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Effect of Chemically Amended Litter and Supplementing Low Protein Diet with Amino Acids on Immune Status, Carcass Characteristics, Biochemical Parameters and Broiler Chicken Behavior during Summer

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ABSTRACT

Background: Ammonia gas present in poultry shed critically affects the health and welfare condition of bird and the labourer which are working at the poultry farm. Good litter management and low protein diet minimize the ammonia concentration resulting in overall improvement of the bird's welfare. The current study was aimed to estimate the effect of alum sulphate in litter and addition of essential amino acids in a low protein diet on immunity status, serum biochemical parameters, carcass parameters and behavior welfare of broiler chicks.

Methods: A total of 240 (day old) Vencobb broiler chicks were randomly distributed into four treatment groups, having 3 replicates of 20 chicks each. Control group (T_c) had no dietary and litter amendments, whereas, experiment groups include litter amendment with alum sulphate @ 90 gm/sq.ft. (T_L) dietary amendment with low protein supplemented with essential amino acids like lysine, methionine, threonine (T_D) and both dietary and litter amendment (T_{LD}).

Result: The results showed that immune status was better in the litter treated groups as compare to control and T_D group. The overall hygiene of the birds was better in treatment groups. It can be concluded that treatment of litter with alum sulphate improved protein metabolism and carcass quality and also help in providing a comfortable environment to the broiler birds.

Key words: Alum sulphate, Amino acids, Broiler behavior, Carcass parameters, Immune status.

INTRODUCTION

In broiler production, chicks are reared on deep litter system of housing using different kinds of bedding materials like rice husk, paddy straw, wood shavings *etc*. which go continuously decomposing with faecal matter, feathers and dander. This litter is the source of volatilized ammonia and its management is a key factor which affects the rate of its emission, health and welfare of birds and in turn environment as well as human health (Beker *et al.*, 2004). Ammonia released from broiler house, whereas, nitrous oxide and other obnoxious gases emitted from the poultry waste may contribute to air pollution. Today there is a need to stabilize or reduce the concentration of these gases in order to reduce climate change. Ammonia concentration in a broiler house can be reduced by dietary and litter amendment practices, thereby, making a comfortable environment for birds to increase their production. The poultry litter nitrogen can be reduced by giving a diet low in crude protein and compensating this by adding essential amino acids and thereby reducing the nitrogen excretion in litter. By adapting these strategies the cost of production can also be reduced. Broiler chicks fed diets marginal in protein and fortified with first and second limiting amino acids have been reported to perform well than those fed diet higher in protein levels (Jensen and Colango, 1991). Litter condition influences bird's performance, which in turn affects the profits of producer and integrators. Dry litter helps control ammonia levels, providing a healthy flock environment, reduces the foot pad burns and breast blister. Dry litter is also important for the health and welfare of the birds as well as persons working on poultry farm. Keeping in view the above facts, the present study was conducted to study the effect of different dietary and litter management practices on immune status, carcass characteristics, serum biochemical

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parameters and behavior of broiler chicken during summer season.

MATERIALS AND METHIODS

Experiment details

Day old, 240 commercial VENCOBB-400 broiler chicks were procured from local market. Each bird was weighed on arrival and randomly assigned to twelve different dietary treatments using completely randomized design. Each dietary treatment had three replicates having twenty broiler birds in each replicate. Before housing the chicks, the pens were thoroughly disinfected using fumigants. Twenty-four-hour light was provided throughout the experimental period. Fresh and clean water were offered ad libitum.

The feeding was done in three phases *i.e.* starter (0-14 days), grower (15-21 days) and finisher (22-42 days) phase as per Anonymous (2013). For each phase, four iso-caloric and iso-nitrogenous diets were formulated as per Anonymous (2013) specification (Table 1). The corn-soybean meal based diet was formulated specifications using various ingredients procured from the local market and was given to control (T_C) and (T_L) group. In T_D and T_{LD} groups, CP was reduced by 2% and essential amino acids (Lysine, methionine, threonine) were added as per Anonymous (2013) standards thus forming 3 treatments which was be tested and compared with control group without implication of any litter or nutritional strategy (T_C) during Summer (May to June, 2016) season. All other feeding management and rearing conditions were similar for all the groups as per the standard except the nutritional strategy and litter abatement under test (Table 1). The amino acid supplementation was calculated according to nutrient requirement of animals – Poultry (Anonymous , 2013) and is shown in Table 2.

