EFFECTS OF SUMAC POWDER (*Rhus coriaria* L.) **ON GROWTH PERFORMANCE, SERUM BIOCHEMISTRY AND INTESTINAL MICROBIOATA IN BROILERS AT DIFFERENT STOCKING DENSITIES**

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Abstract

This study aimed to evaluate the effects of dietary supplementation of sumac powder (Rhus coriaria) on growth performance, serum biochemistry and intestinal microbiota in broilers reared at different stocking densities. A total of 378 one-day-old Ross 308 male broiler chicks were subjected to a 2 stocking densities (10 and 20 chicks/m² floor area; normal and high stocking density) x 3 sumac powder levels (0.0, 0.75 and 1.5 g/kg feed) factorial arrangement of treatments. Body weight and feed intake were significantly lower in chickens reared at high stocking density than normal stocking density groups (P<0.05). Moreover, feed conversion rate was negatively affected by high stocking density (P<0.05). Dietary addition of sumac powder had no effect on these variables in both stocking densities (P>0.05). Both stocking densities and the supplementation of sumac powder to the feeds had a reducing effect on intestinal weight (P<0.05). Serum total protein concentration of chickens reared at high stocking density was higher than those of the control normal groups (P<0.05). There was interaction between stocking density and dietary sumac powder supplementation for only body weight and abdominal fat pad variables at the end of the study (P < 0.05). However, no differences were observed for the relative weight of liver, spleen, gizzard and proventriculus (P>0.05). In addition, alkaline phosphatase (ALP), aspartate amino transferase (AST) activities and high density lipoprotein (HDL), cholesterol and triglyceride concentration in the serum were not influenced by the any stocking density and sumac powder supplementation (P>0.05). In conclusion, our results showed that broilers exhibited low performance when reared at high stocking density and dietary supplementation of sumac powder has not prevented this decline.

Key words: stocking density, sumac powder, growth performance, serum biochemistry, broiler.

INTRODUCTION

Stocking density is the numbers of chickens per unit area reared during grow out. Stocking density has been one of the principal concerns in the welfare of poultry production practices. Presently, there is an ongoing debate at the ideal density for chickens reared on the floor and in the different types of cages. Sorensen et al. (2000) reported that lower growth and fattening performance and higher mortality and prevalence of leg weakness in animals reared in high stocking density. Environmental conditions may have different effects on the productivity of poultry. During the summer season stocking density plays an important in broiler production and low growth performance and high mortality may occur at higher stocking densities in broilers (Türkyilmaz, 2008). High stocking density may cause decreases of growth performance of poultry and can lead to disease or death due to changes in the immune system of animal (Rotllant et al., 1997). Puron et al. (1995) reported that the reduction of feed intake of broiler chickens at high density because of the stress caused by environmental conditions.

Recently, several plant extracts have received considerable attention because of their natural antioxidants effects as feed additives in poultry nutrition.

Sumac *(Rhus coriaria* L.) is a plant, grows widely in Asian countries and it uses as tradetional medicine (Shidfar et al., 2014). Tannins and flavonoids are the main compounds of sumac extracts (Jung, 1998) and it has gallic acid and several group B vitamins (EL Sissi et al., 1972). Some researchers reported that dietary sumac powders improved growth performance in broilers (Gulmez et al, 2006; Ghasemi et al., 2014) and enhanced intestinal characteristics on broiler chicks (Ghasemi et al., 2014).

The aim of this study was to investigate the effects of dietary supplementation of sumac powder on growth performance, serum biochemistry and intestinal microbiota in broilers reared at different stocking densities.

MATERIALS AND METHODS

This study was performed at the Dicle University, Animal Research Center Unit according to the guidelines for animal experimentation of Dicle University and approved by the Ethical Committee (DUHADEK- No: 01.12.2016-5).

Totally, 378 one-day-old Ross 308 male broiler chicks were randomly divided into 6 experimental groups. The 6 experimental treatments subjected to a 2 x 3 factorial arrangement, in which the 2 variation factors were the 2 stocking densities (10 and 20 chicks/m² floor area; normal and high stocking density) and 3 sumac powder levels (0, 0.75 and 1.5 g/kg feed).

Sumac powder was purchased from a local market in Mardin province and it added to experimental diets after grinding.

Diets were formulated based on NRC (1994) recommendations to meet the nutrient requirements of broilers from d 1 to 21 (grower diet) and from d 22 to 42 (finishing diet). The composition of the basal diets is presented in Table 1.

