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Influence of Dietary Supplementation of Medicinal Plants Mixture (Ziziphora, Menta Pulagum and Peppermint) on Some Serum Biochemical and Immunological Measures in Broiler Chickens

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Abstract: An experiment for investigation on effects of dietary supplementation of Ziziphora (*Thymyus valgaris*), Menta pulagum (*Oreganum valgare*) and Peppermint (*Lamiaceae mentha piperit)* on some of serum biochemical and immunological measures of broiler chickens has been conducted. 240 ROSS strain broiler chicks were selected and divided into 4 treatments and 3 replicates based on completely randomized design. Groups as group1 or control (fed basal diet, without medicinal plant supplementation), group2 (fed 1%Ziziphora, 0.5% Menta pulagum and 0.5% Peppermint), group3 (fed 1%Menta pulagum, 0.5 Ziziphora and 0.5% Peppermint) and group4 (fed 1%Peppermint, 0.5% Ziziphora and 0.5% Menta pulagum). At day-42 (end of rearing period), two chicks from each replicate were selected and blood samples (whole blood for hematological measures and serum for biochemical measures) were taken. Supplementation of medicinal plant mixture didn't have significant effect on biochemical measures. About immunological measures, highest lymphocyte counts (94%) and lowest H/L rate (0.05%) were observed in group3 ($p<0.05$). But lowest lymphocyte count (87%) and also highest H/L ratio (0.15) were found for control group. It is concluded that mixture of Menta pulagum, Ziziphora and Peppermint didn't have any considerable effect on serum biochemical measures such as total cholesterol, triglyceride, glucose, total protein and albumen, but whenever mixed as 1%: 0.5:0.5% respectively, could stimulate immune system.

Key words: Dietary supplementation · Medicinal plants · Immunological measures and broiler chickens

INTRODUCTION

In modern poultry farming, dietary supplementation of medicinal plants as antibiotic replacement is a new approach It is recommended not to use antibiotics and chemicals in poultry industry for better human health. Consumers prefer organic meat without chemical or antibiotic residues [1]. The use of natural plant extracts and biocontrol agents provide an opportunity to avoid the harmful effect of chemicals or drugs [2]. Jamroz *et al*. [3] reported that broilers fed plant extracts showed higher body weight gain if compared to control group. Authors referred the positive effects of medicinal plants to some bioactive compound such as caracole that stimulates appetite, secretion of gastrointestinal fluids and improves digestion, absorption and subsequent body weight gain in broiler [4]. Also Javed *et al*.[5] reported that supplementation of mixture of medicinal plant extracts optimized feed conversion ratio.While, Engber *et al*. [6] found that supplementation of plants essential oils was less beneficial than amino acid supplementation to stimulate the body weight gain in broilers Also, supplementation of essential oils from medicinal plants improve the immune-defense in poultry [7] and lower plasma triglyceride [1]. Akiba and Matsumoto [8] reported that the plant fiber controls hypercholesterolemia and elevated triglyceride. Currently Rahimi *et al*. [9] and Khaligh *et al*. [2] traced the efficiency of medicinal plant mixture and herbal extracts in improving performance and immune response of broilers. The objectives of this study was to study the effect of dietary supplementation of medicinal plants; Ziziphora (*Thymyus valgaris*), Menta pulagum (*Oreganum valgare*) and Peppermint (*Lamiaceae mentha piperit)* mixture on some serum biochemical and immunological parameters in broiler chickens.

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MATERIAL AND METHODS

In present study, 240 day old broiler chicks (Ross-308) were divided into 4 treatments and 3 replicate in each one and reared for 42 days. All the experimental groups fed on the basal diet (Table, 1). The first group G1was the control group and fed only the basal diet without medicinal plants supplementation, The dried leaves of medicinal plants were minced and added to the control feed stuff in different ratios where group2 fed on 1%Ziziphora, 0.5% Menta pulagum and 0.5% Peppermint, group3 fed on 1%Menta pulagum, 0.5 Ziziphora and 0.5% Peppermint and group4 fed on 1%Peppermint, 0.5% Ziziphora and 0.5% Menta pulagum. The basal diet was formulated according to NRC-1994 recommendations, based on corn and soybean meal for 1-21 days and 22-42 days chicks. Performed research design was based on completely randomized design (CRD).