Table 1: Plan of work.

Groups	T_C	T_D	T_L	T_{LD}
Number of replications	3	3	3	3
Sub groups	T_{C1} T_{C2} T_{C3}	T_{D1} T_{D2} T_{D3}	T_{L1} T_{L2} T_{L3}	T_{LD1} T_{LD2} T_{LD3}
Number of birds	20x3=60	20x3=60	20x3=60	20x3=60
Strategy under test	Neither dietary nor litter abatement	Dietary management with reduced CP by 2% meeting essential amino acids like lysine, methionine *etc*.	Litter abatement using alum at the dose rate 90 gm/sq ft	Both dietary and litter abatement

Table 2: Ingredient and nutrient composition of broiler starter, grower and finisher rations used in experiment.

Ingredients (%)	0-14 days (Starter)		15-21 days (Grower)		22-42 days (Finisher)	
	Control (T_C and T_L)	Treatment (T_D and T_{LD})	Control (T_C and T_L)	Treatment (T_D and T_{LD})	Control (T_C and T_L)	Treatment (T_D and T_{LD})
Corn yellow	53.70	58.00	52.70	59.20	59.60	66.30
Soybean meal	38.00	32.20	36.60	31.00	31.00	25.20
Rice polish	1.50	3.50	3.00	3.00	2.50	2.50
Oil	3.00	2.50	3.90	3.00	3.60	2.70
LSP	1.00	1.00	1.00	1.00	1.00	1.00
DCP	2.50	2.50	2.50	2.50	2.00	2.00
Salt	0.300	0.300	0.300	0.300	0.300	0.300
Lysine	0.092	0.227	-	0.120	-	0.142
Methionine	0.141	0.159	0.113	0.131	0.056	0.074
Threonine	-	.06	-	.05	-	.02
Analyzed Nutrient composition						
CP %	22.06	20.08	21.50	19.50	19.50	17.50
Calcium (%)	1.02	1.04	1.02	1.04	0.93	0.91
Phosphorus (%)	0.45	0.45	0.50	0.54	0.49	0.48
Calculated value						
ME (Kcal)	3001	3004	3051	3052	3102	3104

*Additives included (per 100 kg): Liver tonic (Superlive TM) 0.25 g, Vitamin C 20 g, Choline chloride 50 g, Trace mineral 50 gm (Iron 4000 mg, Copper 0.5 g, Manganese 6000 mg, Zinc 4600 mg, Selenium 10 mg,Iodine 80 mg) Vitamin A 825000IU, Vitamin D3 165000IU, Vitamin E 500 mg, Vitamin B12 0.015 mg, Vitamin K 100 mg, Thiamine 80 mg, Riboflavin 6 mg, Vitamin B6 160 mg, Niacin 1200 mg, Biotin 0.2 mg, Folic acid 1.0 mg, TM200 25 g, Coccidiost at 50 gm.

better immunity as compared to non-litter amended groups.

On day 7th and 14th post-vaccination, four birds from each treatment were randomly picked up to obtain blood samples. 2 ml of blood was drawn by cardiac puncture and was allowed to clot at room temperature. The serum was collected and used to evaluate the antibody titre. The antibody specific for New Castle Virus (NDV) was detected in sera of chicks by means of haem-agglutination inhibition (HI) test.

On 42nd day, 4 birds from each treatment were randomly sacrificed for recording the data on carcass yield, cut-up parts using the standard procedures (Ricard and Rouvier 1967).

At the age of 3rd and 6th weeks, four birds from each treatment were randomly picked up to obtain blood samples for the evaluation of biochemical parameters. The various biochemical parameters evaluated were SGOT, SGPT, BUN, LDH, creatinine, total protein and blood glucose with the help of BPC BioSed Srl kits.

The behavioural response of the birds in all treatment groups was recorded using handy cam video recorder (SONY cyber-shot) and the responses of the birds in all the treatment groups were examined. The observations were taken for half an hour every day, twice a week throughout the experiment.

The collected data from the experiment was subjected to statistical analysis using Software Package for Social Sciences (SPSS, version 20.0) by analysis of variance (Snedecor and Cochran, 1980) to test the difference between various. The treatment means were compared by Duncan's Multiple Range Test (Duncan, 1995) at 5% level of significance ($P \leq 0.05$).

