ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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International Journal of Poultry Science 7 (3): 234-239, 2008
ISSN 1682-8356

Rosemary Leaves as a Dietary Supplement for Growth in Broiler Chickens

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Abstract: This study was conducted to investigate the use of Rosemary leaves meal as a natural growth promoter in broiler diets on bird performance and immunity. RLM was added in either grower (7-28 day) or finisher (29-49day) diets at three concentrations (0.5, 1.0 and 2.0%). A total of 200 one-day old unsexed Arbor Acres chicks were assigned equally into four treatment groups, with five replicates of 10 chicks each. Chemical analysis of RLM showed a CF content of 19.4% of which 15.89% was present as cellulose. The essential oil of RLM ranged between 1.4-1.6% and the main active components were camphor (11-16%), alpha pinene (15-20%) and cineole (30-35%) which has a high degree of inhibition against many bacteria and fungi. Compared to control, chicks fed 0.5 % RLM exhibited higher body weights, greater weight gain, and better feed conversion during the experimental period as well as better physical properties of the chicken meat. Moreover, 0.5% dietary rosemary increased plasma total protein, albumin and globulin while decreasing glucose, total lipids and cholesterol content. RLM additions did not affect enzymatic activity related to liver and kidney functions. RLM stimulated thyroid function, as evidenced by increased plasma levels of T3, T4 compared to controls. Antibody production against sheep red blood cells was improved and the percentage of the lymphoid organs was increased compared to controls. Increasing the dietary level of RLM more than 0.5% lowered growth and the digestibility of most nutrients. Thus, low levels of dietary RLM could be safely used in broiler diets to promote growth and to impart healthful constituents to the consumer.

Key words: Rosemary leaves, performance, broiler, blood and immunity

Introduction

Natural feed additives of plant origin are generally believed to be safer, healthier and less subject to hazards. Herbs and herbal products are incorporated in livestock feeds instead of chemical products in order to stimulate or promote the effective use of feed nutrients which result in more rapid gain, higher production and better feed efficiency. Moreover, herbs contain active substances that can improve digestion and metabolism and possess bacterial and immunostimulant action of animals (Sabra and Metha, 1990). The word rosemary is derived from the Latin word “rosemarinus", meaning sea dew. It was also called “antos" by the ancient Greeks, meaning the flower of excellence (Giugnolinini, 1985). Oil of *Rosemarinus officinalis* can be used as flavor or perfume, possess carminative properties and has a high degree of inhibition against 25 genera of bacteria and fungi (Montes *et al*., 1998). Limited information is available on the chemical composition of RLM. Tomei *et al*. (1995) reported that the main constituents of the essential oil obtained from flowers and leaves of rosemary in Spain were camphor (32.33%), 1, 8-cineole (14.41%) and α-pinene (11.56%). Mulas *et al*. (1998) found that the essential oil content of rosemary ranged between 0.8 to 2.6 % of the dry powder weight. Wolski *et al*. (2000) reported that the essential oil of RLM ranged from 1.5 to 2.0%, averaging 1.78%, which means that the essential oil and its constituents may differ from place to another due to variations in species, soil, weather, treatments and processing. It can be stated that the main constituents of the essential oil from different species of rosemary are α-pinene (11.5-40%) and camphor (26.0-53.0%) according to El-Amrani *et al*. (2000); Porte *et al*. (2000) and Pintore *et al*. (2002). Biologically, rosemary extract improved feed conversion efficiency of broilers fed diet supplemented with such herb (Singletary and Rokusek, 1997). Rosemary has high amounts of a rosmaric acid (Nielsen *et al*., 1999), Flavonoids and phenolic acids (Ho *et al*., 2000) that have antioxidant capacities. Karpinske *et al*. (2000) also demonstrated that the addition of RLM extract delayed the appearance of rancidity in poultry products. Tekeli *et al*. (2006) determined that the *Rosemarinus officinalis* leaves could be used to decrease blood glucose. The main target of this study was to investigate the effective use of RLM for growth and immune function in broilers.

