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Effect of Aqueous Extract of *Melia Azedarach* L., *Anastatica Hierochuntic* and Enrofloxacin Antibiotic on Live Broiler Performance

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Abstract: The purpose of this trial was to compare between enrofloxacin antibiotic and aqueous extract of Melia azedarach and Anastatica hierochuntica L on live broiler performance, 270 one day unsexed chicks were distributed into 6 treatments which was replicated three times, 45 chicks/tret. (15 chicks/repl.), the control was the 1st treat., Enrofloxacin "antibiotic" at rate of 0.5 mg/ml was the second treatment, aqueous herbal extract (10 and 15 mg herbs per ml of tap water resp.) of Anastatica hierochuntic in 2nd and 3rd treatment , and Melia azedarach in 5th and 6th treatment respectively. Herbal plants were collected locally (Ramady city, AL-Anbar province, Iraq) from October to November, to get 10 and 15 mg per ml concentration, 5 and 7.5 g of powder herbal plants were dissolved in 500 ml of tap water respectively, enrofloxacin antibiotic were used at rate of 0.5 mg per ml according to manufacturing directions. The results of this experiment revealed that the chicks without any treatment have a increase significantly in 4th week of broiler age, weight gain and relative growth rate of 21 days in trial, in comparison with the same traits between the rest treatments at 28 and 49 days of broiler age, herbal extracts significantly decreased body weight, accumulative weight gain and relative growth rate (29-49 days and 1 to 49 days), on the other hand, there was non-significant differences between enrofloxacin treatment and herb treatments in final body weight, accumulative (29 to 49 day and 1-49 days) body weight gain and relative growth weight.

Keywords: *Melia azedarach*, *Anastastica heirochuntica*, Aqueous extract, Enrofloxacin, Broiler performance

Introduction

Treatment with herbal remedy was recently arise in new animal production especially in vast and intense poultry production, this trend were call "veterinary herbal medicine", one of the most herbal which had bactericidal and bacteriostatic actions was *Melia azedarach* (Vishnukanta, 2010), the name *Melia* came from the old Greek name which was mean flowering ash , in addition to common names in several countries (pride of India in England, Bakana Nimb in India …. etc) as referred by Nahak and Sahu (2010), Sharma and Paul (2013) referred that this herbal plant belong to the family Meliaceae and consists of two species (*azedarach* and *azadirachta*).

The active ingredients of *M. azedarach* was responsible for many modes of action to resist some pathogenic microorganisms, Sen and Batra (2012 a) found that extracts (by several solvents; Methanol, Ethanol, Petroleum ether and water) of *M. azedarach* leave had antagonism with 8 pathogenic microorganisms, involved *Bacillus cereus, Staphylococcus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, aspergillus flavus, Fusariumoxisprum, Rhizopus stolonifer*, via petri dish tests using well diffusion assay, later , the same scientist analyze the phytochemical constituents of *M. azedarach* L. leave via methanolic extraction and they found 48 bioactive phytochemical ingredients such as flavonoids, phytosterols, diterpene, alkane hydrocarbon, n-alkanoic acid and vitamin E and Tri-terpene, terpene alcohol (Sen and Batra, 2012 b).

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Some researcher tried to use *M. azedarach* L in veterinary remedy (Akhtar and Riffat, 1985), Muthamilselvan *et. al.* (2016) explored several herbal medicines that used in coccidiosis remedies, they mentioned *M. azedarach* extract was able to enhance birds weight gain and decrease the total count of oocysit of *Emeria tenella* and consequently decrease the lesion scores of coccidian disease, Shekhar *et. al.* (2018) recently confirmed using aqueous extract of *M. azedarach* leave as an alternative to amprolium (anticoccidal drugs) against *E. tenella*, hence, *M. azedarach* attributed in significant decrease of protozoal oocyst total count in the host and participate in healing wounds and antiulcer activity, that's lead to decrease scores lesion of coccidian disease in broiler.