Table 1: Ration ingredients and nutrients for broiler chickens (%)

	Rearing period			
	Starter		Grow	
Feed ingredients %	*control*	*supplemented*	*control*	*supplemented*
Corn	58.76	55.71	54.20	51.14
Wheat	0.00	0.00	15.00	15.00
Soybean oil	32.67	32.92	23.82	24.07
Fish meal	3.00	3.00	3.00	3.00
Medicinal plants	0.00	2.00	0.00	2.00
Vegetable oil	2.45	3.30	1.17	2.02
Bone meal	1.60	1.58	1.58	1.49
oyster shell	0.65	0.62	0.50	0.48
salt	0.25	0.22	0.23	0.22
Mineral supplement	0.25	0.25	0.25	0.25
Vitamin supplement[1]	0.25	0.25	0.25	0.25
Mineral supplement	0.25	0.25	0.25	0.25
methionine	0.15	0.15	0.07	0.07
Estimated nutrients concentrations in ration				
Metabolizable energy (Kcal/kg)	3000	3000	3000	3000
Crude protein (CP)%	21.56	21.56	18.75	18.75
Calcium%	0.94	0.94	0.84	0.84
Available phosphorus%	0.42	0.42	0.38	0.38
Sodium%	0.14	0.14	0.14	0.14
Linoleic acid%	1.43	1.36	1.29	1.23
Crude fiber	3.71	4.36	3.32	3.97
lysine%	1.25	1.25	1.02	1.02
methionone%	0.39	0.39	0.34	0.34
Met + Cys%	0.68	0.68	0.87	0.87
Tryptophan%	0.25	0.24	0.29	0.28

[1] *Vit. A: 22500 IU, Vit. D_3: 5000 IU, Vit. E: 45 IU, Vit.K: 5mg, Vit.B :1 4.3mg, Vit.B_2: 16.5mg, Vit.B_{12}, 0.04mg, pantotenoic acid: 24.5g, folic acid: 2.5mg, niacin: 74mg, pyridoxine: 7.3mg, biotin: 0.04mg, for per Kg of diet.*

At day-42 (end of rearing period), two chicks from each replicate were selected and after 24h starvation period, blood samples (whole blood for hematological measures and serum for biochemical measures) were taken. The laboratory assay of biochemical measures were done via Pars-Azmoon kits and auto analyser Alyson 300, USA. The blood cells were counted by staining and light microscopic observation.

Statistical Analysis: Statistical analyses were performed by Sas 9.1 software. Duncan multiple tests was done for detection of significant difference between experimental groups, $P<0.05$.

RESULTS AND DISCUSSION

Supplementation of medicinal plant mixture didn't have significant effect on some biochemical measures. [table2]. Supplementation of medicinal plant mixture in the third group showed significant increase of lymphocytes ($p<0.05$) while heterophils of the same group showed minor decrease if compared to control group (Table 3).

Menta pulagum is well known immunostimulant used in folk medicine. It has antimicrobial effect and can be used to control microbial infections. It is stomachic as it improves feed digestibility [10].

Table 2: Effects of dietary medicinal plant supplementation on some of serum biochemical measures in 42 day old broiler chickens

	Treatment				
Measure (mg/dl)	1	2	3	4	SEM
glucose	156.33	146.17	157.84	178.5	6.28
Total cholesterol	131.17	123.67	131.17	139.17	8
Triglyceride	55.83	34	46.5	52.83	8.81
Total protein	4.45	4.32	4.14	3.65	0.19
Albumen	1.79	1.95	1.97	1.88	0.04

Table 3: Effects of dietary medicinal plant supplementation on some of immunological measures in 42 day old broiler chickens

	Treatment				
Measure (*percentof total WBC*)	1	2	3	4	SEM
heterophil	13	8	4.16	7.33	2.750
lymphocyte	87[b]	90[ab]	94[a]	92[ab]	1.650
H/L ratio	0.15	0.09	0.05	0.08	0.024

• *different letters (a and b) show significant difference between groups, $p<0.05$.*

Mixture of herbal remedies in certain proportion may optimize their effect. Hardari *et al*. [11] could not trace immunostimulant effect from the combination of Menta pulagum with Nettle (*Urtica dioica*) and Ziziphora (*Thymyus valgaris*). The authors added that the lipid profile is negatively affected when this plant mixture is used for feeding while, Toghyani *et al.,* [12] did not find any significant effect of dietary supplemented peppermint on triglyceride, LDL, HDL and total cholesterol of broilers. In the current study Menta pulagum in combination with Ziziphora and Peppermint showed significant immunostimulant effect and did not elevate the lipid profile. These results for immunological measures are in agreement with Rahimi *et al.* [9]. It seems that Menta pulagum in different medicinal mixture [11] could have different effect on serum biochemical parameters. It was concluded that mixture of Menta pulagum, Ziziphora and Peppermint didn't have any considerable effect on serum biochemical measures such as total cholesterol, triglyceride, glucose, total protein and albumen, but supplementation in these percentages; 1%Menta pulagum, 0.5 Ziziphora and 0.5% Peppermint could stimulate immune system.

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