

Araştırma Makalesi/Research Article (Original Paper)

## Effects of Plantain (*Plantago lanceolata*) Containing Diets of Quails on Growth Performance, Carcass Characteristic, Some Blood Parameters and Mast Cell Numbers

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**Abstract:** Plantain (*Plantago lanceolata*) is used for medicinal purposes as a preventive, therapeutic and metabolic regulator due to its bioactive compounds. With the purpose of determine the effect of this plant on quail, 240 of these 1 day old quail chicks were divided into 4 groups, control, added plantain into rations as 1%, 3% and 5%. Body weight gain, feed intake carcass characteristics, internal organ measurements, blood parameters and mast cells were determined. At the end of the study, the hot carcass yield, cold carcass weight and yield were high in the 1%, gizzard weight in 5% plantain added group ( $p<0.05$ ). The total amount of blood protein was the highest in 3% added group ( $p<0.05$ ). Blood glucose levels were high in all treatment groups compared to the control group ( $p<0.05$ ). The amount of T-testosterone was higher than the others in the control and 3% plantain groups ( $p<0.05$ ). The total number of mast cells increased in the 5% plantain group ( $p<0.05$ ). it was understood that the addition of plantain to quail rations could have a positive effect, but bioactive components and secondary metabolites of plantain and its extracts need identification and isolation by in vitro and in vivo studies for determining effects on metabolism.

**Keywords:** Fattening, Plantain (*Plantago lanceolata*), Quail

### Sinir Otu (*Plantago lanceolata*) İçerikli Rasyonların Bildircinların Büyüme Performansı, Karkas Özellikleri, Bazı kan Parametreleri ve Mast Hücre Sayıları Üzerine Etkileri

**Öz:** Sinir otu (*Plantago lanceolata*) yapısında bulundurduğu biyoaktif bileşikler nedeniyle hastalık önleyici, tedavi edici ve metabolizma düzenleyici olarak tıbbi amaçlarla kullanılmaktadır. Bu bitkinin bildircinlar üzerindeki etkisini belirlemek amacıyla yürütülen bu çalışmada, 240 adet 1 günlük bildircin civcivleri biri kontrol 3'ü muamele grubu olacak şekilde 4 gruba ayrılmıştır. Muamele gruplarının rasyonlarına %1, %3 ve %5 oranlarında sinir otu eklenmiştir. Canlı ağırlık artışları, yem tüketimleri, karkas özellikleri, iç organ ölçümleri, kan parametreleri ve mast hücre sayıları tespit edilmiştir. Araştırma sonunda rasyonlarına %1 sinir otu eklenen grupta sıcak karkas oranı, soğuk karkas ağırlığı ve oranı yüksek çıkmıştır ( $p<0.05$ ). Rasyondaki sinir otu oranı arttıkça taşlık ağırlığı da artmıştır ( $p<0.05$ ). %3 eklenen grupta kan toplam protein miktarı en yüksek olmuştur ( $p<0.05$ ). Kontrol grubuna göre muamele gruplarının tümünde kan glukoz değerleri yüksek olmuştur ( $p<0.05$ ). T-testesteron miktarı kontrol ve %3 sinir otu eklenen gruplarda diğerlerinden yüksek olmuştur ( $p<0.05$ ). %5 sinir otu grubunda toplam mast hücresi sayısı artmıştır ( $p<0.05$ ). Literatür bilgileriyle de birlikte değerlendirildiğinde bildircin rasyonlarına sinir otu katılmasının pozitif etkilerinin olabileceği anlaşılmıştır. Sinir otu bitkisinin ve ekstraktlarının in vitro ve in vivo çalışmalarla içeriğindeki biyoaktif bileşiklerin ve sekonder metabolitlerin tanımı ve izolasyonlarının yapılarak metabolizmadaki etkinliklerinin belirlenmesi gerekmektedir.