Ingredients	Starter (1-22 day)	Finisher (23-42 day)
Maize	58.2	57.0
Soybean meal (48 % CP)	22.0	23.0
Full fat soybean	11.0	12.0
Fish meal (60 % CP)	5.2	-
Sunflower oil	-	3.7
Dicalciumphosphate ^a	1.75	1.60
Limestone	-	1.0
NaCl	0.30	0.35
Vitamin premix ^b	0.10	0.10
Mineral premix ^c	0.15	0.15
L-Lysine HCl	0.15	-
Dl-Methionine	0.15	-
Calculated composition		
Crude Protein	22.9	20.1
ME (kcal/kg)	2,996	3,213
Calcium	0.90	0.98
Available phosphorus	0.45	0.37
L-lysine	1.43	1.10
Methionine+cystine	0.92	0.75

Table 1.	Composition	of experimental	diets (%)
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Contains 240 g Ca and 17.5 g P/kg;

Feed and water were provided ad libitum throughout the experiment. The experiment lasted 42 d, including 21 d on the grower diet and from d 22 to 42 on the finishing diet. Chickens were weighed individually and feed intake determined by pen from 7 to 42 d (n=7). Mortality was checked daily and recorded throughout the experimental period. Feed conversion rate (FCR) was calculated by pen with dividing total feed intake to body weight. At the end of the experiment, blood samples (2 mL per bird) were collected from 10 chickens per treatment for serum biochemical determination. Within 1 h, the serum was obtained by centrifugation $(2.500 \times g \text{ for } 15)$ min) and stored at -80° C until further analysis. Serum biochemical parameters were measured by using Architect System Reagents and an automatic clinical chemistry analyzer. The concentration of total protein (TP) was measured by following the Biuret method; uric acid (UA) by following the uricase method; cholesterol by following the cholesterol esterase-peroxidase method; respectively; triglyceride by following the glycerol phosphate oxidase method; and the enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods. After taking blood samples, chickens were euthanized with an intravenous injection of sodium pentobarbital and immediately intestinal tract, liver and spleen were removed and weighed (data expressed as relative organ weight; grams of organ per 100 g of BW). Small intestine was immediately removed and digesta contents (from final part of small intestine) from 60 chickens (10 chickens per treatment) were collected separately, cooled at once used for microbial assays (Escherichia coli and Lactobacillus).

The data were analyzed by using the ANOVA with the General Linear Model (GLM) procedure of SPSS 16.0 (2011) by using the following model: Yijk = $\mu + \alpha i + \beta j + (\alpha\beta)ij +$ ϵijk , where Yijk is the dependent variable, μ is the overall mean, αi is the effect of stocking density (i = 1,2); βj is the effect of sumac powder (j = 1, 2, 3); ($\alpha\beta$)ij is the interaction between stocking density and sumac powder levels; and $\epsilon \sim N(0,\sigma 2\epsilon)$ represents the

^bProvided (per kg of diet): vitamin Å, 8,000 IU; vitamin D3, 1,200 IU; vitamin E, 10 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; pyroxidine, 0.2 mg; vitamin B12, 0.03 mg; pantothenic acid, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 0.5 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg Provided (per kg of diet): Iron, 80 mg; zinc 40 mg; manganese 60 mg; iodine 0.8

^cProvided (per kg of diet): Iron, 80 mg; zinc 40 mg; manganese 60 mg; iodine 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

unexplained random error. The α -level used for determination of significance for all the analyses was 0.05. Differences between means were tested by Tukey's least significant difference when an interaction between stocking density and sumac powder was significant. Data are presented as means and SEM.

RESULTS AND DISCUSSIONS

Results of the effects of sumac powder (*Rhus* coriaria L.) on growth performance and intestinal microbiota in broilers at different stocking densities are given in Table 2. Body weight gain and feed intake were significantly lower in chickens reared at high stocking density than normal stocking density groups (P<0.05). These results are in agreement with in previous studies by Dozier et al. (2005), who reported that, the negative effect of high stocking density on cumulative body weight gain in broilers. Negative effects of high density on live performance in broilers might

be broilers faced an access difficulties to feed and water difficulties (Sørensen et al., 2000).

On the other hand, dietary supplementation of sumac powder had no significant effect on body weight gain, feed intake and feed conversion rate (P>0.05). In contrast, Ghasemi et al. (2014) reported that addition of different levels of sumac extract (1, 2 and 3 % of diet) at had significant effects on the feed intake. Similarly, Lee et al. (2003) showed that the improvement of feed efficiency in broilers fed diet supplemented sumac extracts. Different results might be partially explained by differences in the sumac extract level supplemented to diet.

Lactobacillus spp. may be considered main microorganisms in the gut of broilers 6 weeks of age (Dumonceaux et al., 2006). Our results indicated that an interaction was existed between stocking density and sumac powder supplementation for body weight gain, total aerobs, *E. coli* and *Lactobacillus* population in gut (Table 2).

