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Effects of Saponin Feed Additive with Oregano Essential Oil on Carcass Characteristics, Antioxidant Status and Immune Response in Broilers.

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Abstract

The objective of this study was conducted to evaluate the effects of adding dietary saponin powder , essential oils (thymol and carvacrol) and their interactions on broiler chicken carcass characteristics, antioxidant status, and immune response. The experiment followed a 4×2 design, with different levels of saponin (0, 10, 20, and 40 ppm/kg diet) and essential oil at 0 (control) and 50 ppm. A total of 240 one-day-old Ross 308 chicks were randomly divided into eight treatment groups, each with three replicates. The results showed that birds fed a basal diet supplemented with saponin at 40 ppm/kg diet and essential oil at 50 ppm had significantly improved live body weight, carcass traits, and total edible parts compared to the control group. The combination of saponin at 40 and 20 ppm, along with essential oil at 50 ppm, resulted in significantly lower levels of serum urea, uric acid, creatinine, AST, and ALT compared to the other groups. Broiler chicks fed diets containing saponin at 20 or 40 ppm, along with essential oil at 50 ppm, showed significantly increased levels of serum GP_X, IgG, and IgM, as well as a significant decrease in serum MDA, compared to the other groups. These findings suggest that incorporating saponin at 40 or 20 ppm, along with essential oil at 50 ppm, is more effective in enhancing the antioxidant response and immune profile compared to other dietary combinations.

Keywords: Broiler, Saponin, Essential oil, carcass, Immunity, Antioxidant

Introduction

Feed expenditures, which make up around 70% of all production costs, are extremely important to the growth of the poultry sector. The rising demand for poultry products is also influenced by other elements such population growth, lifestyle modifications, altering food preferences, urbanization, rising per capita income, and health consciousness. Different alternative feeds have been looked at to fulfil the current demand for increased productivity and decreased feed costs. A common ingredient in the food and beverage sectors as a natural remedy, foaming agent, and flavour enhancer, yucca schidigera extract is also used as a feed supplement in the poultry industry. According to Hristov and Colleagues (1999), it has shown promise in regulating ammonia levels in animal facilities and lowering ammonia concentration and faecal odour in animal excrement. According to Ayasan et al. (2005) and Almuhanna et al. (2011), adding this plant extract to poultry feed improves metabolic efficiency, lowers ambient ammonia levels, and increases body weight, feed conversion, and production. Ammonia is bound by saponin, the main ingredient in *Y. schidigera* extract, which lowers free ammonia levels. Enhancing economic qualities has a substantial effect on grill chicken output and carcass attributes when *Y. schidigera* extract is used. The favourable effects on gut health of oregano essential oil (OEO), which is extracted from plants of the origanum genus and principally contains thymol and carvacrol (Oniga et al., 2018), have drawn attention. However, research on OEO's effects on poultry performance has produced a mixed bag of findings. According to Peng et al. (2016), OEO had a favourable impact on intestinal health, which enhanced broiler development and carcass characteristics. In vitro and in vivo studies have demonstrated the antioxidant activity of oregano, thymol, and carvacrol (Milos et al., 2000; Martinez-Tome et al., 2001; Kulisic et al., 2004). Oregano, thymol, and carvacrol have been shown to increase intestinal barrier function and regulate intestinal flora and morphology in previous studies on different species (Wei et al., 2015; Cheng et al., 2018; Abdel-Latif et al., 2020). The antibacterial properties of carvacrol and thymol, extracted from oregano, were validated in in vitro tests by Liolios *et al.* (2009) against a variety of pathogens, including *Escherichia coli, Staphylococcus aureus, and Streptococcus mutans.* Therefore, this study aimed to investigate the

effects of feeding different level of saponin and essential oil on carcass characteristics, liver function, and immune response of broiler chickens.

Materials and Methods

Ethical approval

The experimental design and procedures complied with the ethical standards of our relevant national and institutional committee on animal experimentation approved by the Scientific Ethics Committee, Animal Production Department, Faculty of Agriculture, Benha University, Egypt.

