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Research Article

Effect of Sweet Wormwood (*Artemisia annua* L.) Leaves Meal Supplementation on Oxidative Status and Immune-Response of Broilers

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Abstract:

This study aimed to assess the impacts of sweet wormwood (*Artemisia annua* L.) leaves meal supplementation on oxidative status and immune-response of broilers. A total of 240 one-day-old unsexed (Ross 308 classic FF) broiler chicks were randomly distributed into 4 equal groups (60 chicks into 3 replicates/group). The 1st group (control) was fed a basal diet without any *A. annua* leaves meal, while *A. annua* leaves meal was added to the basal diet at levels of 0.3, 0.6, and 0.9 %, respectively at the 2nd, 3ed and 4th groups. At the age of 5 wks, broiler fed a diet supplemented with *A. annua* leaves meal up to 0.9 % showed a highly significant ($P \leqslant 0.01$) improvement in the antioxidant status; this evident through a significant increase in the levels of both TAC and SOD and decreasing the amount of MDA in liver tissue. At the same time, there was a highly significant ($P \leqslant 0.01$) improvement in the immune response of broilers fed dietary *A. annua* leaves meal up to 0.9 %, this improvement represented by an increase in the levels of serum IgA, IgG and IgM. It could be recommended that, supplementation of *Artemisia annua* leaves meal in broiler diet at levels up to 0.9% positively affected oxidative status and immune-response during the growing period.

1. Introduction

For the sustainability of the broiler sector and its primary role in providing high-quality animal protein, different approaches have been implemented to increase the return of poultry investment (Akbari et al., 2016, 2018 and El-Senousey et al., 2018). Whereas, the poultry industry, especially the broiler chicken sector, plays a vital role in the Arab Republic of Egypt because it provides a tremendous amount of animal protein quickly with high conversion efficiency (Eldamrawy et al., 2023). The creation of poultry meat and global consumption of poultry products has expanded regularly over the years. This is due to the advantages of chicken meat and products which have numerous attractive dietary qualities, for example, relatively high concentrations of polyunsaturated fatty acids and low lipid substances. In addition, poultry production is relatively not expensive compared to other meats (Saleh et al., 2020).

For many years, a variety of synthetic feed additives, such as drugs and antibiotics, were used as growth promoters in livestock and poultry nutrition to improve the efficiency of production and product quality, modify the gut microflora, and control diseases in poultry (Reda et al., 2021; Alagawany, 2022; Hussein et al., 2023 and Zaki et al., 2023). Considering the spread of different kinds of additives in nutrition and a significant increase in worldwide poultry production, the huge amount of medicine and chemicals that threaten the environment and consumer's health can be easily estimated. Because of the valuable role of these compounds in poultry production efficiency, their usage is almost inevitable (El-Banna et al., 2022). Antimicrobial agents are usually associated with adverse effects on the host, like the development of antibiotic-resistant bacteria (Capita and Alonso-Calleja, 2013). Due to these concerns, in the modern era and over the years, much research around the world has been focused on the development of alternative strategies to maintain poultry health and enhance performance within intensive systems, and numerous substances, commonly known as natural growth promoters (NGPs), have been identified as effective eco-friendly alternatives to antibiotics and synthetic feed additives in poultry production to improve productive efficiency, modify the gut microflora, control diseases, and enhance the immune response to antibiotics (Farag and El-Rayes, 2016; El-Rayes et al., 2023[a, b]).

A variety of nutritional strategies and various studies have been tried by using different herbal plants and their products to overcome these challenges and improve the meat quality of broilers, these herbal substances used sweet wormwood "*Artemisia annua* L." which receives considerable attention due to its nutritional and physiological functions. Sweet wormwood (*Artemisia annua* L.) is one of the natural herbs that belongs to Artemisia species of Asteraceae and is distributed in many countries worldwide. According to Wang et al. (2011); Tayebe et al. (2012) and Wu et al. (2017), *A. annua* is a well-known medicinal herb. The WHO has recognized *A. annua* as a plant medicine and developed it as the benchmark for Western medicine research. It is a well-known plant with a reputation for being extremely effective and having minimal toxicity for treating a variety of ailments.

