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Original Article

Improvement of Growth performance, Biochemical Blood Profiles, and Meat Peroxidation by the Inclusion of Mustard Seed Extract in Broilers' Drinking Water

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Abstract

Growth promoters are used in the broiler industry of Iraq, and herbal plants are believed to be one of the safest growth-promoting agents in modern poultry production. This study aimed to investigate the effect of aqueous mustard (*Brassica juncea*) seed (MS) extract on broiler production. A total of 160 unsexed day-old Ross 308 broiler chicks were selected and exposed to different doses of MS included in drinking water for six weeks. Afterward, MS was added to the treatment groups of T1 (control), T2, T3, and T4 in the dosage of 0mL, 3mL, 5 mL, and 7 mL per liter of drinking water, respectively. Results indicated that the birds that were given extract (T2, T3, T4) for the three weeks were fed significantly more diet, compared to the control treatment group (189.4 g vs. 159.6g) (P<0.05). Accordingly, the Body Weight (BW) of these birds recorded on days 28 (1232, 1252, 1300g) and 35 (1840, 1900, 1960g) were significantly higher, compared to the birds in the control treatment on days 28 (1147g) and 35 (1657g), respectively. On days 28 and 35, the birds in T2, T3, T4 treatment groups had significantly higher Body Weight Gain (BWG) (P<0.05), compared to the control group (G1). Improved Feed Conversion Ratio (FCR) was observed in the 5th week for the birds that were administrated MS extract (5 and 7mL /liter), compared to the control group (P<0.05). The total serum level of protein, phosphorus, and calcium was increased in birds in the treatment group T4 (7.5 g/dL). Moreover, lower cholesterol values and higher values of alkaline phosphatase were obtained in birds in the treatment groups T2, T 3, and T4 (P<0.05). On day 35, higher meat peroxidation was observed in the fresh samples (after 24 h of slaughtering) of birds receiving different levels of MS extract, compared to the birds in the control group. The obtained results revealed that 5-7 mL of MS extract can be considered a functional growth- promoter for commercial broilers, although further studies are needed to confirm MS as a growth promoter.

Keywords: Mustard seeds, growth performance, metabolism

1. Introduction

Poultry producers have succeeded in obtaining maximum weight gain at low production cost using strain selection and growth promoters. Some growth-promoting agents are applied to improve feed efficiency, growth, and meat quality and reduce production costs (1). Medicinal herbs are considered to be safe growth-promoting agents due to their antioxidants, antifungals, and antimicrobial properties. The current study aimed to investigate the safety and acceptability of growth promoters for consumers. Herbs are a potential alternative to antibiotic growth stimulators in the poultry production industry (2, 3) and improve nutrient intake and immune system stimulation (1).

Available herbs are effectively used in poultry diets (4), and mustard seeds (MS) are used as growth stimulants to prevent and control intestinal diseases due

to such components as glucosinolates, dietary fiber, magnesium, selenium, iron, calcium, protein, niacin, zinc, and omega-3 fatty acids (5). The glucosinolates are metabolized into isothiocyanates, which are antimicrobial agents in animal products (6). In addition, MS contains sinigrin as a carcinogenic inhibitor, anti-inflammation, antimicrobial, and antioxidant compound (6), as well as growth stimulants that are involved in improving cellular metabolism (6). Although natural growth stimulants are commonly added in feeds as alternative products, less is known about their possible impression on the health and productivity of the herd. In practical scales, these compounds should be provided in concentrated form, since a high dose of some herbs, spices, and essential oil may lower growth performance, physiological function, and immune response. Therefore, this study aimed to estimate the effect of the new herbal plant, such as mustard seeds, as an alternative to antibiotics in poultry production.

2. Materials and Methods

This study was conducted on 160 unsexed day-old commercial Ross 308 broilers with the mean±SD Body Weight (BW) of 45±3 g. The broiler chicks were weighed individually and randomly classified into four treatment groups. Each treatment was conducted with four replicates on 10 birds that were housed in a 1×1.2 m^2 cage. The temperature was 33˚C during the first week and was gradually reduced by 3 ˚C every week until it was 27 ˚C at the end of the 3rd week. Lighting condition was maintained 24 h/day and each cage was supplied with a feeder and waterer that were partially covered with wood shavings (5 cm deep). Sufficient food and water were available, and the chicks in the experimental groups were fed with the starter diet for three weeks (from 3rd to 6th weeks) with the final diet (Table 1). All feed rations were evaluated using the UFFDA feed formulation package (User-friendly Feed Formulation programmed by J. Hargrave, University of Georgia, Athens, GA, USA) to supply the nutrient requirements of the broiler chicken Ross 308, according to the broiler nutritionSpecifications (7). The broiler chickens were divided into four groups of T1: water without treatment (control group); T2: 3 mL/liter aqueous extract of MS; T3: 5mL/liter aqueous extract of MS; and T4: 7 mL/liter aqueous extract of MS in drinking water.

