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Effect of Low Protein Diet Supplemented with Protease Enzyme and Sodium Bisulphate in Litter on Carcass, Biochemical, Respiratory Tract Lesions and Immunity Status of Birds during Winter Season

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ABSTRACT

The quality of litter is a single major factor in deciding the emission of various harmful gases especially ammonia. Therefore a study was conducted to determine the effect of low protein diet supplemented with protease enzyme and litter amended with sodium bisulphate on carcass parameters, serum biochemical, respiratory tract lesions and immune status of birds during winter season. The experiment was conducted for six week on 240, day old broiler chicks which were randomly distributed into four treatment groups. One bearing control group (Tc) had no dietary and litter amendments and the other three included, litter amendment with sodium bisulphate (Ts), dietary amendment with low protein supplemented with protease enzyme (Tp) and both dietary and litter amendment (Tsp), each having 60 birds in three replications of 20 number. Blood sample for serum biochemical parameters were collected at 3rd and 6th week of age and at the end of experiment, four birds from each treatment were randomly slaughtered for carcass trait measurement and respiratory tract lesions. The results revealed a higher dressing and breast percentage in Ts group as compared to other treatment groups. Total protein level was also significantly higher in Ts and control group. The histopathology revealed that the gross as well as microscopic lesions revealed a better health status of chicks in the treatment groups Ts and Tsp than those in control and Tp group. The immune status was also better in the litter treated groups. Thus it can be concluded that the treatment of litter with sodium bisulphate had significant influence on carcass, serum biochemical, respiratory and foot pad lesions and immune status of birds.

Keywords: Broiler, carcass, litter, serum biochemical, sodium bisulphate

In the modern scenario, poultry farming is gaining strength with fast pace of development to attain mega commercial agro-industrial business in India. Due to changing demands of people, rapid urbanization and population growth, the broiler industry has been the most profitable agro-economic enterprise. Broilers are generally reared in deep litter system of rearing. The broiler house environment is a reflection of overall efficiency of grows out operation. The bedding material used in deep litter system absorbs moisture in order to keep litter dry and ensures a comfortable environment for birds. Wet litter results in high ammonia production which negatively affects productive performance of broilers (Ritz, 2006). Keeping litter dry is a critical part of management of any poultry farm because bird spends most of their lifetime in close contact with the bedding material. Litter quality has a major effect on health and performance of birds. Dry litter helps to control the ammonia level, provide a healthy flock environment, reduces accusation due to hock, foot pad burns and breast blisters. Uric acid and nitrogen in the litter is converted to ammonium (NH4+) by the action of microbes in litter. This ammonium is converted to ammonia depending upon the moisture content, temperature and acidity of litter. Ammonia production is favored by high temperature and high pH (Ritz *et al.,* 2004). Researchers suggested that high ammonia

concentration (>25ppm) can impair performance and immunity of bird and increase susceptibility to respiratory tract infections (Kristensen and Wathes, 2000; Nagaraja *et al.*, 1983). Increased levels of ammonia from litter cause severe irritation to respiratory tract and skin of birds resulting in pododermatitis and breast blister. There are several methods to decrease the nitrogen content in litter and ammonia produced from litter. The main methods include dietary amendment, litter amendment, adequate ventilation, use of acidifier in poultry litter and use of enzymes. Many scientists have reported that the most potent method to inhibit ammonia production in a poultry house is chemical litter amendment. Many litter additives such as alum, clay, $FeSO_4$, $NaHSO_4$ have been used to reduce litter pH, reduce ammonia volatilization and inhibit microbial load on litter. Sodium bisulphate, a dry crystalline acidifier is widely used by the broiler industry to control ammonia levels and to provide a comfortable environment to birds. It is readily soluble in water and has a pH <1. Sodium bisulphate decreases ammonia volatilization by lowering the litter pH, interacting with the uric acid and by lowering the microbial population that produces ammonia. The excretion of nitrogen in litter can also be reduced by supplementation of various enzymes in a low protein diet. The low protein diet supplemented with proteolytic enzymes like protease helps in effective utilization of available protein present in the diet. In view of above facts and figures, the present study was planned to estimate the effect of sodium bisulphate in litter and protease enzyme in a low protein diet on carcass parameters, serum biochemical, respiratory tract lesions and immune status of birds during winter season.

