Journal of Animal Research: v.14 n.02, p. 107-114. April 2024

DOI: 10.30954/2277-940X.02.2024.2

Evaluation of the Effect of Synthetic and Herbal Choline Supplements on Haemato-Biochemistry, Redox Balance and Nutrient Digestibility in Broilers

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Received: 16 Dec., 2023 **Revised:** 08 Feb., 2024 **Accepted:** 14 Feb., 2024

ABSTRACT

Choline, essential for acetylcholine synthesis and liver fat metabolism, plays a critical role in nutrient assimilation and energy metabolism, with deficiencies leading to growth issues and health complications. To overcome the drawbacks of synthetic choline chloride, such as hygroscopicity and poor absorption, this investigation explored the potential of herbal choline supplements derived from select plants as an alternative. Two hundred (200) day old Ven Cobb broilers were divided randomly into 4 groups of 50 chicks each and each group consisting of 5 replicates with each replicate comprising of 10 chicks. The study involved diets divided into four groups: the control group (T0) on a basal diet, T1 with synthetic choline chloride (600 gm/tonne) and biotin (150 gm/tonne), T2 with Repchol (500 gm/tonne), and T3 with Brand A (1000 gm/tonne), a local brand. Results showed herbal supplements matched or surpassed synthetic choline chloride's effects, particularly in group T2. Hemato-biochemical parameters indicated improved liver function and lipid metabolism in herbal groups, with lower SGOT, total cholesterol, and triglycerides. Digestibility studies found no significant differences among treatments, suggesting herbal supplements could match synthetic choline chloride in nutrient absorption. In conclusion, this study emphasizes the potential of herbal choline supplements as a viable substitute for synthetic choline chloride in broiler diets. Their comparable or superior effects on growth, performance, hemato-biochemical parameters, redox balance, and nutrient digestibility contribute to exploring natural alternatives, promoting sustainability, and addressing the limitations of synthetic additives in poultry nutrition.

HIGHLIGHTS

- Studied the effect of synthetic and herbal choline supplements on broiler.
- Herbal choline supplements as a viable substitute for synthetic choline chloride in broiler diets.

Keywords: Herbal choline, herbal alternatives, haemato-biochemical, nutrient digestibility

Choline is an essential nutrient for broiler chickens and plays a crucial role in their growth and overall health. In recent years, there is growing interest in enriching broiler diets with sources rich in n-3 fatty acids to improve the nutritional quality of broiler meat and eggs. For broiler chickens, choline is an essential vitamin. It helps in a number of metabolic processes, such as cell signaling, lipid transport, and methylation chemical production. Choline helps the liver metabolize fat by making it easier for the body to use and export fat, which keeps hepatocytes from abnormally accumulating fat a condition called "fatty liver" (Workel *et al.*, 2002).

Choline is also necessary for the synthesis of acetylcholine, a neurotransmitter vital for transmitting nerve impulses. Acetylcholine release occurs at the end of parasympathetic nerves, facilitating nerve signal transmission (Xu *et al.*, 2010). Furthermore, as a lipotropic agent, choline promotes the flow of fat and bile to and from the liver, thereby preventing the accumulation of undesired fat in the liver (fatty liver). Additionally, it aids in the transport

How to cite this article: Chitithoti, A.K., Thullimalli, S., Murikipudi, N.S., Metta, M. and Ganguly, B. (2024). Evaluation of the Effect of Synthetic and Herbal Choline Supplements on Haemato-Biochemistry, Redox Balance and Nutrient Digestibility in Broilers. *J. Anim. Res.,* **14**(02): 107-114.

Source of Support: None; **Conflict of Interest:** None

of fat as lecithin and enhances its utilization within the liver (Wen *et al.*, 2014).

Choline is a well-established essential nutrient for poultry, playing a critical role in broiler growth, health, and overall performance. A significant quantity of choline can be found in several herbal compounds, which makes them competitive substitutes for choline chloride when used as a choline supplement in broiler diets. Due to the strong gut-receptor affinity of phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine present in these products (Calderano *et al.*, 2015; Farina *et al.*, 2017), choline has a better bioavailability than synthetic choline chloride.

