Journal of Animal Research: v.7 n.5, p. 943-949. October 2017

DOI: 10.5958/2277-940X.2017.00143.7

Effect of Dietary and Litter Amendment on Litter Quality and Broiler Performance during Rainy Season

Ankush Proch[1*], D.S. Malik[1], Amit Sharma[1], Kulvinder Singh[1], A.P.S. Sethi[2], Paviter Kaur[3] and Yashpal Singh[1]

[1]Department of Livestock Production Management, GADVASU, Ludhiana, Punjab, INDIA
[2]Department of Animal Nutrition, GADVASU, Ludhiana, Punjab, INDIA
[3]Department of Veterinary Microbiology, GADVASU, Ludhiana, Punjab, INDIA

**Corresponding author: A Proch; Email: ankushproach@gmail.com*

Received: 12 Aug., 2017 **Revised:** 02 Sept., 2017 **Accepted:** 08 Sept., 2017

ABSTRACT

The study was conducted to determine the effect of low protein diet supplemented with enzyme protease along with litter amendment with sodium bisulphate to assess the effect on litter quality and broiler performance during rainy season. 240 day old Cobb broiler chicks were randomly distributed into four treatment groups, having 3 replicates of 20 chicks each. 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). Production parameters performance assessed by body weight, feed efficiency and survivability and litter parameters like litter pH, litter moisture, litter microbial count and litter nitrogen were studied. The result shows a significant ($p<0.05$) higher average body weight in Ts group (1633g) followed by Tsp group (1581g) than by Tp group (1535) in comparison to control (Tc) group (1515g) during 42 days of study. Best FCR was observed in Ts diet fed group. The survivability was more in all the treatments groups compared to control group. The overall hygiene and growth of broiler chick was better in Ts and Tsp group as compared to Tp and Tc group. It was concluded that that treatment with low protein diet along with protease enzyme supplementation recorded marginally equal body weight and FCR compared to control group however, litter amendment with sodium bisulphate shown significant improvement on the growth rate in the litter amended groups as compared to control group.

Keywords: Broiler performance, dietary amendment, litter amendment, protease, sodium bisulphate

Poultry is one of the fastest growing segments of agriculture sector in India. Eggs and chicken meat production has been growing at the rate of 8-10% per annum (Mehta *et al.,* 2003). However, this sector is accompanied by production of huge quantities of organic waste materials *viz.* faeces, urine, bedding material etc. Agriculture production and especially livestock system have been generally considered to have various negative environmental impacts, including nutrient leaching and significant contribution to global warming. The poultry production has been found to be relatively more environment friendly when compared with other livestock production systems. This does not mean, however, that poultry production systems do not have features that require special attention in terms of their environmental consequences. Poultry litter is the main source of volatilized ammonia contributing towards air, soil and water pollution. Inappropriate litter or waste management by poultry industry is a key factor which affects the rate of its emission, animal welfare, environment and human health (Beker *et al.,* 2004). Ammonia emission from litter, nitrous oxide and methane emission from poultry compost may pollute the air. A better control of nitrogen emissions, such as ammonia and nitrous oxide that contribute to global warming and nitrate leaching is one area of concern. Such emissions can occur at many stages of the poultry production chain, including the growing of crops for feed, bird housing and during manure management.

Pollution with nitrogen originating from animal production has become a major problem in several countries. Poultry excrete more nitrogen. This can be reduced by matching the amino acid composition of diet and by increasing the protein digestibility of the diet. The use of exogenous proteases can help in the reduction of the protein contents of a given feed by improving the digestibility and availability of the protein thereby reducing the cost of feed contributed by the protein feedstuff.