Materials and Methods

Rosemary leaf meal (RLM) samples were finely ground and subjected to proximate analysis according to the methods of AOAC (1980), fiber constituents via procedures of Van Soest and Robertson (1979), macro and micro mineral elements using Atomic Absorption Spectrophotometry, 3300 Perker Elmer and gross energy using an adiabatic bomb calorimeter (IKA-Calorimeter C 4000). Chemical composition of the

Table 1: Composition and calculated analysis of the experimental grower and finisher diets supplemented with different levels of rosemary leaves (RLM)

Ingredients %	---------- Grower diets (7-28d) ----------				---------- Finisher diets (29-49d) ----------			
Rosemary leaves	0.0	0.5	1	2	0.0	0.5	1	2
Yellow corn	57.8	57.4	56.9	56	63.8	63.4	62.9	61.8
Soybean meal (44%)	29.5	29.5	29.5	29	26	25.7	25.9	25.6
Corn gluten meal (60%)	6	6	6	6.4	2.9	3.1	3	3.4
Poultry fat	2.9	2.9	2.9	2.9	4	4	4	4
Bone meal	2.7	2.7	2.7	2.7	2.4	2.5	2.5	2.5
Limestone	0.4	0.3	0.3	0.3	0.2	0.1	0	0
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vit. and Min. mixture *	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL - methionine	0.06	0.06	0.06	0.06	0.1	0.1	0.1	0.1
L - lysine Hcl	0.04	0.04	0.04	0.04	0	0	0	0
Total	100	100	100	100	100	100	100	100
Calculated analysis:								
Crude protein %	22	22.04	22.03	22.05	19	19	19.02	19.02
ME (kcal / kg)	3105	3103	3101	3100	3205	3206	3203	3200
Crude fiber %	3.5	3.58	3.67	3.79	3.34	3.4	3.49	3.63
Ether extract %	5.48	5.54	5.6	5.73	6.7	6.77	6.83	6.95
Calcium %	1.05	1.03	1.01	1.05	0.88	0.88	0.85	0.87
Available phos. %	0.48	0.48	0.48	0.48	0.44	0.45	0.45	0.45
Methionine %	0.51	0.51	0.51	0.51	0.45	0.45	0.45	0.44
Lysine %	1.1	1.1	1.1	1.11	0.94	0.94	0.94	0.94
Meth. + Cyst. %	0.87	0.87	0.87	0.87	0.76	0.76	0.76	0.75

*Each 3 kg of vitamins and minerals mixture contain: Vit. A, 12000000 IU ; Vit. D_3, 2200000 ; Vit. E, 10000mg ; Vit. K, 2000mg; Vit. B_1, 1000mg; Vit. B_2, 5000mg; Niacin, 30000mg; Pantothenic, 10000mg; Vit. B_6, 1500mg; Vit. B_{12}, 10mg; Folic, 1000mg; Biotin, 50mg; Selenium, 100mg; Copper, 4000mg; Iron, 30000mg; Manganese, 60000mg; Zinc, 50000mg; Iodine, 1000mg; Cobalt, 100mg; Choline chloride, 300000mg and Calcium carbonate was the carrier till 3 kg.