Since the discovery of the antimalarial medication artemisinin, *A. annua* has been the focus of extensive

phytochemical analysis (Wang et al. 2011). The chemical composition of A. annua consists of volatile and non-volatile constituents. The volatile components are mainly attributable to essential oils with the content of the latter being 0.2–0.25%. The main compounds, which account for about 70% of the essential oils, appear to be camphene, β-camphene, isoartemisia ketone, 1-camphor, β-caryophyllene and β-pinene. In addition, other minor ingredients, such as artemisia ketone, 1,8-cineole, camphene hydrate, and cuminal are also found in the volatile parts of A. annua. The main non-volatile ingredients include sesquiterpenoids, flavonoids and coumarins, together with proteins (such as β-galactosidase, β-glucosidase), steroids (e.g. β-sitosterol and stigmasterol). The main chemical constituents of A. annua are sesquiterpenoids, including artemisinin, artemisinin I, artemisinin II, artemisinin III, artemisinin IV, artemisinin V, artemisic acid, artemisilactone, artemisinol and epoxyarteannuinic acid (WHO, 2006; Xiao et al., 2002; Cafferata et al., 2010 and Das, 2012). *A. annua* has abundant nutrient profiles such as amino acids, vitamins, and mineral elements, as well as antioxidant compounds including phenolics and flavonoids (Ferreira et al., 2010 and Wan et al., 2016). Besides, it has immune regulation function (Gholamrezaie et al., 2013 and Song et al., 2018).

Previous studies have demonstrated that the inclusion of *A. annua* products to broilers' feed could enhance growth performance (Brisibe et al., 2008; De Almeida et al., 2012), improve antioxidant capacity (Wan et al., 2016) and immune function (Gholamrezaie et al., 2013). In view of the discrepancy between the results of the studies conducted to evaluate the effect of *A. annua* herb on the productive performance, physiological and immune status of broiler chickens, this study was conducted to shed more light on the effect of *A. annua* on oxidative status and immune-response of broilers.

2. Materials and Methods

2.1. Experimental Design

2.1.1. Birds and management

Two hundred and forty one-day-old unsexed (Ross 308 classic FF) broiler chicks were randomly divided into 4 experimental groups with three duplicates of twenty birds. The first group served as control and fed a basal diet without any supplementation, while *A. annua* leaves meal was added to the basal diet at levels of 0.3, 0.6, and 0.9 % in the second, third and fourth, groups, respectively. Throughout the five-week of the study, all experimental groups were raised in floor pens and reared under similar managerial and hygienic conditions according to the recommendations of the breed guide used in the study.

2.1.2. Experimental diet

The basal diet was a commercial corn-soybean meal diet formulated to meet or exceed the nutritional requirement of broilers as recommended by a manual of (Ross 308 classic FF) strain, as shown in Table (1).

Table (1): The composition and calculated analysis of basal diet.

Ingredients	Experimental diets	
	Starter	Grower
Yellow corn	50.48	58.64
Soybean meal (44%)	32.55	30.80
Corn gluten meal (62%)	7.10	2.52
Soybean oil	6.00	4.88
Limestone	1.45	1.30
Dicalcium phosphate	1.69	1.16
Salt	0.30	0.30
Premix*	0.30	0.30
Dl-Methionine	0.10	0.10
L. Lysine	0.03	-
Total	100.00	100.00
Calculated analysis**		
Crude protein (%)	23.01	20.05
ME (Kcal/Kg)	3100	3200
Ether extract (%)	2.40	2.50
Crude fiber (%)	3.50	3.50
Calcium (%)	1.03	0.82
Available phosphorus (%)	0.45	0.37
Methionine (%)	0.50	0.45
Lysine (%)	1.11	1.00

* Each 3kg of premix contained: Vit. A 12000IU, Vit. D 2200IU, Vit. E 10mg, Vit. K_3 2000mg, Vit. B_1 1000mg, Vit. B_2 3000mg, Vit. B_6 1300mg, Vit. B_{12} 10mg, Pantothenic acid 10mg, Niacin 30mg, Folic acid 1000mg, Biotin 50mg, Choline chloride 300mg, Manganese 60mg, Zinc 50mg, Copper 10mg, Iron 30mg, Iodine 1000mg, Selenium 100mg, Cobalt 100mg and $CaCo_3$ to 3g.