**Anahtar kelimeler:** Besi, Sinir otu (*Plantago lanceolata*), Bildircin

### Introduction

Commercial poultry production has specialized in recent years and become a fast enterprise. Nowadays, this industry has paid more attention to attracting the public's consideration for the environment and food safety. Demand of livestock products throughout the world is increasing by population. The use of synthetic antibiotic growth enhancers in animal nutrition has been a frequent practice for more than 40 years, but since 2006 the use of antibiotic growth promoters in the European Union (EU) has been prohibited. The aim of using antibiotics in poultry feeds is to protect animals against diseases and to increase the utilization of feed. However, over time, microorganisms that cause disease are at risk of developing resistance to these antibiotics. Therefore, researches on feed additives that can be used instead of synthetic antibiotics are increasing. More resources have been devoted to research to determine the effects of natural and safe aromatic plants and their extracts on poultry with various bioactive compounds and secondary metabolites.

Research in this area has shown that the use of aromatic plants in poultry significantly improves live weight gain, feed efficiency, egg weight and production. This also applies to Japanese quails (Şahin 2012; Chacrabati et al. 2013).

In the feeding of quail, many different materials have been tested. Some of them are *Yucca* (*Yucca schidigera*) extract (Erdoğan et al. 2001), nigella seed (*Nigella sativa*) and nigella seed oil (Tufan et al. 2015), silkworm pupa extract and residue (Anggraenia et al. 2011), *Morinda citrifolia* fibre extract (Retnani et al. 2014), Sage (*Salviya triloba*), laurel (*laurus nobilis*) oil (Bülbul et al. 2015), weed extract (Çiftçi et al. 2016).

Plantain (*Plantago lanceolata*) as known narrow-leaved plantain, ribwort plantain, narrowleaf plantain is a species of plants that are widely distributed in pastures and green areas in the temperate world. It has been used for various medicinal purposes for centuries such as related to the skin, wound healing, inflammation, disorders of respiratory and digestive organs, reproductive system, blood circulation and cancer because of contained a number of exceptional properties. Previous studies have shown that the plantago genus contains five chemical classes of biologically active compounds, namely flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds (Stewart 1996; Chiang et al. 2003; Moore et al. 2006). Bioactive substances identified in the study of Beara et al. (2012) on the phenolic profile, antioxidant, anti-inflammatory and cytotoxic activity of *Plantago lanceolata* L are Phenolic acids (p-Hydroxybenzoic acid, 2,5-Dihydroxybenzoic acid, Protocatechuic acid, Vanillic acid, Gallic acid, Syringic acid, Cinnamic acid, p-Coumaric acid, Caffeic acid, Ferulic acid, Sinapic acid and Chlorogenic acid), Flavonoids (Apigenin, Apigenin-7-O-glucoside, Apiin, Vitexin, Amentoflavone, Kaempferol-3-O-glucoside and Chrysoeriol, Luteolin, Luteolin-7-O-glucoside, Quercetin, Quercitrin, Quercetin-3-O-glucoside, Hyperoside, Rutin and Naringenin) and Coumarins (Aesculetin and Scopoletin).

In the Gonda et al.'s study (2013) focused on iridoid glycosides (aucubin and catalpol) and caffeoyl phenylethanoid glycoside (akteositis) as the major bioactive metabolites of *Plantago lanceolata* L.

This trial was established to evaluate the effect of Plantain (*Plantago lanceolata*), added to ratio, on live weight gain, feed consuming, feed conversion efficiency, biochemical parameters of serum and number of mast cells in some organs of the digestive system in Japanese quails (*Coturnix coturnix japonica*), since the quails are one of the types of birds that holds the fastest production cycle compared to other birds.

## Materials and Methods

Dried plant material collected flowering period in Bitlis were purchased from the shop selling herbal medicines. After extracting stem parts, it was milled to exceed through a 2 mm sieve.

