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Efficacy of Ziziphus mauritiana leaves extract as antibiotic alternatives in broiler chicken

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Abstract

The current experiment was conducted to evaluate the efficacy of oral administration of hydroalcholic Ziziphus mauritiana leaves extract (AZL) as antibiotic alternatives on growth performances, internal organs, lymphoid organs (spleen, Bursa) and blood biochemical characters of broiler during the period from 20 November to 26 December 2016 at the farm of Al Qasim Green University, Babylon, Iraq. A total of 160 one- day old male Ross broilers were divided randomly into 4 treatments with 4 replicate (10 birds per pen). The AZL was added at 0, 3, 7, and 10 ml / liter to water during starter (1-21 day) and grower (22-35) periods. The results showed that AZL increased the body weight and body weight gain at the all AZL groups but with no significant differences. During the grower period, the feed consumption was significantly higher (P < 0.05) in the AZL group of 10 ml/litter (1910 gm) as compared with control (1700 gm). The feed conversion ratio (FCR) was significantly lower (P < 0.04) at the 3ml/litter (1.38) as compared with control (1.59). Regarding, the internal organs, AZL showed no significant effects on lung, heart, gizzard, spleen, Bursa, and intestinal weight at the end of experimental period. On day35, Hemoglobin (Hb) and Total protein (TP) were found significantly higher (P < 0.002) in AZL group of 10 ml /liter than control. The blood cholesterol level was significantly declined (P<0.001) for all AZL groups. It can be concluded that AZL shows a good results especially in low dose in growth performance, blood biochemical characters.

Keywords: antibiotic, broiler, blood, organ, performance, Ziziphus

1. Introduction

Historically, biological active substances in herbal plants are considered the main sources for preventing and treating infectious diseases. They are also applied as protective drugs in human. Many investigators have explained that some herbal plants have biological active materials are able to increase animal production and act as antibacterial drugs ^[1,2]. The attention on herbal plants as growth promoter has gained more focus especially after banning antibiotic growth promoter and spreading multiple antimicrobial-resistant bacteria that has become the one of the world's public health risks. These challenges promote the researchers to seek for alternative solutions. As a suggested solution, herbal plant is a potential solution for these risks. According to WHO, herbal plant may be the best substitute of antibiotic ^[3]. Therefore, herbs must be investigated deeply to know their therapeutic traits, safety, and efficacy as antimicrobial drugs ^[4,5]. The Ziziphus spp leaves are considered as one of the most significant due to their use as antibiotic or antiseptic and traditional arabic Medicine. Ziziphus mauritiana leaves contain many types of biological ingredients substances like flavonoids, biflavones, proanthocyanidins, alkylphenols, carboxylic acids, sterols, polyprenols, beutic acid, ceanothic acid, cyclopeptides, saponin glycoside, flavonoids, lipids, protein, free sugar and mucilage ^[6]. These ingredient materials are promising source to improve animal and human health status^[7]. Ziziphus mauritiana was antibacterial drug against E. coli, S. pyogenes and S. aureus while Ziziphus spinachristi was very strong only against S. pyogenes ^[8]. In vitro and Vivo, the antimicrobial and anti-fungi effects of the Ziziphus leaves extract were also studied [9].

To date, no study has explained the biological effect of *Ziziphus mauritiana* on internal organs and growth performance of broiler chickens. Considering all above and depending on previous study ^[7, 8]. The current study helps us to understand how the aqueous *Ziziphus mouritiana* leaves extract affect growth performance, internal organs and blood biochemical characters. Based on that, we need more studies to reach a permission of using it in broiler production.

2. Materials and Methods

2.1. Experimental animals and housing

A total of 160 -one day old male broilers Ross 308 were taken from a commercial hatchery for this study. The chicks were housed in 16 pens (10 broilers per pen). They were reared in wood shaving littered cages (the cage area was 1 m^2). The chicks were maintained in a controlled condition. The experiment was conducted during the period from 20 November to 26 December 2016 at the farm of Al-Qasim Green University, Babylon, Iraq.

2.2. Design and Diet

The chicks were divided randomly into four groups (four replicated pens per group): control group (group A) without any additives. Groups B, C, and, D were given a water containing AZL (20% w/v) at 3,7, and 10 ml/liter respectively. All diets were balanced to be iso-caloric and iso nitrogenous to meet nutritional requirements of broiler chicken according to NRC ¹⁰ (Table 1). Water and feed were *adlibitium* during the experimental period (35 days).

