



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(6): 1054-1058

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Received: 26-09-2018

Accepted: 27-10-2018

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Supplementation herbal choline in diets on haematological indices and development of vital organ in broilers

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Abstract

The present study was carried out to evaluate the effect of supplementation of herbal choline in diets on haematological indices and development of vital organ in broilers. Total three hundred Cobb-400 straight-run day-old broiler chicks were randomly distributed into five treatment groups with three replicates containing 20 chicks in each and reared up to 6 weeks. The group T1 (control) offered basal diet, while group T2 basal diet with choline chloride-60% @ 1 kg/ton of feed, groups T3, T4 and T5 offered basal diets with herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed, respectively. The results revealed that the values of haematological parameters at 21st day as well as internal organ weights at 42nd day in all treatment groups were statistically non-significant. The treatment group T5 showed significantly ($P < 0.01$) higher hemoglobin values when compared with groups T1, T3 and T4 groups at 42nd day. The mean values of mean corpuscular haemoglobin concentration in T2 and T5 were significantly ($P < 0.01$) increased as compared to control group. The total leucocyte and lymphocyte count was found to be significantly ($P < 0.01$) increased in treatment groups T2, T4 and T5 as compared to T1 and T3. It is thus concluded that the broiler diet supplemented with synthetic choline or herbal choline did not alter the haematological parameters at the 21st day and internal organ weight at 42nd day. However, at 42nd day the haematological values of haemoglobin mean corpuscular haemoglobin concentration, total leucocyte count and lymphocytes were significantly increased in treatment groups supplemented with synthetic choline (1 kg/ton) or herbal choline (0.500 kg/ton) compared to control group. Thus, the supplementation of synthetic choline @ 1 kg/ton or herbal choline @ 0.500 kg/ton in broiler diets showed beneficial effect on haemopoiesis.

Keywords: Herbal choline, choline chloride, haematology, organ weight, broilers

1. Introduction

Choline is a beta-hydroxy ethyl trimethyl ammonium hydroxide. It is essential for the formation of acetylcholine, a substance that makes possible the transmission of nerve impulses. Acetylcholine is an agent released at the termination of the parasympathetic nerves. The acetylcholine is responsible for transmission of nerve impulses from presynaptic to postsynaptic fibers of sympathetic and parasympathetic nervous systems. In addition, choline plays an essential role in fat metabolism in the liver. It prevents abnormal accumulation of fat (fatty livers) by promoting its transport as lecithin or by increasing the utilization of fatty acids in the liver itself [1]. Choline is thus referred as a "lipotropic" factor due to its function of acting on fat metabolism by hastening removal or decreasing deposition of fat in liver. Choline also acts as a methyl group donor, after oxidized to betaine. In poultry diet, Betaine can also be incorporated which helps to convert homocysteine to methionine in the transmethylation pathway. The use of high energy diets aim at shortening the rearing period may increase metabolic disorders such as fatty liver syndrome (FLS) in broiler chickens [2]. Increased abdominal fat pad [3], the incidence of leg problems [4] and hypertension [5] are some other detrimental responses associated with high energy diets [6].

Choline is a rediscovered critical amino acid for poultry and usually added to poultry diets in the form of synthetic choline chloride. However, synthetic choline chloride has several drawbacks. It is highly hygroscopic and the acceleration of oxidative loss of vitamins in the diet, and the formation of tri-methylamine in the gastrointestinal tract of the birds [7]. Synthetic choline chloride is very less absorbed from intestine. Supplementation of choline in poultry ration is well established to improve growth, performance and carcass quality in broilers [8]. A plant also contains choline in the form of phosphatidylcholine, free choline and

sphingomyelin. Currently there are natural products, produced from selected plants, with high content of choline in esterified form and with high bioavailability, which may be an important alternative to synthetic choline chloride. Previous work showed that these products can replace choline chloride in diets for poultry^[9, 10]. The present study was undertaken to evaluate the efficacy of herbal choline as a replacement of synthetic choline chloride in diets on haematological indices and development of internal organ in broilers.

2. Materials and Methods

2.1 Experimental design and management of birds

The experiment was conducted on three hundred 'Cobb.400' straight run day-old broiler chicks. The chicks were randomly distributed into five treatment groups with three replicates of 20 chicks in each and reared up to 6 weeks on deep litter housing system. The treatment group T1 (control) offered basal diet prepared as per National Standard^[11] without supplementation of synthetic choline chloride-60% (SC) or herbal choline (HC), group T2 offered basal diet with choline

chloride-60% @ 1 kg/ton of feed, groups T3, T4 and T5 offered basal diet with supplementation of herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed, respectively.