2.1. Measurements of Growth Production, Hematological and Biochemical Traits, and Meat Peroxidation

2.1.1. Measurements of Growth Production

The birds were weighed biweekly to estimate the mean BW and Body Weight Gain (BWG). The mean feed intake (FI) and the mean feed conversion ratio (FCR) were estimated on days 1-14, 15-28, and 29-35. On the morning of day 35, two female birds from each replication were randomly selected after 4 h of feed starvation.

2.1.2. Haematological and Biochemical Analysis

Two blood samples were collected from the jugular vein with Ethylenediaminetetraacetic acid (EDTA) and without the anticoagulant tube. The sample with anticoagulant EDTA was used to determine red blood cells (RBC) and hemoglobin (Hb) using a blood analyzer (Nihon Cohen Co.). The samples without EDTA were centrifuged at 4000 rpm for 15 min to collect serum, which was then stored at -20 ℃ for later total protein analysis (TP), total cholesterol, calcium (Ca), Hb, phosphorus (P), and alkaline phosphatase (ALP), using Reflotron Plus analyzer (Roche).

2.1.3. Meat Peroxidation Analysis

After analysis of blood parameters, the same chicks were individually weighed, euthanized by cervical dislocation (Halal method), eviscerated, and chilled in cold water (4°C) for 24 h. The bone was removed from the carcasses and breast muscle was cut longitudinally into two halves that underwent eight treatments per muscle. Each sample was weighed, and five grams of each slice were wrapped in foil, vacuum-sealed, and stored at -18 °C for meat oxidation evaluation. Meat peroxidation was determined using thiobarbituric acid assay (TBARs) on the 1st day (fresh sample) and after 30 days of freezing at -18 ºC. The oxidation tests were

estimated using TBARS value, according to the study conducted by Jung, Jung (8). Three grams of breast meat were dipped in 9 mL of deionized water and added to 50 µl of butylated hydroxyanisole (7.2%), and the mixture was homogenized for 30 s. A total of 1mL of the homogenated fluid was transferred to a test tube with 2 mL of TBA-TCA solution (fluorometric thiobarbituric acid [20 mm] in trichloracetic acid 15 %). The tubes were heated using a boiling water bath at 90 ℃ for 30 min, cooled, and centrifuged for 10 min (2.90xG). The absorbance of the supernatant was measured at 532 nm with a spectrophotometer, and the percentage of fat oxidation was calculated using the following formula.

Percentage of fat oxidation= (Absorption control - Sample uptake) ÷ Absorption control × 100.

2.2. Plant Extract

The used MS was obtained from a local market in Hilla city, middle south of Iraq (Figure 1), and the extract was prepared. Afterward, MS was ground into a powder using a blender, and 250 g of ground seeds were dipped in 1 L of water for 3 days at room temperature. The mixture was then filtered using a Whatman Filter Paper No.1, and the filtered fluid was dried in a water bath at 40 ℃ for 96 h to obtain the stock solutions diluted with distilled water 10% (w/v).

2.3. Statistical Analysis

The data were analyzed using SPSS software version 23 through one–way ANOVA test with a completely randomized design between experimental groups (by LSD tests; $P<0.05$). The following formula is used for the mathematical and statistical model:

$Yij = \mu + Ti + eij$

Where Yij presents the mean variables (BW, DWG, FI, FCR)

Moreover, µ is defined as the overall mean, Ti is considered to be treatment responses, and eij is a random error. A *p*-value less than 0.05 ($P \leq 0.05$) was considered statistically significant.