Experimental animals and design:

Birds and Housing Management

A total number of 240 (one-day old) chicks from Ross 308 strain were weighed equally and randomly divided and distributed in eight dietary treatments groups having three replicate in each. Each dietary treatment group consists of 30 chicks distributed in three replicated pens, with 10 chicks in each. The birds in the control group (T1) were fed a diet without saponin while, saponin 10 ppm (T2), 20 ppm (T3) and 40 ppm (T4), however birds in group 5 (T5) fed diet without saponin and drink water with 50 ppm essential oil, from hatching day to the end of experimental work, saponin 10 % with 50 ppm EO (T6), 20 ppm with 50 EO (T7) and 40 ppm with 50 EO (T8). Chicks were weighed individually at hatch and wing banded, placed in the rearing farm to perform the post-hatching experiment. Chicks were kept under similar hygienic and environmental conditions. All chicks were brooded and reared at 32-33 °C from hatch to 7 d of age, 28-30°C from 8 to 14 d, 24-26°C from 15 to 21 d, and 21-24°C from 22 day of age to the end of the experiment. The light program was 24h light at the first 5 days of age, then from 6 to 35 days of age (the end of the experiment) 23 h light and 1 h dark was applied. The relative humidity was within 50 and 65%.

Diets and feeding regime

Standard commercial broiler diets consisted of a crumbled starter (232 g/kg crude protein and 3,000 kcal metabolizable energy/kg diet from 1 to 14 d of age, pelleted grower (211 g/kg crude protein and 3,100 kcal metabolizable energy/kg diet from 15 to 28 d of age and pelleted finisher (195 g/kg crude protein and 3,219 kcal metabolizable energy/kg diet from 29 to 35 d of age. were used for each group. Feed and water were offered ad-libitum.

Slaughtering and carcass measurements

At the end the of experimental period 35 days, 6 birds from each dietary treatment (2 bird/ replicate) were randomly selected and slaughtered after 12 hr of fasting with free assess to drinking water. At slaughter time the jugular vein was cut using a sharp knife to guarantee maximum and rapid blood loss for humane slaughtering. Then, the feathers, heads, shanks, viscera, and abdominal fat were removed, and the remaining carcasses were dissected .The carcass and giblets (empty gizzard, liver and heart) were separately weighed and expressed as a percentage of live body weight. The proportional weights to live weight of giblets, carcass and total edible parts were calculated as follows: giblets weight (%) = (GW/LBW) ×100, edible parts (%) = ((EW+GW)/LBW) ×100, whereas: LBW = live weight, EW= eviscerated weigh and GW= giblets weight. the breast and thigh meat was sampled.

Biochemical blood parameters, including, aspartate aminotransferase (AST, U/I), and alanine aminotransferase (ALT, U/I) concentrations were measured; kidney function tests; creatinine , uric acid; immunoglobulin IgG and IgM levels; antioxidant capacities of plasma; glutathione peroxidase (GPx) and malondialdehyde (MDA) were also determined .

Statistical analysis:

Data were analyzed using SAS, 2004 software **(SAS, 2004)** by using two way ANOVA factorial design. Tests of significance for the differences between means were carried out according to **Duncan (1955)**. According to the following liner model:

$$X_{ij} = \mu + S_i + O_j + SO_{ij} + e_{ij}$$

Whereas: X_{ij} = the observation of traits for ij^{th} birds; μ = Overall mean; Si = Effect of the i^{th} aponin levels. (i, 1-4); Oj = the effect of the k^{th} oil. (j, 1-2); SO_{ij}= Interaction between i^{th} saponin level and j^{th} levels of oil (4×2); and e_{ij}= the experimental error.

Results and Discussion

Carcass traits

Results presented in Tables 1 showed that chicks fed diet with saponin at a level of 40 ppm had the higher absolute and relative weights of carcass, Giblets and total edible parts compared with different levels applied of saponin and control group. The results obtained were agree with those reported by **Patoary *et al.* (2020)** demonstrated that eviscerated weight percentage were significantly higher in Yucca treated group ($p<0.05$) than the control group in broiler chickens.

Chicks supplemented water with EO at a level of 50 ppm had the lower averages of absolute and relative weights of carcass compared with control group which showed the higher average of absolute weight of giblets and total edible parts. The results obtained disagree with those reported by **Ocak *et al.* (2008)** showed that no impact of feeding thyme leaves on carcass weight, carcass yield, or relative weights of the edible organs of broiler chicks .