The standard and uniform managerial practices were followed for all treatment groups throughout the experimental period. The birds were offered *ad-lib* fresh and clean drinking water throughout the experiment. The immunization against Ranikhet Disease (B1) and Infectious Bursal Disease (IBD, standard strain) was carried out on 7th and 14th day, respectively, followed by booster doses on 18th day and 24th day through drinking water.

2.2 Procurement of Ingredients and Feed Formulation

The good quality feed ingredients were procured from local market for preparation of experimental diets. The herbal choline was procured from M/s. Vamso Biotec Pvt. Ltd., Gurgaon, Haryana, India. All the diets were prepared isocaloric and isonitrogenous. The rations were formulated as per National standards^[11] for pre-starter, starter and finisher phases as presented in Table 1.

Table 1: Ingredient (%) and nutrient composition of basal diet for pre-starter, starter and finisher feed

Ingredient (%)	Pre-starter	Starter	Finisher
Maize	52.180	53.440	57.930
Soybean meal	40.700	38.200	32.900
Vegetable oil	3.100	4.400	5.250
Dicalcium phosphate (DCP)	1.800	1.800	1.800
Limestone powder (LSP)	1.200	1.200	1.200
Salt	0.270	0.250	0.250
Trace mineral mixture*	0.150	0.150	0.150
Vitamin premix**	0.050	0.050	0.050
DL-Methionine	0.200	0.180	0.150
L-Lysine	0.050	0.030	0.020
Toxin binder (UTPP)	0.100	0.100	0.100
Coccidiostat	0.050	0.050	0.050
Sodium bicarbonate	0.150	0.150	0.150
Total	100.00	100.00	100.00
Nutrient composition (% DM)			
Metabolizable energy (kcal/kg)	3003.83	3103.04	3202.76
Crude protein (%)	23.01	22.000	20.02
Ether extract (%)	5.48	6.80	7.78
Crude fiber (%)	4.21	4.09	3.88
Calcium (%)	1.01	1.00	0.98
Total phosphorus (%)	0.70	0.69	0.67
Available phosphorus (%)	0.42	0.42	0.42
Total lysine (%)	1.31	1.22	1.08
Total methionine (%)	0.55	0.51	0.46

The treatment group T2 incorporated synthetic choline chloride 60% at 1 kg/ton of feed and treatment groups T3, T4 and T5 incorporated herbal choline at 0.250, 0.350 and 0.500 kg/ton of feed, respectively in the basal diet.

*Trace Mineral Mixture: - Each kg contains: Copper-15g, Iodine-2g, Iron-90g, Manganese-100g, Selenium-0.3g and Zinc-80g.

**Vitamin Premix: -Each 500g contains: Vit. A12.50MIU, Vit. D3-2.50 MIU, Vit. E-12g, Vit. K-1.50g, Thiamine (B1)-1.50g, Riboflavin (B2)-5g, Pyridoxine (B6)-2g, Cyanocobalamin (B12)- 0.015g, Niacin-15g, Cal D Pantothenate-10g and Folic acid-0.50g.

2.3 Haematological Indices

For haematological observations, blood samples from two birds under each replicates i.e. 6 birds from each treatment group were collected on 21st and 42nd day of experiment by random selection. About 2 ml of blood in sterilized EDTA vial was collected aseptically from wing vein. Haematological parameters studied were Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC) and Differential Leucocytes Count (DLC) as per the standard procedure described by Medway *et al.*^[12]. TEC and TLC

were carried out in Neubauer's chamber as per method described by Pierson^[13] by using Nat and Harrick diluting fluid^[14].

2.4 Development of vital organs

At the end of experiment, the development of vital organ weights of liver, spleen, heart and gizzard were measured. Two birds from each replicated i.e. six birds from each treatment were selected at the end of 6th week of age. The birds were fasted for a period of twelve hours prior to slaughter. These birds were slaughtered using the standard procedure and to measure internal organ weight in broilers.

2.5 Statistical Analysis

Data generated on various parameters was subjected to statistical analysis by using Complete Randomized Design and the treatment means were compared by critical differences [15].

