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Influence of *Satureja sahandica* Bornm extract on darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weight in broiler chickens

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ABSTRACT

A study with 240-day-old broiler chickens was conducted to investigate the effects of Satureja sahandica Bornm extract in diet on plasma cholesterol, triglyceride, high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), proportional abdominal fat, liver weights and liver color index. The chickens were divided into four groups and received 0.0%, 0.3%, 0.6% and 0.9% alcoholic extract of Staureja sahandica Bornm from day one to day 42 of age. All the birds receiving the Satureja sahandica extract had lower plasma total cholesterol, triglyceride, LDL-c concentrations and proportional fat weights compared with the control group (P<0.05). No differences were observed for liver proportional weight and liver absolute weight. Liver color index of 0.6% and 0.9% alcoholic extract of Staureja sahandica groups was higher than that of control group. In comparison, Satureja sahandica extract supplementation diminished the plasma triglyceride, total cholesterol and LDL-c decreased the and abdominal fat weight (P<0.05) and increased the liver colour index as compared to control (P<0.05). Significant negative correlation (P<0.01) was found between the Satureja sahandica extract supplementation and plasma cholesterol, abdominal fat weight and positive correlation (P<0.01) between the Satureja sahandica extract supplementation and liver colour index. In conclusion, Satureja sahandica consumption in broiler chickens could improve the carcass quality to the consumers and net returns of the producers.

Key words: Satureja sahandica, extract, Blood cholesterol, Liver colour index, broiler chickens.

INTRODUCTION

Heart disease is an important occurring case in modern human societies and is directly related with increased levels of plasma cholesterol. Hypercholesterolemia and low high density lipoprotein (HDL-c) levels are often associated with endothelium dysfunction and inflammation, which are often followed by atherosclerosis [1]. Moreover, cellular cholesterol concentration is as well related to other disease such as Alzheimer [2]. Animal products with high fat content are one of the risk factors for cardiovascular diseases and are not desirable for consumers [3]. Although chicken meat is a healthier product, compared with other animal protein sources for human diet, having low cholesterol and fat content, and several different dietary treatments have been used to attempt a further decrease of these ingredients in poultry meat [4]. Nowadays, medicinal plants are receiving high attention as feed additives, due to their depressing effects on fat contents of animal products. In this regard, beneficial effects of dietary alfalfa, rosemary, thyme and garlic have been revealed in human and animals [4, 5, 6 and 7]. Satureja sahandica Bornm is a perennial medicinal herb in the *Lamiaceae* family, cultivated worldwide for culinary, cosmetic and medical purposes. This species has special activities such as antispasmodic, expectorant, antiseptic, antimicrobial and

antioxidant [8 and 9]. Thymol (5-methyl-1-2-isopropyl phenol) and para-Cymene (5-isopropyl-2-methyl phenol) are the main phenolic components in Satureja sahandica that have hypocholesterolemic effects [8]. Hypocholesterolemic and performance improvement effects of thyme herb [10] or its essential oil [11] have been revealed previously. Nevertheless there is no information about Satureja sahandica extract effects on abdominal fat (as a negative carcass characteristic), liver color index and weight (as the liver lipid content indices). Fat accumulation in carcasses of broilers, particularly in abdominal and visceral areas, represents a waste product to consumers from the dietal and health points of view. Such obese broilers are unpleasant to the consumers, lead to decreased saleability and reduce the net returns of the producers [12].

Moreover, colour intensity indicates the cholesterol or lipid contents of the liver and indirectly meat quality. The liver colour associates to liver lipid contents in broiler chickens, and high dietary lipids [13] or cholesterol [14] have resulted in lighter colour of the liver. So far, few studies are available regarding the potential effects of medicinal plants on these important indices in broiler chickens. In an experiment, dietary supplementation of 1.5% dried thyme herb alleviated the abdominal fat weight and reddened the skin colour [15].

Therefore, this experiment was conducted to evaluate the effects of Satureja sahandica extract supplementation in diet on abdominal fat weight, liver colour, rather than plasma lipoproteins.

MATERIALS AND METHODS

240 one-day-old broiler chicks (Ross 308) were purchased from a local hatchery, weighed on arrival and randomly allocated to 16 pens (1x1 m) of 15 birds each with four replicates per each treatment. All chickens were fed the similar starter (day 1-21 of age) and grower (day 22-42 of age) diets in pellet form (Table 1), but received 0.0% (ZT), 0.3% (LT), 0.6% (MT) and 0.9% (HT) alcoholic extract of Staureja sahandica Bornm (0.06% thymol and pH=5) in diet during the experimental period, respectively. *Staureja sahandica* alcoholic extract was prepared using a standard maceration method [16]. For this purpose, vegetative parts of the shade dried Staureja sahandica full bloom stage were crushed and soaked in ethanol 80% in 1:5 ratios (w/v) for 72 h on a shaker. The extract strained afterwards and its thymol content was determined by TLC (thin layer chromatography) method, the pH value by using a pH meter instrument (HQ40D, Hach Co., Loveland, CO, USA). At days 42 of age, two birds per pen (a male and a female) were selected, weighed and killed by decapitation to obtain the blood samples, abdominal fat and liver weights, and liver colors. Liver and abdominal fat were removed, weighed and their proportional (percentage of live body weight) or absolute weights were reported. Immediately after slaughter, livers surface area colors were grouped into five grades, namely 1=light, 2=reddish light, 3=red, 4=dark red and 5=dark visually.