Another herbal medicines was a Rose of Jericho (*Anastatica hierochuntica* L) , this herb plant known in Arabic region as Kaff Maryam, many of people in that region consumed as a tea beverage (El-Ghazali, *et. al.* ,2010), Daur (2012) found that *A. hierochunitca* had high levels of minerals (Mg, Ca, and Mn) in addition to phenolic ingredients, also, he referred to its antioxidant abilities, and he stated that may be the reason of using that herb as traditional folk medicine to treat vast of human disorders, Rosemary *et. al.* (2017) had a concurrent information and reviewed a good deal of medicine herbal plants and stated that aqueous extract of this herb (*A. hierochuntica* L) had many activities (antioxidant, antifungal, and antimicrobial actions), Benyagoub *et. al.* (2018) confirmed the ability of *A. hierochuntica* leave aqueous extract has antimicrobial action when they noticed the inhibition zones in petri dishes (well diffusion assay) were increased in *A. hierochuntica* treatments during testing several microorganisms that had resistance to some antibiotics.

Enrofloxacin was antimicrobial medicines belong to Quinolones drugs family, which had a broad spectrum antimicrobial action against Gram positive and negative microorganisms, it was used to prevent or/and treat many diseases in poultry production, enrofloxacin effect on the activities of topoisomerease IV enzyme and DNA-gyrase of microorganisms and consequently lead to kill these microorganisms (Abd ElAziz, *et. al.* 1997) , but enrofloxacin may participate in arising resistant heredity in pathogenic microorganisms and that leads to establish a new generation of these microorganisms which was resistant to enrofloxacin (Assis , *et. al.*, 2016), and that was dangerous to end consumed poultry products (humankind), with high risks of residuals of antimicrobial drugs in animal tissues (Attari, *et. al.*, 2014), so, the purpose of this experiment was to investigate the ability of substitute enrofloxacin antibiotic with herbal aqueous extract of *M. azedarach* and *A. hierochuntica* in broiler live performance.

Method

This experiment was conducted at poultry farm belong to Animal production department, College of Agriculture, University of Anbar. Unsexed one day old chicks were used via distributing 270 chicks into 6 treatments and the treatments were replicated three times, 45 chicks/tret. (15 chicks/repl.), the control was the 1st treat., Enrofloxacin "antibiotic" at rate of 0.5 mg/ml was the second treatment, aqueous herbal extract (10 and 15 mg herbs per ml of tap water resp.) of *Anastatica hierochuntic* in 2nd and 3rd treatment , and *Melia azedarach* in 5th and 6th treatment respectively.

Herbal plants used in this experiment were collected from AL-Anbar province, Ramadi city, Iraq, from October to November, the aqueous extract were prepared according to Harborne (1974, 1984) via dissolving 5 g of herbal plant in 500 ml of tap water to have a final concentration 10 mg/ml, to obtain concentration 15 mg/ml , 7.5 g herbal plant were dissolving in 500 L of tab water, these herbal plant doses were used according to antibacterial (G^+ and G^-) lethal dose which was *in-vivo* conducted by Saleh and Hassan (2007).

Herbal plant aqueous extract were added to water fountain drinker for full time of first day every week (1st to 7th week), and the water in fountain drinker were modulate according to age progress, chicks were raised in litter pens (1.5m long X 1 m width), nipples drinkers were used except the day of giving aqueous herbal extract, fountain drinker were used to give aqueous herbal extract, feed and water were free access (*ad libtum*).

Requirements of nutrients were calculated according to NRC (1994), by composing two diets (table 1), first one, was the starter diets, were given to chicks from first day till 28 days of chicks age, the second diet were used from 29th day till 49th day of broiler age as showed in table (1).

Chicks were raised after recommendations and requirements of modern ROSS 308 , according to the manual of company directions (ROSS, 2014), also, the live broiler performance were calculated weekly, then results showed as accumulative pattern (1 to 28, 29 to 49 , and 1 to 49 days) ; weight gain, feed consumption, feed conversion ratio, mortality (ROSS, 2014) and Relative Growth Rate (RGR) (Gondwe and Wollny ,2005)

To evaluate the effects of aqueous herbal extract, One-Way Analysis system were used to analyze experimental data via General Linear Model (GLM) procedure belong to statistical package SAS version 9.1 (SAS Institute, 2004), the values of probability below than 0.05 and 0.01 represent the significant status between experimental treatments, the results showed in tables as means/pooled SEM.