Table 1. Composition of feed and nutrients fed to the broilers (%, as fed-basis)[1]

Feed %	Starter-diet (days 1-21)	Finisher-diet (days 22-35)
white corn	58.50	63.10
Soybean meal	35.99	31.39
Oil (vegetable)	1.25	2.21
Di-calcium phosphate	1.75	1.25
$CaCo_3$	1.57	1.39
Lysine-HCL	0.02	0.03
Dl-Methionine	0.37	0.23
Salt	0.25	0.20
Mineral and vitamin premix¥	0.30	0.21
Nutrient's composition		
Metabolizable energy (kcal/kg)	2,960	3,085
Crude protein (%)	22	20
Calcium (%)	1	0.94
Available phosphorus (%)	0.71	0.63
Methionine + cysteine (%)	0.92	0.77
Lysine (%)	1.13	1.05

¥ Mineral-vitamin premix provided the following per kg of diet:
Vitamin A, 9,000 IU; vitamin D3, 2,100 IU; vitamin E, 30 mg; nicotinic acid, 30 mg, vitamin B12, 0.12 mg; calcium pantothenate, 10 mg; vitamin K3, 5 mg; thiamine, 1.1 mg; riboflavin, 4.5 mg, vitamin B6, 2.0 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 50 mg; Cu, 10 mg; Mn, 70 mg; Zn, 50 mg; I, 1.0 mg; Se, 0.3 mg; butylated hydroxytoluene,150 mg.
[1]Estimated from NRC (1994) composition table.

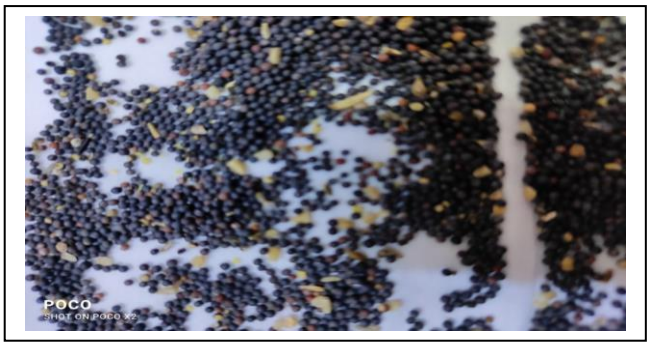

Figure 1. Mustard seed (*Brassica juncea*)

3. Results

3.1. Growth Production

A significant difference was observed between the groups (P<0.05) in terms of mean BW, BWG, FI, and FCR (Table 2). The effect of treatments on BW and BWG was observed during days 15-28 and 29-35 (P<0.05), and a more significant increase was observed in the broiler's weight in the T3 and T4 treatment groups (P<0.05), compared to the control treatment (T1). Although no significant differences were observed during days 1-14, a higher feed intake was observed in the groups that received MS (T2, T3, and T4), compared to the control group (T1) after these days (P<0.05). The FCR was significantly increased in chickens in T2 and T3 treatment groups on days 29-35, compared to the control group (P<0.05).

3.2. Blood Biochemical Parameters

Based on the obtained results, MS doses had no significant effect (P>0.05) on TP (Table 3); however, the groups that received MS had numerically higher TP values, compared to the control group (T1). Elevated levels of ALP, Ca, and P were observed in all MS-treated groups, compared with the controls (P<0.05) (Table 3). The increased level of MS was associated with a decrease in total cholesterol serum, while the level of treatments had no significant effect on the number of red blood cells and Hb (P≤0.05) (Table 4).

3.3. Meat Peroxidation

Based on the results, TBARS was increased in the fresh samples of MS-treated birds (0.224, 0.178, and 0.188, respectively), and TBARS value was obtained at 0.148 for samples in the control treatment group (Table 5). In the frozen samples, the high doses of MS (5 and 7 mL) led to the highest TBARS (P<0.05), compared to the low dose of MS (3mL) or the lack of MS (control group).

Table 2. Effect of inclusion of mustard seed in drinking water of broiler chickens on their growth performance

Items (g)	T1	T2	T3	T4	SEM
Body weight (g)					
Starter period (days 1-14)	334	340	333.7	358.7	6.4
Grower period (days 15-28)	1147^{C}	1232.5^{bc}	1252^{ab}	1300^{ab}	19
Finisher period (days 29-35)	1657.5^{c}	1840^{b}	1900^{ab}	1960^{a}	31
Bodyweight gain (g)					
Starter period (days 1-14)	275	279.7	274.7	298	6.3
Grower period (days 15-28)	813.5^{b}	892.5^{a}	918^{a}	941^{a}	16.8
Finisher period (days 29-35)	515^{c}	607^{bc}	650^{ab}	662^{ab}	24
Total feed intake (g)					
Starter period (days 1 -14)	394	400	394.7	410.7	4.4
Grower period (days15-28)	701^{b}	$800.^{a}$	815^{a}	843^{a}	17.8
Finisher period (days 29-35)	1100.5	1112	1137	1143	18
Feed conversion ratio (g feed/ g gain)					
Starter period (days1 -14)	1.4	1.4	1.44	1.3	0.036
Grower period (day15-28)	0.85	0.91.	0.91	0.97	0.016
Finisher period (days 29-35)	2.1^{cb}	1.79^{ba}	1.71^{a}	1.7^{a}	0.067