The nitrogen excreted from the poultry litter is the main source of obnoxious gases in a broiler house. This nitrogen excretion can be reduced by dietary supplementation of various enzymes responsible for protein digestion. The use of low crude protein (CP) diets with supplementation of protease enzyme may be used for economic advantage, improved performance of chicken, cost saving on feed, usage of less digestible protein sources and for environmental concerns. When the diets are already low or marginal in crude protein, protease enzyme can be added in the diet for effective utilization of the available protein (Robert Gauthier, 2007). Another approach may be through adequate ventilation and use of litter amendments that help in control of ammonia volatilization (Payne *et al.*, 2007). Sodium bisulphate in a dry granular form is used extensively by the poultry industry for ammonia control and litter acidification. Sodium bisulphate eliminates ammonia by converting litter ammonium to ammonium sulphate and lowers litter pH to acidify litter (McWard and Taylor, 2000). Keeping in view all of the above facts and figures, the present study is planned using low CP diet supplemented with protease enzyme and litter amendment with sodium bisulphate either individually or in combination on microclimatic condition of broiler house in conjugation with quality of litter and performance of broiler chicks.

MATERIALS AND METHODS

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

Experimental birds

Day old, commercial broiler chicks with similar body weight range and average group weight procured from Venky's India (Ltd.) were used for the experiment. The experiment involved 240, day old broiler chicks (vencobb), which were randomly assigned to four equal groups. In the experiment 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}), which was tested and compared with control group without implication of any litter or nutritional strategy (T_C), thus forming total 4 groups (Table 1). All other feeding management and rearing conditions were similar for all the groups as per the standard except the diet supplementation and litter amendment. The experiment was conducted at the Poultry Research Farm of college of veterinary science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana during rainy season (July-Sep 2016). Mean shed temperature and relative humidity (RH) were recorded as 30.9°C & 74.28% respectively during the starter phase, 29.7°C & 79.14% during grower phase and 30.1°C & 73.5% during finisher phase. The average temperature humidity index (THI) was calculated as 83.42 from the average temperature and relative humidity values prevailing in the shed for the starter phase, 82.31 during grower phase and 82.07 during finisher phase.

Weekly live weight and feed intake were recorded for calculation of weight gain and feed conversion ratio (feed/ gain in body weight), and protein efficiency ratio (gain in body weight/ protein intake) during each week.

Litter pH and Nitrogen content: 10 gm of litter sample was taken in a 100 ml beaker from individual pen at weekly interval and 50 ml of distilled water was added to it and properly stirred with a glass rod. The content was kept at room temperature for 30 minutes. Then the pH was recorded using the portable pH meter (BOECO Germany PT-380) which was calibrated using 7 and 4 standard buffer at room temperature. Litter nitrogen was estimated as per the standard protocol of AOAC International (2000).

Bacterial Count: Litter samples were collected in alternate weekly basis for bacterial count. The litter samples were collected from all the pens individually from six different locations and mixed. The bacterial load was calculated using Pour Plate Technique. Three media *viz.*

Table 1: Experiment Details

	T_C	T_S	T_P	T_{SP}
Number of replication	3 T_{C1} T_{C2} T_{C3}	3 T_{S1} T_{S2} T_{S3}	3 T_{P1} T_{P2} T_{P3}	3 T_{SP1} T_{SP2} T_{SP3}
Number of birds	60	60	60	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. $T_{SP} = T_S + T_P$
Starter CP%	22	22	20	20
Grower CP%	21.5	21.5	19.5	19.5
Finisher CP%	19.5	19.5	17.5	17.5

Mac Conkey agar (HiMedia® Mumbai)- to differentiate lactose fermenter and non-lactose fermenter, Hichrome *Escherichia coli* agar media (HiMedia® Mumbai) for the identification of *E. coli* and Brain Heart Infusion agar media (HiMedia® Mumbai) for total bacterial count (White colonies) was used. The number of colony forming units (CFU) per gram from the original aliquot/sample calculated as CFU per gram = Average number of colonies for a dilution × dilution factor.

Parasitic Load: The fecal samples were collected from each pen and screened regularly at bi-weekly interval for *Eimeria* oocysts. The fecal examination was carried out on fresh fecal material collected immediately after defecation. Simple floatation method was used to qualitative examination of faecal sample. The number of oocysts per field was counted as per Stoll's dilution method (Soulsby, 1982).

The broiler chicks under each treatment group were examined on daily basis to access the general health. Four birds were randomly picked up from each group in every week and examined for general cleanliness. The data was recorded in the form of foot pad score and breast blister score relative to litter quality of each group (McWard and Taylor, 2000).