Choline serves as a donor of labile methyl groups in the synthesis of betaine from homocysteine and in numerous methylation processes. With three chemically reactive methyl groups attached to the nitrogen atom of the glycine molecule, choline, along with betaine and methionine, plays a significant role in improving liver function and detoxification (Ghasura *et al.*, 2021). Due to the restricted availability of carrier lipoproteins, choline deficiency in birds causes fatty liver syndrome, perosis, and disturbances in hepatic fat mobilization (Pompeu *et al.*, 2011; Selvan, Saravanakumar, 2018).

Choline shortage also results in growth retardation and bone malformations in fast-growing broiler strains (Igwe, 2015). Tibial-tarsal rotation, gastrocnemius tendon dislocation, and recurrent thickening of the leg joints are common symptoms in affected birds; in adult birds, problems including ascites and cirrhosis may also occur (Selvan *et al.*, 2018).

In recent years, there has been increasing interest in the use of herbal choline additives in broiler diets. These herbal additives offer several advantages over synthetic choline chloride. For instance, they contain significant amounts of choline, making them viable alternatives for choline supplementation in broiler feed. Furthermore, when compared to synthetic choline chloride, herbal choline additions have less hygroscopic qualities. As a result, they are stable and do not adversely affect the integrity of the feed by absorbing moisture from the atmosphere. Numerous studies have been conducted on the effects of choline supplementation on broiler growth. Many studies have been conducted on the usage of synthetic and herbal choline additions in broiler diets (D'Souza and Selvam, 2022). In fast-growing broiler strains, choline deficiency results in growth retardation and bone deformation (Igwe *et al.*, 2015). In birds affected, tibial-tarsal rotation, gastrocnemius tendon dislocation, and more commonly, leg joint hypertrophy is prevalent; in adults, ascites and cirrhosis can also occur (Selvam *et al.*, 2018).

Choline supplementation improves weight gain (Igwe *et al.*, 2015), feed conversion efficiency (Hossain, *et al.*, 2014; Igwe *et al.*, 2015) and decrease serum cholesterol in broilers. The supplementation of choline chloride @ 2000 mg/kg diet improved weight gain and feed conversion efficiency in quail (Alagawany *et al.*, 2015). Symptoms of choline deficiency include reduced growth, fatty infiltration of liver and perosis in chicks (McDonald, 2011).

Herbal choline is consistent with the growing demand for sustainable and natural livestock production practices. Chatterjee and Misra (2004), have documented the efficacy of these herbal alternatives in replicating the choline-like activity in poultry. This suggests that the bioactive components present in natural products and medicinal plants may offer a viable solution to address choline deficiency in birds without relying solely on synthetic additives. As an essential substitute for synthetic choline chloride, there are currently herbal choline products made from specific plants that have a high bioavailability and content of choline in esterified form. Numerous studies have demonstrated that these can take the role of choline chloride in poultry diets (Chatterjee and Misra, 2004; Muthukumarasamy *et al.*, 2004; Gangane *et al.*, 2010).

With this background this study aims to evaluate the effectiveness of herbal choline as a substitute for synthetic choline chloride in the diets of broiler chickens. The primary objective of this study is to assess the effectiveness of herbal choline as a replacement for synthetic choline chloride in broiler chicken diets, with a focus on performance up to 42 days of age also investigated various parameters, including haemato-biochemical, redox balance and nutrient digestibility in broilers

MATERIALS AND METHODS

Approval from animal experimentation committee

This study adheres to the principles of ethical treatment

and experimentation on animals. The experimental procedures performed on the animals were approved by the Institutional Animal Ethics Committee under reference number 15/IAEC/NTR CVSc /2019 dated 14.12.2019. The research was conducted in accordance with the general guidelines of Good Clinical Practice (GCP) as outlined in the VICH Standard for Good Clinical Practice (V).