Table 1. Composition of experimental diets

Ingredients (%)	Starter 1-21 d	Grower 22-42 d
Corn	**54.87**	**61.78**
Soybean meal	**36.72**	**26.36**
Fish meal	**1.31**	**4.5**
Vegetable oil	**3**	**4**
Limestone	**1.15**	**1.05**
Dicalcium phosphate	**1.94**	**1.49**
Vitamin and mineral premix	**0.50**	**0.50**
Salt	**0.30**	**0.30**
DL- Methionine	**0.21**	**0.02**
Total	**100**	**100**
Calculated analysis		
Metabolizable energy (kcal/kg)	**2937**	**3100**
Crude protein	**21.44**	**19.37**
Calcium	**1.05**	**1**
Available phosphorus	**0.51**	**0.50**
Sodium	**0.16**	**0.14**
Arginin	**1.41**	**1.23**
Methionine + Cystine	**0.91**	**0.69**
Lysine	**1.20**	**1.10**
Tryptophan	**0.31**	**0.26**

Provide per kg of diet: retional, 15,000 U; colecalciferol, 8000 U; menadione, 3 mg; cyanocobalamin 15 µg; niacin, 32 mg; choline, 840 mg; biotin, 40 µg; thiamine, 4 mg; riboflavin, 6.6 mg; pyridoxine, 5 mg; folic Acid, 1 mg; zinc, 80 mg; manganese, 100 mg; selenium, 200 mg; iron, 80 mg; magnesium, 12 mg; copper,10 mg; calcium,15 mg; iodeine,1 mg.

The liver colour data were considered as the non-parametric data and transformed to √x+0.5. Then the transformed data were used for statistic analyses. Blood samples were collected in heparinzed tubes (citrate sodium 3.6% solution) during a forty min period; blood plasma was separated and plasma triglyceride, total cholesterol and plasma lipoproteins were determined by enzymatic method using commercial kit technique (Man Co., Tehran, Iran). Means of all variables for both samples of day 42 of age were calculated and used for statistic analyses. The data were subjected to SAS [17] statistical software (version 9.1) and analyzed based on a completely randomized design using the general linear model (GLM) procedure. When the overall model was statistically different (P<0.05), the Tukey-Kramer multiple comparison test was used to compare the mean values (P<0.05).

RESULTS

Plasma lipoproteins, total cholesterol and triglyceride

The effect of Staureja sahandica extract on these parameters is shown in Table 2. There was no significant difference between the treatments for plasma HDL-c but plasma total cholesterol , Triglyceride and LDL-c concentration of Staureja sahandica extract received birds were lower (P<0.05) compared with those of ZT birds. Moreover, compared with the control, Staureja sahandica extract supplementation in diet decreased (P<0.05) the plasma triglyceride, total cholesterol, LDL-c.

Table. 2: Effect of different levels of Staureja sahandica extract on blood parameters in broilers (mg/dl)

Treatments	Total cholesterol	Triglyceride	HDL-c	LDL-c
ZT	48.75^{a}	74.75	73.61^{a}	150^{a}
LT	32.25^{b}	70.75	48.74^{b}	126^{b}
MT	33.75^{ab}	76.75	46.24^{b}	132.75^{ab}
HT	37.5^{ab}	74.25	47.75^{b}	130^{ab}
P value	0.045	0.354	0.002	0.039
SEM	0.56	2/92	6.65	4.94

HDL-c, high density lipoprotein; LDL-c low density lipoprotein; ZT, 0% Satureja sahandica extract treatment; LT, 0.3% Satureja sahandica extract treatment; MT, 0.6% Satureja sahandica extract treatment; HT, 0.9% Satureja sahandica extract treatment; ^{a,b} means with no common superscript letter in each columns differ significantly (P<0.05); Two birds per replicate (8 per treatment) were taken for the analyses.

Liver colour index, liver and abdominal fat proportional and absolute weights

Satureja sahandica extract received birds had a no differences proportional liver weight and absolute liver weight compared with control birds (Table 3). No differences were observed between the treatments for abdominal fat absolute weight. Although all the Satureja sahandica extract received birds had a lower proportional fat weights compared with control birds, but only HT birds had a significantly lower value (P<0.05). HT and MT birds had a higher liver colour index compared with ZT birds, while no difference was observed between the liver colour index of LT and ZT birds (P>0.05).