 Table 2. Effects of sumac powder (*Rhus coriaria* L.) on growth performance and intestinal microbiota in broilers at different stocking densities

Treatments ¹		Gr	owth performan	Bacteriacolony		
Stocking density (bird/m ²)	Sumac powder (g/kg feed)	BW gain (g)	Feed intake (g)	Feed conversion rate	<i>E. coli</i> (logCFU ^{g-1})	Lactic acid bacteria (log CFU ^{g-1})
10	0	2773.0 ^{ab}	4536.8 ^a	1.66	4.73 ^a	3.56 ^b
10	0.75	2863.7 ^a	4529.4 ^a	1.63	4.08 ^{ab}	3.97 ^{ab}
10	10 1.5		4569.5 ^a	1.65	3.68 ^b	3.98 ^{ab}
20	20 0		4452.9 ^{ab}	1.68	4.88 ^a	3.30 ^b
20	0.75	2481.6 ^d	4205.3 ^b	1.66	3.87 ^b	4.14 ^a
20	1.5	2596.0 ^{cd}	4364.8 ^{ab}	1.66	3.64 ^b	4.22 ^a
SEM		16.92	30.41	0.004		
Main effect			Probability			
Stockingdensity		**	**	NS	*	NS
Sumacpowder		NS	NS	NS	**	*
StockingdensityxSur	nacpowder	** NS		NS	*	*

SEM: Pooled standard error of mean

¹Each value represents the least square mean from 7 pens per each treatment

 $^{a-d}$ Means within a column without a common superscripts differ statistically (P < 0.05).

NS: No significant (P>0.05), *: P<0.05, **: P<0.01

¹Results are reported as means for 5 replicates of 3 broilers each.

The effects of stocking density and dietary sumac powder supplementation on serum biochemistry in broilers are presented in Table 3. At the end of the experiment, none of the serum biochemistry variables was affected by dietary sumac powder (P>0.05) (Table 3). Although there was no interaction between stocking density and sumac powder in serum biochemistry variables (P>0.05) Serum uric acid, total protein and albumin concentrations were affected by stocking density (P<0.05). However, ALP, and AST activities, serum HDL, cholesterol and triglyceride concentration of broilers were not affected by any treatment (P>0.05).

Table 3. Effects of sumac powder (Rhus coriaria L.) on serum biochemistry in broilers at different stocking densities

Treatm	ients ¹	Measurements						
Stocking density (bird/m ²)	Sumac powder (g/kg feed)	ALP (U/L)	AST (U/L)	CHOL (mg/dL)	UA (g/dL)	TP (g/dL)	HDL (mg/dL)	ALB (mg/dL)
10	0	1744.6	392.5	121.1	3.46	3.65	64.8	0.56
10	0.75	1757.0	344.1	126.7	4.28	3.78	69.65	0.53
10	1.5	1470.0	362.6	122.0	3.63	3.65	64.33	0.48
20	0	1467.3	313.5	129.7	4.17	4.13	73.08	0.48
20	0.75	1626.8	274.0	127.2	4.93	4.24	69.68	0.47
20	1.5	1811.0	311.2	131.0	5.06	4.11	74.03	0.49
SEM		68.71 14.92 1.91 0.21 0.62 1.20 0.				0.008		
Main effect		Probability						
Stocking density		NS NS NS * ** NS *				*		
Sumac powder		NS NS NS NS NS NS NS				NS		
Stocking density x	Sumacpowder	NS NS NS NS NS NS NS					NS	

SEM: Pooled standard error of mean, ALP: Alkaline phosphatise, AST: Aspartate amino transferase, CHOL: Cholesterol, UA: Uric acid, TP: Total protein: HDL: High density lipoprotein, ALB: Albumin

¹Fach value represents the least square mean from 7 pens per each treatment

^dMeans within a column without a common superscripts differ statistically (P < 0.05).

NS: No significant (P>0.05), *: P<0.05, **: P<0.01

1Results are reported as means for 5 replicates of 3 broilers each.

Our findings demonstrate that stocking density had significant effects on spleen, proventriculus, intestine weight and abdominal fat pat (P<0.05) (Table 4). However, internal organ weights of broilers were not affected by any levels of supplemental sumac powder. A higher stocking density significantly decreased percentage gizzard, intestine weight and

abdominal fat pad at 42 days (P<0.05). Thaxton et al. (2006), similarly, observed that lower internal organ weight of broilers reared at high stocking density. However, the interaction of sumac powder supplementation and stocking density was not significant for the internal organ weights checked.