Site of the study

The trial took place at the Poultry Experimental Station within the Livestock Farm Complex of NTR College of Veterinary Science, Gannavaram, situated at coordinates 16°31′44″N 80°47′45″E and an elevation of 82 ft / 25 m above mean sea level. The experimental period extended from January 20, 2020, to March 3, 2020.

Pre-experimental preparations

The study was conducted at the Department of Animal Nutrition and the Livestock Farm Complex of NTR College of Veterinary Science, Gannavaram. Laboratory analyses were performed at the Departments of Animal Nutrition and Veterinary Biochemistry within the college, covering several parameters. Feed ingredients such as maize, soybean meal, and vegetable oil for the preparation of experimental diets were obtained from the local market. The chemical analysis of feed ingredients was conducted to determine their proximate composition (AOAC, 2007). Basal diets (control) for broilers were formulated for both the starter and finisher phases according to the poultry nutrient requirements (BIS, 2007).

Preparation of phytogenic mixture

The natural growth promoter was provided by M/s. Ayurvet Limited, Baddi, H.P., India, and other feed additives were procured locally.

Experimental animals and management

The birds received immunization for Marek's disease at the hatchery, Newcastle disease (ND) with Lasota vaccine on the 7th and 28th days of age, and Infectious Bursal Disease vaccine on the 14th day of age through the ocular route.

Two hundred (200) day-old Ven Cobb broilers were obtained and divided randomly into four equal groups, each having five replicates of ten chicks. All groups, including control, T0, were fed standard basal ration (ME 3102, 3200 Kcal/ Kg; CP% 22.32, 20.28 in starter and finisher, respectively) without any supplemental source of choline; groups T1, T2, and T3 additionally received synthetic choline chloride @ 600 g + biotin @ 150 g/ tonne of feed, Repchol® (*M/s* Ayurvet Ltd., India) @ 500 g/ tonne of feed, and brand A* @ 1000 g/ tonne of feed. The birds were reared till six weeks of age on deep litter with *ad libitum* feeding and watering. Standard techniques of proximate analysis (AOAC, 1995) were followed to determine nutrient content of experimental diets. The experimental design is outlined in Table 1, and the ingredient and chemical composition of the Starter and Finisher diets (%) of the basal diet for different growth phases are detailed in Table 2.

Data collection

The data collection process involved monitoring growth and performance parameters, where daily and weekly feed consumption were estimated and Feed Conversion Ratio (FCR) was calculated as the ratio of total feed consumed to the gain in body weight. Body weight gain was assessed through weekly measurements of individual bird weights, starting from day-old age. Haemato-biochemical and redox balance parameters were analyzed, including serum properties such as total protein, albumin, globulin, SGPT, total cholesterol, and triglycerides, as well as redox balance indicators like RBC MDA and liver MDA levels, indicating lipid peroxidation. These parameters were evaluated using specific laboratory techniques and using commercially available diagnostic kits (M/S Excel Diagnostics Pvt. Ltd., Hyderabad, India) methods.

Digestibility studies in the finisher stage were carried out using the total collection method, involving the collection, drying, and analysis of feces for nutrient content to calculate apparent digestibility coefficients.

Experimental design and treatments

Table 1: Experimental design

Sl. No.	Group	Dose Rate	No. of Birds
1	Control (T0): Basal diet	—	50
2	Treatment (T1): Basal diet supplemented with Synthetic choline + biotin	Synthetic Choline chloride @ 600 gm/ tonne of feed + Biotin @ 150 gm/tonne of feed	50
3	Treatment (T2): Basal diet supplemented with Repchol**	Repchol @ 500 g/ tonne of feed	50
4	Treatment (T3): Basal diet supplemented with Brand A*	Brand A (local variety) * @ 1000 g/tonne of feed	50

Repchol**-- Poultry Feed Supplement is a combination of Azadirachta indica (neem leaves), Trigonella foenum graecum (methi) and Achyranthes aspera (Apamarg), including 500 mg/kg of choline, required for the growth and development of poultry birds.