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, 1989) 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

During the six week of experimental study, the results revealed a significant difference ($P \leq 0.05$) in average body weight of Ts and Tsp group when compared with control group i.e. Tc (Table 3). However the average body weight of Tp group was higher than control group but it was non-significant. The highest average body weight was seen in Ts group followed by Tsp, then Tp and then Tc group. The FCR in the treatment group was 1.99 in Ts, 2.04 in Tsp and 2.16 in Tp group when compared with control group which is having a FCR of 2.14. Significantly higher FCR was recorded in Ts group and Tsp groups as compare to Tc and Tp groups. Higher protein, energy and net feed consumption by the chicks of Ts group resulted in more body weight gain and because of this there was better FCR, PER, EER values which indicates higher efficiency of utilization of feed, protein and energy in Ts group as compared to control group (Tc). The FCR of Tp group was lower as compare to control group and this might be due to the low protein content of the diet given to them. Percent % survivability was recorded low in the control group (Tc) as compared to the other treatment groups. These results are in concurrence with findings of Guo and Song (2009) who reported that broilers grown on chemically treated acidified litter had better weight gain in comparison with birds raised on untreated litter. Broilers raised on using litter amendment products exhibited

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

Ingredients	0-14 days		15-21 days		22-42 days	
	Control (Tc &Ts)	Treatment (Tp*& Tsp*)	Control (Tc &Ts)	Treatment (Tp*& Tsp*)	Control (Tc &Ts)	Treatment (Tp*& Tsp*)
Corn yellow	54.2	58	52.5	59.2	59.6	66.3
Soyabean meal	38	32.2	36.7	31	31	25.2
Rice polish	01	3.5	03	03	2.5	2.5
Oil	03	2.5	04	03	3.6	2.7
LSP	01	01	01	01	01	01
DCP	2.5	2.5	2.5	2.5	02	02
Salt	0.300	0.300	0.300	0.300	0.300	0.300
Protease	—	0.12	—	0.12	—	0.12
Lysine	0.092	0.227	—	0.120	—	0.142
Methionine	0.141	0.159	0.113	0.131	0.056	0.074
CP %	22.04	20.08	21.5	19.5	19.5	17.5
ME (Kcal)	3002	3004	3055	3052	3102	3104
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

*Additives included (per 100 kg): Liver tonic (Superlive TM) 0.25g, Vitamin C 20g, Choline chloride 50g, Trace mineral 50 gm (Iron 4000mg, Copper 0.5g, 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, Coccidiost at 50gm.

Table 3: Production parameters of broiler chicks under different treatment group

Parameters	Tc	Ts	Tp	Tsp
Initial average Body weight (g)	40.04±0.01	40.03±0.00	40.03±0.00	40.03±0.00
Final average body weight (g)	1556^{a} **±5.10**	1673.13^{c} **± 4.14**	1575.30^{ab} **±2.95**	1621.93^{bc} **±3.92**
Average weight gain (g)	1515.96^{a} **±5.12**	1633.10^{c} **±4.20**	1535.27^{ab} **±2.91**	1581.90^{bc} **±3.88**
Average feed intake (g)	3339.26±36.72	3333.12±32.15	3410.12±24.18	3312.24±28.12
FCR	2.14^{c} **±0.02**	1.99^{ab} **±0.01**	2.16^{cd} **±0.01**	2.04^{b} **±0.02**
PER	2.21^{a}**±0.01**	2.39^{ab}**±0.02**	2.43^{ab}**±0.02**	2.57^{b}**±0.03**
Survivability %	96.66±2.78	98.33±1.69	98.33±1.69	98.33±1.69

significantly enhanced weight gain, better feed conversion in comparison to birds raised on untreated litter and this might be due to low ammonia production in litter treated group i.e. Ts. Moreover the addition of enzyme protease in the diet of Tp group resulted in more body weight but there was not any significant difference when compared to control group (Tc). These results are in agreement with the findings of Angel *et al*. (2011) and Rada *et al.* (2013) who reported that addition of enzyme protease regardless of its concentration in the low CP feed diet produced almost similar body weight gain when compared with high CP diet.