The interaction between either 40 ppm saponin x EO increased average of absolute weights of carcass, giblet and total edible when compared with different interactions applied.

Table 1. Least – square means and standard error (X̄±S.E) for carcass traits of different experimental groups as affected by the studied factors.

Items	Live body weight (g)	carcass		Giblets		Total edible parts	
		(g)	(%)	(g)	(%)	(g)	(%)
Saponin (ppm)							
Control	1751.6	1261.2	72.0	98.5	5.68	1359.8	77.7
10	1792.5	1294.1	72.0	106.1	5.95	1400.3	78.0
20	1797.9	1286.6	71.6	100.2	5.55	1386.8	77.2
40	1891.5	1387.2	73.4	109.1	5.76	1496.3	79.2
MSE	66.9	48.4	0.68	5.07	0.22	51.5	0.71
Essential oils (ppm)							
Control	1782.4	1277.3	72.2	103.0	5.85	1380.4	78.1
50	1849.3	1337.2	72.3	103.9	5.62	1441.2	77.9
MSE	46.7	34.3	0.49	3.62	0.15	36.6	0.51
Saponins × Essential oils							
Control x Without	1586.7^{c}	1166.7	73.50	84.60	5.34	1251.3	78.85
10 × without EO	1800.0^{abc}	1296.7	71.74	97.43	5.38	1394.1	77.12
20 × without EO	1801.7^{abc}	1273.3	70.73	88.83	4.79	1359.2	75.53
40 × without EO	1826.7^{abc}	1336.7	73.11	110.17	6.02	1446.8	79.14
Control × plus EO	1825.2^{abc}	1328.3	72.3	102.3	5.62	1435.2	77.81
10 × plus EO	1973.3^{ab}	1440.0	72.97	104.43	5.30	1544.4	78.27
20 × plus EO	1920.0^{abc}	1373.3	71.53	106.30	5.53	1479.6	77.06
40 × plus EO	2040.0^{a}	1456.7	71.33	108.1	5.32	1564.8	76.64
MSE	1.70	89.2	1.18	7.93	0.37	93.4	1.0

$^{a,b,c,\cdots}$Means with different superscript in the same column are significantly different at (P0.05)

Kidney function

Data in the Table 2 shows the effect of saponin and EO on serum urea, uric acid and creatinine in broilers chicks. Results obtained showed that treatments applied and interaction between saponin and EO significantly ($p \geq 0.05$) decreased kidney function against other treatment and control group.

Chicks fed diet supplemented with saponin at a level of 40 ppm showed significantly the lowest levels of urea , uric acid and creatinine (16.91 , 4.67 and 0.56 mg/dl, respectively) followed by those fed diet supplemented with saponin at a level of 20 ppm compared with different treatments applied and control group. These results were agreed with those reported by **Ayoub *et al.* (2019**) showed that the creatinine revealed that Yucca had no adverse effect on liver and kidney functions. Chicks supplemented water with EO at a level of 50 ppm had significantly the lowest average of serum urea (18.07), uric acid (4.85) and creatinine (0.61 mg/dl) compared with control group. The interaction between saponin at a level 40 ppm x EO had significantly the lower averages of serum urea, uric acid and creatinine when compared with different interactions applied and control group.

Table 2. Least – square means and standard error (X̄±S.E) for kidney function (urea, uric acid and creatinine) as affected by the studied factors.

