musa et al., 2012).

Micro nutrients such as trace minerals and vitamins act as activators of metabolic systems or components of organic compounds. There is a complex interaction between trace minerals and vitamins. In particular, vitamins protect organs from the negative effects of free radicals (Dede et al., 2002; Dede et al., 2008; Gill and Tuteja, 2010).

The objective of the present study was to investigate the effects of the substitution of soybean meal protein with pea in soybean meal based diet on serum antioxidant vitamin and certain minerals levels in broiler chicken.

MATERIAL AND METHODS

Animals and feed

A total of sixty, one-day-old male broiler chicks (ROSS 308) procured from a commercial hatchery were divided into three groups, including control and two experimental groups with a similar mean live weight (average 61g). Each group included 4 sub-groups with 5 chicks each. Each group consumed two different diets throughout the experiment, namely the broiler starter (240 g/kg CP and 13.2 MJ/kg, between 1st and 3rd weeks) and finisher (210 g/kg CP and 13.5 MJ/kg, between 4th and 6th weeks of the experiment). A corn-soybean meal based diet was formulated as the control diet (Control=C) (NRC, 1994), and then pea was added into the control diet to replace 20% (P20) or 40% (P40) of the crude soybean protein in the control diet. The composition and chemical analyses of the starter and finisher diets are presented in Tables 1 and 2. The temperature was maintained at 35°C during the first week of the experiment and gradually reduced to 22°C on the 35th day of the experiment. The chickens received 23 h of fluorescent illumination per day and had free access to the feed and clean water.

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 D3: 1500 IU; Vitamin E: 30 mg; Vitamin K3: 5 mg; Vitamin B1: 3 mg; Vitamin B2: 6 mg; Vitamin B6: 5 mg; Vitamin B12: 0.03 mg; Nicotinamide: 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 was 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 D3: 1500 IU; Vitamin E: 30 mg; Vitamin K3: 5 mg; Vitamin B1: 3 mg; Vitamin B2: 6 mg; Vitamin B6: 5 mg; Vitamin B12: 0.03 mg; Nicotinamide: 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 was calculated according to Titus and Fritz (1971).

Collection of blood samples

Blood samples were collected from the wing vein of the chickens. Three chickens from each sub-group, a total of 36 birds (3 chickens/per pen x 4 pens x3 treatments) were used to collect the blood samples. Serum samples were separated by centrifuge at 500 g for 10 min.

Biochemical analysis

Serum Ca, Fe, Mg, Mn and Zn concentrations were determined with a UNICAM Atomic Absorption Spectrometer using the standard laboratory procedures (Greenberg et al., 1994; Longbottom et al., 1994).

The retinol, tocopherol and vitamins D levels were determined using HPLC (Agilent-1100, Germany), following the procedures described by Miller and Yang (1985) and Karatepe (2004).

Statistical Analysis

The data were analyzed with a One-Way Analysis of Variance. Duncan test was used in multiple comparisons. The mean and standard deviation are used as descriptive statistics. Differences were considered significant when the p value was less than 0.05 in SPSS 22.0 statistics software.

RESULTS

Serum vitamin and mineral levels are presented in Table 3. Although serum retinol, α tocopherol and vitamin D levels increased with the addition of pea into the diets, only the increases in retinol and vitamin D were statistically significant ($P<0.05$). Among the minerals, only serum Ca levels significantly increased with high level pea substitution. Forty percent substitution of soybean meal protein with pea led to numerically higher serum Mg, Fe, Mn and Zn levels when compared to those of the control; however, the differences were not statistically significant ($P>0.05$). Serum Mg and Zn levels in the chicks fed the 20% pea diet were significantly lower when compared to those fed the 40% pea diet ($P<0.05$).