By the end of 2nd week the *E. coli* count was significantly higher in the control group (Tc) when compared with treatment group, the salmonella count was not significant between the control and treatment group and the total bacterial count was significantly higher in the control

group when compared with the litter treated group (Table 4). The reduction in bacterial count of the treatment group (Ts & Tsp) might be due to low pH of the litter. Moreover by the end of 4th week the *E.coli* count, salmonella count and total bacterial count was significantly ($p<0.05$) higher in control group as compared to treatment group. At the end of experiment i.e 6th week the *E.coli* count, salmonella count and total bacterial count was not significant in all the treatments (Ts, Tp & Tsp) and control group (Tc) and this might be due to the shift of pH from acidic to alkaline. Similar results were also obtained by Lines (2002) and Line and Bailey (2006) who reported that lower litter pH results in lower microbial levels in litter.

The *Eimeria* oocyst count was almost same in all the groups during the start of experiment. However with the advancement of time the count was more in the control group as compared to treatment but it was non-significant.

The present study shows a low moisture content in the treatment groups as compared to control group at the end of 2nd, 4th and 6th week of experiment period (Table 5). Among the treatment group the Tsp group has lowest moisture content. The treatment group with low protein diet and enzyme supplementation recorded less moisture compared to control group. During the whole experiment trial, the moisture content of the control group was always on the higher side. The finding was in agreement with

Table 4: Microbial load of different treatments

Periods	Parameters	Tc	Ts	Tp	Tsp
End of 2nd Week	*E. coli*, CFU in log10	1.84^{b}±0.04	1.54^{a}±0.03	1.74^{b}±0.01	1.53^{a}±0.11
	Salmonella and salmonella like microbes, CFU log 10	2.29±0.04	1.96±0.22	2.24±0.01	1.89±0.14
	TBC, CFU in log 10	2.52^{b}±0.02	1.88^{ab}±0.01	1.98^{ab}±0.08	1.61^{a}±0.13
	Parasitic count, oocyst/gm	3325±55.28	2974±48.42	3436±52.34	3102±44.20
End of 4th Week	*E. coli*, CFU in log10	2.09^{b}±0.02	1.71^{a}±0.05	1.99^{b}±0.01	1.79^{a}±0.02
	Salmonella and salmonella like microbes, CFU log 10	2.45^{b}±0.03	2.03^{a}±0.02	2.43^{b}±0.04	2.07^{a}±0.02
	TBC, CFU in log 10	2.55^{b}±0.07	2.11^{a}±0.05	2.42^{ab}±0.04	2.04^{a}±0.04
	Parasitic count, oocyst/gm	7662±45.36	4546±38.14	7543±42.14	4676±36.12
End of 6th Week	*E coli*, CFU in log10	2.18±0.01	2.11±0.02	2.16±0.03	2.13±0.02
	Salmonella and salmonella like microbes, CFU in log 10	2.45±0.06	2.41±0.16	2.45±0.08	2.43±0.12
	TBC, CFU in log 10	2.72±0.04	2.70±0.03	2.71±0.01	2.66±0.04
	Parasitic count, oocyst/gm	9459±143.70	7520±210.14	9312±160.90	7688±152.16

Table 5: Litter quality of different treatments

Period	Parameters	Tc	Ts	Tp	Tsp
End of 2nd week	pH	7.3^{b}**±0.13**	2.5^{a}**±0.08**	6.9^{a}**±0.12**	2.5^{a}**±0.06**
	Moisture %	18.48^{b}**±2.98**	14.18^{a}**±1.12**	17.72^{b}**±3.12**	13.25^{a}**±0.98**
	Nitrogen %	2.86±0.23	3.42±0.54	2.74±0.18	2.96±0.28
End of 4th week	pH	7.4^{b}**±0.15**	3.5^{a}**±0.18**	7.2^{b}**±0.08**	3.40^{a}**±0.12**
	Moisture %	38.10^{b}**±2.56**	25.16^{a}**±2.62**	36.18^{b}**±2.14**	23.13^{a}**±2.78**
	Nitrogen %	4.38±0.12	4.56±0.11	3.98±0.18	4.06±0.08
End of 6th week	pH	9.3^{bc}**±0.24**	9.0^{ab}**±0.22**	9.4^{c}**±0.18**	8.9^{a}**±0.14**
	Moisture%	39.27^{b}**±1.64**	26.18^{a}**±1.22**	34.41^{b}**±0.98**	23.98^{a}**±1.10**
	Nitrogen %	5.12±0.18	5.66±0.27	4.94±0.42	5.08±0.14