MATERIALS AND METHODS

The whole experiment was carried out as per the code of practices approved by Institute of Animal Ethics Committee GADVASU, Ludhiana, Punjab-141004, India (Permission no: GADVSAU/2016/IAEC/32/15).

Research Methodology

The experiment was conducted at poultry research farm of department of Livestock Production Management, Guru Angad Dev Veterinary and Animal Science University, Ludhiana. All preparation in the poultry house was made much before the arrival of chicks. After removal of all equipments and old litter, the house was thoroughly cleaned, washed and disinfected. Around two inches layer of litter of rice husk was spread all over the floor. Sodium bisulphate @ 25gm/sq.ft was mixed in the litter of treatment groups Ts and Tsp. The brooders were kept in the centre of each pen to ensure uniform heating and were switched on 24 hours before the arrival of chicks to obtain the required temperature. Day old, 240 commercial VENCOBB-400 broiler chicks with similar body weight range and average group weight were randomly divided into four groups, each having 60 birds in 3 replications of 20 birds each. In the experiment (Table 1), four various management strategies *viz*; litter manipulation with sodium bisulphate (T_S), dietary manipulation with low CP and protease supplementation (T_P) and a combination of both litter manipulation with sodium bisulphate and dietary manipulation with low CP and protease supplementation (T_{SP}), was tested and compared with control group without implication of any litter or nutritional strategy (T_C) during December 2016 to January 2017.

Table 1: Research Methodology

Number of replication	**T_C**			**T_S**			**T_P**			**T_{SP}**		
	3			**3**			**3**			**3**		
Sub groups	**T_{C1}**	**T_{C2}**	**T_{C3}**	**T_{S1}**	**T_{S2}**	**T_{S3}**	**T_{P1}**	**T_{P2}**	**T_{P3}**	**T_{SP1}**	**T_{SP2}**	**T_{SP3}**
Number of birds	**20×3 = 60**			**20×3 = 60**			**20×3 = 60**			**20×3 = 60**		
Strategy under test	No dietary or litter amendment			Litter amendment with sodium bisulphate @ 25 gm/sq.ft.			Dietary management with protease supplementation (Crude protein level will be reduced by 2% in each phase and diet will be supplemented with protease enzyme @ 15000 PROT units/Kg of feed).			Both dietary and litter amendment. $T_{SP} = T_S + T_P$		

Table 2: Ingredient composition of broiler starter, grower and finisher diet (additives*)

Ingredients (%)	0-14 days (Starter)		15-21 days (Grower)		22-42 days (Finisher)	
	(Tc&Ts)	(Tp&Tsp)	(Tc&Ts)	(Tp&Tsp)	(Tc&Ts)	(Tp&Tsp)
Corn yellow	54.20	58	52.50	59.20	59.60	66.30
Soyabean meal	38.00	32.20	36.70	31.00	31.00	25.20
Rice polish	1.00	3.50	3.00	3.00	2.50	2.50
Oil	3.00	2.50	4.00	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
Protease	—	0.10	—	0.10	—	0.10
Lysine	0.092	0.227	—	0.120	—	0.142
Methionine	0.141	0.159	0.113	0.131	0.056	0.074
Lysine %	1.20	1.20	1.08	1.06	0.93	0.93
Methionine %	0.51	0.51	0.48	0.48	0.41	0.41
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
Analyzed chemical composition						
CP (%)	22	20	21.5	19.5	19.5	17.5
ME (Kcal/Kg)	3000	3000	3050	3050	3100	3100

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

At that time mean shed temperature and relative humidity (RH) were recorded as 28.6°C & 49.34% during grower phase and 25.2°C & 62.61 % during finisher phase. The average temperature humidity index (THI) was calculated as 70.22 from the average temperature and relative humidity values prevailing in the shed for the grower phase and 68.79 during finisher phase. In both the phases of rearing i.e. grower and finisher, average THI value indicated the comfortable ambient environment for growth of broiler chicks (Silva, 2007). The ingredient composition of the diet given to different treatment group is presented in Table 2 prepared as per ICAR (2013) schedule. All other management and rearing practices were similar for all the groups.