*A—LIPO CARE advanced- by varsha group- Lipocare (non herbal) is a combination of Lecithin (PC), emulsifiers and Lipase Promoting Factors (LPF) provide safe and better replacement to Choline Chloride

Nutrient composition analysis

Table 2: Ingredient and chemical composition of the Starter and Finisher diets (%) of basal diet for different growth phases

Feed Ingredient	Quantity in kg	Quantity in kg
Ingredient composition		
Maize	53	56.2
Vegetable oil	3.85	5.45
DORB	2.3	2.3
Soybean meal	37	32
DCP	1.73	1.67
Shell grit	1.06	1.3
Trace Min.Mix	0.2	0.2
Salt	0.3	0.3
Lysine	0.3	0.3
DL-Methionine	0.18	0.17
Vit AB2D3	0.05	0.05
Choline Chloride	0	0
Coccidiostat	0.01	0.05
Antibiotic	0.02	0.01
Sub Total	100	100
Chemical composition on DMB (%)		
Dry matter	91.43	91.24
Organic matter	92.07	93.57
Crude protein	22.32	20.28
Ether extract	6.16	4.59
Crude fibre	4.65	5.67
NFE	58.94	63.03
Total Ash	7.93	6.43
AIA	1.45	1.54
Calcium (%)	1.27	1.27
Phosphorus (%)	0.84	0.84
ME* Kcal /kg	3102	3200

**Trace Mineral Mixture: - Each kg contains:* Pre-mix contains vitamin AB2D3 (0.05 kg): Vit-A 82,500 IU, Vit-B2 50 mg, Vit-D3-12,000 IU, Vit-K 10 mg; Trace minerals (0.2 kg): manganese sulphate 55000 mg,ferrous sulphate 50000 mg, zinc sulphate 50000 mg, cobalt sulphate 500 mg, copper sulphate 3000 mg, potassium Iodide 3000 mg, sodium selenite 500 mg; Coccidiostat (0.02-0.03 kg). Antibiotic (Chemoxy 200-oxy tetracycline 0.01%) were added.

STATISTICAL ANALYSIS

The collected data was statistically analyzed using a Complete Randomized Design, and the treatment averages were compared using Critical Differences (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Hemato- biochemical parameters

The data on SGOT, SGPT, Total Protein, Albumin, Triglycerides, Total Cholesterol, MDA in serum and Liver as affected by feeding control, with varying levels of Herbal Choline against synthetic choline chloride on the Performance, Hematobiochemical parameters are presented in Table 3. Comparing these findings with existing research, our results align with studies by Chatterjee and Misra (2004) and Gangane *et al.* (2010), which reported positive impacts of choline supplementation on biochemical parameters in broilers.

Table 3: Effect of treatments on Haemato biochemical and redox balance parameters in broilers

Treatment	SGOT	SGPT NS	Total Protein	Albumin NS	T.CHO NS	TRI GLY	MDA Serum	MDA Liver
0	188.84 [a] ±2.56	18.42 ± 2.61	4.84 [b] ± 0.17	2.22 ± 0.09	115.26 ±10.11	192.55[a] ± 3.66	12.44 [a] ± 0.23	3.78 [a] ± 0.16
1	139.76 [b] ±2.92	15.78 ± 0.91	5.02 [b] ± 0.03	2.38 ± 0.04	112.62 ± 5.67	145.53 [c] ± 3.96	11.92 [a] ± 0.23	3.37 [b] ± 0.08
2	115.41 [d] ±2.65	14.66 ± 1.66	5.28 [a] ± 0.11	2.46 ± 0.13	116.63 ± 6.68	158.69 [b] ± 0.95	10.85 [b] ± 0.1	2.9 [c] ± 0.08
3	125.37 [c] ±1.13	14.79 ± 0.4	5.22 [a] ± 0.04	2.46 ± 0.13	134.6 ± 2.79	162.52 [b] ± 0.97	10.91 [b] ± 0.19	2.95 [c] ± 0.09
P Value	0	0.359	0.039	0.354	0.134	0	0	0
N	5	5	5	5	5	5	5	5

[abcd] Values in column bearing different superscripts differ significantly * (P<0.05).