2.3. The parameters

The body weight (BW) and feed consumption (FC) were recorded on 21, 35 day. The body weight gain (BWG) and feed conversion ratio (FCR) were measured. On day 35 (in the morning), two birds from each pen were selected randomly and blood sample were collected from the jugular vein using an EDTA tube for heamatological parametrs or non EDTA tube for sera analysis. After centrifugation process at 1500xg per 20 minute, sera was stored at -20 C⁰ until measurement of total protein (T.P), cholesterol and hemoglobin (Hb). These parameters were determined according to Kelly, ^[11].

The Red blood cells (RBC) were performed by blood analyzer apparatus (Nihon Cohden Centlac. Japan). After blood parameters were completed, the same chicks were weighed individually and euthanized by cervical dislocation (Halah method). The carcass dressing, Bursa, spleen, liver, gizzard, and whole intestine were weighed and the relative weight to live body weight of these was recoded.

2.4. Extract procedure

Ziziphus mauritiana leaves were obtained from a local market, cleaned, dried and ground into powder. Hydroalcholic extract was achieved according to Nagappan ^[12] with some modifications. Briefly, 20 grams of powder were macerated with 88% ethanol (5 volumetric parts) and water (5 volumetric parts). The ratio between herbs and the mixture was 2: 8.

The mixture was kept in a dark room for 48 hours and filtered. The concentrated mixture was allowed to dry at 40 C^0 . After drying, the mixtures were added to distilled water up to 100 ml.

2.5. Statistical analysis

Data were evaluated using one way ANOVA by using SPSS ^[13]. The differences among treatments were assessed by using LSD. Significant levels were declared when the statistically analysis was less or equal to 5 % ($P \leq 0.05$).

3. Results

The results of BW and BWG (gram /bird) were presented in Table (2) that showed no significant differences between treated and control groups during the experimental period (p= 0.06).

3.1. Feed consumption (FC) (g/bird)

The highest FC (P<0.05) was recorded in the birds that were received the *Ziziphus* leaves extract with 10 ml/liter as compared to the control group, whereas the FC with other concentrations was numerically higher (p=0. 4) relative to control. At the end of experiment the best FCR that was found in the birds giving water containing 3 ml /liter (treatment B) during grower and finisher periods (P<0.04) as compared with control. The other groups (C, D) have shown numerical improvement in FCR compared to control (P=0.08).

3.2. Internal organs

There were no differences in lung, gizzard, spleen, bursa, liver, and whole intestinal weight among the four groups for lasts 35 days (P>0.05) (table 3). Crucial findings from our study suggest that additive of *Ziziphus maurtiana* leaf extract did not have any deleterious effect on internal organs according these doses.

3.3. Blood parameters

The effects of Ziziphus maurtiana leaves extract on blood biochemical parameters were shown in table (4). T.P. has (*P*<0.05) with shown improvement AZL concentrations, where TP has (4.2,4.9, 6.3) for 3, 7, 10 ml/litter water. The Hb level was significantly higher in broiler that were received 10 ml /liter of Ziziphus leaf extract than the control group (P < 0.008) while other groups did not show any effect on Hb. The birds that received the Ziziphus leaves extract with 10ml /liter had the lowest cholesterol (81.5) (P<0.001) followed by treatment of 7ml /liter (91.7 g) (P < 0.01) and finally with treatment of 3ml /liter (98) (p = 0.06) in comparison with the control group (110.5). The number of red blood cells didnot show any significant differences between treatments at significant level 5%.

Table 1: Ingredients and	composition of t	he basal diet
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Item	Starter (0-21	Grower (21-				
item	d)	35d)				
Diet composition, g/kg as fed basis						
maize (8% CP)	536.5	628.8				
soyabean meal (43% CP)	407	327.4				
Vegetable oil	21.7	12.6				
calcium carbonate	12.1	12.8				
dicalcium phosphate	14.4	10.7				
DL-methionine	1.3	0.2				
vitamin premix1	2.5	2.5				
mineral premix2	2.5	2.5				
NaCl	2.5	2.5				
Calculated ch	nemical composit	ion				
metabolizable energy, kcal/kg	2900	2950				
crude protein, g/kg	208.4	189.9				
Ca, g/kg	9	8.28				
available phosphorous, g/kg	4.05	3.22				
methionine + cystine, g/kg	8.1	6.6				
lysine, g/kg	9.9	9.2				

