

## Effects of *Plantago major* extract on serum levels of antioxidant vitamins and minerals in broiler

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### ABSTRACT

The aim of this study was to evaluate the effects of *Plantago major* liquid extract on serum antioxidant vitamin and mineral concentrations of broiler. The experiment consisted of the control and 2 treatment groups, composed of 28 Ross 308 broiler chicks (Total, 84). A basal (control) diet was formulated. *P. Major* was added to the control diet as following: PM1 (5 g/kg feed), PM2 (10 g/kg feed). The chicks were feed for 42 days *ad libitum*. The serum micronutrients (Retinol,  $\alpha$ -tocopherol, vitamin D, calcium, magnesium, iron, manganese, and zinc) levels were determined. The retinol,  $\alpha$ -tocopherol, Fe, Mg, Mn levels were not affected, and the Zn levels decreased in the PM1 and PM2. The supplementation of *P. major* has affected the serum vitamin D and Zn levels for this study, and should be analyzed for the alterations on the metabolism in subsequent studies.

**Key words:** Broiler, Diet, Micronutrients, *Plantago major*.

In EU countries, since 2006, the use of antibiotics as growth promoters in feeds has been prohibited, in order to protect the society against the emergence of the antibiotic resistance (Buchanan *et al.*, 2008). Many alternative products (organic acids, probiotics, prebiotics, plant extracts, essential oils etc.) have been tried to obtain the effects of the antibiotics to a certain extent (Nemereshina *et al.*, 2015).

*P. major* is a perennial plant, consisting of alkaloids, caffeic acid derivatives, flavonoids, iridoid glycosides etc. (Samuelsen, 2000). Using *P. major* has led to beneficial effects on the immune system and antibiotic properties in birds (Dorhoi *et al.*, 2006).

In the previous studies about the effect of *Plantago major* extract in broiler chicks, it was noted that it did not significantly influence performance parameters (live weight gain, live weight increase, feed consumption, the feed conversion ratio, carcass, empty carcass, viscera, abdominal fat and the weight of the intestines), some serum proteins (Bingol *et al.*, 2010; Bingol *et al.*, 2017).

Micronutrients (trace elements, vitamins) function as activators of enzyme or as constituents of organic compounds (Dede *et al.*, 2002). However, there is lack of information regarding the effects of *Plantago major* plant on some micronutrients of the serum of broiler chicks.

The aim of this study was to evaluate the effects of *Plantago major* liquid extract on serum antioxidant vitamin and mineral concentrations of broiler.

In the study, Ross 308 type broiler chicks (n=84) have been used. Two experimental groups, a control group were set up, each with 28 chicks. Each experimental group was divided into 4 sub-groups with 7 chicks each.

Two different diets (starter and finisher) diets were used. Diets were calculated to meet nutrient requirements of chicks based on recommendation of NRC (1994). Both botanical and chemical compositions of diets are given in Table 1.

The daily prepared *P. major* water extract samples were prepared from 5 g of *P. major* (PM1), 10 g of *P. major* (PM2). Then, prepared feed was dried before given to the animals *ad libitum* (Bingol *et al.* (2010). A basal (control) diet was formulated and *P. Major* was added. Starter-diet was fed to the all chicks up to 3 weeks of life and followed by grower ration up to 6 weeks *ad libitum* of the study.

For determination of mineral and vitamin after completion of the study, serum samples were collected from the birds. The serum mineral concentrations (AAS (UNICAM) Longbottom *et al.*, 1994), and, serum vitamins levels were determined (HPLC, Agilent-1100 series,

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Germany) (Miller and Yang, 1985; Reynolds and Judd, 1984).

The data was analyzed with ANOVA test, differences were considered as significant ( $p < 0.05$ ), (SPSS 22.0).

The obtained results were summarized at Table 2. It was determined that retinol,  $\alpha$  tocopherol, Ca, Fe, Mg, Mn levels did not differentiate significantly between the groups. While vitamin D levels decreased in PM2, there has been no alteration in PM1 group. Zn concentrations were lower in the PM1 and PM2 groups than in the control group.

**Table 1:** The chemical composition of the experimental diet.

Content (kg/100kg)	Starter	Finisher
Corn	49.00	57.50
Soybean Meal	42.00	33.70
Dicalcium phosphate	0.90	0.13
Limestone	1.50	2.00
Vegetable oil	5.80	6.00
Vitamin-Mineral Premixes*	0.30	0.30
Methionine-Cystine	0.15	0.07
Salt	0.35	0.30
<b>Chemical compositions of mixed feeds, calculated</b>		
Crude protein	<b>22.95</b>	<b>19.75</b>
Metabolizable energy (Kcal/kg)	<b>3105</b>	<b>3207</b>
Calcium	<b>1.00</b>	<b>0.90</b>
Usable phosphorus	<b>0.46</b>	<b>0.40</b>
<b>Chemical compositions, determined by analysis of mixed feeds</b>		
Dry Matter	<b>92.72</b>	<b>97.71</b>
Crude Protein	<b>24.23</b>	<b>21.49</b>
Crude Oil	<b>6.19</b>	<b>6.41</b>
Crude Cellulose	<b>5.26</b>	<b>5.04</b>
Crude Ash	<b>7.91</b>	<b>5.04</b>
Self-Nitrogen-free Substances	<b>49.13</b>	<b>54.39</b>
Metabolic Energy (Kcal/kg)**	<b>3230</b>	<b>3356</b>