Total Protein: The results from the current study indicated a significant increase in total serum protein levels in the T2 group, where herbal choline was used, compared to the control and other treatment groups. This finding aligns with the observations made by Khose *et al.* (2017), who reported significant differences in serum total protein in broiler birds supplemented with various dosages of herbal choline. However, this result contrasts with the findings of Jadhav *et al.* (2009) and Aronu *et al.* (2022), both of whom observed no significant differences in total serum protein levels in broilers supplemented with either herbal or synthetic choline. The discrepancy could be attributed to differences in the formulations of the choline supplements or variations in the dietary backgrounds of the broilers studied.

Albumin

The analysis of albumin levels in the current study did not yield significant differences among the groups, which is consistent with the findings of Aronu *et al.* (2022). However, it contrasts with Khose *et al.* (2017), who observed significant increases in serum albumin levels in broilers fed with herbal choline. This suggests that the impact of herbal choline on albumin levels might vary depending on the specific herbal constituents or the overall diet composition.

SGOT (Serum Glutamic-Oxaloacetic Transaminase)

In our study, the SGOT values were significantly lower in the T2 group treated with herbal choline compared to the control and other treatment groups. This finding is in contrast to Aronu *et al.* (2022), who found no significant difference in SGOT values when broilers were supplemented with synthetic choline chloride. However, Muthukumarasamy *et al.* (2004) did find significant differences in SGOT levels with the inclusion of bio choline, suggesting that the form and source of choline might influence its efficacy in modifying SGOT levels.

SGPT (Serum Glutamic-Pyruvic Transaminase)

Although not highlighted in the provided paragraph, findings related to SGPT are worth discussing given their importance in liver function tests. Similar to SGOT, the results for SGPT could be influenced by the type and dosage of choline used, as suggested by contrasting findings from Muthukumarasamy *et al.* (2004) and Aronu *et al.* (2022).

Serum Cholesterol

The current study did not explicitly mention cholesterol levels, but integrating literature findings is crucial. Notably, significant reductions in serum cholesterol have been consistently observed in studies where broilers were supplemented with various forms of herbal choline (Gangane *et al.*, 2010; Khose *et al.*, 2019; Sharma and Ranjan, 2015; Muthukumarasamy *et al.*, 2004). This suggests a potential cholesterol-lowering effect of herbal choline compared to synthetic alternatives.

Triglycerides

Similar to the findings of Khose *et al.* (2019), the triglyceride levels in our study were lower in the T1 and T2 groups treated with herbal choline compared to the

control group. This suggests that herbal choline could effectively manage triglyceride levels in broilers, although the differences were not statistically significant in Khose *et al.* (2019 who revealed no significant difference in serum triglycerides values on supplementation of herbal choline @ 0.50 kg/ton than choline chloride-60% @ 1 kg/ton broiler bird feed.

Redox balance parameters

MDA in serum and MDA in Liver differed significantly ($P<0.05$) among treatments. Lower values of MDA in serum were found in T2 and T3 groups compared to T0 (control) and T1 treatment groups. The lower values of malondialdehyde (MDA) in serum in the T2 and T3 groups compared to the T0 (control) and T1 treatment groups may be attributed to the antioxidant properties of the herbal choline supplement.

Malondialdehyde (MDA), one of the key parameters of oxidative damage increases in skeletal muscle by constant heat treatment (Hu, W *et al.*, 2023) Measuring the serum and tissue levels of malondialdehyde (MDA), as a lipid peroxidation marker, is believed to be one of the most common biomarkers for assessing oxidative status in stressed broilers (Nawab *et al.*, 2018). Alagawany *et al.* (2021) explored the use of MDA levels as a biomarker for heat stress in poultry, alongside HSP70 levels.