3. Results and Discussion

The haematological indices of broiler were carried out on 21st

and 42nd day and are presented in Table-2 and Table-3, respectively. The internal organ weights of broilers at 42nd was measured and presented in Table-4.

3.1 Haematological Indices on 21st day

The haematological parameters analyzed on 21st day of age in broilers fed with different levels of herbal choline (Table 2).

Table 2: Haematological indices of broilers on 21st day fed different levels of herbal choline

Sr. No.	Parameters	T1 (without SC/HC)	T2 (SC@1kg/T)	T3 (HC@0.25kg/T)	T4 (HC@0.35kg/T)	T5 (HC@0.5kg/T)	CD	CV%
1	Haemoglobin (g/dl)	9.58±0.24	9.83±0.21	9.42±0.24	9.67±0.40	10.58±0.58	NS	9.06
2	Packed cell volume (%)	32.67±0.88	32.33±0.84	31.33±1.41	31.67±1.26	33.17±1.17	NS	8.60
3	Total erythrocyte count (10 ⁶ /cu mm)	3.77±0.19	3.95±0.17	3.85±0.32	3.96±0.25	4.08±0.27	NS	15.52
4	MCV (fL)	87.34±3.02	82.39±3.13	84.48±8.08	81.57±6.10	83.80±7.95	NS	17.74
5	MCH (pg)	25.62±0.86	25.09±1.02	25.43±2.33	24.93±1.98	26.83±2.90	NS	18.94
6	MCHC (%)	29.36±0.45	30.44±0.35	30.19±0.70	30.52±0.24	31.81±0.87	NS	4.59
7	Total leucocyte count (10 ³ /cu mm)	12.43±0.44	13.70±0.67	12.20±0.83	12.60±0.45	13.80±0.53	NS	11.41
8	Heterophil (%)	33.83±1.80	31.50±1.12	30.83±2.32	31.17±2.66	31.67±1.87	NS	15.58
9	Lymphocyte (%)	59.50±1.84	62.00±1.10	62.33±2.35	62.67±2.88	61.67±1.61	NS	8.14
10	Monocyte (%)	3.33±0.33	3.50±0.34	4.17±0.31	3.00±0.45	3.50±0.22	NS	23.68
11	Eosinophil (%)	2.33±0.21	2.50±0.34	2.17±0.31	2.17±0.31	2.50±0.43	NS	34.29
12	Basophil (%)	1.00±0.26	0.50±0.22	0.50±0.22	1.00±0.37	0.67±0.33	NS	95.78

NS- Non-significant, CD-Critical difference, CV-Coefficient of variance.

The data revealed that mean values of haemoglobin, PCV, TEC, MCV, MCH, MCHC, TLC, heterophils, lymphocytes, monocyte, eosinophils and basophil in all treatment groups were statistically non-significant and were within the normal physiological range. The results indicated that the choline chloride @ 1kg/ton of feed and herbal choline @ 0.250, 0.350 and 0.500 kg per ton of feed supplementation in broiler diet did not alter the haematological parameters at 21st day of age. The findings are in accordance with Onu *et al.* [16] who reported that the non significant increase of haemoglobin on feeding ginger and garlic to broiler. The normal PCV, Hb, TLC and DLC of the white blood cells in the normal range is in agreement with the findings of Thange [17] who also observed non-significant effect of *Ocimum sanctum* and other herbal preparation on monocytes, lymphocytes, esinophils and heterophils.

3.2 Haematological Indices on 42nd day

The haematological parameters on 42nd day of age in broilers fed with different levels of herbal choline presented in Table 3. The treatment group T5 receiving diet with herbal choline at 0.500 g per ton of feed showed significantly ($P<0.01$) higher hemoglobin values as compared to treatment groups T1, T3 and T4 groups. The mean values of PCV, TEC, MCV, MCH, heterophil, monocyte, eosinophil and basophil corresponding to all the different treatments differed non-significantly among different groups. The mean values of

