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Growth performance, carcass characteristics, and immune responses in broiler chickens fed dietary oregano (*Origanum vulgare* L.) leaves as an alternative for antibiotic growth promoter

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ABSTRACT

Background & Aim: Since the use of antibiotic growth promoters (AGP) have been banned in many countries, there is an urgent need to find a suitable alternative for them. Meanwhile, in recent years, special attention has been paid to herbal compounds. The present study was carried out to evaluate the effects of oregano (*Origanum vulgare* L.) as an alternative for AGPs on growth performance, carcass characteristics, and immune responses in broiler chickens.

Experimental: A total of 192 one-d-old female broiler chicks (Ross 308) were weighted and randomly allotted to 16 cages (12 chicks/cage). The dietary treatments were as follows: basal diet as a control, control + 4.5 mg flavophospholipol/kg, or control + 5 or 10 g oregano leaves powder/kg of diet.

Results: Final body weight of broilers were not significantly affected by the dietary treatments, although it tended to increase in broilers fed diets containing antibiotic or 10 g oregano/kg of diet ($P>0.05$). Daily feed intake and feed conversion ratio (FCR) of broilers were not significantly affected by the dietary treatments; although during the whole experiment (0-42 d) FCR tended to improve in broilers fed diets containing antibiotic or 10 g oregano/kg of diet ($P>0.05$). The antibody titers against Newcastle disease virus (NDV) were significantly higher in broilers fed diets containing 5 g oregano/kg of diet ($P<0.05$).

Recommended applications/industries: In conclusion, the results indicate that supplementation of broiler diet with 5 g oregano/kg could enhance antibody titers against NDV without adverse effects on performance criteria.

1. Introduction

In-feed antibiotics (IFA) have been consumed at dosage below therapeutic levels in poultry farming to improve performance and preserve health (Afiouni *et al*., 2023; Moradpour *et al*., 2024; Landy and Kheiri, 2023). IFAs were expected to promote performance of the birds as a consequence of promoted gut microbiome and limited digestive symptoms, resulting in greater nutrient consumption and improved feed: gain ratio (Foroutankhah *et al*., 2019). Without regard to positive effects of IFAs, there is fear of increasing proliferation of resistant bacteria in the gut, which can be unfavorable to humans (Gheisari *et al*., 2017). As a consequence, attempts have been made in different parts of the world to prohibit supplementation IFAs in poultry production. As a result of the ban on the consumption of IFAs, and the increasing pressure on farmers to produce antibiotic-free meat; probiotics (Landy and Kavyani, 2013), prebiotics (Ceylan and Ciftc, 2003), medicinal plants (Landy and Kheiri, 2019; Kavyani *et al*., 2014) and biologically active peptides (Landy *et al*., 2020) have been investigated. Medicinal herb and their products have received enhanced

consideration in the last few years for the reason that they have been approved by users as organic ingredient (Shokraneh *et al*., 2010).

Oregano (*Origanum vulgare* L.) is aromatic perennial herb native to temperate Western and Southwestern Eurasia and the Mediterranean region. Oregano possess rosmarinic acid, linalool, thymol, carvacrol, tannins, flavonoids, triterpenes, phenol carvacrol, and thymol (Ansari *et al*., 2021). Oregano leaves contain compounds with proven cytotoxic, antioxidant, and antibacterial properties (Coccimiglio *et al*., 2016). Oregano extract has been used in animal production as appetite, and prevention of pathological damages due to its antioxidant activity (Fr An Kis *et al*., 2009). Aglipay *et al*. (2023) reported that administration of oregano extract in drinking water could induce favorable influences on performance criteria. In contrast Lee *et al*. (2003) and Jang *et al*. (2007) investigated the effects of oregano essential oils on broilers performance; the researchers reported that it had not any marked effects on broilers performance. On the basis of these investigations, the goal of this experiment was to give a contribute on the efficacy of dietary administration of different levels of oregano on growth performance, carcass traits and immune responses of broiler chickens.

2. Materials and Methods

2.1. Animals and dietary treatments

A total of 192 one-d-old female broiler chicks (Ross 308) were individually weighted and accidently allotted to 16 cages (12 chicks/cage). The dietary treatments were as follows: basal diet as a control, control + 4.5 mg flavophospholipol/kg, or control + 5 or 10 g oregano leaves powder/kg of diet. The starter (0 to 14 day), grower (14 to 28 day) and finisher (28 to 42 day) diets were formulated to meet nutrient requirements of Ross 308 strain (Aviagen, 2019) and the formula and calculated composition have been shown in Table 1. The antibiotic or oregano were supplemented to the formulated diets at the expense of maize. The birds were reared on cages (120 × 120 × 80 cm) for 42 d and feed and water were provided *ad libitum* during the experiment. The light schedule includes full of light for the first day and a period of 23 h light and 1 h absence of light thereafter. The temperature of broiler house was preserved at 32ºC for the beginning week and circumspectly diminished by 3ºC in the second and third weeks, and eventually set at 22ºC afterwards.