Carcass parameters

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

Biochemical parameters

At the age of 3rd and 6th weeks, four birds from each treatment were randomly selected for collection of blood samples. The serum biochemical parameters evaluated were SGOT, SGPT, LDH, Creatinine, BUN, total protein and blood glucose by using BPC BioSed Srl kits (Roma, Italy).

Histopathological evaluation

All tissues samples viz. trachea, lungs, liver and kidney were collected and fixed in 10% neutral buffered formalin solution at the time of sacrifice. After fixation tissue samples were given overnight washings under tap water.

Then, dehydration of samples was done through ascending grades of alcohol (70%, 80%, 90%, and absolute alcohol) followed by clearing with acetone and benzene. Tissues were embedded in paraffin wax (Leica Microsystem, Paraplast tissue embedding medium, 56°C for further processing) and 4-5µ thick sections were cut. The paraffin sections were stained with routine hematoxylin technique (Luna, 1968). Slides were examined by BX6I Research Photomicrograph Microscope System of Olympus Corporation, USA. The tissues were screened for the presence of any histopathological changes in the organ system.

Immune status

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

Foot Pad Score

All the birds in each treatment were examined from 4th week at weekly interval to record the occurrence of foot pad lesion if any.

Foot Pad score

0 = normal (no burn, scab or lesion)

1 = Pad burn (dermis only)

2 = Pad scab (healing) on one or both feet

3 = Pad lesion (open sore) on one or both feet

Statistical analysis

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 among treatments. 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 on carcass yield and cut-up part of the meat in various treatment groups have been presented in Table 3. The data revealed that dressing percentage of different treatments varied from 61 to 65%. The Ts group had significantly ($p \leq 0.05$) higher dressing percentage followed closely by Tsp group then by Tc and Tp group. The yield of breast meat was also significantly ($p \leq 0.05$) higher in Ts group as compared to control group. The abdominal fat % although non significant but was numerically higher in control group as compared to other treatment groups. This might be due to the amendment of litter with sodium bisulphate because it has antibacterial and bacteriostatic properties. Similar findings were found by Karimi *et al*. (2011) and Younis *et al*. (2016) who stated that dressing percentage and giblet percentage is more in litter treated groups as compared to control group.

Table 3: Effect of various treatments on carcass parameters of broiler chicks during winter season

Parameters	Tc	Ts	Tp	Tsp
Dressing %	61.75^{b}±0.83	65.40^{a}±0.40	61.10^{b}±1.07	62.10^{ab}±2.02
Breast %	27.39^{b}±1.03	29.35^{a}±0.73	26.64^{b}±0.54	27.14^{b}±0.66
Drumstick %	14.73±0.85	14.13±0.63	14.80±0.34	14.32±0.23
Thigh %	14.91±0.34	13.60±0.18	15.42±0.16	14.37±0.3
Back %	13.79±0.37	14.12±0.18	13.90±0.24	13.88±0.24
Neck %	9.68±1.16	9.12±2.72	9.42±1.37	10.12±1.71
Wings %	9.52±0.47	9.73±0.41	9.52±0.18	9.60±0.12
Giblet %	7.65±0.35	7.78±0.23	7.94±0.26	7.99±0.23
Abdominal fat %	2.17±0.13	1.98±0.17	1.91±0.19	1.95±0.16

Table 4: Effect of various treatments on biochemical parameters of broiler chicks during winter season