Items	Serum (mg/dl)		
	Urea	Uric acid	Creatinine
Saponin (ppm)			
Control (without)	23.89±0.29^{a}	5.64±0.04^{a}	**0.77±0.00^{a}**
10	20.56±0.29^{b}	5.22±0.04^{b}	**0.69±0.00^{b}**
20	18.86±0.29^{c}	4.90±0.04^{c}	**0.62±0.00^{c}**
40	16.91±0.29^{d}	4.67±0.04^{d}	**0.56±0.00^{d}**
Essential oils (ppm)			
Control (without EO)	22.05±0.21^{a}	5.36±0.03^{a}	**0.71±0.00^{a}**
50	18.07±0.21^{b}	4.85±0.03^{b}	**0.61±0.00^{b}**
Saponin × Essential oils			
Control × without EO	25.93±0.45^{a}	5.76±0.08^{a}	**0.77±0.01^{a}**
10 × without EO	21.53±0.45^{b}	5.36±0.08^{b}	**0.71±0.01^{b}**
20 × without EO	20.10±0.45^{c}	5.03±0.08^{cd}	**0.63±0.01^{c}**
40 × without EO	17.60±0.45^{de}	4.76±0.08^{de}	**0.58±0.01^{de}**
Control × plus EO	18.17±0.45^{d}	4.81±0.08^{de}	**0.60±0.01^{cd}**
10 × plus EO	18.23±0.45^{d}	4.83±0.08^{de}	**0.59±0.01^{cd}**
20 × plus EO	16.46±0.45^{ef}	4.56±0.08^{ef}	**0.54±0.01^{e}**
40 × plus EO	15.16±0.45^{f}	4.36±0.08^{f}	**0.48±0.01^{f}**

$^{a,b,c,\cdots}$Means with different superscript in the same column are significantly different at (P<0.05).

Liver function

Data in the Table 3 shows the effect of saponin and EO on serum AST and ALT in Ross 308 broilers chicks. Obtained data showed that treated groups with essential oils plus saponin significantly(P≤0.005) decrease serum AST and ALT when compared with control groups.

Chicks fed diet supplemented with saponin at level of 40 ppm showed significantly (P≤0.005)the lowest levels of serum AST (28.27) and ALT (16.70 U/L) respectively, followed by those fed diet with 20 ppm saponin for serum AST and ALT (31.87 and 18.87 U/L, respectively) compared with chicks supplemented with saponin at a level 10 ppm and control group which showed significantly the higher average of serum AST and ALT, respectively. Result obtained disagree with those reported by **Bera *et al.* (2019)** who noted that the AST and ALT were not influenced main effect of saponins levels and duration of supplementation or their interaction thereof in broiler chicken. **He *et al.* (2021)** who showed that serum ALB, ALT, and GGT levels of Ross 308 male broiler chicks supplemented with red ginseng root powder containing saponin at concentrations of 0. 75, 150, and 225 mg/kg also showed no significant changes**.**

Chicks fed diet with EO had significantly the lower average of serum AST (30.98U/L) and ALT (18.48U/L) compared with control group which showed the highest serum AST (37.68) and ALT (23.12U/L, respectively).The interaction between saponin at a level of 40 ppm plus EO or saponin at a level of 20 ppm plus EO had the lower values of serum AST and ALT when compared with different interactions applied and control group which showed the higher average of serum ST and ALT.

Table 3. Least – square means and standard error (X̄±S.E) for serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as affected by the studied factors

Items	Serum (mg/dl)	
	AST	ALT
Saponin (ppm)		
Control	41.05±0.31[a]	**25.40±0.25[a]**
10	36.12±0.31[b]	**22.21±0.25[b]**
20	31.87±0.31[c]	**18.87±0.25[c]**
40	28.27±0.31[d]	**16.70±0.25[d]**
Essential oils (ppm)		
Control (without EO)	37.68±0.22[a]	**23.12±0.18[a]**
50	30.98±0.22[b]	**18.48±0.18[b]**
Saponin × Essential oils		
Control × without EO	43.50±0.62[a]	**27.86±0.53[a]**
10 × without EO	37.83±0.62[b]	**23.63±0.53[b]**
20 × without EO	34.13±0.62[c]	**20.33±0.53[cd]**
40 × without EO	80.26±0.62[d]	**18.00±0.53[e]**
Control × plus EO	31.51±0.62[d]	**18.52±0.53[e]**
10 × plus EO	31.63±0.62[d]	**19.26±0.53[de]**
20 × plus EO	27.20±0.62[e]	**15.90±0.53[f]**
40 × plus EO	24.10±0.62[f]	**14.06±0.53[g]**

[a,b,c....]Means with different superscript in the same column are significantly different at (P<0.05).