(Alleman and Leclercq, 1997), who reported that excessive dietary protein level in diet increases moisture content of litter. Alleman and Leclercq (1997) also reported that increase moisture content of litter may be due to increased heat production due to excessive dietary protein level in diet.

Statistical analysis for pH value in litter samples as influenced by different treatment was significant at 2nd, 4th and 6th week of experiment trial (Table 5). At the start of experiment the pH of litter used in all group was 7.8. The lowest pH was recorded in litter treated group (Ts and Tsp) as compared to Tp and Tc group. The acidic pH in the litter treated group was maintained up to 2nd and 4th week only. The pH of low protein diet group (Tp) was also on the lower side as compared to control group. This is in agreement with a previous study (Namroud *et al.,* 2008) which reported that low protein diet reduced the pH level there by making litter more acidic. At the end of 6th week, although the pH in the different treatment was not acidic but it was low as compared to control group. As per Carr *et al.* (1990), ammonia emission has been positively correlated with higher litter pH. However, litter pH for the litter treated groups (Ts & Tsp) were lower than the untreated control group at the end of experiment. Similar type findings were reported by Mc Ward and Taylor, (2000) while using sodium bisulphate as litter amendment.

The percentage available nitrogen is a direct indicator of the crude protein content. Statistical analysis revealed a non significant difference of nitrogen content in different treatment groups. The nitrogen content of the litter from litter treated group was higher than that of control group. The crude protein content of litter was numerically higher in the Ts group than the control group and then closely followed by Tsp group throughout the study. The nitrogen content of low protein diet group supplemented with protease enzyme was numerically less as compared to control group. The present finding was in accordance to the Sklan and Noy (2005) and O'Connell *et al.* (2006). The acidic nature of the litter did not allow the free ammonium ion to convert to ammonia resulting in more nitrogen retention. Choi and Moore (2008) also observed the improved nitrogen percentage due to litter amendment. Similarly, Burgess *et al*., 1998 reported that regardless of litter source, the treatment of litter samples with acidifier resulted in significantly higher nitrogen values.

The hygiene of broiler chicks was overall better in the treatment groups as compared to control group. There was more cake formation of litter in the control group as compared to treatment group. The foot pad score of control group was 3, followed by 2 in low protein diet group (Tp) and was 0 in litter treated group (Ts & Tsp). Haslam *et al*., 2007 reported an increase in the prevalence of foot pad dermatitis, hock burn and breast blister lesions in broiler chicken due to the decrease in litter quality.

CONCLUSION

The present study revealed that treatment with low protein diet along with protease enzyme supplementation recorded marginally equal body weight and FCR compared to control group. Low dietary protein treatment with enzyme supplementation recorded low moisture, pH and litter nitrogen values. Moreover litter amendment has also an important role on broiler growth, survivability and efficiency of feed and protein utilization. Sodium bisulphate in litter @ 25gm/sq.ft was more effective in controlling the microbial load and pH of litter. So, the use of low protein diet along with protease supplementation and sodium bisulphate in litter is beneficial to maintain the litter quality which directly influences the production of broiler.

ACKNOWLEDGEMNTS

The author thanks Guru Angad Dev Veterinary and Animal Science University, Ludhiana, India.

REFERENCES

Alleman, F. and Leclercq, B. 1997. Dietary protein and environmental temperature on growth performance and water consumption of male broiler chicken. *Br. Poult. Sci.,* **38**: 607-610.