Period	Treatments			
	Tc	Ts	Tp	Tsp
		SGOT (IU/L)		
End of 3rd week	167.50±2.5	146.75±4.50	163.50±6.48	132.18±2.48
End of 6th week	184.45±10.54	185.85±5.21	167.20±15.02	170.20±4.24
		SGPT (IU/L)		
End of 3rd week	20.65^{a}±4.85	16.40^{a}±2.30	12.05^{b}±1.15	13.60^{b}±1.20
End of 6th week	15.20±1.20	17.40±1.32	16.60±1.60	14.63±1.56
		Creatinine (mg/dl)		
End of 3rd week	0.53±0.27	0.71±0.45	0.47±0.02	0.57±0.03
End of 6th week	0.25±0.01	0.29±0.03	0.67±0.04	0.31±0.01
		BUN (mg/dl)		
End of 3rd week	14.49±1.15	14.66±0.52	13.79±1.53	13.01±0.79
End of 6th week	11.62±0.44	12.96±0.23	10.34±1.59	10.65±0.15
		LDH (IU/L)		
End of 3rd week	198.95±22.25	147.50±40.01	157.90±7.10	170.20±58.20
End of 6th week	149.15±13.55	151.20±22.20	153.55±12.55	143.35±28.85
		Total Protein (g/dl)		
End of 3rd week	2.78^{a}±0.11	2.81^{a}±0.12	2.51^{b}±0.19	2.56^{b}±0.16
End of 6th week	2.89^{a}±0.08	2.94^{a}±0.11	2.74^{b}±0.10	2.78^{b}±0.20
		Glucose (mg/dl)		
End of 3rd week	240.25±67.75	191.95±20.95	184.22±19.01	179.30±3.60
End of 6th week	178.10±5.80	179.65±4.65	172.55±4.65	159.05±14.65

Enzyme activities of SGOT and SGPT are an indirect assessment for the feed consumed and these activities are used as an index for liver tissue integrity. The data for all biochemical parameters is presented in Table 4 and revealed that during initial phases of growth (3rd week), the level of SGPT was significantly ($p \leq 0.05$) higher in Ts and control group as compared to Tp and Tsp group. Total protein level was also higher in Ts and control group. However in later stages of growth (6th week), SGPT level was numerically higher in Ts group as compared to all other treatment groups. The total protein level was significantly ($p \leq 0.05$) higher in Ts and control group as compared to Tp and Tsp. These findings are in concurrent with the findings of Soliman and Hassan (2017) who reported that there occurs significant improvement in biochemical parameters of the birds which were reared in litter treated groups.

The changes in the histopathology of trachea, lungs and kidney of broiler chicks is shown in Fig. 1, 2 and 3.

Tezrich *et al.* (1998) also reported significantly betterment of respiratory lesion in sodium bisulphate treated litter chicks than that of untreated groups. The reduction in respiratory tract lesions among broilers raised on litter treated groups was associated with reduction in atmospheric ammonia concentration.

The antibody titre data for immune status of the birds is presented in Table 5.

Table 5: Immune status of broiler chicks under different treatments during winter season

Age	Antibody Titre of NDV (Log 2)			
	Tc	Ts	Tp	Tsp
On 7th day post Vaccination	1.80^{b} ± 0.12	2.20^{a} ± 0.16	1.95^{b} ± 0.14	2.40^{a} ± 0.20
On 14th day post vaccination	2.20^{b} ± 0.17	2.95^{a} ± 0.25	2.30^{b} ± 0.26	3.00^{a} ± 0.12