MCHC in treatment groups T2 and T5 were significantly ($P<0.01$) increased as compared to treatment groups T1 and T3 indicating increased haemoglobin concentration which again proved on the basis of values observed in treatment groups T2 and T5. The mean values of TLC and lymphocytes count were significantly ($P<0.01$) increased in treatment groups T2, T4 and T5 as compared to treatment groups T1 and T3. The significant increase in the value of lymphocytes in treatment groups T2, T4 and T5 indicated that choline chloride and herbal choline stimulates the growth rate and functioning of the immune system in broilers. The supplementation of choline chloride @ 1 kg/ton of feed and herbal chloline @ 0.500 kg/ton of feed in broiler diet showed non-significant difference for all haematological parameters at 42nd day. The findings are accordance with Igwe *et al.* [18] reported that there was non-significant difference for PCV, MCV, MCH, MCHC and eosinophil, whereas, significantly increased total leucocyte and lymphocyte count was recorded in choline chloride supplemented groups when compared with control at the end of 6th week in broilers. Kanduri *et al.* [19] reported significantly higher haemoglobin content in treatment groups supplemented with herbal phytoadditive product and synthetic choline compared to control group diet without any supplementation of natural or synthetic source of choline, Methionine, lysine and biotin. However, PCV, TEC, and DLC revealed non significant variation in broilers.

Table 3: Haematological indices of broilers on 42nd day fed different levels of herbal choline

Sr. No.	Parameters	T1 (without SC/HC)	T2 (SC@1kg/T)	T3 (HC@0.25kg/T)	T4 (HC@0.35kg/T)	T5 (HC@0.5kg/T)	CD	CV%
1	Haemoglobin (g/dl)	9.20 ^c ±0.15	10.15 ^{ab} ±0.17	9.08 ^c ±0.29	9.47 ^{bc} ±0.28	10.85 ^a ±0.45	1.14**	7.29
2	Packed cell volume (%)	31.50±0.89	31.83±0.60	30.83±1.01	30.33±0.84	32.67±0.92	NS	6.73
3	Total erythrocyte count (10 ⁶ /cu mm)	2.92±0.15	3.14±0.08	2.69±0.07	2.98±0.31	3.06±0.12	NS	14.07
4	MCV (fL)	109.72±8.07	101.68±3.60	115.33±5.45	106.56±9.69	107.58±4.98	NS	15.24
5	MCH (pg)	32.06±2.25	32.41±1.07	33.86±1.01	33.19±2.83	35.67±1.74	NS	14.02
6	MCHC (%)	29.29 ^b ±0.75	31.91 ^a ±0.51	29.52 ^b ±0.88	31.23 ^{ab} ±0.64	33.16 ^a ±0.64	2.73**	5.47
7	Total leucocyte count (10 ³ /cu mm)	11.73 ^b ±0.35	13.45 ^a ±0.32	11.62 ^b ±0.37	13.55 ^a ±0.60	14.19 ^a ±0.41	1.67**	8.05
8	Heterophil (%)	40.67±1.05	37.33±0.76	39.67±1.65	39.33±1.17	36.50±1.73	NS	8.38
9	Lymphocyte (%)	52.83 ^b ±1.25	57.33 ^a ±0.71	52.00 ^b ±0.89	55.00 ^{ab} ±1.13	57.17 ^a ±1.47	4.42**	5.01
10	Monocyte (%)	3.17±0.48	3.17±0.48	4.50±0.56	2.83±0.40	3.17±0.31	NS	32.99
11	Eosinophil (%)	2.33±0.33	1.67±0.33	3.17±0.83	2.50±0.99	2.67±0.56	NS	66.04
12	Basophil (%)	1.00±0.37	0.50±0.22	0.67±0.33	0.33±0.21	0.50±0.34	NS	123.23

Means bearing different superscripts within a row differ significantly. ** $P < 0.01$, NS- Non-significant, CD-Critical difference, CV-Coefficient of variance.

Similar to the findings of Kumar [20] who reported that non significant differences for haemoglobin, PCV, total erythrocyte count, MCV, MCH, MCHC, total leucocyte count, lymphocyte, monocyte, eosinophil and basophil count in broilers on the supplementation of choline chloride (1.4 kg/ton) and Biocholine (0.300, 0.400 and 0.500 kg per ton) at 7th week of age. Vivian *et al.* [21], Ajit *et al.* [22] and Sakthi *et al.* [23] reported that the mean haemoglobin Hb (%), TEC ($\times 10^6/\text{cu.}\mu\text{l}$) and the mean TLC ($\times 10^3/\text{cu.}\mu\text{l}$) count was also found to be significantly ($P < 0.05$) increased in the treatment groups suggesting that the herbal preparation is found to stimulate RBC production at a slightly increased level of supplementation. However, there is no significant difference

in heterophils (%), monocytes (%), eosinophil (%) and basophil (%) values between the control groups and the treatment groups.