Table 3: Serum vitamin and mineral levels of chickens under different diets

Parameters	Control	P20	P40
Retinol (µg/ml)	0.601±0.096^{b}	1.116±0.199^{a}	0.966±0.213^{a}
α Tocopherol (µg/ml)	0.956±0.169	1.023±0.188	1.237±0.275
Vitamin D (µg/ml)	0.0185±0.0035^{b}	0.0258±0.0036^{a}	0.0223±0.0060^{ab}
Calcium (mg/dl)	15.122±3.674^{b}	12.409±2.452^{b}	20.674±5.640^{a}
Magnesium (mg/dl)	5.278±1.689^{ab}	4.009±1.281^{b}	6.060±1.745^{a}
Iron (mg/dl)	0.327±0.126	0.208±0.065	0.332±0.134
Manganese (mg/dl)	0.0085±0.0042	0.0084±0.0016	0.0119±0.0019
Zinc (mg/dl)	0.1221±0.0434^{a}	0.0675±0.0104^{b}	0.1512±0.0356^{a}

DISCUSSION

Pea (*Pisum sativum L.*) is a good source of both of energy and protein. Dried pea grain contains protein, complex starch and some non-starch carbohydrates. Furthermore, pea also includes micro-nutrients such as minerals, vitamins, phytic acid, isoflavone, saponins, alkaloids, secondary metabolites, and bioactive carbohydrates. It was reported that the addition of pea into broiler diets improved broiler performance and meat quality (Cowieson et al., 2003; Wilson et al., 2003; Rochfort and Panozzo, 2007; Laudadio and Tufarelli, 2010; Dahll et al., 2012; Ebsim, 2013).

Pulse crops (pea, bean, lentils, chickpea, and fava bean) are important sources of both macro-nutrients and minerals (Rochfort and Panozzo, 2007). Pea may play an important role in preventing diseases associated with vitamin and mineral, particularly Se and folate deficiencies (Dahl et al., 2012). In an experiment carried out to determine the key minerals in four pea varieties, while it was found that the most common macro minerals were K, P and Ca, the most common trace minerals were Fe, Se, Zn, Mo, Mn, Cu and B, respectively (CatootjieLusjeNalle, 2009).

There is a gap in the literature on the studies conducted on pea regarding the effects of serum vitamin and mineral levels. Only Arif et al. (2017), reported that addition of pigeon pea into broiler diet did not significantly affect serum calcium levels when compared to the control group.

Trace minerals, known as enzyme and hormone activators, are necessary for several metabolic system functions and maintenance. The metabolisms of Zn and Fe, Cu, Mn, Co and Mo are interactive. Zinc deficiency may lead to growth retardation, bad feathering, and slow development in leg and wing bones. Fe is a part of several proteins and enzymes and necessary for the sustenance of normal physiological functions in humans (Reddy et al., 2004; Herzig et al., 2009). Age, nutrition, physiological status of the poultry could easily affect the mineral metabolism (Dede and Deger, 2000; Dede and Camas, 2001; Deger et al., 2007). Phytate, which is found in high concentrations in pulse crop, may prevent absorption of Zn, Fe and Ca. Furthermore, when serum Fe levels are low, serum Ca and Zn levels increase (Sandberg 2002; Dahll et al., 2012). In the current study, serum Fe and Mn levels did not significantly differ between control and treatment groups. However, serum Mg, Ca and Zn levels were the lowest in chicks that were fed the 20% pea diet.

Vitamin A is one of the essential vitamins and vitamin A deficiency leads to a decrease in animal performance, infertility or impairment in reproduction (Clagett-Dame and DeLuca, 2002). Vitamin A supplementation of the diet significantly increases mRNA expression of Vitamin D receptors in the duodenum mucosa (Yuan et al., 2014). Furthermore, use of Vitamin E in chicken diet is recommended due to anti-oxidant effects and positive effects on metabolic pathways (NRC, 1994). Vitamin D requirement is dependent highly on diet Ca and P concentrations (Rodriguez-Lecompte et al., 2016). Several studies reported that there was a correlation between Vitamin D and Ca levels in broiler tissue. Addition of certain forms of Vitamin D into the diet of broilers with Ca and P deficiency improved the Ca and P levels in bone ash (Bello et al., 2014; Han et al., 2016). While serum α-tocopherol levels were similar between the groups, serum retinol and Vitamin D levels increased with the addition of pea into the control diet.

