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# Effect of Vincholine Supplementation on CHDH, PEMT-1, BHMT and MAT1 Gene Regulation in Commercial Broilers

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**A B S T R A C T**

Choline is an essential nutrient for broiler chickens, vital for lipid metabolism, methyl group donation, and liver health. This study examined the impact of Vincholine, a natural choline source developed by Vinayak Ingredients (India) Pvt. Ltd., on the expression of key genes—CHDH, PEMT-1, BHMT, and MAT1 in broilers. Birds were assigned to three groups: a control group fed a basal diet with synthetic choline chloride, and two treatment groups receiving 300 g/tonne (T1) and no choline chloride (T2), respectively, over 42 days. Gene expression was measured via real-time PCR from liver samples. The T1 group showed significantly lower expression of all four genes compared to the T2 group, indicating better choline availability and utilization. Expression levels in T1 were close to those of healthy birds, suggesting a hepatoprotective effect. These results suggest Vincholine supplementation may support liver health and improve choline metabolism in broiler production.

## Introduction

Choline is an essential micronutrient with various roles in animal metabolism, especially in rapidly growing commercial broiler chickens. As a precursor to phosphatidylcholine (PC), a key part of very low-density lipoproteins (VLDL), choline is vital for lipid transport from the liver to peripheral tissues (1). Without sufficient dietary choline, lipid export is blocked, leading to fat accumulation in the liver and the development of fatty liver syndrome, a common issue in high-energy poultry diets (2).

Beyond its lipotropic function, choline serves as a crucial methyl group donor, supporting the production of methionine, DNA, and histone methylation, as well as overall protein synthesis. These processes are vital for the fast tissue growth and metabolic activity typical of modern broiler breeds. Choline is also essential for creating acetylcholine, a neurotransmitter necessary for neuromuscular function, and for maintaining the structural integrity of cell membranes. Additionally, choline has been linked to better feed efficiency, higher carcass yield, and the prevention of skeletal issues such as perosis, through its role in cartilage and bone development. At the molecular level, several genes control the metabolic processing of choline and its derivatives. These include choline dehydrogenase

(CHDH), phosphatidylethanolamine N-methyltransferase (PEMT-1), betaine-homocysteine methyltransferase (BHMT), and methionine adenosyltransferase 1 (MAT1). The CHDH gene encodes a mitochondrial enzyme that oxidizes choline to betaine, which acts as both a methyl donor and an osmolyte (6). This pathway is crucial for maintaining a balance of methyl groups and liver health during metabolic stress.

The PEMT-1 gene facilitates the methylation of phosphatidylethanolamine (PE) to form PC, using S-adenosylmethionine (SAM) as a methyl donor. This alternative pathway for PC biosynthesis is vital for lipid transport and cellular membrane formation in broilers (7). The BHMT gene encodes an enzyme that remethylates homocysteine back to methionine using betaine, thereby supporting methionine regeneration in diets where methionine is often a limiting amino acid (8). Finally, the MAT1 gene encodes methionine adenosyltransferase, which converts methionine into SAM—the essential methyl donor in many transmethylation reactions necessary for cellular function, growth, and epigenetic regulation (9).

Collectively, these genes operate within a tightly regulated network that controls choline metabolism, lipid transport, and methylation processes. Understanding the expression and regulation of CHDH, PEMT-1, BHMT, and MAT1 is crucial for optimizing nutrient utilization, preventing metabolic disorders, and supporting the high growth performance expected in commercial broiler production systems. The current trial was conducted to evaluate the effect of Vincholine, a natural choline formulation developed by Vinayak Ingredients (India) Pvt. Ltd., at various concentrations in commercial broiler birds.

## Materials and Methods

## Bird, dosing, and sampling

Day-old broiler chicks were obtained from a reputable hatchery and housed at Omega Laboratories, Satara, India. The birds were cared for and handled in accordance with national and international ethical guidelines. Standard brooding, feeding, and watering practices were followed throughout the trial's duration. All birds were vaccinated according to an appropriate and recommended vaccination schedule.

The broiler birds were randomly assigned to three groups: a control group given a basal diet with synthetic choline chloride and two treatment groups. Treatment Group 1 received 300 g/ton of Vincholine (herbal choline), while Treatment Group 2 did not receive choline chloride. The control group was fed the standard basal broiler diet throughout the study. The experiment lasted 42 days. Weekly observations included measuring average body weight, feed intake, and feed conversion ratio (FCR), as well as monitoring for clinical signs, morbidity, and mortality. At the end of the 42-day trial, five birds from each group were humanely sacrificed for gene expression analysis. Liver samples were collected aseptically, immediately placed on dry ice, and subsequently stored in a deep freezer for mRNA extraction and further molecular analysis.