Table 1. diets compositions and calculated chemical analysis

ingredients	Starter (%)	Finisher (%)
Corn	59	55
Soybean meal	30	24
Protein concentrate [1]	10	10
Wheat	0	10
Lime stone	0.7	0.7
Salt	0.3	0.3
Total	100	100
Calculated chemical analysis		
Crude protein (%)	23.1	21.55
Metabolizable Energy (kcal/kg)	2935	2958
C/P ratio	126.97	137.25
Lysine (%)	0.61	0.61
Methionine (%)	0.53	0.51
Meth+ Cys (%)	0.57	0.60
Ca (%)	1.05	1.04
Available Phosphor (%)	0.44	0.45
Crud fiber (%)	3.40	3.40
Linoleic acid (%)	1.42	1.42

[1]Protein concentrate contains; 49% crud protein, 2900 kcal/kg ME, 3.1% available phosphor, 0.6% Ca, , 13% Ether Extract, 3.2% methionine+cys., 2.4% methionine and 3.4% Lysine.

Results and Discussion

The probability row showed in table (2) revealed a significant difference between treatments in accumulative performance (1-28 days), it's obvious that the chicks without any treatment have a significant improvement in body weight, weight gain and relative growth rate in comparison with the rest treatments, herbal extracts significantly decreased body weight, weight gain and relative growth rate (table 2), on the other hand, from table (2) there was non-significant differences between enrofloxacin treatment and herb treatments in body weight, body weight gain and relative growth weight.

Table 2. Impact of *M. azedarach* L., *A. hierochuntic* aqueous extract and enrofloxacin on live broiler accumulative performance (1-28 days)

treatments	Body weight (g)	Weight gain (g/b/28 day)	Feed consumption (g feed/b/28 day)	Feed conversion ratio (g feed/b/g wg/28 day)	Mortality (%)	Relative Growth Rate (%)
Control	814.04 a	777.34 a	1595.2	2.050	3.84	182.74 a
Enrofloxacin (0.5 ml/L)	734.29 bc	697.59 bc	1540.2	2.210	3.57	180.95 bc
M. azedarach (10 mg/ml)	763.87 b	727.17 b	1634.4	2.246	10.31	181.66 b
M. azedarach (15 mg/ml)	713.40 c	676.70 c	1619.9	2.390	7.50	180.42 c
A.hierochuntic (10 mg/ml)	718.18 c	681.48 c	1564.3	2.295	11.85	180.55 c
A.hierochuntic (15 *mg/ml*)	743.62 bc	706.92 bc	1624.6	2.295	3.57	181.18 bc
Total mean	746.57	709.87	1600.83	2.257	7.08	181.22
Probability	0.0837	0.0837	0.8812	0.2611	0.2097	0.0975
SEM	9.7718	9.7718	34.0884	0.0488	1.5410	0.2273

Accumulative broiler live performance from 29 to 49 days were illustrated in table (3), there were a significant differences between treatments in weight gain and feed consumption, *M. azedarach* aqueous extract and control treatments continues their improvement in the second period (29 to 49 days), also, there was non-significant differences between enrofloxacin treatment and herb treatments in weight gain and feed consumption.

Table 3. Influence of *M. azedarach* L., *A. hierochuntic* aqueous extract and enrofloxacin on live broiler accumulative performance (29-49 days)

treatments	Weight gain (g/b/21 day)	Feed consumption (g feed/b/21 day)	Feed conversion ratio (g feed/b/g wg/21 day)	Mortality (%)	Relative Growth Rate (%)
Control	1390.38 a	3473.0 a	2.507	14.16	92.05
Enrofloxacin (0.5 ml/L)	1288.24 ab	3091.2 ab	2.404	3.84	93.44
M. azedarach (10 mg/ml)	1379.80 a	3217.3 ab	2.332	5.12	94.90
M. azedarach (15 mg/ml)	1289.51 ab	3228.4 ab	2.510	2.77	94.88
A.hierochuntic (10 mg/ml)	1300.14 ab	3000.0 b	2.309	0.00	95.02
A.hierochuntic (15 *mg/ml*)	1163.05 b	2826.0 b	2.458	8.33	87.56
Total mean	1306.53	3151.25	2.420	5.45	93.25
Probability	0.2112	0.0622	0.8947	0.3647	0.3552
SEM	27.3756	63.8177	0.0555	1.7807	1.0534

The accumulative live performance for 49 days of broiler age were illustrated in table (4), there were a significant differences between treatments in final body weight, weight gain and relative growth weight, the control and *M. azedarach* treatments were increased significantly in comparison with the rest treatment, whereas, there were non-significant differences between enrofloxacin treatment and herb treatments.