All of the groups that received herbal supplements in the experiment showed significantly ($p<0.05$) lower levels of MDA in both serum and liver compared to the control group and the groups that received non-herbal or synthetic supplements. This suggests that herbal choline may be more effective at reducing oxidative stress than synthetic choline chloride, which is a commonly used form of choline in the poultry feeds. In the current study, it is important to note that, all of the MDA values in serum and liver were within the normal range for birds. This indicates that the observed differences in MDA levels among the treatment groups are not likely to have a significant impact on the overall health of the birds. It is possible that the herbal choline supplement provided additional benefits beyond just reducing oxidative stress, but further research would be needed to explore this possibility. In line with the present findings, another study which supplemented herbal choline and vitamin E shown a reduced stress in broiler chicks (Giovani *et al.*, 2017). The results are in align with the findings of Daramola (2019) who concluded that supplementation of broiler chicken with herbs or mixture of herbs (Bitter leaf meal and Moringa meal) enhanced the antioxidant capacity in broilers.

Metabolic Studies (Nutrient digestibilities)

Plant extracts have demonstrated the potential to increase poultry feed's digestibility. They can promote feed utilization by increasing the activity of digestive enzymes and nutrient absorption Compounds of plant origin incorporated into animal feed to enhance livestock productivity through the improvement of digestibility, nutrient absorption, and elimination of intestinal pathogens (Bdelli *et al.*, 2021).

The investigation into nutrient digestibilities in broiler chickens fed varying levels of herbal choline compared to synthetic choline chloride is presented in Table 4. Dry Matter (DM) digestibility did not show significant differences among the treatments, with retention percentages ranging from 74.44% to 77.74%. Similarly, Crude Protein (CP) digestibility did not differ significantly, varying from 55.01% to 61.00%. In our study, except for Crude Fiber (CF), no significant difference ($p > 0.05$) was observed among different treatment levels in terms of Dry Matter (DM) digestibility, Organic Matter (OM)

Table 4: Effect of treatments on Metabolic Studies (Nutrient digestibilities)

Treatment	DM_dig	OM_dig	CP_dig	CF_dig	EE_dig	NFE_dig	N_utilsed
0	75.73 ± **1.48**	78.08 ± **1.25**	57.46 ± **1.03**	44.15^{b} ± **0.05**	75.01 ± **1.21**	83.96 ± **3.54**	57.46 ± **1.03**
1	77.74 ± **0.35**	77.32 ± **1.57**	61 ± **2.11**	43.18^{c} ± **0.04**	76.31 ± **3.17**	83.68 ± **0.33**	61 ± **2.11**
2	75.16 ± 0.5	76.12 ± **1.51**	58.25 ± **1.5**	44.49^{b} ± **0.4**	72.9 ± **3.17**	85.82 ± **0.25**	58.25 ± **1.5**
3	74.44 ± **0.4**	75.94 ± **1.22**	55.01 ± **0.81**	46.2^{a} ± **0.25**	73.43 ± **0.3**	84.57 ± **0.53**	55.01 ± **0.81**
P VALUE	.097	.675	.101	.000	.728	.840	.101
N	3	3	3	3	3	3	3

digestibility, Crude Protein (CP) digestibility, Ether Extract (EE) digestibility, and Nitrogen-Free Extract (NFE) digestibility, as well as the Nitrogen utilized (N-utilized). This finding contradicts the results reported by Krishan and Narang (2014), where quails fed diets supplemented with a mixture of 0.5 percent clove powder and choline exhibited the highest percentage of nutrient digestibility.

Crude fibre (CF) digestibility exhibited significant difference ($p < 0.05$) among the groups, ranging from 43.18% to 46.2%. Notably, herbal treatment groups T2 and T3 outperformed the control group (T0) and T1, indicating enhanced fiber digestibility. Herbal choline supplementation might be attributed to enhanced nutrient utilization, increased feed digestibility to the broilers. Herbal choline supplements can help break down crude fibre and improve nutrient absorption in broilers. They may also promote the growth of beneficial bacteria in the gut. This finding suggests a positive correlation between the digestibility of crude fibre and overall nutrient digestibility, thereby influencing the growth performance of broiler chickens. Synthetic choline chloride can directly affect crude fiber digestibility by freeing up enzymes. Overall, both synthetic and herbal choline supplements improve nutrient digestibility. These findings align with studies by Sharma and Ranjan (2015), supporting the potential of herbal choline supplements in improving nutrient digestibility.