T1: control, T2: drinking water + 3% MS extract, T3: drinking water + 5 % MS extract, T4: drinking water + 7% MS extract; SEM: standard error of mean; the values in the same row with no superscripts are significantly different (P<0.05)

Table 3. Effect of mustard seed in drinking water on serum biochemical parameters of broilers

Blood parameter	T1	T2	T3	T4	Total Sem
Total Protein(g/dL)	5.4±0.34	4.2±0.5	6.3±1.0	7.5±0.47	0.42
Alkaline phostase(µ/L)	16.5^{c} ±1.5	20.3^{ab}±0.4	22.5^{a}±1.4	26.2^{a}±0.9	1.2
Calciumm mg/dL	8.8^{ab} ±0.54	7^{b}±0.77	8.7^{a}±0.62	9.2^{a}±0.6	0.39
Phosphor mg/dL	5.91^{b}±0.35	5.3^{b}±0.45	5.2b ±0.2	7.5^{a} ±0.5	0.31
Cholestrolmg/dL	181.2±26	189.1±17	170±8	150±15	9.1

T1: control, T2: drinking water +3% MS extract, T3: drinking water+5% MS extract, T4: drinking water+7% MS extract; SEM: standard error of mean; means in the same row with no superscript or with common superscripts are significantly different (P<0.05).

Table 4. Effect of mustard seed in drinking water on blood parameters of broilers on the day 35

Treatments	*Hb (g/dL)	*RBC(m/1mL)
T1	3.4^{b}	2.6
T2	3.9^{b}	2.5
T3	3.4^{b}	2.5
T4	3.5^{b}	2.7
Pooled SEM	0.29	0.13
P-value	0.008	0.08

$^{a-b}$ Values within a column in each parameter with the same superscripts are significantly different (P≤0.05); *RBC: red blood cell (million cells per mL); Hb: hemoglobin

Table 5. Effect of mustard seed added to the drinking water on lipid-peroxidation in breast muscle of broilers

Experimental treatments		Malondialdehyde (µg/g)[3]	
Effects		Fresh meat	After 1 month keeping in -20˚ C
MS	Control	0.148^{c}	0.356^{b}
	3mL	0.224^{a}	0.270^{a}
	5mL	0.178^{b}	0.368^{b}
	7mL	0.188^{b}	0.375^{b}
Pooled SEM		0.010	0.013
Pooled SEM		0.011	0.014

abcd: values in a column without any common superscripts have statistically significant differences (P≤0.05).

4. Discussion

The nutrients in the poultry industry contain chemical and natural compounds. Due to the negative effect of chemical residues on the nutritional value of bird carcasses, medicinal plants are used to improve the quality and growth of this industry. The present study aimed to minimize the chemical additives in the broiler production industry. The obtained results suggested that the application of herbal plant extract in poultry production increased gastrointestinal function and enzymatic excretion of the birds (9). Moreover, MS-treated diet resulted in an increase in BW, FI, and BWG during the grower and finisher stages, compared with the control group. This increase can be attributed to improved gastrointestinal function and enhanced enzymatic excretion of broilers. Improvement in body weight gains can be attributed to the bio-agent components of herbal plants that improve metabolic

activities and absorption of macro and micronutrients. Moreover, these bioactive components boost birds' appetite, which results in the improvement of overall growth performance (2, 10). Phenolic and flavonoid compounds in MS have been widely used as digestive stimulants (11, 12). Based on the results, MS could improve the organoleptic properties of the feed. This finding was in line with those reported by Adegbeye, Oloruntola (13), showing that MS improved metabolic mechanisms by increasing thyroid activity due to its positive impact on the digestive system. The results of the study conducted by Abd El-Hack, Attia (12) in 2016 demonstrated that MS improved the organoleptic properties of drinking water and induced appetite (12). Low-feed conversion rate is an index of good feed consumption of broilers, indicating that the broiler chicken benefitted greatly from the feeds.