essential oil and its active ingredients was conducted using gas liquid chromatography (Masada, 1976). Our experiment was conducted using a total of 200 unsexed one-day old Arbor Acres broiler chicks, distributed randomly into four experimental groups, with five replicates of 10 chicken each. The first group was fed a control diet without addition of RLM while the other groups, were fed diets supplemented with 0.5, 1.0 and 2.0 % of dried RLM (*Rosmarinus officinalis L.*). Chicks were housed in battery cages and artificial lighting was provided 24 hours daily for the 7 week experimental period. Treatment grower diets were formulated to contain about 22%CP and 3100 Kcal ME / kg and was used from 7 to 28 days of age, while finisher diets were formulated to contain about 19% CP and 3200 Kcal ME/kg and was used from 29 to 49 days of age (Table 1). Methionine, lysine, vitamins and minerals mixture were added to cover the dietary requirements of chicks in accord once with the Arbor Acres management guide. Diets and water were offered at libitum over the experimental period, chicks in all treatments were kept under the same management system. Mortality was recorded daily, while live body weights and feed intake was recorded at 28 and 49 days of age. Body weight gain and feed conversion were calculated. At 50 days of age individual blood samples were collected from the jugular vein and plasma was separated for determination of total protein (Weichselbaum, 1946), albumin (Doumas and Biggs, 1972), total lipids (Zollner and Kirsch, 1962), HDL-cholesterol (Warnick *et al.*, 1983) , LDL - cholesterol (Assmann *et al.*, 1984), total cholesterol (Allain *et al.*, 1974), glucose (Trinder, 1969), AST and ALT (Reitman and Frankel, 1957), alkaline phosphatese (Young, 1975), creatinine (Jaffe, 1986), uric acid (Fossati, 1980) and thyroid hormones (Mardell, 1978 for T_3 and Dunn and Foster, 1973 for T_4) using commercial kits. Birds were used to measure carcass characteristics as well as to measure organoleptic properties including meat color, taste, aroma, texture and overall acceptability. Broiler chicks fed RLM were immunized with SRBC to determine immune response and antibody titers (Van Der Zipp and Leenstra, 1980) also the differences in the lymphoid organs weights including thymus and spleen (Bread, 1980). Data obtained from the study were tested for significance by one-way ANOVA using the GLM procedures of SAS (1990). Differences among treatment means were separated by Duncan's new multiple range test (Duncan, 1955).

Results and Discussion

Chemical evaluation of the experimental RLM: Results from chemical analysis shown in Table 2 indicate that RLM contains 8.5% moisture, 5.12% crude protein, 15.4% ether extract, 7.06% ash, 19.4% crude fiber and 44.52% nitrogen free extra. The gross energy content amounted to 3.593 kcal/kg. The main chemical constituents of the cell wall are cellulose, hemicelluloses, lignin, pectin and silica. RLM contained high level of hemicelluloses (6.79%) cellulose (15.89%)

Table 2: Chemical analysis of rosemary leaves used in the experiment

Proximate analysis, %		Mineral Elements %		ppm		Active components of essential oil, %		Gross energy Kcal/g
Moist.	8.50	K	1.1	Cu	2.0	Camphor	11-16	3.593
CP	5.12	Ca	2.2	Zn	29.9	α-pinene	15-20	
EE	15.40	P	0.5	Mn	13.8	Cineole	30-35	
CF	19.40							
Ash	7.06							
NFE	44.52							
Cellulose	15.59							
Hemicellu.	6.79							
Lignin	5.94							
Essential oil	1.4-1.6							

Table 3: Effects of different dietary concentrations of RML on the performance of broiler chicks

Item	Rosemary leaves levels (RLM), %				
	0.0	0.5	1.0	2.0	SEM
Live body weight (g) :					
At 7 days	120.16^{a}	119.43^{a}	119.33^{a}	119.20^{a}	0.500
At 28 days	956.63^{a}	915.93^{ab}	885.23^{bc}	848.37^{c}	21.703
At 49 days	1741.67^{b}	1874.67^{a}	1847.33^{ab}	1787.00^{ab}	64.350
Body weight gain (g) :					
7 – 28 days	836.47^{a}	796.50^{ab}	765.90^{c}	729.17^{c}	21.705
28 – 49 days	785.03^{b}	958.73^{a}	962.10^{a}	938.63^{a}	54.518
7 – 49 days	1621.50^{b}	1755.23^{a}	1728.00^{ab}	1667.80^{ab}	64.380
Feed intake (g / Bird) :					
7 – 28 days	1364.93^{a}	1284.77^{ab}	1256.00^{b}	1261.00^{b}	44.666
28 – 49 days	1997.40^{a}	2186.50^{a}	2221.30^{a}	2167.70^{a}	191.435
7 – 49 days	3362.33^{a}	3471.30^{a}	3477.30^{a}	3428.70^{a}	163.655
Feed conversion (feed / gain) :					
7 – 28 days	1.63^{b}	1.61^{b}	1.64^{b}	1.73^{a}	0.017
28 – 49 days	2.54^{a}	2.28^{b}	2.30^{b}	2.31^{b}	0.067
7 – 49 days	2.07^{a}	1.97^{b}	2.01^{ab}	2.05^{ab}	0.042