1 vitamin premix per kg of diet: mg: vit. A, 2.7 vit. D3 0.05; vit. E, 18; vit. K3 2; thiamine 1.8; riboflavin 6.6; panthothenic acid 10; pyridoxine 3; cyanocobalamin 0.015; niacin 30; biotin 0.1; folic acid 1; choline chloride 250; antioxidant 100

 $^{^2}$ mineral premix per kg of diet: mg: Fe 50; Mn 100; Zn 100; Cu 10; I 1; Se 0.2

Table 2: Effe	ect of Ziziphus	leaves extract on	growth p	performance (of broiler ¹	(1-35day	s of age)
	- 1					\	

			Parameters ¹		
experimental	Treatment ²	Body weight g	Body weight gain g	Feed consumption	Feed conversion ratio
period	I reatment-	/bird	/chick	g/bird	g/g
1-21 days	А	513.8	474	758	1.59 ^b
	В	621	582.5	809.7	1.38 ^a
	С	512	488.7	720	1.56 ^{ab}
	D	562	518	782	1.50 ^{ab}
	Pooled SEM	20.4	20.6	21.7	0.03
	P-value	0.06	0.43	0.43	0.04
22-35 days	А	845	805.7	1700 ^b	2.6
	В	950	911	1670 ^b	1.7
	С	922.5	876.7	1797.5 ^{ab}	2.0
	D	912	867	1910 ^a	2.2
	Pooled SEM	24.9	24.7	38.2	0.07
	P-value	0.17	0.16	0.04	0.16
1-35 days	А	1358		2458	
	В	1563		2479.7	
	С	1435		2517	
	D	1475		2692	
	Pooled SEM	39.0		50	
	P-value	0.08		0.3	

¹Values represent the means of six replicated pens of ten chicks each, SEM: standard error of mean.² A: control group without any additive in feed or water; B group: *Ziziphus mauritiana* leaves extract at 3 ml / liter water; C: Ziziphus mauritiana leaves extract at 7 ml / liter water; D: *Ziziphus mauritiana* leaves extract at 10 ml / liter water. ^{a-b} Means within a column in each growth period with the same superscripts are not significantly different at $P \leq 0.05$.

Table 3: Relative organs weight (g/100 g live body weight) with /without Ziziphus leaves extract of broiler (1-35days of age)

Items ¹ Relative weight of organs %								
Treatments Carcass	yield%	Liver*	Gizzard [*]	Heart*	Lung*	Bursa*	Spleen*	Whole intestinal weight [*]
А	67.7	1.54	1.0	0.83	0.78	0.11	0.13	7.7
В	67.8	1.33	1.2	0.82	0.76	0.13	0.14	6.9
С	68.0	1.86	1.25	0.56	0.73	0.14	0.17	7.7
D	68.3	1.82	1.2	0.6	0.88	0.13	0.20	8.0
Pooled SEM	0.24	0.05	0.04	0.08	0.05	0.08	0.01	0.24
P-value	0.07	0.4	0.24	0.5	0.4	0.25	0.08	0.2

¹Values represent the means of six replicated pens of ten chicks each, SEM: standard error of mean. A: control group without any additive in feed or water; B group: *Ziziphus mauritiana* leaves extract at 3 ml / liter water; C : *Ziziphus mauritiana* leaves extract at 7 ml / liter water; D: *Ziziphus mauritiana* leaves extract at 10 ml / liter water. ^{a-b} Means within a column in each growth period with the same superscripts are not significantly different at $P \leq 0.05$. *Percentage of live body weight.

 Table 4: Effect of Ziziphus leaves extract on blood parameters of broilers at 35 days.

	Biochemical Blood Parameters ¹						
Treatments	*T.P (mg/dl)	*Cholestrol (mg/dl)	*Hb (mg/dl)	*RBC (m/1ml)			
А	4.1 ^b	110.0 ^a	3.4 ^b	2.4			
В	4.2 ^b	98.0 ^{ab}	3.9 ^b	2.9			
С	4.9 ^b	91.7 ^{bc}	3.4 ^b	2.7			
D	6.3ª	81.5°	5.5ª	3.0			
Pooled SEM	0.28	3.4	0.29	0.13			
P-value	0.002	0.001	0.008	0.08			

¹Values represent the means of six replicated pens of ten chicks each, SEM: standard error of mean.² A: control group without any additive in feed or water; B group: *Ziziphus mauritiana* leaves extract at 3 ml / liter water; C : Ziziphus mauritiana leaves extract at 7 ml / liter water ; D: *Ziziphus mauritiana* leaves extract at 10 ml / liter water. ^{a-} ^b Means within a column in each growth period with the same superscripts are not significantly different at *P*≤0.05. *T.P:Total protein ; *Hb: Haemoglobulin ; *RBC: red blood cell, million cell per ml