In the study total of 240 one-day age Japanese quail (*Coturnix coturnix japonica*) were used. The chicks were randomly distributed into 4 groups including 60 birds each. Thus research was carried out with three experimental and one control groups with two replicate containing 30 chicks in each.

Broiler starter diet was used in the feeding of animals. Plantain was stirred as 0% (Control), 1% (P%1), 3% (P%3) and 5% (P%5) in to diet for each group. The composition of the diets and *Plantago lanceolata* used in the experiment were determined (AOAC 1990; Van Soest et al. 1991; AOCS 2004) and given in Table 1.

Table 1. Composition of the diets and plantain, %

Diets	DM	CP	EE	NDF	ADF	Ash
Cont	95.14	16.78	7.09	6.76	3.29	7.09
P%1	95.65	16.78	6.74	8.51	7.63	6.74
P%3	95.25	16.48	6.95	13.17	10.21	6.95
P%5	95.64	16.00	6.78	19.44	12.89	6.78
Plantain	95.02	6.92	2.68	45.40	34.74	2.68

DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, Cont: Control group not added to plantain, P%1: Group containing 1% plantain, P%3: Group containing 3% plantain, P%5: Group containing 5% plantain.

The animals in each cage were subjugated to group feeding and watering as ad libitum with experimental diets during 6 weeks. The Live weight gains were determined by weighing at the beginning and each week of trial. Feed intake was calculated daily.

At the end of the experiment, 8 male and 8 female quails were sacrificed from each group and blood of the quails was taken off. Serum total cholesterol, triglyceride, glucose and testosterone analyzes were performed in the AMS (Autolab otoanalyzer device with Audit Diagnostics kits).

The hot carcass and cold carcass yield of 16 animals slaughtered from each group were determined. Heart, anterior stomach, liver, abdominal fat weights, small intestine, duodenum, large intestine lengths were measured as internal organs.

For identification of the mast cells, 6 male quails were slaughtered on from each group randomly. The appropriate size pieces of digestive tract organs, pre-stomach, duodenum, jejunum, and ileum intestinal sections were taken after slaughter immediately. The samples taken were fixed according to Becker and Chung's (1985) method. After dehydration and transparency processes by routine histological techniques samples were blocked at paraplast. Serial sections with a thickness of 6 µm were obtained from the blocked tissues. These sections were painted with toluidine blue for 5-8 min. (Enerback 1966; Uslu and Yörük 2008). Then, these preparations were examined under light microscope (Leica DM500, Wetzlar, Germany); Mast cell distribution counts were made in the sections of *Lamina propria*, *Tunica submucosa* and *Tunica muskularis* + *Tunica serosa* (Böck 1989).

### Statistical analysis

Analysis of variance was performed for compare means of factor using SAS statistical programs (proc GLM procedure). In order to determine the data that are significantly different from each other, Duncan multiple range test method was applied.

Necessary permissions were obtained from the Local Ethics Committee of Yüzüncü Yıl University (Date: 25.12.2015, Number 2015/14).

## Results

Table 2. Body weight weekly, g

weeks	0	1	2	3	4	5	6
Cont	14.22±0.31	29.49±0.70	66.40±10.79	92.95±5.56	122.19±1.90	151.95±1.97	178.53±2.77
P%1	14.40±0.25	37.50±6.09	55.26±1.04	89.37±1.19	122.11±1.98	157.18±2.18	179.81±2.89
P%3	14.37±0.28	29.80±0.66	64.95±8.59	87.41±2.08	121.08±2.13	154.73±2.16	173.15±3.83
P%5	14.04±0.24	28.94±0.60	54.91±1.17	86.70±1.74	120.01±2.05	152.78±2.21	173.66±2.79
P	NS	NS	NS	NS	NS	NS	NS

a, b, c: The values of the different letters in the same column is significant, (p < 0.05).

P: Statistical significance, NS: No significant, \*: P < 0.05.