a , b , c : Means on the same row within each parameter bearing common superscripts are not significantly different (P < 0.05).

and 5.94% lignin. The last two components comprise the acid detergent fiber (ADF) which is the most suitable estimate of fiber, since it represents the indigestible fraction. Results indicate (Table 2) the presence of moderate amounts of some macro (Potassium, 1.1%; Calcium, 2.2%; Phosphorus, 0.5%) and micronutrients (Copper, 2 mg/kg; Zinc, 29.9 mg/kg; Manganese, 13.8 mg/kg). The essential oil of RLM (Tale 2) ranged between 1.4 - 1.6% and the main active components were camphor (11-16%), α-pinene (15-20%) and cineole (30-35%). These findings agree with those obtained by Wolski *et al.* (2000) and Porte *et al.* (2000). Moreover, Farag *et al.* (1989) stated that these active compounds have high antioxidant activity due to the presence of phenolic groups in their structure. They so could be considered as a safe and natural method to retard auto-oxidation, considered harmful to the consumer. Moreover, Farag (2007) stated that RLM is more a process effective in retarding lipid oxidation in both raw and cooked meat by decreasing the formation of malondialdehyde (MDA).

Performance of broiler chicks: Mean live body weight (LBW) and body weight gain (BWG) of chicks during the experimental period are summarized in Table 3. Highly significant differences (P<0.01) were observed between dietary treatments at 28 days of age. However, no significant differences were recorded between chicks given 0.5% RLM and the control group. While, both LBW and BWG decreased significantly for chicks fed diets supplemented with either 1.0 or 2.0% of RLM. Such decrease could be attributed to the decrease in feed intake for birds of these groups during 7-28 days of age. At 49 days of age, the addition of different levels of RLM significantly (P<0.01) increased LBW and BWG compared to the control group.

Compared to the control group. Over the entire experimental period (7-49 days of age),significant differences (P<0.05) were only found between the group of chicks having 0.5% RLM and controls, while no significant differences were observed among the other dietary treatments. This means that 0.5% RLM is the best dietary concentration that could be used to promote growth in broiler chick, as previously reported by Lopez-Bore *et al.* (1998). The decrease in growth as dietary RLM increased could be caused by the high crude fiber content in particular cellulose from the cell walls of RLM, which may impede the utilization of nutrients by chicks.

Table 4: The effect of levels different concentration of RLM on blood parameters of broiler chickens