In addition, folic acid (Vitamin B9) is one of the main components of MS which stimulates the secretion of digestive juices and improves the feed conversion ratio (14). Furthermore, MS contains polysaccharides (e.g., acemannan) that boost the immune system and improve the birds' health status (15). The improved health status of the birds, in turn, enhances the BWG and feed intake (16). These results are consistent with those in the study conducted by Adegbeye, Asaniyan (17) showing that growth was enhanced in broilers fed on an MS-containing diet. Additionally, the results obtained in this study were in line with those reported by Grioui, Boukhris (18) suggesting that leaf extract of the herbal plants could improve growth performance. In this study, increased growth performance and decreased FCR with MS may be due to the effects of bioagents on the improvement of health and antioxidant factors. Furthermore, the growth performance in this study is in line with the results of the study conducted by Adegbeye, Oloruntola (13) that reported an increase in BW and weekly BWG of broilers treated with MS on day 45 of age. Ripon, Rashid (19) in 2019 also hypothesized that the addition of natural herbs did not influence the growth performance of broilers.

In this study, the alpha-linolenic acid in MS led to low-blood cholesterol concentrations and reduced the impacts of stress. The cellular activity and overall health may be measured using the ALP level in the plasma (20). A high level of ALP in the broiler chickens with MS levels in the diet may result from bioagents in MS (e.g., flavonoids and phenol) which enhance hepatocellular function. The results are consistent with those reported by Adegbeye, Oloruntola (13), indicating that blood ALP was enhanced after feeding with MS supplements (200 mg/kg BW). The results also revealed that a high dose of MS (up to 300 mg/kg) elevated the serum concentration of ALP. The efficacy of MS in elevating the serum TP might be due to polyphenolic compounds that acted as scavengers for free radicals and enhanced cellular metabolic mechanisms (21). The high serum Ca and P concentration after supplementation with MS could be explained by the high mineral composition in the MS-treated meal. Caballero, Finglas (22) mentioned that the MS meal contained high levels of Ca, P, Na (sodium), and Fe (iron), low level of K (potassium), Mg (manganese), Zinc, and Cu (copper). Based on the evidence, and in line with the findings of the present study, high doses of MS could change the serum calcium, phosphorus, and alkaline phosphatase (AKP) (13, 23). Furthermore, Abd El-Hady, El Ashry (24) showed the positive role of essential oils in drinking water in absorbing minerals (e.g., Ca and P) and increasing the activity of hepatic enzymes. The unchanged level of Hb and RBC indicated that MS was safe at these levels for the blood circulation system. According to the findings of the previous study, the addition of MS (15 g/kg) to feed did not affect the biochemical blood parameters (25).

Fat oxidation affects the sensory scores and quality of meat, and poultry meat exhibits a higher degree of unsaturated fatty acids due to the relative content of phospholipids, compared to red meat (26). The high level of polyunsaturated fatty acids in chicken meat may be the main reason for the increased concentration

of MDA in the fresh muscle sample. The phospholipid fraction contributes to 90% malondialdehyde (26). Fat is the main contributor to animal rancidity (27). Poultry meat has a high content of phospholipids which makes it hypersensitive to oxidation (28). The results of the study conducted by Aluwong, Kawu (29) showed that feed additives increased lipid oxidation in broiler chicken.

Moreover, storage conditions are essential for promoting lipid oxidation. This finding which was consistent with those reported by Bidarnamani, Shargh (30) showed that prolonged meat storage under refrigeration led to increased MDA levels in chicken in the first month after preservation. (28).

5. Conclusion

Based on the obtained results, MS extract enhanced the growth performance of broilers through the improvement of oxidative effects and blood biochemical parameters. This finding can promote the use of MS extract as an effective growth promoter; however, more research is needed for the alternative use of this plant extract. Eventually, the recommended level of MS supplements is up to 7 mL per litre of drinking water in broilers.

Authors' Contribution

Study concept and design: I. A. A.
Acquisition of data: Y. S. A.
Analysis and interpretation of data: Y. S. A.
Drafting of the manuscript: Y. S. A.
Critical revision of the manuscript for important intellectual content: Y. S. A.
Statistical analysis: I. A. A.
Administrative, technical, and material support: Y. S. A. and I. A. A.

Ethics

The study was approved by the Research Ethics Committee of the University of Al-Qasim Green University, Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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