** Calculated according to (NRC, 1994).

2.2. Measurements

2.2.1. Oxidative status:

At the end of the trial (5 wks of age), nine chicks from each treatment (3/replicate) were randomly chosen according to the overall mean of the treatment to collect the liver samples after slaughter, for determines antioxidant activity. Liver samples (250) mg were homogenized with 1 ml PBS (phosphate buffer saline) by tissue homogenizer until obtaining homogenous mixture. This solution was centrifuged at 10000 rpm for 15 minutes at 4C° in a cooling centrifuge. The supernatant was carefully aspired and stored in Epindorff at -20 c° until analysis. Assay of the activities superoxide dismutase (SOD), total antioxidant capacity (TAC) and malonaldehyde (MDA) were measured using diagnostic kits (Bio-diagnostics, Giza, Egypt) following the manufacturer's instructions based on the methodology of Claiborne (1985), Koracevic et al. (2001), and Ohkawa et al. (1979).

2.2.2. Immune-response:

At the end of the experimental period, 3 chicks from each replicate were randomly chosen and blood samples were collected from the wing vein without anticoagulant. Blood serum was separated by centrifuga-

tion at 3000 rpm for 20 min. The collected serum was kept frozen at - 20ºC until immunoglobulins were assayed. Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentrations were determined appropriately diluted samples by a sandwich ELISA using microtiter plats and chicken-specific IgA, IgG, and IgM ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX). The ELISA procedure was carried out according to the protocol of the manufacturer and absorbance was measured at 450 nm. The concentration of IgA, IgG, and IgM were determined using standard curves constructed from respective immunoglobulin standards run on the assay microtiter plate and were expressed as milligrams of IgA, IgG, or IgM per milliliter of serum (Piquer et al., 1991).

2.3. Statistical analysis

Data were statistically analyzed by one-way ANOVA, using the general linear model procedure (SAS, 1996). Shapiro-Wilk and Levene tests confirmed variance normality and homogeneity. Tests of significance for differences among treatments were done according to Duncan (1955). The statistical model was used for the analysis of variance to estimate the effect of *A. annua* leaves meal supplementation levels on broilers oxidative status and immune-response as follows:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = The observations

μ = Overall mean

T_i = Effect treatments (i = 1, 2, 3and 4)

e_{ij} = Residual effects (Random error).

3. Results

3.1. Oxidative status

Data illustrated in Table (2) shows the effect of *A. annua* leaves meal supplementation levels on the oxidative status of broilers. Results indicated a highly significant ($P \leqslant 0.01$) improvement in the antioxidant status; this is evident through a significant increase in the levels of both TAC and SOD and decreasing the amount of MDA in liver tissue. The amount of TAC was significantly ($P \leqslant 0.01$) increased with increasing the supplementation level of *A. annua* leaves meal from 0 up to 0.9 %. chicks fed a diet supplemented with *A. annua* leaves meal at the level of 0.6 or 0.9 % possessed the highest amount of TAC followed by those fed dietary 0.3 % by 17.08 and 10.13% respectively, as compared to the control. The same direction was observed for the activity of SOD enzyme, broilers fed a diet supplemented with A. annua leaves meal at the level of 0.9 % possessed the highest activity of SOD by 5.82 %, as compared to the control. No significant differences were observed between groups treated with 0.3 or 0.6% and the control. On the other hand, the concentration of MDA was significantly ($P \leqslant 0.01$) decreased by increasing A. *annua* leaves meal supplementation level from 0 up to 0.9 %. Broiler chicks fed diet supplemented with A. *annua* leaves meal at the level of 0.9 % possessed the lowest content of MDA followed by those received 0.6 % and then those received 0.3 % by 51.85, 46.91and 39.51 % respectively, as compared to the control.

3.2. Immune-response

Data on broiler's immune response as influenced by *A. annua* leaves meal supplementation levels (0, 0.3, 0.6, and 0.9%) are illustrated in Table (3). Generally, all of immunoglobulin fractions (IgA, IgG, and IgM) were significantly ($P \leq 0.01$) increased by increasing *A. annua* leaves meal supplementation levels from 0.3 up to 0.9 %. Broiler chicks fed diet supplemented with *A. annua* leaves meal at the level of 0.9 % had significantly ($P \leq 0.01$) the highest percent concentration of serum IgA, IgG, and IgM by 40.22, 60.78, and 226.05% respectively, as compared to the control group.

4. Discussion

The poultry industry has been recognized as a fast-developing sector aiming to produce low-cost and high-nutrient foods for human consumption. This industry is always at risk of infectious and non-infectious agents that cause adverse losses. Many potential feed additives have been investigated as health enhancers, immune stimulants, and antimicrobials (Ferdous et al., 2019 and Rafiq et al., 2022). Among different feed additives, phytogenic feed additives have been widely used to boost immunity and relieve stress (Mehdi et al., 2018). Sweet wormwood (*Artemisia annua* L.) is considered one of the phytogenic feed additives with great a reputation for being extremely effective and having minimal toxicity for treating a variety of diseases (Wu et al., 2017), improving antioxidant capacity (Wan et al., 2016) and immune function (Gholamrezaie et al., 2013).