Table 1. The ingredient and calculated composition of basal starter, grower, and finisher diets.

Item	Starter	Grower	Finisher
Ingredient, g/kg			
Corn	549.5	555.3	557.4
Soybean meal	387.5	366.4	343.7
Soybean oil	13.6	33.9	57.0
Dicalcium phosphate	20.8	18.0	16.5
$CaCO_3$	10.5	9.9	9.3
NaCl	1.4	1.7	1.7
NaHCO3	2.9	2.6	2.5
Trace mineral premix[1]	2.5	2.5	2.5
Vitamin premix[2]	2.5	2.5	2.5
DL-Methionine	3.7	3.3	3.3
L-Lysine-HCl	2.8	2.1	2.0
L-Threonine	1.0	0.7	0.6
Choline chloride	1.2	1.1	1.0
Calculated composition			
Metabolizable energy (kcal/kg)	2.870	3.027	3.200
Crude protein (g/kg)	220	210	200
Calcium (g/kg)	9.6	8.7	8.1
Available phosphorus (g/kg)	4.8	4.3	4.0
Methionine + cysteine (g/kg)	10.3	9.6	9.4
Lysine (g/kg)	13.7	12.6	11.9
Threonine (g/kg)	9.3	8.6	8.1

[1] Provided the following per kg of diet: Mg, 120 mg; Fe, 20 mg; Cu, 16 mg; Zn, 110 mg; Se, 0.3 mg; I, 1.25 mg.
[2] Provided the following per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 5000 IU; vitamin E, 65 IU; vitamin K, 3.2 mg; riboflavin, 8.60 mg; vitamin B_{12}, 0.017 mg; pantothenic acid, 20 mg; nicotinic acid, 65 mg; folic acid, 2.2 mg.

2.2. Performance and carcass traits

Body weights (BW) of broilers were measured at termination of starter, grower and finisher phases. Daily feed intake (DFI) and daily weight gain (DWG) were registered in starter, grower and finisher phases as well as the whole experiment. Feed conversion ratio was calculated by dividing DFI to DWG.

At 42 day of age, two broilers/cage were accidently selected, on the basis of middle weight of the cages and sacrificed. Carcass yield was computed by dividing eviscerated weight to live BW. Empty proventriculus, liver, abdominal fat, gizzard, heart and small intestine were too separated, weighted, and computed as a proportion of live BW. The length of small intestine and ceca were also determined (Landy *et al*., 2011).

2.3. Immunity

At 9 d of age, the broilers were vaccinated against Newcastle Disease virus (NDV) and Avian Influenza virus (AIV) via an oil-adjuvant injectable emulsion. Furthermore, at 21 d of age the broilers were orally vaccinated against NDV (Lasota). Antibodies against NDV, AIV, and sheep red blood cells (SRBC), were determined as humoral immune responses. Albumin to globulin (A/G) ratios were also assessed. At 25 d of age, the broilers (2 birds/cage) were inoculated with 1 mL of 1% SRBC, and at 31 d of age the blood samples were taken, serum were separated and take the measurement of antibodies against SRBC as described by Wegmann and Smithies (1966). Antibodies were expressed as the $\log_2$. At 28 d of age, the blood samples were taken, serum were separated and serum samples take the measurement of antibodies against NDV and AIV as described by Abbas *et al*. (2011).

At 42 d of age the blood samples (2 broilers per pen) were taken to determine albumin to globulin ratio. After obtaining plasma from blood, the concentrations of albumin and protein were measured using spectrophotometer and the kit (Pars Azmoon Company; Tehran, Iran). The concentration of globulin in serum was calculated by subtracting albumin concentration from proteins.

2.4. Statistical analysis

The obtained data were submitted to undergo analysis of variance technique suitable for a completely randomized design using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Comparison of difference between means were done using Duncan's multiple range test. Statements of statistical significance are based on $P<0.05$.