Table 3: Effect of different levels of Staureja sahandica extract on carcass quality in broilers (%)

Treatments	Liver weight		Abdominal fat		Liver Colour Index
	Proportional	Absolute	Proportional	Absolute	
ZT	**2.23**	**40.50**	**2.42^{a}**	**36.8**	**1.51^{b}**
LT	**2.28**	**39.60**	**1.99^{ab}**	**36.3**	**1.83^{ab}**
MT	**2.19**	**40.20**	**1.25^{b}**	**38.6**	**1.95^{a}**
HT	**2.15**	**39.59**	**1.05^{b}**	**33.1**	**1.98^{a}**
P value	**0.41**	**0.345**	**0.02**	**0.14**	**0.02**
SEM	**0.41**	**1.60**	**0.40**	**2.37**	**0.02**

ZT, 0% Satureja sahandica extract treatment; LT, 0.3% Satureja sahandica extract treatment; MT, 0.6% Satureja sahandica extract treatment; HT, 0.9% Satureja sahandica extract treatment; ^{a,b}means with no common superscript letter in each columns differ significantly (P<0.05); Liver and abdominal fat of two birds per replicate (8 per treatment) were taken for the analyses.

DISCUSSION

The results of the present study indicate that Satureja sahandica extract consumption in diet reduces the plasma total cholesterol, LDL-c and triglyceride in broiler chickens, which is in agreement with the results of previous studies in laying hens by dietary supplementation of 0.25% thyme plant [18] and in broiler chickens by dietary levels of 0.5, 1.0, 1.5 and 2.0% crushed thyme [10]. In the same way, Case *et al* [19] reported 9% reduced serum cholesterol in 21-day-old leghorn chicks fed 15 ppm thyme essential oil. These results are also consistent with those of a previous study [14], where reduced plasma triglyceride was found in female broiler chickens at day 28 of age by dietary

carvacrol supplementation. Nevertheless, higher plasma levels of triglyceride, HDL-cholesterol and LDL-cholesterol were reported in broilers by dietary supplementation of 100 and 200 ppm thyme essential oil [11]. Undoubtedly different additives (Satureja essential oil and Satureja extract) and supplementation ways (dietary and drinking water) should be considered between the studies. Depressing effects of Saturej sahandica extract supplementation on plasma total cholesterol, LDL-c and triglyceride of recent experiment may possibly be related to the reduced activity of HMG-CoA reductase by active components of Satureja sahandica extract (thymol and p-cymene) in gastrointestinal tract. Satureja sahandica extract has essential oil, tannins, glycosides and other components [8]. Dietary thymol inhibit the hepatic 3-hydroxy-3-methyle glutaryl coenzyme A (HMG-CoA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis. The non-sterol products derived from mevalonate, which modulate HMG-CoA reductase activity [20], thymol and carvacrol might induce putative regulatory non-sterol products [19 and 21]. This action results in a reduction of the plasma cholesterol concentration [10, 14, 19 and 22]. A positive correlation has been reported between the activity of HMG-CoA reductase and total cholesterol or LDL-cholesterol in broiler chickens [23]. Satureja sahandica extract supplementation reduced the proportional abdominal fat weight in the recent study, which is in agreement with the results of [24], who found in broiler chickens fed 200 ppm thyme essential oil. This is related to the decreased cholesterol and triglycerides absorption from the gut or synthesis in liver, because a significant correlation obtained between the plasma cholesterol and proportional abdominal fat weight.

No changes of liver weight were reported in broiler chickens fed 1 g/kg thyme powder [25]. An increase in relative liver mass of broiler chickens was observed after the thyme essential oil consumption [14]. The higher liver colour index in Satureja sahandica extract received birds further indicates the decreased liver fat content. A lower plasma cholesterol concentration (mg/dL) and liver proportional weight in broilers fed 100 ppm thyme essential oil have been reported already [24]. Lee *et al* [14] observed higher plasma and liver cholesterol, and liver weight, in broiler chickens fed 1% cholesterol in diet. Moreover, they observed the yellowish livers in cholesterol fed broilers and pinkish livers in birds fed the control diet (without cholesterol). In other consistent study [13] indicated the lighter colour in broiler chickens fed high dietary lipids and the redder colour in liver with low cholesterol content.

CONCLUSION

Based on the results of our experiment, we can conclude that Satureja sahandica extract supplementation in diet decreases the plasma triglyceride, total cholesterol and LDL-c, which in turn lower the abdominal lipids, reduces the proportional abdominal fat weights, and increases as well the liver colour intensity in broiler chickens. These effects of the Satureja sahandica extract consumption are supposed to be exerted by the lower activity of HMG-CoA reductase enzyme, reduced fat absorption from the gastrointestinal tract or the lipid catabolism for gluconeogenesis process. Consequently, it can be concluded that Satureja sahandica consumption in broiler chickens could improve the carcass quality, for a higher appreciation by the end users and profitable net returns for the producers.

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