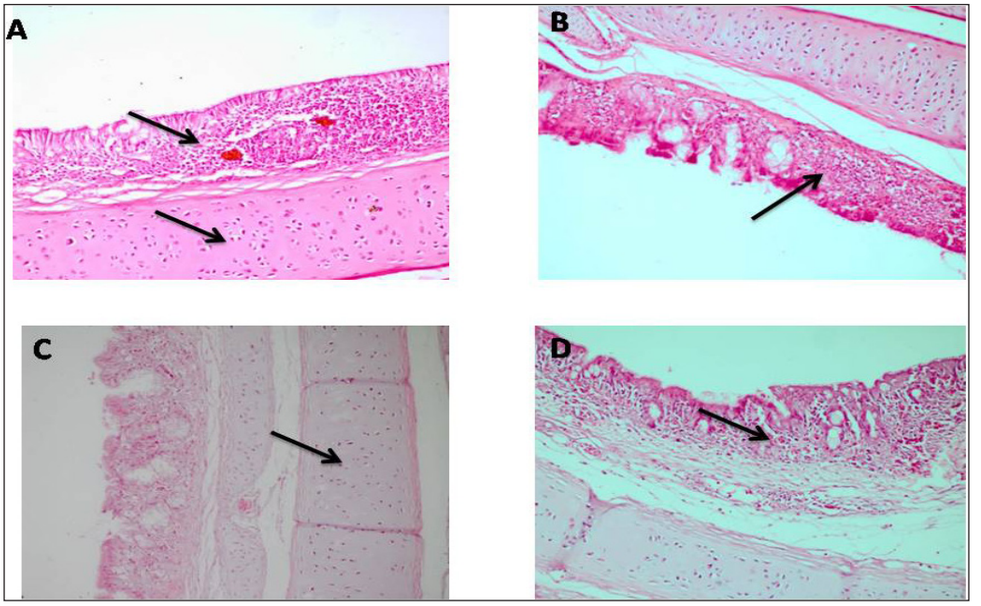

Fig. 1: Histopathology of trachea in different treatment groups during winter season. A (Tc)- Massive infiltration of inflammatory cell along with increased goblet cells; B (Ts)- Mild infiltration in the sub mucosa of trachea; C (Tp)- Increased goblet cell hyperplasia; D (Tsp). Mild infiltration of cell in sub mucosa

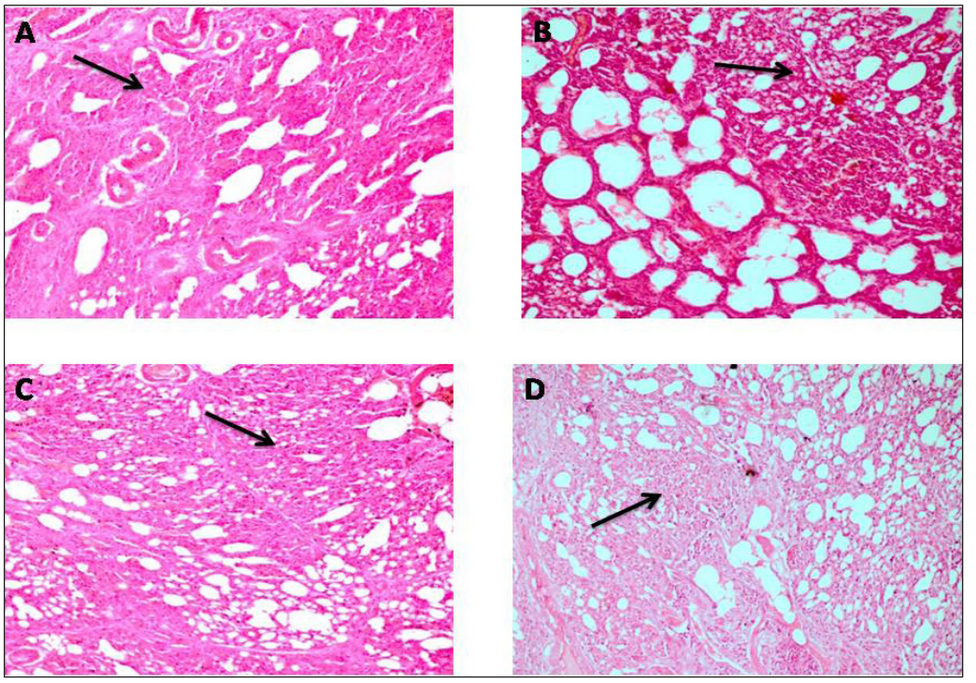

Fig. 2: Histopathology of lungs in different treatment groups during winter season. A (Tc)- Massive infiltration of cell in the interstitium; B (Ts)- Moderate infiltration of cell in the interstitium; C (Tp)- Comparatively more infiltration of cell in the interstitium; D (Tsp)- Mild infiltration of cell in the interstitium