Table 3. Feed intake, Body weight gain (BWG) (g/bird) and feed conversion ratio (FCR)

	Cont.	P%1	P%3	P%5	P	
Feed intake	544.25±34.65	530.15±33.57	530.36±34.45	530.08±33.97	NS	
	Weeks					
BWG	1	15.26±1.20	26.09±7.23	15.36±0.42	14.90±0.56	NS
	2	26.40±0.39	26.24±1.28	26.09±1.22	25.77±0.47	NS
	3	37.06±3.41	34.10±3.67	31.05±0.08	31.79±0.33	NS
	4	29.24±5.87	32.76±0.83	33.44±4.93	33.31±1.02	NS
	5	29.76±5.44	35.07±0.20	33.76±1.33	32.77±2.39	NS
	6	26.57±2.48	26.57±2.48	29.99±1.02	20.79±2.97	NS
	$\bar{X}$	164.30±3.83	173.88±17.08	161.69±1.86	159.54±5.02	NS
FCR	1	2.69±0.12	1.97±0.64	2.58±0.14	2.83±0.16	NS
	2	3.28±0.15	3.33±0.22	3.17±0.01	3.22±0.01	NS
	3	3.62±0.18	3.96±0.62	4.37±0.23	3.97±0.12	NS
	4	5.32±1.58	4.39±0.16	4.37±0.86	4.34±0.01	NS
	5	6.16±1.25	5.05±0.04	5.15±0.25	5.40±0.35	NS
	6	6.73±0.41	4.42±0.36	7.73±0.69	8.19±0.41	NS
	$\bar{X}$	4.64±0.28	4.42±0.36	4.59±0.07	4.65±0.05	NS

a, b, c: The values of the different letters in the same line significant, (p < 0.05).

P: Statistical significance, NS: No significant, \*: P < 0.05.

Table 4. Effects of dietary treatments on carcass, organs, blood and mast Cell values

	Control	P%1	P%3	P%5	P
<b>Carcass</b>					
Slaughter weight,g	180.22±5.59	185.37±5.53	189.54±5.74	184.48±5.59	NS
Hot carcass,g	122.86±2.62	130.52±3.28	126.30±2.96	122.11±3.05	NS
Hot dressing, %	68.56±1.34ab	70.62±0.88a	66.96±1.12b	66.48±1.07b	*
Cold carcass,g	122.56±2.55ab	130.76±3.42a	126.04±3.02ab	121.88±3.05b	*
Cold dressing, %	68.41±1.13ab	70.73±0.95a	66.81±1.13b	66.35±1.06b	*
<b>Organs</b>					
Heart,g	1.64±0.04ab	1.75±0.07ab	1.56±0.06bc	1.44±0.06c	*
Heart, %	0.92±0.02ab	0.95±0.04a	0.83±0.04bc	0.79±0.03c	*
Liver,g	4.46±0.30	4.74±0.37	5.08±0.38	4.86±0.39	NS
Liver, %	2.46±0.12	2.52±0.14	2.65±1.14	2.59±0.16	NS
Gizzard,g	2.77±0.10b	2.85±0.10b	3.18±0.14a	3.28±0.11a	*
Gizzard, %	1.56±0.07b	1.54±0.05b	1.68±0.06ab	1.79±0.05a	*
Duodenum, cm	11.92±0.39	12.55±0.25	12.28±0.36	11.95±0.39	NS
Small intestine, cm	47.44±1.31	46.594±1.00	49.17±1.78	45.84±1.51	NS
Large intestine, cm	4.69±0.22	4.90±0.24	4.79±0.33	4.39±0.20	NS
Abdominal fat,g	1.79±0.19	1.75±0.07	2.29±0.34	1.72±0.35	NS
Abdominal fat, %	0.98±0.09	0.95±0.04	1.18±0.16	0.89±0.16	NS
Testis,g	5.11±0.37	4.26±0.47	4.04±0.32	4.37±0.51	NS
<b>Blood</b>					
Triglyceride, mg/dl	439.65±147.53	602.01±156.02	617.29±132.04	532.28±122.02	NS
Total protein, mg/dl	1.26±0.04b	1.37±0.55ab	1.59±0.14a	1.29±0.09ab	*
Cholesterol, mg/dl	185.69±8.33	194.80±16.16	193.53±14.10	176.00±12.46	NS
Glucose, mg/dl	287.56±12.69b	314.80±11.16ab	334.73±7.70a	322.37±13.55a	*
T- testosterone, mg/dl	5.99±1.31a	2.96±2.31b	5.72±1.17a	3.39±1.02ab	*
<b>Mast Cell</b>					
İleum	39.91±4.11	39.56±4.26	40.15±4.33	43.52±4.51	NS
Jejunum	39.93±3.98	37.70±4.53	38.94±4.21	40.35±3.89	NS
duodenum	44.30±4.95	44.21±4.89	44.17±5.34	47.84±5.43	NS
pre-stomach	62.60±4.14	62.71±4.17	62.06±4.13	66.51±4.52	NS
Total	46.68±2.38ab	46.04±2.48b	46.33±2.47ab	49.55±2.56a	*