3. Results and discussion

3.1. Performance and carcass traits

As indicated in Table 2 addition of antibiotic or different levels of oregano leaves had not any significant effects on BW of broilers at 14 d of age although it tended to increase in broilers fed diets containing antibiotic ($P>0.05$). The dietary treatments had not any significant effects on BW of broilers at 28 d of age whereas it tended to enhance in broiler chickens fed diets containing oregano or antibiotic ($P>0.05$). At 42 d of age broilers fed diets containing antibiotic or 10 g oregano/kg of diet had higher BW compare to those fed basal diet or basal diet supplemented with 5 g oregano/kg of diet; although the results were not statistically significant ($P>0.05$). The dietary treatments had not any significant effects on DFI ($P>0.05$). The dietary treatments had not any significant effect on FCR although it tended to improve in broiler chickens fed diets containing antibiotic or 10 g oregano/kg of diet ($P>0.05$).

Table 2. Effect of experimental diets on performance indices of broilers at different ages.

Variables	Dietary treatments				
	Control	**Flavophospholipol**	**5 g Oregano/kg**	**10 g Oregano/kg**	**SEM[1]**
Body weight (g)					
14 d	285	307	294	292	6.5
28 d	881	941	937	957	32.4
42 d	1808	1868	1774	1859	42.2
Daily feed intake (g/d)					
0-14 d	29	29	30	29	0.7
14-28 d	94	70	70	70	10
28-42 d	126	128	125	126	2.3
0-42 d	75	75	75	75	1
Feed:gain (g:g)					
0-14 d	1.72	1.56	1.72	1.68	0.1
14-28 d	1.63	1.55	1.52	1.48	0.4
28-42 d	1.92	1.94	2.11	1.97	0.1
0-42 d	1.79	1.74	1.83	1.75	0.03

[1] Standard error of mean.

IFAs may possibly control and restrict the spread and development of two main types of bacteria in chicks' gut (Ferket, 2004). A further balanced flora in the small intestinal of the birds may perhaps cause a higher efficaciousness in digestibility and absorption of feed, leading to an increased growth and promoted feed efficiency (Bedford, 2000). Aglipay *et al.* (2023) reported that administration of oregano extract in drinking water could induce favorable influences on final BW of broilers. Roofchaee *et al.* (2011) reported that supplementation of oregano essential oils in broiler diet could induce favorable influences on growth performance and also displayed potent antibacterial effects against cecal *E. coli*. Since the major components of oregano oil, have been indicated antioxidant, antibacterial and anticoccidial activities (Ivanov and Bozakova, 2022) in the current study an improvement in growth performance of broilers was anticipated. As the performance of broiler chickens was not significantly affected by the addition of antibiotics or different levels of oregano, it may be due to the suitable conditions of the experiment and application of easy-to-digest diet, so it is better to test the performance of broilers in response to the addition of oregano in other conditions, such as receiving indigestible or hard-to-digest diets.

As indicated in Table 3 administration of antibiotic or different levels of oregano had not any significant effects on carcass yield, relative weight of internal organs, and small intestine and ceca lengths (P>0.05). Peng *et al.* (2016) reported that addition of avilamycin and oregano to broilers diet could not induce any marked effects on carcass traits. Kirkpinar *et al.* (2014) reported that dietary oregano supplementation did not affect carcass yields, and the relative weight of internal organs. These are consistent with the results obtained by Eler *et al.* (2019) who reported that supplementation of oregano essential oils in broilers diet did not induce any marked effects on carcass characteristics.

Table 3. Effect of experimental diets on carcass yield and internal relative organ weight of broilers at 42 d.

Relative organ weight	Dietary treatments				
	Control	Flavophospholipol	5 g Oregano/kg	10 g Oregano/kg	SEM[1]
Carcass (%)	77.3	78.2	79.1	79.2	0.6
Heart (%)	0.47	0.48	0.45	0.51	0.2
Liver (%)	2.37	2.45	2.32	2.58	0.2
Abdominal fat (%)	0.97	0.96	1.01	1.13	0.6
Gizzard (%)	1.94	1.73	1.95	1.78	0.03
Proventriculus (%)	0.43	0.43	0.52	0.5	0.07
Small intestine (%)	6.44	5.87	6.52	6.52	2.1
Small intestine (cm)	247.0	231.0	225.0	226.0	11.1
Cecum (cm)	41	42	43	41	1.7

[1] Standard error of mean.

3.2. Immune responses

The effect of dietary treatments on antibody titers against NDV, AIV and SRBC and A/G ratio are summarized in Table 4.

Table 4. Effect of experimental diets on immune responses of broiler chickens.

Variables	Dietary treatments				
	Control	Flavophospholipol	5 g Oregano/kg	10 g Oregano/kg	SEM[1]
New castle (log_2)	2.74^{ab}	2.25^{b}	3.5^{a}	2.75^{ab}	0.2
Influenza (log_2)	2.37	2.00	2.00	2.50	2.2
SRBC (log_2)	7.75	7.62	8.37	7.75	0.5
Albumin to globulin ratio	0.89	0.92	0.83	0.94	0.1

a,b Values in the same row not sharing a common superscript differ significantly (P<0.05).
[1] Standard error of mean.