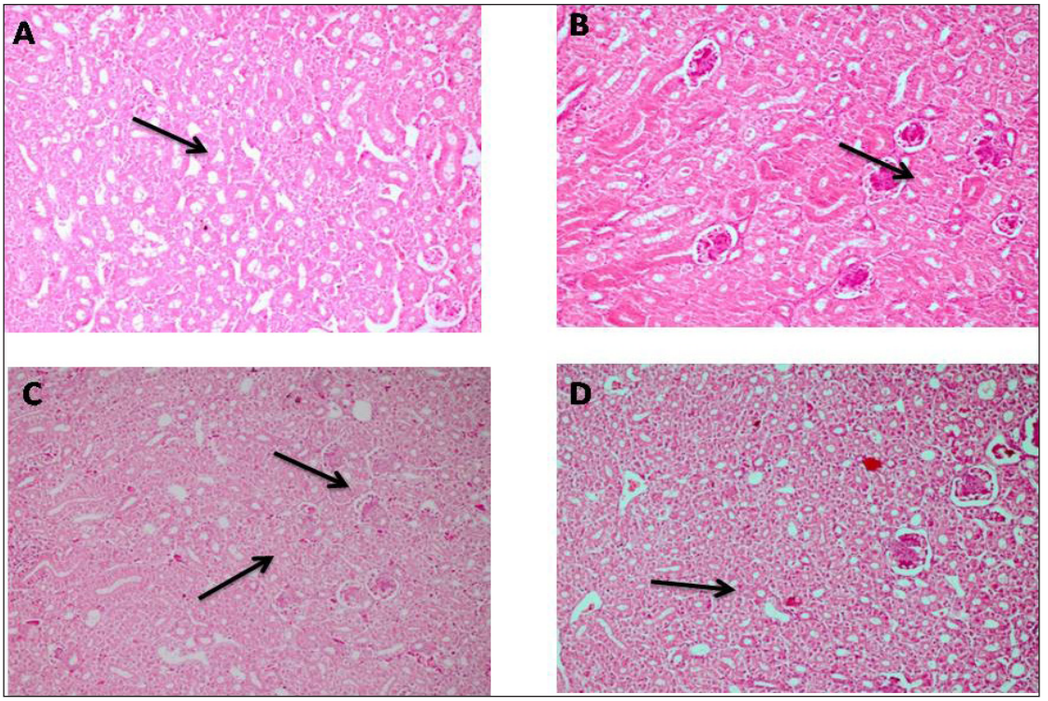

Fig. 3: Histopathology of kidney in different treatment groups during winter season. A (Tc)- Massive tubular degeneration and necrosis of kidney tubules; B (Ts)- Moderate granular to vacuolar degeneration; C (Tp)- Moderate to severe granular to vacuolar degeneration; D (Tsp)- Mild granular to vacuolar degeneration

These data revealed that at 7th day post vaccination, the antibody titre of New Castle Disease (NDV) in Ts and Tsp groups had significant ($p \leq 0.05$) higher titre as compared to Tc and Tp group. On 14th day post vaccination, both litter treated groups Ts and Tsp had significantly ($p \leq 0.05$) higher value than the control and Tp group indicating poor immune response in the later group. The results are in agreement with the findings of Sahoo *et al.* (2015) who reported that antibody titre of treated litter group was higher as compared to untreated litter group. As reported by Abbasi *et al.* (2014) diets with different CP and threonine levels had no marked effect on antibody titers against NDV.

The foot pad score numerically had lower values in litter amended groups i.e. Ts and Tsp as compared to Tp and control group (Table 6). No incidence of leg abnormality was observed in the control as well as treatment group. There was zero breast blister score in the control as well as treatment group. The cake formation in the litter was more in Tp and control group as compared to Ts and Tsp group. The results are in agreement with the findings of Nagraj *et al.* (2007) who reported that sodium bisulphate as a litter amendment numerically reduced the incidence of pododermatitis by 10 or more percentage points.

Table 6: Average foot pad score of different treatment groups during winter season

Week	T_C	T_S	T_P	T_{SP}
4th week	0.20±0.06	0.17±0.03	0.17±0.09	0.13±0.07
5th week	0.43±0.03	0.33±0.07	0.37±0.07	0.33±0.07
6th week	0.67±0.03	0.57±0.09	0.53±0.09	0.57±0.09

CONCLUSION

Addition of sodium bisulphate in litter had a significant ($p \leq 0.05$) effect on dressing and breast percentage of the birds. Low protein diet with protease supplementation had no significant effect on carcass parameters. There was no change in serum biochemical levels except SGPT and total protein levels probably by improving nutrient digestibility, mainly protein. The immune status of the birds reared

in litter treated groups had better immunity status as compared to untreated litter group. Moreover, the foot pad score was also better in the litter amended groups. So, the use of sodium bisulphate in litter is beneficial to maintain the litter quality to have a profitable broiler production.

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