a, b, c: The values of the different letters in the same line is significant, ( $p < 0.05$ ).

P: Statistical significance, NS: No significant, \*:  $P < 0.05$ .

When the weekly body weight was examined, the differences between the groups were not statistically significant (table 2). Even though there was no statistical difference in the weekly BWG and in total, there was an increase in BW of more than 5.1% in the P%1 group compared to the control group and no difference was found in feed conversion rate (FCR) and feed intake values (table 3).

In Table 4, whilst there was no difference between slaughter weight and hot carcass weights, there was a significant increase in hot dressing percentage, cold carcass and cold dressing percentage in the P%1 group ( $p < 0.05$ ). Heart weight percentages were significantly higher in the P%1 group than the control group but were lower in the P%3 and P%5 groups compared to the control group ( $p < 0.05$ ). Gizzard weight was significantly higher in control and P%1 groups than others ( $p < 0.05$ ). As the amount of the plantain increased, the weight of the gizzard increased. There was no difference among duodenum, small intestine, large intestine lengths and abdominal fat and testis weights. When blood values were examined, triglyceride and cholesterol differences between groups were insignificant, while the highest value of total protein was found in P%3 groups ( $p < 0.05$ ). Blood glucose values were higher in all treatment groups compared to the control group and the highest in the P%3 and P%5 groups. ( $p < 0.05$ ). T-testosterone values were the highest in control and P%3 group ( $p < 0.05$ ). When the mast cells in the tissues of different organs were examined, the total number of mast cells in P%5 group was significantly higher ( $p < 0.05$ ).

## Discussion

In this study, differences among the groups for feed intake, body weight weekly, body weight gain and feed conversion ratio values were insignificant but the addition of 0.5% plantain in the diet had positive effect on growth, feed intake, feed conversion ratio, performance index and carcass characteristics of broilers (Chacrabati et al., 2013). In another experiment by the effects of *Plantago major* water extract obtained from 5, 10, 15g dried and grilled plant for per kg