The dietary treatments had not any significant effects on antibody titers against AIV and A/G ratio. The antibody titers against NDV increased in broiler chickens fed diets containing 5 g oregano/kg of diet (P<0.05). The dietary treatments had not any significant effects of antibody titers against SRBC, although it tended to increase in broiler chickens fed diets containing 5 g oregano/kg of diet (P>0.05). Franciosini *et al.* (2016) reported that supplementation of aqueous extracts of oregano in broilers diet could

improve growth performance, immune function and intestinal microbial population. Hashemipour *et al.* (2013) reported that supplementation of broilers diet with thymol and carvacrol enhanced total IgG and IgG anti-SRBC and it also decreased heterophil to lymphocyte ratio. Abd El-Ghaffar Galal *et al*. (2016) reported that administration of oregano essential oils in broilers could improve humoral immune responses to NDV and AIV. Du *et al*. (2016) stated that supplementation of broilers diet with thymol and carvacrol could induce favorable influences on intestinal integrity and modulate immune responses in the Clostridium perfringens-challenged broiler chickens. In the present study supplementation of broilers diet with antibiotic had not any significant effects on humoral immune responses. Belay *et al*. (1992) stated that supplementation of virginiamycin in broilers diet had not any marked effects on the total IgG, IgM antibody titers. Therefore, the impact of enteric conditioners and their effects on intestinal flora could be mostly restricted to the mucosal immune complement and not the systemic portion of the immune system

4. Conclusion

In conclusion, the results indicate that supplementation of broiler diet with 5 g oregano/kg could enhance antibody titers against NDV without adverse effects on performance criteria.

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6. References

Abd El-Ghaffar Galal, A.A., El-Sayed El-Araby, I., Hassanin, O., El-Said Omar, A. 2016. Positive impact of oregano essential oil on growth performance, humoral immune responses and chicken interferon alpha signalling pathway in broilers. *Advances in Animal and Veterinary Sciences*, 4: 57-65.

Abbas, M., Spackman, E., Fouchier, R., Smith, D., Ahmed, Z., Siddique, N., Sarmento, L., Naeem, K., McKinley, E., Hameed, A., Rehmani, S. and Swayne, D. 2011. H7 avian influenza virus vaccines protect chickens against challenge with antigenically diverse isolates. *Vaccine*, 29: 7424-7429.

Afiouni, A., Toghyani, M. and Landy N. 2023. Performance, morphological responses of the small intestine, and humoral immunity of broilers fed oak acorn (*Quercus brantii*) as a substitution for antibiotic growth promoters. *Iranian Journal of Applied Animal Science*, 13: 321-331.

Ansari, I., Varma, S. and Gupta N. 2021. A Review on phytochemical and pharmacological study on origanum vulgare. *International Journal of Pharmaceutical research and Applications*, 6: 408-416.

Aglipay, M., Rodriguez, C., Libunao, L., Hufalar, P. and Sagun, E. 2023. Performance of broilers given oregano (*Origanum vulgare*) extract as drinking supplement. *International Journal of Biosciences*, 3: 126-131.

Aviagen. 2019. Ross Broiler Management Manual. Aviagen Ltd., Midlothian, UK.

Bedford, M. 2000. Removal of antibiotic growth promoters from poultry diets. *World's Poultry Science Journal*, 56: 347-365.

Belay, T., Deyhim, F. and Teeter, R.G. 1992. Effect of virginiamycin supplementation on growth and humoral mediated immunity of broilers. Poultry Science, 71 (Suppl.): 137. (Abstr.).

Ceylan, N. and Ciftc, I. 2003. The Effects of some alternative feed additives for antibiotic growth promoters on the performance and gut microflora of broiler chicks. *Turkish Journal of Veterinary and Animal Science*. 27:727–733.

Coccimiglio, J., Alipour, M., Jiang, Z., Gottardo, C. and Suntres, Z. 2016. Antioxidant, antibacterial, and cytotoxic activities of the ethanolic origanum vulgare extract and its major constituents. *Oxidative Medicine and Cellular Longevity*, 2016: 1-8.

Eler, G., Gomes, A.V.C., Trindade, B.S., Almeida, L.S.L., Dilelis, F., Cardoso, V.S. and Lima, C.A.R. 2019. Oregano essential oil in the diet of broilers: performance, carcass characteristics, and blood parameters. *South African Journal of Animal Science*, 49: 753-762.