The above findings indicated that the increase in the vital haematological constituents like Hb (%), MCHC (%), PCV (%), RBC ($10^6/\text{cu.}\mu\text{l}$) and TLC ($10^3/\text{cu.}\mu\text{l}$) in broilers fed with the herbal choline, ginger, garlic and tulsi is an indication of improved oxygen carrying capacity of the cells which translated to a better availability of nutrients for utilization to the birds consequently affecting their well being with an active immune system.

3.3 Development of vital organs

The means for the percent organ weight to relative body weights of different treatment groups are presented in Table 4.

Table 4: Internal organ weight (%) relative to body weight of broilers at the end of 6th week fed different levels of herbal choline

Groups	Liver	Heart	Gizzard	Spleen	Proventriculus
T1	1.88 ± 0.03	0.54 ± 0.01	2.02 ± 0.12	0.12 ± 0.01	0.35 ± 0.01
T2	1.85 ± 0.04	0.58 ± 0.02	1.97 ± 0.12	0.14 ± 0.01	0.41 ± 0.02
T3	1.87 ± 0.06	0.56 ± 0.02	2.00 ± 0.05	0.15 ± 0.03	0.40 ± 0.02
T4	1.82 ± 0.07	0.55 ± 0.02	2.18 ± 0.08	0.14 ± 0.02	0.40 ± 0.02
T5	1.81 ± 0.08	0.52 ± 0.01	1.98 ± 0.12	0.13 ± 0.01	0.39 ± 0.02
CD	NS	NS	NS	NS	NS
CV%	7.87	6.99	12.47	29.47	10.25

NS- Non-significant, CD-Critical difference, CV-Coefficient of variance.

The percent liver weight was numerically increased in non-supplemented choline chloride or herbal choline supplemented group (control) compared to all other treatment groups. The data revealed that the percent of liver weight, heart weight, gizzard weight, spleen weight and proventriculus weight were non-significant differences in all treatment groups. The findings are accordance with Igwe *et al.* [18] who reported that the internal organs did not differ significantly in control group and various levels of choline used could not have interfered negatively with the muscle development. Rao *et al.* [24] observed that liver fat content decreased in broiler breeders fed a methionine deficient diet supplemented with choline. In contrast with Fouladi *et al.* [25] reported that the choline chloride supplemented with levels of 1000 and 500 mg kg⁻¹ significantly decreased the livers, spleen and hearts weight ($P < 0.0001$) in relationship to basal

diet in broilers.

4. Conclusion

It is thus concluded that the broiler diet supplemented with synthetic choline or herbal choline did not alter the haematological parameters at the 21st day and internal organ weight at 42nd day. However, at 42nd day the haematological values of Hb, MCHC, TLC and Lymphocytes were significantly increased in treatment groups supplemented with synthetic choline (1 kg/ton) or herbal choline (0.500 kg/ton) compared to control group. Thus, the supplementation of synthetic choline @ 1 kg/ton or herbal choline @ 0.500 kg/ton in broiler diets showed beneficial effect on haemopoiesis.

5. Acknowledgements

The authors are highly grateful to Associate Dean, Post Graduate Institute of Veterinary and Animal Sciences, Akola for providing necessary facilities and for their help at various stages of the experiment. The authors are also thankful to M/s. Vamso Biotech Pvt. Ltd., Gurgaon, Haryana, India for supplying the herbal choline for research.