## RNA isolation

Total RNA was isolated from liver samples from each group using the GETTM Total RNA extraction kit (GBiosciences, USA; Cat No. 786-132). Extraction was performed following the manufacturer's instructions. Briefly, add 1 mL of lysis buffer for every 100 mg of tissue. Homogenize using a Dounce hand homogenizer. Incubate the homogenized samples at room temperature for 5 minutes to allow complete dissociation of the nucleoprotein complex. Add 200 µL of chloroform per 1 mL of lysis buffer used for homogenization. Cap the tube securely and vortex for 15 seconds. Incubate for 3-5 minutes at room temperature. Centrifuge the sample for 10 minutes at 12,000 rpm at 2-8°C. RNA remains solely in the aqueous phase. Pipette the aqueous phase into a new tube.

Preparation of RNase-Free Collection Column: Add 500 µL of Balance Buffer to the collection column tube and incubate for 2 minutes at room temperature. Then, centrifuge for 2 minutes at 12,000 rpm at 4°C. Discard the flow-through. Add 200 µL of ethanol to the aqueous phase from step 4, vortex, then transfer the aqueous phase to the collection column. Add 600 µL of Wash Buffer to the collection column tube and centrifuge for 2 minutes at 12,000 rpm at 4°C. Discard the flow-through. Add 500 µL of Wash Buffer to the collection column tube and centrifuge for 2 minutes at 12,000 rpm at 4°C. Discard the flow-through. Centrifuge for 2 minutes at 12,000 rpm, then discard the collection tube. Incubate the spin column membrane for a few minutes to allow it to dry. Ensure no ethanol remains during RNA elution.

Place the RNase-Free spin column into a new 1.5 mL collection tube. Add 50-100 µL of RNase-free water directly onto the spin column membrane. Incubate for 5 minutes at room temperature and centrifuge for 2 minutes at 12,000 rpm to elute the RNA.

## Quality of RNA

RNA concentration was measured using the Take3 plate on an Epoch instrument (BioTek, USA). The RNA concentration should exceed 1000 ng for cDNA preparation. A total of 2000 ng of RNA was used to synthesize cDNA. The absorbance ratio (260/280) should be between 1.9 and 2.0 for RNA samples.

## First strand cDNA synthesis

The RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Cat. No. 4368814) according to the manufacturer's instructions. Briefly, the purified RNA was incubated at 42°C for 2 minutes in the genomic DNA Wipeout Buffer to remove any contaminating DNA. After this, reverse transcription was performed using a master mix composed of Quantiscript Reverse Transcriptase, RT Buffer, and RT Primer Mix.

Quantiscript Reverse Transcriptase has a high affinity for RNA and is optimized for efficient, sensitive cDNA synthesis from 10 pg to 2 µg of RNA. The reaction occurs at 42°C and is inactivated at 95°C. Unlike other methods, this process does not require additional steps such as RNA denaturation, primer annealing, or RNase H digestion.

## Real Time PCR amplification

The primer listed in Table 1 was used in conjunction with the following PCR protocol: an initial activation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 1 minute. The GAPDH gene was amplified separately as an endogenous control to quantify the mRNA target. The baseline and threshold for fluorescence signals were manually set for each detector, allowing calculation of the cycle threshold (Ct) value for each sample. Each group was tested with five replicates, including the endogenous control and a no-template control (NTC).

The expression levels of the target genes were analyzed using the base 2 logarithm-transformed $2^{-\Delta Ct}$ values. Results are presented as fold changes calculated with the $2^{-\Delta\Delta Ct}$ method (10).

Pairwise comparisons were then conducted using GraphPad Prism Software (Version 6.01) with the General Linear Model, and the results are shown as mean ± standard deviation. Differences were deemed significant at $P \leq 0.05$.

## Results and Discussion

Gene expression of CHDH, BHMT, MAT-1, and PEMT was assessed between the fatty liver control group (T2) and treatment group (T1), as well as between normal chicken liver (Ctrl) and fatty liver control (T2). Five samples per group were analyzed.

All four genes showed decreased expression levels by 0.832 ± 0.138, 0.633 ± 0.198, 0.465 ± 0.08, and 0.8 ± 0.181 fold, respectively, compared to the no-synthetic choline chloride T2 group (Figure 1).

BHMT and MAT-1 gene expressions in T1 were significantly ($p \leq 0.05$) lower than in T2 controls. The dotted line indicates the T2 group's expression level, standardized at 1. In contrast, the no-synthetic choline chloride T2 group displayed significantly ($p \leq 0.05$) higher expression levels of CHDH, BHMT, MAT-1, and PEMT (1.601 ± 0.561, 1.374 ± 0.31, 1.285 ± 0.488, and 1.448 ± 0.515, respectively), compared to untreated normal chicken liver (Figure 2).