Angel, C.R., Saylor, W., Vieira, S.L. and Ward, N. 2011. Effect of a monocomponent protease on performance and protein utilization in 7 to 22 day old broiler chickens. *Poult. Sci.,* **90**: 2281-2286.

AOAC International.2000. Official methods of analysis. 17th Ed. Pp 2004. Analytical chemists. Washington, DC: *Association of Official Analytical Chemists*.

Beker, A., Vanhooser, J.H., Swartzlander, H. and Teeter, R.G. 2004. Atmospheric ammonia concentration effects on broiler growth and performance. *J. App. Poult. Res.,* **13**: 5-9.

Burgess, R.P., Carey, J.B. and Shafer, D.J. 1998. The impact of pH on nitrogen retention in laboratory analysis of broiler litter. *Poult. Sci.,* **77**: 1620–1622.

Carr, L.E., Wheaton, F.W. and Douglass, L.W. 1990. Empirical models to determine ammonia concentrations from broiler chicken litter. *Trans. ASAE*., **33**: 1337–1342.

Choi, I.H. and Moore Jr. PA. 2008. Effects of liquid aluminum chloride additions to poultry litter on broiler performance, ammonia emissions, soluble phosphorus, total volatile fatty acids, and nitrogen contents of litter. *Poult. Sci.,* **87:** 1955–1963.

Duncan, D.B. 1955. Multiple range and multiple 'F' tests. *Biometrics,* **11**: 1–42.

Guo, M. and Song, W. 2009. Nutrient value of alum-treated poultry litter for land application. *Poult. Sci*., **88**: 1782–1792.

Haslam, S.M., Knowles, T.G., Brown, S.N., Wilkins, L.J., Kestin, S.C., Warriss, P.D. and Nicol, C.J. 2007. Factors affecting the prevalence of foot pad dermatitis, hock burn and breast burn in broiler chicken. *Br. Poult. Sci.,* **48**: 264–275.

Line, J.E. 2002. Campylobacter and salmonella populations associated with chickens raised on acidified litter. *Poult. Sci.,* **81:**1473–1477.

Line, J.E and Bailey, J.S. 2006. Effect of on-farm litter acidification treatments on campylobacter and salmonella populations in commercial broiler houses in Northeast Georgia. *Poult. Sci.,* **85**: 1529–1534.

McWard, G.W. and Taylor, D.R. 2000. Acidified clay litter amendment. *J. App. Poult. Res.,* **9**: *518*-29.

Mehta, R., Nambiar, R.G., Delgado, C.L. and Subramanyam, S. 2003. Livestock Industralization Project: Phase (II) – Policy, technical and environmental determinants and implications of the scaling up of broiler and egg production in India. Final research report of project on livestock industrialization, trade and social-health-environment impact in developing countries, *International Food Policy Research Institute*, Washington, D C.

Namroud, N.F., Shivazad, M. and Zaghari, M. 2008. Effect of fortifying low crude protein diet with crystalline amino acid on performance, blood ammonia level and excreta characteristic of broiler chicks. *Poult. Sci.,* **87:** 2250-2258.

Payne, J.B., Osborne, J.A., Jenkins, P.K. and Sheldon, B.W. 2007. Modeling the growth and death kinetics of *salmonella* in poultry litter as a function of pH and water activity. *Poult. Sci.,* **86**: 191-01.

Rada, V., Foltyn, M., Lichovnikova, M. and Musilova, A. 2013. Effect of protease supplement of low protein broiler diets on growth parameters and carcass characteristic. Mendel Net : 268-272.

Robert Gauthier. 2007. The use of protected organic acids (Galliacid^{TM}) and a protease enzyme in poultry feeds. *Brochure of Alinat Colombia Ltda & Jefo Latino America Ltda*, pp. 1-21.

Sklan, D. and Noy, Y. 2005. Direct determination of optimal amino acid intake for maintenance and growth in broilers. *Poult. Sci*., **84**: 412-418.

Soulsby, E.J.L. 1982. A text books of Helminths, Arthopods and Protozoa in domesticated animals. 7th ed. London: E.L.B.S. and Bailliere and Tindall, pp. 776.