6. References

- Xu CF, Yu CH, Xu L, Sa XY, Li YM. Hypouricemic therapy: A novel potential therapeutic option for nonalcoholic fatty liver disease. *Hepatology*. 2010; 1865-1866.
- Leeson S, Diaz G, Gonzalo J, Summers JD. *Poultry Metabolic Disorders and Mycotoxins*. University Books Publishing, Guelph, Ontario, Canada, 1995.
- Corduk M, Ceylan N, Ildiz F. Effects of dietary energy density and L-carnitine supplementation on growth performance, carcass traits and blood parameters of broiler chickens. *South African Journal of Animal Science*. 2007; 37(2):65-73.
- Van Emous RA, Kwakkel RP, Van Krimpen MM, Hendriks WH. Effects of dietary protein levels during rearing and dietary energy levels during lay on body composition and reproduction in broiler breeder females. *Poultry Science*. 2015; 94(5):1030-1042.
- Gopi M, Purushothaman MR, Chandrasekaran D. Effect of dietary coenzyme Q10 supplementation on the growth rate, carcass characters and cost effectiveness of broiler fed with three energy levels. *Springer Plus*. 2014; 3(1):518.
- Buyse J, Janssens GP, Decuypere E. The effects of dietary L-carnitine supplementation on the performance, organ weights and circulating hormone and metabolite concentrations of broiler chickens reared under a normal or low temperature schedule. *British Poultry Science*. 2001; 42(2):230-241.
- Zeisel SH, Dacosta KA, Youssef M, Hensey S. Conversion of dietary choline to trimethylamine and dimethylamine in rats: dose-response relationship. *Journal of Nutrition*. 1989; 119(5):800-804.
- Attia WA, El-Ganzory EH, Hassan RA. Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to and diet containing on different levels of choline. *Journal of Poultry Science*. 2005; 4(11):840-850.
- Muthukumarasamy B, Sahu BK, Swain RK, Samantaray DP. Studies on the effect of biocholine supplementation in commercial broilers. *Indian Journal of Poultry Science*. 2004; 39(3):246-251.
- Gangane GR, Gaikwad NZ, Ravikanth K, Maini S. The comparative effects of synthetic choline and herbal choline on hepatic lipid metabolism in broilers. *Veterinary World*. 2010; 3(7):318-320.
- BIS (Bureau of Indian Standards). *Poultry Feeds Specification*. 5th Revision, Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi, 2007.
- Medway W, Prier JE, Wilkinson JS. *A textbook of Veterinary Clinical Pathology*. The Williams and Wilkins Co. Baltimore, 1969.
- Pierson FW. *Laboratory technique for Avian hematology* In Schalm's *Veterinary Hematology*, 5th ed. Lippincott Williams and Wilkins, Landon, 2000.
- Natt MP, Herrick CA. A new blood diluents for counting the erythrocytes and leucocytes of the chicken. *Poultry Science*. 1952; 31(4):735-738.
- Snedecor GW, Cochran WG. *Statistical methods*, 8th edition. *Journal of Educational and Behavioral Statistics*. 1994; 19(3):304-307.
- Onu PN. Evaluation of two herbal spices as feed additives for finisher broilers. *Biotechnology in Animal Husbandry*. 2010; 26(5, 6):383-392.
- Thange HP. Efficacy of different herbal preparation in broiler diet for immunomodulation. Thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur, 2009.
- Igwe IR, Okonkwo CJ, Uzoukwu UG, Onyenegecha CO. The effect of choline chloride on the performance of broiler chickens. *Annual Research and Review in Biology*. 2015; 8(3):1-8.
- Kanduri AB, Saxena MJ, Ravikanth K, Maini S, Dandale M, Kokane SS. Performance assessment of broiler poultry birds fed on herbal and synthetic amino acids. *Advances in Bioresearch*. 2013; 4(3):26-28.
- Kumar V. Biocholine as a replacer of choline chloride in commercial broilers. M. V. Sc. Thesis submitted to U.P. Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan Mathura, Uttar Pradesh, India, 2004.
- Vivian UOO, Ndofo-Foleng HM, Olorunleke SO, Uguru JO. Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. *Journal of Agricultural Sciences*. 2015; 7(4):167-173.
- Ajit S, Doley P, Neeraj, Prasad J. Effect of dietary supplementation of tulsi (*Ocimum sanctum*) leaf powder on haematology and serum biochemistry of broiler chicks. *International Journal of Biological and Pharmaceutical Research*. 2014; 5(1):89-92.
- Sakthi Priya M, Gopala Krishna Murthy TR, Vijayanand T. Effect of blend herbal supplement on haematology and serum biochemistry in commercial layer chicken. *Journal World's Poultry Research*. 2017; 7(2):48-56.
- Rao SV, Sunder GS, Reddy MR, Praharaj NK, Raju MV, Panda AK. Effect of supplementary choline on the performance of broiler breeders fed on different energy sources. *British Poultry Science*. 2001; 42:362-367.
- Fouladi P, Salamat Doust Nobar R, Ahmadzade A, Aghdam Shahriar H, Noshadi A. Effect of choline chloride supplement on the internal organs and carcass weight of broilers chickens. *Journal of Animal and Veterinary Advances*. 2008; 7(9):1164-1167.