Table 4. Impact of *M. azedarach* L., *A. hierochuntic* aqueous extract and enrofloxacin on live broiler accumulative performance (1-49 days)

treatments	Body weight (g)	Weight gain (g/b/49 day)	Feed consumption (g feed/b/49day)	Feed conversion ratio (g feed/b/g wg/49 day)	Mortality (%)	Relative Growth Rate (%)	Production Efficiency Factor
Control	2204.42 a	2167.72 a	5370.0	2.485	19.17	193.43 a	149.86
Enrofloxacin (0.5 ml/L)	2022.53 ab	1985.83 ab	4688.0	2.363	7.42	192.86 ab	162.23
M. azedarach (10 mg/ml)	2143.67 a	2106.97 a	5051.8	2.398	16.92	193.26 a	152.55
M. azedarach (15 mg/ml)	2002.91 ab	1966.21 ab	4947.7	2.519	10.71	192.79 ab	145.14
A.hierochuntic (10 mg/ml)	2018.32 ab	1981.62 ab	4564.3	2.304	11.86	192.85 ab	157.98
A.hierochuntic (15 *mg/ml*)	1906.67 b	1869.97 b	4572.9	2.455	12.50	192.42 b	137.42
Total mean	2053.112	2016.41	4884.91	2.426	13.19	192.95	150.57
Probability	0.0799	0.0799	0.2585	0.9153	0.8665	0.0891	0.9079
SEM	32.4286	32.4286	106.729	0.0530	2.4816	0.1107	5.4775

From the previous tables (2, 3 and 4), the live broiler performance were significantly increased in control and *M. azedarach* treatments, the improvement of *M. azedarach* treatment could be attributed to the active ingredients in this herb which take part in several modes of actions, Selvaraj and Mosses (2011) stated that *M. azedarach* leave had active ingredients, such as limonoids, but whole leave extract had better efficiency than the active ingredients individually, and that because of several active constituents in *M. azedarach* leave, Fufa *et.al.* (2018) found that phytochemicals ingredients in *M. azedarach* leave was alkaloids, flavonoids, phenols, tannins, saponins, terpenoids and steroids, and these compounds were responsible for antagonisms with four bacteria strains (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Esecherichia coli*), that could be the explanation of boiler live performance improvement throughout decreasing the total counts of these pathogenic bacteria in the host, leads to improve health status and consequently improve performance.

The non-significant differences between enrofloxacin and herbs treatments referred to the ability of replacing antibiotic with herbs without any differences in broiler live performance (table 2, 3 and 4), in addition, these herbs were able to enhance and promote growth parameters of broilers as antibiotic enrofloxacin did, AL-Dhanki and AL-Hamadani (2012) referred to a significant increase in total protein in serum of broiler gave aqueous extract *M. azedarach* and *A. hierochuntic* and that's lead to drive the excessive energy from health status to build and growth energy, and consequently make the hypothesis of this experiment come true, by ensuring that antibiotic enrofloxacin could be replaced by herbs (*M. azedarach* and *A. hierochuntic*) without any significant differences in live broiler performance, in addition to the beneficial uses of these herbs to prevent and treat some pathogenic microorganisms, Shekhar *et.al.* (2018) use *M. azedarach* in coccidiosis challenge as alternative remedy to amprolium and they found that *M. azedarach* could reduce the viable count of oocyst of cocidia and leads to improve health status of broiler infected with this parasite and decrease lesions occurred.

Conclusion

It can be concluded that using enrofloxacin antibiotic could be replaced by herbal aqueous extract of *M. azedarach* and *A. hierochuntic* without any significant differnces in live broiler performance, hence, these herbs had many active ingredients that could prevent broad spectram of microorganisms from being alive and profelrate in the host.

Recommendations

The authors recommend to use aqueous extract of *M. azedarach* and *A. hierochuntic* in broiler raising as pre and post-exposure prophylaxis instead of enrofloxacin antibiotic to prevent its side effect and arise of resistance microorganisims , in addition, to avoid antiboitic resdual in animal tissues that could effect the end consumer (humankinde).

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