RESULTS AND DISCUSSION

The data for immune status of bird in form of antibody titre is presented in Table 3. The data for antibody titre of New Castle Disease affirmed poor performance of the control than all the treatment groups. However, the immune response of birds in the form of antibody titre among all groups did not vary significantly but it was more in the litter amended groups (T_L and T_{LD}) as compared to control and T_D groups. High antibody titre in the litter treated group might be due to low ammonia concentration in the shed and birds feels more comfortable These results are in agreement with the findings of Sahoo *et al.* (2015) and Younis *et al.* (2016) who reported that birds reared on litter amended with acidifiers have high antibody titre as compared to untreated litter group.

The data on the dressed yield and cut-up part of the meat in various treatment groups have been presented in Table 4. These data indicated that dressing percentage for different treatment varied from 59.34% to 62.53%. Though there was no significant difference between the treatment and control group w.r.t dressing percentage but numerically higher values were obtained in T_L group and T_{LD} groups than by T_C and T_D group. The yield of breast meat was higher ($P \leq 0.05$) in T_L group followed by T_{LD} group. Thigh weight was found higher ($P \leq 0.05$) in T_C than all the treatment groups. Non-significant differences were observed for rest all carcass parameters between different groups. These findings are in agreement with the results of McWard and Taylor (2000) and Proch *et al.* (2018) who noticed significant yield in carcass parameters of birds raised over acidified litter amendments.

Blood serum SGOT, SGPT and LDH enzymes concentration is an indirect indicator of antioxidant status of bird in response to any environmental stress. These activities are used an index for liver tissue integrity. The data for all the biochemical parameters is presented in (Table 5) and revealed that at the end of 3rd week SGOT concentration among different treatment groups vary from 159.50 to 163.75

Table 3: Immune status of broiler chicks under different treatments during summer season.

Age	Antibody Titre Log 2			
	T_C	T_L	T_D	T_{LD}
On 7th day post vaccination	1.48±0.22	1.84±0.47	1.34±0.24	1.92±0.16
On 14th day post vaccination	2.26±0.14	2.39±0.11	2.22±0.12	2.34±0.13

Means with different superscripts vary significantly ($p \leq 0.05$).

Table 4: Effect of various treatments on carcass parameters of broiler chicks during summer season.

Parameters	T_C	T_L	T_D	T_{LD}
Dressing %	59.72±1.01	62.53±0.85	59.34±1.25	61.28±0.62
Breast %	21.26^{b}±0.56	24.97^{a}±0.59	21.66^{b}±0.92	22.74^{b}±0.22
Drumstick %	15.69±0.49	15.12±0.28	14.83±0.22	15.17±0.23
Thigh %	14.90^{a}±0.25	14.14^{ab}±0.19	13.89^{b}±0.38	13.37^{b}±0.15
Back %	13.76±0.21	13.71±0.35	13.46±0.66	13.78±0.23
Wings %	8.26±0.49	8.76±0.55	8.51±0.67	9.05±0.64
Giblet %	7.15±0.09	7.87±0.12	7.73±0.10	7.76±0.15
Abdominal fat %	1.32±0.12	1.17±0.03	1.56±0.08	1.34±0.19

Means with different superscripts vary significantly ($p \leq 0.05$).

IU/L among different treatment groups, which was highest in T_C followed by T_L, T_D and T_{LD} group. Similar observation was found in 6th week at the end of experiment.

TP, BUN and Creatinine are the protein metabolites and indicates status of protein metabolism in response to nutrient availability and quality. The level of Creatinine, BUN and total protein were non-significantly lower in T_D and T_{LD} in which dietary protein was reduced as compared to T_C and T_L during the 3rd and 6th week. Glucose is an indicator of energy metabolism; glucose values were lower in T_D and T_{LD} groups as compare to T_C and T_L at the end of 3rd and 6th week. However, these values were in normal range. These results are in agreement with the findings of Proch *et al.* (2018) and Soliman and Hassan (2017) who stated that improvement occurs in biochemical parameters of birds reared on litter treated with some chemicals.

Table 5: Effect of various treatments on biochemical parameters of broiler chicks during summer season.