Table 4. Effects of sumac powder (Rhus coriaria L.) on internal organ weights in broilers at different stocking densities

Treatn	nents ¹	Measurements (g/100 g body weight)					
Stocking density (bird/m ²)	Sumac powder (g/kg feed)	Liver weight	Spleen weight	Gizzard weight	Proventriculus weight	Intestine weight	Abdominal fat pad
10	0	2.27	0.136	1.91	0.38	4.69	0.81
10	0.75	2.18	0.129	1.70	0.42	4.59	1.20
10	1.5	2.26	0.127	1.92	0.41	4.18	0.95
20	0	2.20	0.136	1.72	0.39	3.94	0.72
20	0.75	2.42	0.126	1.57	0.36	3.95	0.75
20	1.5	2.11	0.112	1.56	0.32	3.44	0.77
SEM		0.51 0.005 0.05 0.01 0.08 0				0.04	
Main effect		Probability					
Stocking density	NS	NS	*	NS	**	*	
Sumac powder	Sumac powder		NS NS NS NS NS NS				NS
Stocking density x S	Sumac powder	NS NS NS NS *					*

SEM: Pooled standard error of mean

¹Each value represents the least square mean from 7 pens per each treatment

^{a-d}Means within a column without a common superscripts differ statistically (P < 0.05). NS: No significant (P>0.05), *: P<0.05, **: P<0.01</p>

1Results are reported as means for 5 replicates of 3 broilers each.

CONCLUSIONS

In conclusion, our results showed that broilers exhibited low performance when raised in high stocking density and dietary supplementation of sumac powder has not prevented this decline.

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REFERENCES

- Dozier W.A., Thaxton J.P., Branton S.L., Morgan G.W., Miles D.M., Roush W.B., Lott B.D., Vizzier-Thaxton Y., 2005. Stocking density effects on growth performance and processing yields of heavy broilers. Poultry Science, 84(8):1332-1338.
- Dumonceaux T.J., Hill J.E., Hemmingsen S.M., Van Kessel A.G., 2006. Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. Applied and Environmental Microbiology, 72(4):2815-2823.
- EL Sissi H.I., Ishak S. Abd El Wahid M.S., 1972. Polyphenolic components of Rhuscoriaria leaves. In Planta Med. 21(1):67–71.
- Ghasemi R., Faghani M., Reza J.P., Khonmirzaie N., Rahimian Y., 2014. Using Sumac (*Rhus coriaria* L.) extract affect performance and intestinal characteristics of broiler chicks. Scholarly Journal of Agricultural Science Vol. 4(8):442-445.
- Golzadeh M., Farhoomand P., Daneshyar M., 2012. Dietary Rhuscoriaria L. powder reduces the blood cholesterol, VLDL-c and glucose, but increases abdominal fat in broilers. South African Journal of Animal Science, 42(4):398-405.
- Gulmez M. Oral N., Vatanever L., 2006. The effect of water powder of sumac (*Rhus coriaria* L.) and lactic acid on decontamination and shelf life of raw broiler wing. Poultry Sci. 85, 1466-1471.
- Jung N.C., 1998. Biological activity of urushiol and flavanoid from Lac tree (*Rhus verniciflua* Stokes).

Ph.D. Thesis Chonnam National University, Kwang-Ju, South Korea.

- Lee J.C., Kim J., Jang Y.S., 2003. Ethanol eluted extract of *Rhus verniciflua* Stokes inhibits cell growth and induces apotosis in human lymphoma cells. J. Biochem. Mol. Bio. 36, 337-343.
- Puron D., Santamaria R., Segura J.C., Alamilla J.L., 1995. Broiler performance at different stocking densities. The Journal of Applied Poultry Research, 4(1):55-60.
- Rotllant J., Pavlidis M., Kentouri M., Abad M.E., Tort L., 1997. Non-specific immune responses in the red porgy Pagruspagrus after crowding stress. Aquaculture 156, 279-290.
- Shidfar F., Rahideh S.T., Rajab A. et al., 2014. The Effect of Sumac (*Rhus coriaria* L.) Powder on Serum Glycemic Status, ApoB, ApoA-I and Total Antioxidant Capacity in Type 2 Diabetic Patients. Iranian Journal of Pharmaceutical Research. 13(4):1249-1255.
- Sørensen P., Su G., Kestin S.C., 2000. Effects of age and stocking density on leg weakness in broiler chickens. Poultry Science, 79(6):864-870.
- Thaxton J.P., Dozier W.A., Branton S.L., Morgan G.W., Miles D.W., Roush W.B., Lott B.D., Vizzier-Thaxton Y., 2006. Stocking density and physiological adaptive responses of broilers. Poultry science, 85(5):819-824.
- Türkyilmaz M.K., 2008. The effect of stocking density on stress reaction in broiler chickens during summer. Turkish Journal of Veterinary and Animal Sciences, 32(1):31-36.