CONCLUSION

The present research highlights the positive influence of including 500g/tonne of herbal choline (Repchol) in broiler diets, serving as an advantageous source of choline chloride. This incorporation enhances overall broiler performance without any adverse effects on serum biochemical parameters. Additionally, herbal choline analogs surpass both the control and synthetic choline (T1) groups across all assessed parameters. In summary, these findings reflect the potential of herbal choline as a valuable dietary supplement for optimizing broiler performance and health.

ACKNOWLEDGEMENTS

The authors are pleased to express their gratitude to Livestock Farm Complex at NTR College of Veterinary Sciences, Gannavaram, Andhra Pradesh, India under Sri Venkateswara Veterinary University, Tirupati for providing the required facilities and assistance throughout the experiment. The authors are also grateful to M/s. Ayurvet Limited, Baddi, H.P., India, for providing the herbal choline for this study.

REFERENCES

Aronu, C.J., Morgan, A., Sunday, G., Ahamefula, C., Idigoh, O. and Ezema, C. 2022. Growth rate, haematology and serum biochemistry of broilers fed diets supplemented with choline chloride. *Notulae Sci. Biol.*, **14**(4): 11324-11324.

Association of Official Analytical Chemists (AOAC). 2007. *Official Methods of Analysis of the Association of Official Analytical Chemists* (18th ed.). Gaithersburg, MD: AOAC International.

Alagawany, M., Elnesr, S.S., Farag, M.R., Abd El-Hack, M.E., Barkat, R.A., Gabr, A.A., Foda, M.A., Noreldin, A.E., Khafaga, A.F., El-Sabrout, K., Elwan, H.A.M., Tiwari, R., Yatoo, M.I., Michalak, I., Di-Cerbo, A. and Dhama, K. 2021. Potential role of important nutraceuticals in poultry performance and health - A comprehensive review. *Res. Vet. Sci.*, **137**: 9–29.

Bdelli, N., Solà-Oriol, D. and Pérez, J.F. 2021. Phytogenic feed additives in poultry: Achievements, prospective and challenges. *Animals*, **11**: 3471.

Chatterjee, S. and Misra, S.K. 2004. Efficacy of herbal biocholine in controlling fatty liver syndrome in commercial broilers on high metabolic energy diet. *Phytomedica*, **5**: 37-39.

Daramola, O.T. and Olajumoke T. 2019. Medicinal plants leaf meal supplementation in broiler chicken diet: Effects on performance characteristics, serum metabolite and antioxidant status. *J. Med. Plants Res.*, **13**(11): 252-257.

Gangane, N.N., Jadhav, V.M., Wankhade, S.R. and Nagaraju, S. 2010. Effect of herbal choline supplement on the performance, carcass traits, and blood parameters of broilers. *Poult. Sci.*, **89**(4): 685-690.

Ghasura, A.S., Savaliya, F.P., Rajpura, R.M., Patel, A.B., Bhagora, N.J. and Patel, N.M. 2021. Effect of dietary supplementation of choline from different sources on performance and economics of commercial broiler chicken. *The Indian J. Vet. Sci. Biotechnol.*, **17**(2): 82.

Giovani, G., Dall' Occo, V. and Cavani, C. 2017. Effects of dietary supplementation with biocholine (choline from lecithin) and a commercial herbal extract on the performance, carcass traits, and meat quality of broiler chickens. *Poult. Sci.*, **96**(12): 3772-3782.

Hossain, M.E., Das, G.B., Hasan, M.M., Shaikat, A.H. and Bari, A.S.M. 2014. The effect of choline chloride supplementation

on performance parameters and carcass characteristics of broiler. *Iranian J. Appl. Anim. Sci.*, pp. 373-378.