Serum glutathione peroxidase (GPX) and malondialdehyde (MDA)

Data in Table4 illustrates the effect of saponin and essential oil supplementation on glutathione peroxidase (GP_X) and malondialdehyde (MDA).Birds fed diet supplementation with saponin at a level of 40 ppm showed significantly the highest average of GP_X (461.58nmol/ml) and the lowest average of MDA averaged (1.32mg/dl) compared with different treatments applied and control group. While chicks diet supplemented of control group showed the lowest value of GP_X (432.42) and the highest average of MDA (1.88mg/dl). These results were agreed with those reported by **Alghirani *et al.* (2021)**who noted that the steroidal saponin in *Y.schidigera* demonstrating antioxidant activity to prevent, delay and protect against cell deterioration could be applied against heat stress effect to maintain the pH value in broilers. **Shi *et al.*(2014)** who showed that addition of the alfalfa saponin extract to the diet (5-15.1kg diet) increased glutathione peroxidase, super oxide dismutase activities in serum, liver and muscle in dose dependent manner suggesting that saponin promoted antioxidant activity in the body to coverage free radicals and prevent the action of lipid per oxidation.

Chicks supplemented water with 50 ppm EO (thymol and carvacrol) showed the highest value of GPX (463.37) and the lowest value of serum MDA (1.44mg/dl) compared with control group. Oregano essential oil added in doses of 50 to 100 mg/kg to the diet of chickens exerted an antioxidant effect in animal tissues **Bostoglou *et al.* (2002)**such antioxidant effects would be expected to improve the health of poultry. **Lin *et al.* (2003)** stated that the intake of herbs which contains of essential oils in chickens results in an increase in serum antioxidant enzyme activities and decrease in MDA level. **Ruberto *et al.* (2000); Alma *et al.* (2003) and Luna *et al.* (2010)** reported that the diet supplemented with carvacrol or thymol has similar effectiveness to inhibit the oxidation of lipids than the synthetic antioxidant supplementation such as but ylatedhydroxyl toluene. The interaction between saponin at a level of 40 ppm x EO had the higher averages of GP_X and the lowest average of MDA when compared with different interactions applied and control group.

Table 4. Least – square means and standard error (X̄±S.E) for antioxidant status and immunity response as affected by the studied factors.

Items	Serum (u/l)		Serum (Ug/dl)	
	GPX	MDA	IgG	IgM
Saponin (ppm)				
Control	432.42 ±8.93^{c}	1.88± 0.03^{a}	25.79±0.20^{d}	**42.33±0.50^{d}**
10	437.33 ±8.93^{bc}	1.65±0.03^{b}	27.84±0.20^{c}	**47.81±0.50^{c}**
20	462.17 ±8.93^{ba}	1.48±0.03^{c}	30.20±0.20^{b}	**51.83±0.50^{b}**
40	466.58 ±8.93^{a}	1.32±0.03^{d}	32.55±0.20^{a}	**55.36±0.50^{a}**
Essential oils (ppm)				
Control (without EO)	435.87±6.31^{b}	1.72±0.02^{a}	27.64 ± 0.14^{b}	**45.78 ± 0.35^{b}**
50	463.37±6.31^{a}	1.44±0.02^{b}	30.54 ± 0.14^{a}	**52.90±0.35^{a}**
Saponin × Essential oils				
control×without EO	410.6 ±14.8^{d}	1.96±0.06^{a}	25.46±0.38^{f}	**39.03±0.91^{e}**
10 × without EO	430.3 ±14.8^{cd}	1.74±0.06^{b}	27.06±0.38^{e}	**45.16±0.91^{d}**
20 × without EO	451.0 ±14.8^{bcd}	1.56±0.06^{bc}	28.96±0.38^{d}	**48.16±0.91^{c}**
40 × without EO	446.0±14.8^{bcd}	1.33±0.06^{de}	31.46±0.38^{b}	**54.16±0.91^{b}**
control× plus EO	462.35±14.8^{abc}	1.43±0.06^{cd}	29.25±0.38^{c}	**51.42±0.91^{b}**
10 × plus EO	463.0±14.8^{abc}	1.40±0.06^{cd}	30.13±0.38^{c}	**52.13±0.91^{b}**
20 × plus EO	490.6±14.8^{ba}	1.23±0.06^{de}	32.60±0.38^{b}	**56.96±0.91^{a}**
40 × plus EO	508.3±20.2^{a}	1.16±0.06^{e}	34.43 ± 0.38^{a}	**58.10 ± 0.91^{a}**

$^{a,b,c,\cdots}$Means with different superscript in the same column are significantly different at (P<0.05).