In addition to its high protein and lipid content, pea is also rich in vitamins (especially vitamin E) and minerals (particularly Ca, Mg, Fe, Mn, Zn) (Hickling, 2003) and addition of pulse crop into broiler diet may improve the diet quality (Johansson et al, 2014; Mudryj et al., 2014).

In conclusion, serum vitamin levels increased with pea addition into the diet probably due to higher vitamin content in pea. Serum mineral levels decreased with the addition of low-level pea into the diet. These decreases might have resulted from the presence of compounds that bind certain minerals in the pea. However, serum mineral levels increased with the addition of pea in high doses. Further studies should be conducted to analyze the underlying reasons in this mechanism. It can be concluded that the pea levels in soybean meal based diet might affect serum mineral and vitamin levels in various ways.

REFERENCES

Arif M, Rehman A, Saeed M, El-Hack MEA, Alagawany M, Abbas H, Arian MA, Fazlani SA, Hussain A, Ayasan T. Effect of different processing methods of pigeon pea (*Cajanuscajan*) on growth performance, carcass traits, and blood biochemical and haematological parameters of broiler chickens. Turk J Vet Anim Sci 2017;41:38-45.

Bello A, Hester PY, Gerard PD, Zhai W, Peebles ED. Effects of commercial *in ovo* injection of 25-hydroxycholecalciferol on bone development and mineralization in male and female broilers. Poult Sci 2014:93(11);2734-9.

CatootjieLusjeNalle. Nutritional Evaluation of Grain Legumes for Poultry. A thesis Doctor of Philosophy in Poultry Nutrition at Massey University, Palmerston North, New Zealand, 2009.

Clagett-Dame M, DeLuca HF. The role of vitamin A in mammalian reproduction and embryonic development. Annu Rev Nutr 2002:22;347-81.

Cowieson AJ, Acamovic T, Bedford MR. Supplementation of diets containing pea meal with exogenous enzymes: effects on weight gain, feed conversion, nutrient digestibility and gross morphology of the gastrointestinal tract of growing broiler chicks. Br Poult Sci 2003;44(3):427-37.

Dahll WJ, Foster LM, Tyler RT. Review of the health benefits of peas (*Pisum sativum L.*). Br J Nutr 2012;108Suppl 1:S3-10.

Dede S, Deger Y, Kahraman T, Deger S, Alkan M, Cemek M. Determination of oxidation products of nitric oxide and the concentrations of

antioxidant vitamins in goats with parasitic invasions. Acta Vet Brno 2002:71;341-5.

Dede S, H Çamaş. Yumurta tavuklarında bazı biyokimyasal kan parametrelerinin (GSH, Hb ve Tf tipleri ve Mn) tayin edilmesi ve yumurta verimi üzerine etkilerinin araştırılması. YYÜ Sağ Bil Derg 2001;7:131-8.

Dede S, Bingol T, Kilicalp Kilinc D, Deger Y, Yoruk IH, Tanritanir Ekici P. Effects of *Plantago major* extract on serum levels of antioxidant vitamins and minerals in broiler. Ind J Anim Res 2018:B-855; 1661-3.

Dede S, Değer Y. Yumurta tavuklarının kan plazması ve tüylerinde iz element (Cu, Zn) konsantrasyonlarının karşılıklı incelenmesi ve düzeyler üzerine yaşın etkisi. FÜ Sağ Bil Derg 2000:14(1);61-4.

Dede S, Değer Y, Kahraman T, N Dönmez. Suplemental olarak çinko uygulanan tavuklarda nitric oksit oksidasyon ürünleri ve antioksidan vitamin düzeyleri. Vet Bil Derg 2001;17(2), 119-22.