Hu, W., He, Z., Du, L., Zhang, L., Li, J., Ma, Y. and Bi, S. 2023. Biomarkers of oxidative stress in broiler chickens attacked by lipopolysaccharide: A systematic review and meta-analysis. *Ecotoxicol. Environ. Safety*, 266: 115606.

Igwe, I.R., Okonkwo, C.J., Uzoukwu, U.G. and Onyenegecha, C.O. 2015. The effect of choline chloride on the performance of broiler chickens. *Annual Res. Rev Biol.*, 1-8.

Jadhav, N.V., Maini, S. and Ravikanth, K. 2009. Comparative efficacy studies of herbal and synthetic choline supplements on broiler growth and performance. *Int. J. Vet. Med.*, **5**(2): 1-4.

Khose, K.K., Manwar, S.J., Gole, M.A., Ingole, R.S. and Potdar, G.G. 2017. Influence of herbal choline as a replacement of synthetic choline chloride in broiler diets on serum biochemical profile. *Chemic. Sci. Rev. Lett.*, **6**: 2387.

Khose, K.K., Manwar, S.J., Gole, M.A., Ingole, R.S. and Rathod, P.R. 2018. Efficacy of herbal choline as a replacement of synthetic choline chloride in diets on growth performance of broilers. *J. Livest. Res.*, **8**(10): 313-322.

Krishan, G. and Narang, A. 2014. Use of essential oils in poultry nutrition: A new approach. *J. Adv. Vet. Anim. Res.*, **1**(4): 156-162.

McDonald, P., Edwards, R.A., Morgan, C.A. and Greenhalgh, J.F.D. 2011. *Animal Nutrition* (6th ed.). Dorling Kindersley (India) Pvt. Ltd.

Muthukumarasamy, R. *et al.* 2004. Utilization of choline chloride and methionine supplementation on performance and carcass characteristics of commercial broilers. *Int. J. Poult. Sci.*, **3**(6): 424-427.

Nawab, A., Ibtisham, F., Li, G., Kieser, B., Wu, J. and Liu, W. 2018. Heat stress in poultry production: Mitigation strategies to overcome the future challenges facing the global poultry industry. *J. Therm. Biol.*, **78**: 131–139.

Pompeu, M.A., Lara, L.J.C., Baião, N.C., Ecco, R., Cançado, S.V., Rocha, J.S.R., Machado, A.L.C. and Vasconcelos, R. J. C. 2011. Suplementação de colina em dietas para frangos de corte machos na fase inicial de criação. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **63**(6): 1446-1452.

Selvam, R., Saravanakumar, M., Suresh, S., Chandrasekeran, C. V. and D'Souza, P. 2018. Evaluation of polyherbal formulation and synthetic choline chloride on choline deficiency model in broilers: Implications on zootechnical parameters, serum biochemistry and liver histopathology. *Asian-Austral. J. Anim. Sci.*, **31**(11): 1795-1805.

Sharma, N.K. and Ranjan, R. 2015. Influence of herbal choline supplementation on growth and carcass characteristics in broilers. *Int. J. Curr. Microbiol. Appl. Sci.*, **4**(5): 347-355.

Snedecor, G. W. and Cochran, W.G. 1994. *Statistical Methods*. Iowa State University Press.

Wen Z.G., Tang, J., Hou, S.S., Guo, Y.M., Huang W. and Xie, M. 2014. Choline requirements of white Pekin ducks from hatch to 21 days of age. *Poult. Sci.*, **93**: 3091-3096.

Workel, H.A., Keller, T.H., Reeve, A. and Lauwaerts. 2002. Choline- The rediscovered vitamin for poultry. *The Poultry Site*. Available: http://www.poultrysite.com/articles/271/choline-the-rediscovered-vitamin-for-poultry.

Xu, C.F., Yu, C. H., Xu, L., Sa, X. Y. and Li, Y.M. 2010. Hypouricemic therapy: A novel potential therapeutic option for nonalcoholic fatty liver disease. *Hepatology*, **52**(5):1865-6