	Rosemary leaves levels (RLM)%				
Item	0.0	0.5	1.0	2.0	SEM
Protein fractions:					
Total protein (g/dl)	3.29^{b}	4.65^{a}	3.56^{b}	3.23^{b}	0.427
Albumin (g/dl)	1.61^{a}	1.80^{a}	1.59^{a}	1.59^{a}	0.194
Globulin (g/dl)	1.67^{b}	2.85^{a}	1.96^{ab}	1.64^{b}	0.894
A / G ratio	0.993^{a}	0.643^{b}	0.813^{ab}	0.990^{a}	0.185
Lipid fractions:					
Total lipids (g/dl)	0.754^{a}	0.747^{a}	0.675^{a}	0.658^{a}	0.108
Total Choles. (mg/dl)	124.71^{a}	95.83^{b}	84.40^{b}	82.24^{b}	14.012
LDL-Choles.(mg/dl)	54.00^{a}	38.00^{ab}	36.25^{b}	36.31^{b}	8.672
HDL-Choles.(mg/dl)	51.43^{a}	40.06^{a}	38.30^{a}	37.46^{a}	14.574
Liver Function:					
AST (U/L)	41.00^{b}	45.00^{ab}	49.66^{a}	53.00^{a}	8.163
ALT (U/L)	19.00^{a}	18.33^{a}	23.00^{a}	19.00^{a}	4.886
Alk. Phos.(IU/L)	283.17^{a}	342.75^{a}	319.60^{a}	302.01^{a}	58.443
Kidney Function:					
Creatinine (mg/dl)	2.54^{a}	1.12^{b}	1.14^{b}	1.22^{b}	0.135
Uric acid (mg/dl)	5.41^{a}	3.49^{b}	4.49^{ab}	3.56^{b}	0.988
Thyroid Activity:					
T_3 (ng/ml)	1.81^{b}	2.67^{a}	2.77^{a}	2.62^{a}	0.544
T_4 (ng/ml)	5.79^{b}	9.30^{a}	9.66^{a}	9.65^{a}	0.54
Glucose (mg/dl)	172.51^{a}	148.13^{b}	144.60^{b}	143.05^{b}	12.67

^{a,b}Means on the same row within each parameter bearing common superscripts are not significantly different (P < 0.05).

The data in Table 3 indicated that feed conversion (FC) was not significantly affected by RLM up to 1.0% concentrations during growing period (7-28), while raising the level to 2.0% the poorest, FC. During the finishing period (29-49 days of age), data showed an improvement in FC by adding RLM compared to controls. During the overall period from 7-49 days of age, FC of control birds was significantly poorer when birds were fed 0.5 % RLM, while 1 and 2% levels were intermediate and not significantly different from the control and 0.5% treatments. This improvement could be attributed to the higher BWG of these groups. In general, chicks fed the control diet had inferior to those fed diets supplemented with 0.5% RLM. This result be attributed to the essential oil present in RLM and its active constituents which have possess antibacterial, antifungal and antioxidant activities due to the presence of phenolic compounds (Nielsen *et al*., 1999 and Ho *et al*., 2000). Alternatively high levels of RLM (1.0 and 2.0%) had an adverse effect on broiler performance from 7-28 days of age as feed consumption decreased, possibly do to low palatability in young chicks compared to the control, although this effect disappeared during finishing period. This may be attributed to the capacity of chicks at older ages to adapt to eating diets with greater cellulose content. Either during the growing (7-28d) or finishing (29-49d) periods, the livability rate of chicks fed experimental diets with RLM were better than the controls. The highest livability was recorded for chicks fed 0.5% RLM, at 100%. Although the mortality of most birds from the study might be account for natural cases. The mortality may also be attributed to the growth promoter action against pathogenic micro-organisms that can attack young chicks (Montes *et al*., 1998). In general, the improvement in growth associated with supplementing diets with RLM have been associated with changes in enteric flora and reduced *E. coli* populations. According to Wenk, (2002) lowering gastric pH can stimulate favorable micro-organism and the synthesis of catabolic enzymes that help in the digestion and absorption of amino acids, sugars and fatty acids.

Blood analysis: Inspection of the blood data showed (Table 4), that average values of total protein and globulin fractions were significantly increased (P<0.05) with the addition of 0.5%RLM compared to controls. Higher dietary levels of RLM had no appreciable benefit. No significant differences were observed in blood albumin, however the A/G ratio was reduced among 0.5% RLM fed birds compared to the control and other treatment groups (P<0.05). This reflects the ability of chicks to store reserve protein even after the body has reached its maximum capacity for depositing protein to tissues. In addition, the increase in the globulin fraction indicates the effective role of rosemary in increasing immunity due to its role in developing and protecting cells and inhibiting non-enzymatic oxidation (Houghton *et al*., 1995). The reduced plasma content of total cholesterol and LDL may reflect the hypocholesterolemic properties attributed to the defatted part of the leaves which are rich in fibrous content and may block intestinal cholesterol absorption (Lanksy *et al*., 1993).