\*Vitamin-Mineral premixes (1U or mg/kg diet): vitamin A 12000 IU; vitamin D3 > 1500 IU; vitamin E 30 mg; vitamin K3 > 5mg; vitamin B1 > 3mg; vitamin B2 6 mg; vitamin B6 > 5mg; vitamin B12 > 0.03mg; nicotinamide 40 mg; calcium-D-pantotenat 10mg; folic acid 0.075mg; cholinechloride 375mg; antioxidant 10mg manganese, 80mg; ferrous 80mg; zinc 60mg; copper 8mg; iodine 0.5mg; cobalt 0.2mg; selenium 0.15mg (\*\*Calculated according to Titus and Fritz (1971).

**Table 2:** Blood micronutrients levels.

Parameters	Control	PM1 (5 g/kg feed)	PM2 (10 g/kg feed)
Retinol ( $\mu\text{g/ml}$ )	0.874 $\pm$ 0.177	0.772 $\pm$ 0.096	0.689 $\pm$ 0.057
$\alpha$ -Tocopherol ( $\mu\text{g/ml}$ )	2.086 $\pm$ 0.113	1.969 $\pm$ 0.239	1.918 $\pm$ 0.087
Vitamin D ( $\mu\text{g/ml}$ )	0.0284 $\pm$ 0.0023b	0.0233 $\pm$ 0.0025ab	0.0190 $\pm$ 0.0021a
Calcium (mg/dl)	24.92 $\pm$ 1.82	21.41 $\pm$ 1.71	23.53 $\pm$ 2.35
Magnesium (mg/dl)	7.371 $\pm$ 0.266	5.966 $\pm$ 0.693	7.157 $\pm$ 0.741
Iron (mg/dl)	0.310 $\pm$ 0.015	0.253 $\pm$ 0.035	0.258 $\pm$ 0.044
Manganese (mg/dl)	0.043 $\pm$ 0.027	0.0106 $\pm$ 0.002	0.014 $\pm$ 0.002
Zinc (mg/dl)	0.130 $\pm$ 0.019a	0.052 $\pm$ 0.009b	0.053 $\pm$ 0.014b

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

*P. major* (Plantaginaceae family) have various chemical constituents which can affect blood composition and metabolic pathways in treated animals. It can be used as therapeutic and preventive agent in many conditions (anti-inflammatory, analgesic, antimicrobial, anesthetic, antiviral, anthelmintic, analeptic, etc.), in popular and traditional medicine (Samuelsen, 2000; Harput *et al.*, 2012; Jongberg *et al.*, 2014).

The minerals concentrations affected with age, feeding and physiological conditions in birds (Dede and Deger, 2000; Dede and Çamaş, 2001; Deger *et al.*, 2007). We did not find a study about the effects of the *P. major* in broilers, specifically the serum on antioxidant vitamins and minerals levels in literature.

*Plantago* species have a number of polyphenols that have iron-binding capacity and reducing activities and positively correlated with iron-binding activity (Nemereshina *et al.*, 2015). The serum Fe levels were not significantly different in groups that *P. Major* was supplemented (Oto *et al.*, 2012). But, we observed that retinol,  $\alpha$  tocopherol, Fe, Mg, Mn concentrations were not affected by the using doses and duration of this study.

Zinc (Zn), are in fact involved in both humoral and cellular immunity and decreased generation of free radicals and oxidative stress (Herzig *et al.*, 2009; Oto *et al.*, 2012). In this study, using PM at low levels can decrease Zn concentrations. It would be possible to declare that *P. major* has a significant effect on the avian immune system; hence, we need to pay attention for possible immunosuppressive effect of *P. major* in broiler diet.

Vitamin A and vitamin E are essential micronutrients throughout the life cycle and have many effects with antioxidant activities, recommended for chickens (NRC, 1994; Yuan *et al.*, 2014). But, in this study, we can say that using *P. major* extract have no effect on serum vitamin A and E levels.

Vitamin D requirement is heavily dependent on the concentrations of Ca and P in the diet. There is a relationship between vitamin D and calcium in some tissues in broiler chickens (Han *et al.*, 2016). In this study, while vitamin D is

decreasing at PM2 group, the level of Ca has not changed. This may be originated from the using dose of PM2 (10 g/kg feed).

According to the obtained results, it was determined that the concentrations of retinol,  $\alpha$  tocopherol, Fe, Mg, Ca, Mn did not significantly change among the groups. However,

vitamin D levels decreased in PM2 and Zn levels decreased in PM1 and PM2. The using doses and durations of *P. major* extract have no effect of serum micronutrients levels except vitamin D and zinc on broiler diet. It is suggested that to be useful to investigate in detail the causes of the decrease in vitamin D and zinc levels due to using of *P. major*.

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