Dede S, Değer Y, Değer S, Tanritanir P. Plasma levels of zinc, copper, copper/zinc ratio and activity of carbonic anhydrase in equine piroplasmosis. Biol Trace Elem Res 2008;125:41-5.

Ebsim S. Establishing the nutritional value of field pea as affected by feed processing and pea cultivar for poultry. Thesis doctoral, Department of Animal and Poultry Science University of Saskatchewan Saskatoon, SK. Canada, 2013.

Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Physiol Biochem 2010;48:909-30.

Greenberg WE, Trusell RR, Clescer LS. Standard Methods for the Examination of Water and Wastewater, 60th ed. American Public Health Association, Washington, DC, pp. 143-179, 1994.

Herzig M. Navrátilová I, Totušek J, Suchý P, Večerek V, Blahová J, Zralý Z. The effect of humic acid on zinc accumulation in chicken broiler tissues. Czech J Anim Sci 2009:54:121-7.

Johansson B, Persson Waller P, Jensen SK, Lindqvist H, Nadeau E. Status of vitamins E and A and β-carotene and health in organic dairy cows fed a diet without synthetic vitamins. J Dairy Sci 2014;97;1682-92.

Karatepe M. Simultaneous determination of ascorbic acid and free malondialdehyde in human by HPLC-UV. LC-GC North America. 2004:22;365.

Laudadio V, Tufarelli V. Growth performance and carcass and meat quality of broiler chickens fed diets containing micronized-dehulled peas (*Pisum sativum* cv. Spirale) as a substitute of soybean meal. Poult Sci 2010;89:1537-43.

Longbottom JE, Martin TD, Edgell KW, Long SE, Plantz MR, Warden BE. Determination of trace elements in water by inductively coupled plasma-mass spectrometry: Collaborative study. J AOAC Int 1994;77:1004-23.

Longstaff M, McNab JM. Digestion of starch and fibre carbohydrates in peas by adult cockerels. Br Poult Sci 1987;28:261-85.

Mariotti F, Pueyo ME, Tomé D, Bérot S, Benamouzig R, Mahé S. The influence of albumin fraction on bioavailability and postprandial utilization of pea protein given selectively to humans. J Nutr 2001;131:1706-13.

Miller KW, Yang CS. An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, α-tocopherol and various carotenoids. Analy Biochem 1985;145:21-6.

Mudryj AN, Yu N, Aukema HM. Nutritional and health benefits of pulses. Appl Physiol Nutr Metab 2014;39(11):1197-204.

Musa MM, Elamin KM, Ati KA, Elagib HA, Musa AM. Effect of dietary levels of decorticated cow pea (*Vigna unguiculata*) supplemented with molasses on broiler chicks performance and carcass traits. Pak J Biol Sci 2012;15(21):1010-8.

NRC (National Research Council). Nutrient Requirement for Poultry. USA, Ninth Revised, http://www.pjbs.org/ijps/fin182.pdf

Ravindran V, Blair R. Feed resources for poultry production in Asia and Pacific. 2. Plant protein sources. World Poult Sci J 1992;48:205-31.

Reddy SB, Charles MJ, Raju GJN, et al. Trace elemental analysis of cancer-afflicted intestine by PIXE technique. Biol Trace Elem Res 2004;102:265-81.

Rochfort S, Panozzo J. Phytochemicals for health, the role of pulses. J Agric Food Chem 2007, 55(20):7981-94.

Rodriguez-Lecompte JC, Yitbarek A, Cuperus T, Echeverry H, Van Dijk A. The immunomodulatory effect of vitamin Din chickens is dose-dependent and influenced by calcium and phosphorus levels. Poult Sci 2016;95(11):2547-56.

Sandberg AS. Bioavailability of minerals in legumes. Br J Nutr 2002, 88 Suppl 3:S281-5.

Wilson RD, Davies G, Désilets V, Reid GJ, Summers A, Wyatt P, Young D. The use of folic acid for the prevention of neural tube defects and other congenital anomalies. J Obstet Gynaecol Can 2003, 25(11):959-73.