Dietary RLM did not affect the plasma content of alkaline phosphates however AST levels of the control were reduced compared to birds fed 1 and 2 % RLM.

Table 5: Effects of RLM on immunization and physiological parameters of broiler chicks

	Rosemary leaves levels, %				
Item	0	0.5	1	2	SEM
Spleen,%	0.16	0.14	0.2	0.15	0.086
Thymus,%	0.34[a]	0.27[a]	0.28[a]	0.17[b]	0.084
Antibody titter	8	8	7.66	8	0.763

[a,b]Means on the same row within each parameter bearing common superscripts are not significantly different (P < 0.05).

Table 6: Slaughter characteristics and organoleptic properties of chicken meat from broilers fed different concentrations of RLM

	Rosemary leaves levels , %				
Item	0.0	0.5	1	2	SEM
Slaughter characteristics, %					
Carcass	68.62	70.05	68.42	68.68	1.683
Liver	2.19	2.2	2.47	2.31	0.283
Gizzard	2.05[a]	1.52[b]	1.61[ab]	1.38[b]	0.489
Heart	0.47	0.4	0.45	0.37	0.155
Abdominal fat	2.5	2.31	2.42	1.99	0.594
Organoleptic properties:					
Color	7.20[ab]	7.63[a]	7.46[a]	7.00[b]	0.440
Taste	7.13[b]	8.40[a]	8.26[a]	6.96[b]	0.239
Aroma	6.96[c]	8.06[a]	7.80[ab]	7.43[b]	0.379
Texture	7.30[b]	8.20[a]	7.93[a]	7.36[b]	0.272
Overall acceptability	7.26[b]	8.23[a]	8.00[a]	7.13[b]	0.278

[a,b,c]Means on the same row within each parameter bearing common superscripts are not significantly different (P < 0.05).

Creatinine and uric acid levels were all reduced by dietary RLM compared to controls (P<0.05). Results of thyroid activity indicated that RLM may stimulate the thyroid gland directly, as T_3 and T_4 levels in plasma increased significantly compared to controls. In addition, similar to the reports of Erenmemisoglu *et al.* (1997), plasma glucose of chicks fed diets with RLM were reduced compared to controls group. All the above mentioned effects may consequently improve the metabolic processes in the body leading to greater weight and FC.

Immunization and physiological parameters: It is well known that globulin has a considerable role in bird's immunity and is formed by lymphatic tissues including the spleen, thymus and Bursa of Fabricius. Dietary RLM showed no change in the total of spleen or thymus, with the exception that 2% inclusion significant reduced thymus size (Table 5). Antibody titer to SRBC was reduced by 1% dietary RLM compared to the control and other treatments.

Carcass characteristics and organoleptic properties of meat: There were no significant differences in carcass, liver, heart and abdominal fat percentages of chicks fed different concentrations of RLM compared to the control group (Table 6). However, gizzard percentages tended to decrease as the level of RLM in the diet increased (P<0.05). Numerically the addition of 0.5% RLM to the diet increased carcass % almost 1.5% and adding 2% RLM reduced abdominal fat more than 0.5%. Significant improvements (P<0.05) in organoleptic properties were detected among treatment groups given 0.5 or 1.0% RLM compared to the control and 2.0% RLM diet. Taste, texture, aroma and overall acceptability of the meat was improved by 0.5% and 1.0% dietary addition (P<0.05). The negative effect of the higher 2% level of RLM could be attributed to the presence of high concentration of essential oil and probably a great part of its components are metabolized and then precipitated in the chicken meat. These results are in agreement with those obtained by Farag (2007).

Generally speaking the best overall bird performance and consumer acceptability of the meat was obtained by feeding broiler chicken diets supplemented with 0.5% RLM. This treatment level exhibited the best growth and feed utilization, in addition to increase immunological responses of the birds.

Acknowledgements

The author acknowledges the help of Poul. H. Patterson and Paul Bartell. (Dep. Poult. Sci. Pennsylvania State University).

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