Ferket, P.R. 2004. Alternatives to antibiotics in poultry production, in: Lyons, T.P., Jacques, K.A. (Eds), Nutritional Biotechnology in the Feed and Food Industries. E-publishing Inc., Nottingham, UK, pp. 57-67.

Foroutankhah, M., Toghyani, M. and Landy, N. 2019. Evaluation of *Calendula officinalis* L. (marigold) flower as a natural growth promoter in comparison with an antibiotic growth promoter on growth performance, carcass traits and humoral immune responses of broilers. *Animal Nutrition*, 5: 314-318.

Fr An KIČ, T., Voljč, M., Salobir, J. and Rezar, V. 2009. Use of herbs and spices and their extracts in animal nutrition. *Acta Agriculturae Slovenica*, 94: 95-102.

Franciosini, M. P., Casagrande, P., Proietti, C., Forte, D., Beghelli, G., Acuti, D., Zanichelli, M., and Marinucc, T. 2016. Effects of oregano (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis* L.) aqueous extracts on broiler performance, immune function and intestinal microbial population. *Journal of Appllied Animal Research*, 44: 474-479.

Hashemipour, H., Kermanshahi, H., Golian, A. and Veldkamp, T. 2013. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry Science*, 92: 2059–2069.

Ivanov, V. and Bozakova, N. 2022. Possibilities of using oregano (origanum vulgare l.) as a dietary supplement in broiler chicken and hens production. Journal of Hygienic Engineering and Design. 226-271.

Jang, I.S., Ko, Y.H., Kang, S.Y. and Lee, C.Y. 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Animal Feed Science and Technology*, 134: 304-315.

Kavyani, A., Shahne, A., Pourreza, J., Jalali, M.A., Nikkhah, M., Landy, N. 2014. Efficiency of different levels of mushroom (*Agaricus bisporus*) on intestinal morphology and microflora of broiler chickens. *Journal of Farm Animal Nutrition and Physiology*, 91: 23-30.

Landy, N., Ghalamkari, G.H., Toghyani, M. 2011. Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (Azadirachta indica) as alternative for an antibiotic growth promoter. *Livestock Science*, 142: 305-309

Landy, N. and Kheiri, F. 2023. Effects of hydrolyzed cottonseed protein on growth performances, carcass traits, immunity, microbial and morphological responses of the small intestine and total antioxidant capacity of serum, and small intestine in broiler chickens. *Iranian Journal of Applied Animal Science*, 13: 121-132.

Landy, N. and Kavyani A. 2013. Effects of using a multi-strain probiotic on performance, immune responses and cecal microflora composition in broiler chickens reared under cyclic heat stress condition. *Iranian Journal of Applied Animal Science*, 3: 703-708.

Landy, N. Kheiri F. 2019. Use of Echinacea purpurea (L.) Moench as an additive in poultry nutrition. *Journal of Medicinal Herbs*, 9: 79-85.

Landy, N., Kheiri, F. and Faghani M. 2020. Evaluation of cottonseed bioactive peptides on growth performance, carcase traits, immunity, total antioxidant activity of serum and intestinal morphology in broiler chickens. *Italian journal of animal science*, 19: 1375-1386.

Lee, K.W., Everts, H., Kapperst, H.J., Yeom, K.H. and Beynen, A.C. 2003. Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. *Journal of Applied Poultry Research*, 12: 394-399.

Moradpour, H., Kheiri, F., Faghani, M. and Landy N. 2024. Influence of dietary hydrolyzed cottonseed protein supplementation on physiology and metabolism of Japanese quails. *Journal of the Hellenic Veterinary Medical Society*, 75: 6925-6936.

Peng, Q.Y., Li, J.D., Li, Z., Duan, Z.Y. and Wu, Y.P. 2016. Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. *Animal Feed Science and Technology*, 214: 148-153.

Roofchaee, A., Irani, M., Ebrahimzadeh, M.A. and Akbari, M.R. 2011. Effect of dietary oregano (*Origanum vulgare* L.) essential oil on growth performance, cecal microflora and serum antioxidant activity of broiler chickens. *African Journal of Biotechnology*, 10: 6177-6183.

Shokraneh, M., Ghalamkari, G., Toghyani, M. and Landy N. 2016. Influence of drinking water containing Aloe vera (Aloe barbadensis Miller) gel on growth performance, intestinal microflora, and humoral immune responses of broilers. *Veterinary World*, 9: 1197.

Wegmann, T.G. and Smithies, O. 1966. A simple hemagglutination system requiring small amounts of red blood cells and antibodies. *Transfusion*, 6: 67-73.