From our results, the supplementation of a poultry diet with *Artemisia annua* can enhance the antioxidative status, including the levels of TAC, MDA, and the activity of SOD enzyme in broiler chickens. *Artemisia annua* beneficial effect on antioxidant indicators may be related to its phenolic and flavonoid compounds, which are able to scavenge free radicals. Whereas the total phenols and flavonoid content of *A. annua* leave powder were 32.5 ± 0.67 mg Equivalent Gallic Acid/100 mg and 11.3 ± 1.52 mg Equivalent Quercetin/100 mg (Adjogblé et al., 2019). Furthermore, the oxygen radical absorbance capacity (ORAC) value was 2123 µmole TE/g for *Artemisia annua* water extract, while 70% ethanol extracts of the same plant material resulted in 2535 µmoles TE/g (Ferreira et al., 2010). Also, Zheng and Wang, (2001) reported that total phenolic content and antioxidant ability of A. annua were 0.154 g GAE/100 g and 15.69 mol TE/g. Our results of the oxidative status are compatible with that observed by Cherian et al. (2013) who found that the MDA concentration was decreased in breast and thigh muscles of broilers fed diets supplemented with 20 or 40 g/kg *A. annua* leaves. Moreover, Studies have shown that the

extracts of *Artemisia* species increased SOD, CAT and GSH-Px activities, as well as decreased MDA production in liver of rats (Ryu et al., 1998; Kim et al., 2003; Ryu et al., 2013).

Table (2): Antioxidant status of broiler chicks as affected by *A. annua* leaves meal supplementation levels.

Items	*A. annua* leaves meal supplementation levels %				SEM	Significant
	0	0.3	0.6	0.9		
TAC (mm/L)	1.58^{b}	1.74^{ab}	1.85^{a}	1.85^{a}	±0.02	**
SOD (U⁄ml)	72.50^{b}	72.67^{b}	73.00^{b}	76.72^{a}	±0.08	**
MDA (nmol/mL)	0.81^{a}	0.49^{b}	0.43^{b}	0.39^{c}	±0.09	**

TAC=Total antioxidants capacity; SOD= Super oxide dismutase MDA= Malondialdehyde
-Means of each raw followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.
-** indicate significance at P<0.01

Table (3): Immune-response of broiler chicks as affected by *A. annua* leaves meal supplementation levels.

Items	*A. annua* leaves meal supplementation levels %				SEM	Significant
	0	0.3	0.6	0.9		
IgA (mg/mL)	20.04^{b}	26.32^{a}	26.48^{a}	28.10^{a}	±3.23	**
IgG (mg/mL)	25.19^{c}	27.63^{bc}	32.91^{b}	40.50^{a}	±5.04	**
IgM (mg/mL)	15.01^{c}	19.60^{c}	29.93^{b}	48.94^{a}	±2.26	**

-Means of each raw followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.
-** indicate significance at P<0.01

As for immune response, phytogenic feed additive, as an alternative to antibiotics, improved immune response in chickens (Attia et al., 2018; Hesabi et al., 2019). *A. annua* is an immune modulator because of its chemical composition which consists of volatile constituents such as, camphene, β-camphene, isoartemisia ketone, 1-camphor, β-caryophyllene, β-pinene. artemisia ketone, 1,8-cineole, camphene hydrate, and cuminal. Alsom ther were non-volatile ingredients include sesquiterpenoids, flavonoids and coumarins, together with proteins (such as β-galactosidase, β-glucosidase), steroids (e.g. β-sitosterol and stigmasterol). All of these active phytochemical compounds act as immune-stimulants (Xiao and Yang, 2002). The flavonoids present in *A. annua* leaves have been linked to a beneficial immunomodulatory activity in subjects afflicted with parasitic and chronic diseases (Ferreira et al., 2010). Our results are compatible with those observed by Guo et al. (2022) and Song et al. (2018) who found that dietary supplementation with enzymatically treated *A. annua* improved intestinal sIgA and IgG content.

5. Conclusions

In conclusion, it could be recommended that, supplementation of *A. annua* leaves meal in broiler diet at the level of 0.9% affected positively antioxidant activity and immune response during the growing period.

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