diet were demonstrated that addition of *Plantago major* extract at differing levels into broiler diet did not affected animal performance and carcass parameters (Bingöl et al., 2010). Obtaining different results in this way may result from the use of different species, different dosing and extracting methods in the studies. The hot carcass and cold carcass yield, heart weight and yield increased significantly in P%1 group ( $p < 0.05$ ). Heart weight ratio decreased compared to control as plantain increases in ration ( $p < 0.05$ ). In fact, similar results were obtained when 0.5% plantain was added to broiler rations (Chakrabarti et al., 2013). In our study, there was no difference in feed consumption, weekly live weight gain and feed conversion rates in the control and treatment groups, but increased in hot carcass, cold carcass, heart weight in P1% group. In addition, gizzard weight and ratio, blood glucose values were in P%3 and P%5 in group, blood total protein was in P%3 group, T-testosterone values were in control and 3%, total mast cells were significantly higher in P%5 group ( $p < 0.05$ ). Roberts et al. (2009) applied the testosterone implant to the quails and reported that the high testosterone reduced the body mass. In our study, P% 1 group which has the lowest serum testosterone level has the highest cold carcass weight and confirms this situation. All these findings suggest that the plantain increases anabolism by affecting the digestion, absorption, energy, protein, hormone and immune metabolism. Various studies have been conducted in which such anabolic effects have been determined. When these studies are examined, it is understood that plantain plants may have very positive effects on poultry, which are mostly compatible with the results of this study. In this way, different results are reported to be effective in factors such as triterpenoids, saponins, glycosides, alkaloids, phenolic compounds and metabolites, the amount or dose used type of animal, maintenance conditions, health status and intestinal microflora (Jin et al. 1996; Retnani et al. 2014). In addition, these substances have an antibacterial effect and regulate intestinal flora, enhances the release of endogenous digestive enzymes, thus enhancing the digestive quality effects (Lee 2003; Skomorucha and Sosnowka-Czajka 2013). In studies, iridoid glycosides and derivatives with significant biological activity in the plantain were identified. These are antimicrobial, laxative, tissue enhancer, non-steroidal anti-inflammatory, liver activity regulating, antioxidant and uric acid excretion regulators (Stewart et al. 1996). Based on these characteristics, different animal species were found to be resistant to diseases by participation in rations and patents were obtained (USA patent). In vivo and in vitro studies have also been shown to have immune system regulatory properties (Chiang et al. 2003; Ghule and Yeole 2012).

Dorhoi et al. (2006) reported that ethanol extracts from plantain (*Plantago major*) has a beneficial effect on cell-mediated immunity in layers. Marchesan et al. (1998) investigated the anti-inflammatory effect of liquid Extracts of *Plantago lanceolata* L. to inhibit membrane irritation on the chick chorioallantoic membrane and concluded that these extracts showed a potent activity in the inhibition of membrane irritation. Additionally, Kojima and Takahashi (1999) were demonstrated and patented that ternary combination of plantain (*Plantago asiatica* L.), Cucurbita Seed (*Cucurbita moschata* Duch.) and Lonicera (*Lonicera japonica* THUNB.) plants were caused to increasing of resistance against to diseases in animals like swine, milking cows, beef cattle, broilers, layers, quails, and the like and cultured fish, such as yellowtail (*Seriola quinquerata* iata), Hamachi, eels, sea breams.

In a study of broilers, 3-day-old chicks were infected with *Eimeria tenella* and determined the effect of *Plantago asiatica* extract. As a result of the study, it was determined that the extract had an excellent antioxidant effect against *Eimeria* parasite and could be used and improved instead of anticoccidial drugs (Hong et al. 2016).

*Plantago major* seed added to the broiler rations by P% 1 increased live weight and carcass weight, decreased serum cholesterol and triglyceride levels, decreased heterophile / lymphocyte ratio and improved immune system and ileal microflora (Mehrparvar et al. 2016). In this study, a significant increase in the total number of mast cells in P%5 of the group is an indicator of strengthening the immune system. In addition, mast cells are involved in many physiological and physio-pathological events. It is reported that histamine and heparin secreted from these cells play a role in maintaining the integrity of the bones, in wound healing and in the regulation of blood flow (Huntley, 1992; Eren et al. 1999).

In consequence of this study, it was understood that the addition of plantain to quail rations could have a positive effect. When the literature and results are evaluated together, in vitro and in vivo studies should be done for determination, identification and isolation of bioactive substances and secondary metabolites of plantain (*Plantago lanceolata*) and its extracts for the most appropriate dose for rations. To clarify the effects on the immune system, it would be appropriate to work with more animals. Thereby, the main active compounds and their mechanisms of action should be determined and studies on the usability in animal nutrition should be carried out.

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