Discussion

The adding of AZL could be useful as an alternative to antibiotics because of its positive effects on FC and FCR

especially with low dose. These findings supported the hypothesis that herbal plants could improve livestock production. Although, the increase in the BW & BWG of the AZL was not significant, these results could be given an indication of new alternative antibiotics in animal production. It is known that the significant effects of the herbal plants are more effective when the chickens are incubated under an unhealthy condition [14, 15, 16, 17]. Additionally, Botsoglou.[18] referred that the birds reared in poor conditions have a significant responses with growth promoter supplementations. It is fact that the potential activities of herbal plants as growth promoters are highly dependent on environmental condition like floor litter and intensive rearing ^[19]. Moreover, because of the limited space associated with rearing, we used a small number of replications which may be reflected on the absence of a significant and obvious effect on BW and BWG. However, we have found that the FC and FCR were improved by AZL. These results can provide insight into the function and role of these herbals as antibiotic alternative. A possible explanation for these results might be due to the antimicrobial activity of the AZL [20] or the role of the AZL as antioxidant materials ^[21]. Several reports have shown that the AZL had beneficial effects on the growth performance particularly with

unhygienic condition ^[19, 22, 23]. Other researchers have pointed out the possibility of having antimicrobial properties of the AZL against pathogenic bacteria [24]. Addionally, the following results are in agreement with Son, ^[25] who found that feed additives of Zizyphus jujuba seed meal with 0.3 to 0.6 % has increased digestibility of feed, reduced the NH₃ emissions from feces, and enhanced the growth of broiler chickens. It was surprising to note that the low dose of AZL (3ml) was more significant than high doses (7and 10). This observation is particularly important as it gives a typical dose of AZL as growth promoter in chickens. It can be said that a high dose of AZL may have limited factors on growth gain, or contain on some metabolites that affect metabolic pathway of lipid ^[26]. This result are similar with Ganachari, & Kumar ^[27]; Ashour, ^[28] who found that the high doses and long administrative periods of Ziziphus extract increased food consumption, but didnot affect body weight gain. It is highly recommended that AZL may give promising effects that resemble the antibiotic growth promoters under commercial fields.

Surprisingly, no differences were observed in the internal organs. These findings provide further support for the hypothesis that the AZL did not have any deleterious effect on internal organs. Thus, this study raises the possibility for the use of AZL as an antibiotic alternative without any side effects. The current paper is in consistent with Son ²⁵ finding which showed no effects of *Ziziphius* extract on internal organ except gizzard. It is thought that the AZL extract did not have any toxic effects on the tissues ^[29]. Because of the toxic effects of some herbal plants and to avoid any drawback in the treatment with AZL, intensive researches are required to proof their safe use.

The blood constituents of the animals are very sensitive to the Physiopathological and nourishment condition [30, 31, ^{32]}.Therefore, blood parameters including RBC, Hb, PCV, proteins, and cholesterol, among others are main indicator of disorder in immune pathological and nutritional status of animal ^[33,34,35]. On the other hand, serum proteins are good indices of the feed metabolism and the biological status of body cells ^[36, 37]. Also, there is a correlation between dietary components, stress response and serum cholesterol level in the animals ^[38, 39]. The T.P, cholesterol and Hb in current study indicate that the AZL caused a significant impact on healthy status of animal and may act as anti-hepatic toxic factors [40] this result may be attributed to the natural metabolites of the AZL like tannin, glycoside, saponine, and alkaloids which act as antimicrobial drug and protect body cells from oxidant injury [40]. The lower cholesterol in all treatments of AZL may be attributed to vitamin Ascorbic acid (vit. c), which is shown in its constituents ^[42]. Several studies have shown that cholesterol in the blood has been reduced by vit. C [43].

5. Conclusion

This study is very successful in pointing out that AZI can be used as a substitute for antibiotics and can reduce the use of antibiotic in broiler farms. We can recommend that orally administrated AZL enhances FCR, FC especially with a low dose and the inclusion of concentration up to 7 ml /liter of AZL improves some blood biochemical characters like, TP, Hb and RB. Furthermore. This study was successful to point out to a new antibiotic alternative and at the end, further researches on the current topic are therefore recommended.

6. Conflict of interest statement

This manuscript has been written by above authors and it does not publish previously. We do not have any financial support.

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