Period	Treatments			
	T_C	T_L	T_D	T_{LD}
		SGOT (IU/L)		
End of 3rd week	163.75±3.84	161.25±3.25	160.25±2.78	159.50±2.90
End of 6th week	167.75±4.21	165.75±3.66	163.25±2.50	165±3.34
		SGPT (IU/L)		
End of 3rd week	13.25±1.03	13.75±.85	13.50±1.19	13.50±1.19
End of 6th week	11.5±1.04	12.50±1.04	11.25±1.18	10.75±1.18
		Creatinine (mg/L)		
End of 3rd week	0.51±.10	0.54±.11	0.39±.12	0.47±.13
End of 6th week	0.59±.09	0.57±.09	0.44±.09	0.44±.08
		BUN (mg/L)		
End of 3rd week	13.33±1.41	12.63±1.22	12.45±1.18	12.43±1.23
End of 6th week	11.73±1.29	11.93±.87	10.33±1.03	10.33±.93
		LDH (IU/L)		
End of 3rd week	154.50±9.22	146.25±4.27	148±7.43	147±7.14
End of 6th week	143.25±9.98	145.25±9.42	145.75±8.04	142.75±6.34
		Total Protein (g/L)		
End of 3rd week	2.45±.14	2.48±.17	2.28±.19	2.25±.19
End of 6th week	2.00±.16	2.07±.18	1.53±.18	1.95±.16
		Glucose (mg/L)		
End of 3rd week	168±11.05	170.25±6.64	160.50±7.24	156.25±5.01
End of 6th week	152.25±5.66	155.50±6.24	147±6.4	154±6.26

Means with different superscripts vary significantly ($p \leq 0.05$).

Table 6: Behavioral parameters of broiler chicks under different treatments during summer season.

Behavioral activities	T_C	T_L	T_D	T_{LD}
Feeding	9.98^{c}±0.51	12.79^{a}±0.53	11.43^{b}±0.37	12.62^{a}±0.58
Drinking	2.32±0.10	2.98±0.11	2.34±0.11	2.78±0.09
Dozing	13.47±0.39	14.12±0.34	14.12±0.18	14.77±0.31
Resting	64.73^{a}±0.67	61.37^{b}±0.24	62.52^{b}±0.19	61.84^{b}±0.29
Preening	0.18±0.03	0.34±0.02	0.32±0.03	0.23±0.01
Wing flapping	0.43±0.02	0.41±0.02	0.38±0.04	0.37±0.02
Head and litter scratching	0.42±0.03	0.57±0.03	0.59±0.05	0.59±0.05
Pecking	0.13±0.01	0.15±0.01	0.15±0.01	0.14±0.01
Avoiding	0.05±0.01	0.04±0.00	0.04±0.00	0.05±0.00
Pushing	0.05±0.00	0.04±0.00	0.05±0.00	0.04±0.01
Dust bathing	0.12±0.01	0.10±0.01	0.10±0.01	0.10±0.01
Leg stretching	0.05±0.01	0.05±0.00	0.04±0.00	0.05±0.01
Neck stretching	0.10±0.00	0.12±0.00	0.11±0.00	0.13±0.00
Threats or fights	0.04±0.00	0.04±0.00	0.05±0.00	0.05±0.00
Panting	7.93±0.43	6.88±0.45	7.78±0.41	6.24±0.71

Means with different superscripts vary significantly ($p \leq 0.05$).

The feeding behavior was highest (P≤0.05) in T_L and T_{LD} group as compared to T_D and control group (Tc) (Table 6). The poorest (P≤0.05) feeding behavior was observed in control group. The expression of resting was mostly shown in the control group as compared to other treatment group. This might be due to non-engagement of birds under control groups in their other comfortable activities as compared to rest treatment groups. The panting behavior which indicates environmental stress was more in control group birds as compared to other treatment group. This might be due to the positive effect of alum sulphate in litter as it reduces the ammonia concentration of shed and birds were under less stress. The percent duration time spent on head and litter scratching was higher in treatment groups as compared to control group. Rest other behaviors like preening, wing flapping, avoiding, pushing, dust bathing, leg scratching and threats were found to be statistically similar in all groups.

CONCLUSION

The present study revealed that treatment of litter with alum sulphate helps in improving the immune status of birds. This in turn had a positive effect on birds as indicated by improved dressing percentage in litter treated groups. Alum sulphate in litter also helps in providing a comfortable environment to the broiler birds by reducing the ammonia gas in the shed. Therefore the practice of litter treatment with alum sulphate must be encouraged for beneficial broiler production.

Ethical approval

The experiment was carried out as per the code of practice approved by Institute of Animal ethics committee, GADVASU, Ludhiana-141 004, Punjab, India (Permission no: GADVASU/2016/IAEC/32/14).

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