Serum immunogloblin G (IgG) and immunogloblin M (IgM):

The effect of dietary supplementation of saponin and essential oils or in combination on the immunogloblin G (IgG) and immunogloblin M (IgM) is shown in Table 4.Chicks fed diet with 40 ppm saponin showed the highest average of IgG (32.55 mg/dl) and IgM averaged (55.37 mg/dl) compared with different treatments applied and control group. These results agree with those reported by **Ranjbar *et al*. (2014)** who reported that the effect of Y. schidigera powder at 100 or 200 ppm showed a better effect on cellular and hum oral immune responses in broilers. **Su *et al*. (2016)** found that dietary supplementation of 100ppm Y.schidigera powder increased IgG, and IgM. At a level of 200 ppm, a better effect on cellular and hum oral immune responses in broilers.

Chicks supplement of water with 50 ppm EO had highest average of serum IgG and IgM (30.54 and 52.90 mg/dl, respectively) compared with control group. These results agree with those reported by **colleagues (2016)** who challenged the broiler birds with Clostridium perfringens and supplemented the thymol and carvacol to investigate the effect of EO in intestinal morphology and on immune system of broiler. The interaction between saponin at a level 40 ppm × EO had the higher averages of IgG and IgM when compared with different interactions applied and control group which showed the lower average of IgG and IgM.

Conclusions

The results can be summarized that the supplementation of saponin and essential oil to broiler chicken diets and water improved carcass characteristics, liver function and antioxidant status. Birds fed diet with saponin at a level 40 ppm with EO at 50 ppm improved most traits than other groups.

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تأثير إضافة الصابونين مع زيت عضوي أساسي على خصائص الذبيحة ومضادات الأكسدة والإستجابة المناعية في دجاج التسمين

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إجريت هذه الدراسة لتقييم تأثير إضافة الصابونين الغذائي والزيوت الأساسية (الثيمول والكارفاكرول) وتفاعلاتها علي خصائص الذبيحة لدجاج التسمين،ومستويات مضادات الاكسدة، والجهاز المناعي. تم تصميم تجربة عاملية 4 × 2 ،مع مستويات مختلفة من الصابونين (0 ،10 ،20 و 40 جزء في المليون / كجم عليقة) والزيوت الأساسية بمعدل 0 (كنترول) و 50 جزء في المليون. تم تقسيم عدد 240 كتكوت روس 308عمريوم واحد بشكل عشوائي إلى ثمانية مجموعات تجريبية، كل منها ثلاثة مكررات. أظهرت النتائج أن الطيور التي غذيت على عليقة أساس مضاف اليها الصابونين بمستوى 40 جزء في المليون / كجم من العلف مع الزيت العطري عند 50 جزء في المليون،أظهرت تحسنًا ملحوظًا في وزن الجسم الحي،وصفات الذبيحة، وإجمالي الأجزاء الصالحة للأكل مقارنة بمجموعة الكنترول. أدى التداخل بين الصابونين عند 20 و 40 جزء في المليون مع الزيت العطري عند مستوي 50 جزء في المليون إلى إنخفاض مستويات اليوريا في االسيرم وحمض البوليك والكرياتينين و AST و ALT بشكل ملحوظ مقارنة بالمجموعات الأخرى. أظهر الدجاج الذي غذي على عليقة تحتوي على مادة الصابونين بنسبة 20 أو 40 جزء في المليون مع الزيت العطري عند 50 جزء في المليون إلي زيادة كبيرة في مستويات السيرم من ال GP_X وIgG وIgM، بالإضافة إلى إنخفاض كبير بمستوي MDA في السيرم، مقارنة بالمجاميع الأخرى. تشير هذه النتائج إلى أن استخدام الصابونين عند 20 أو 40 جزء في المليون،إلي جانب الزيت العطري عند 50 جزء في المليون، يعززالاستجابة المضادة للأكسدة والإستجابة المناعية بشكل أكثر فعالية من التركيبات الغذائية الأخرى.

الكلمات الدالة: دجاج التسمين –الصابونين– الزيت العضوي – الذبيحة – المناعة – مضادات الاكسدة