All CHDH, BHMT, MAT-1, and PEMT genes play key roles in choline metabolism. Choline is used as a treatment for metabolic problems related to fat metabolism or conditions such as fatty liver. When dietary synthetic choline intake increases, these genes quickly recycle free choline, preventing its levels from staying high and reducing its pharmacological effects (11).

Feeding the T1 group helps lower the expression of these four genes, allowing free choline to serve its therapeutic purpose in liver issues or fatty liver disease. This is supported by comparing gene expression in normal chicken (control) liver to that in the fatty liver (T2) group, where these genes were significantly upregulated (12). After T1 treatment, their expression levels decreased significantly, approaching normal levels.

**Table.1** Primers used in this study

| Gene Name | Forward primer | Reverse Primer |
|---|---|---|
| **CHDH** | GGCTGACTGAAGACCCTCAC | TCGGGGCCAGTACATAATTC |
| **MAT1** | TCATACCAGTGCGTGTCCAT | CTGAGGCCCTCCAATAACAA |
| **PEMT-1** | CCCTTCAGCATCCTGGATAA | GCTCCCTTCTGTTTCTGACG |
| **BHMT** | GCTTGTGACATTGCCAGAGA | GCCCAGACAGCTTCTTCAAC |
| **GAPDH** | CCCAGCAACATCAAATGGGC | TCACAAACATGGGGGCATCA |

**Figure.1** Differential expression of CHDH, BHMT, MAT-1, and PEMT genes in T1 compared to T2 region in commercial broilers.

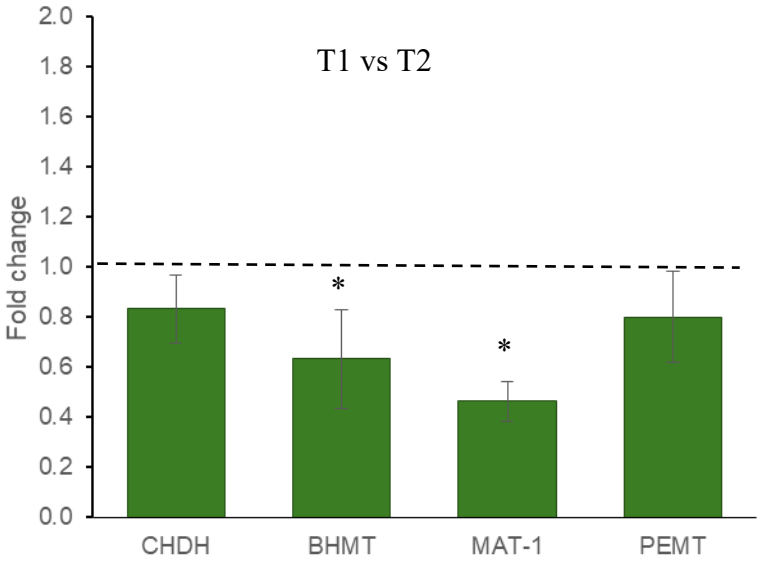



**Figure.2** Differential expression of CHDH, BHMT, MAT-1, and PEMT genes in the T2 group compared to the control ration group in commercial broilers.

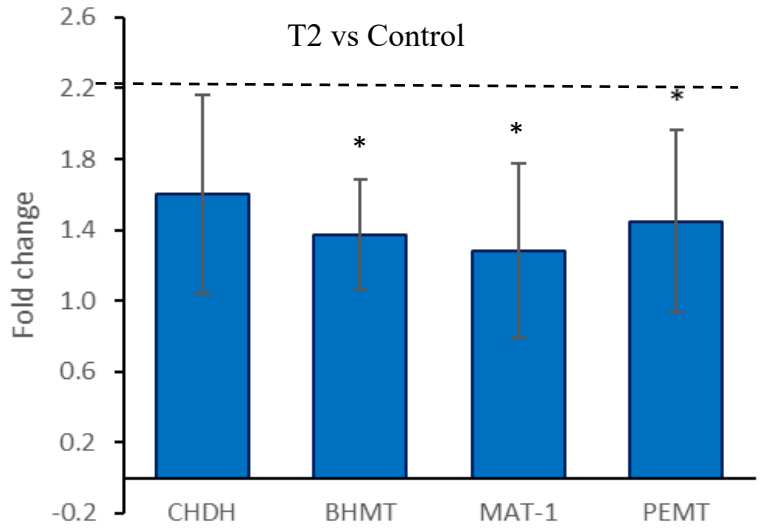
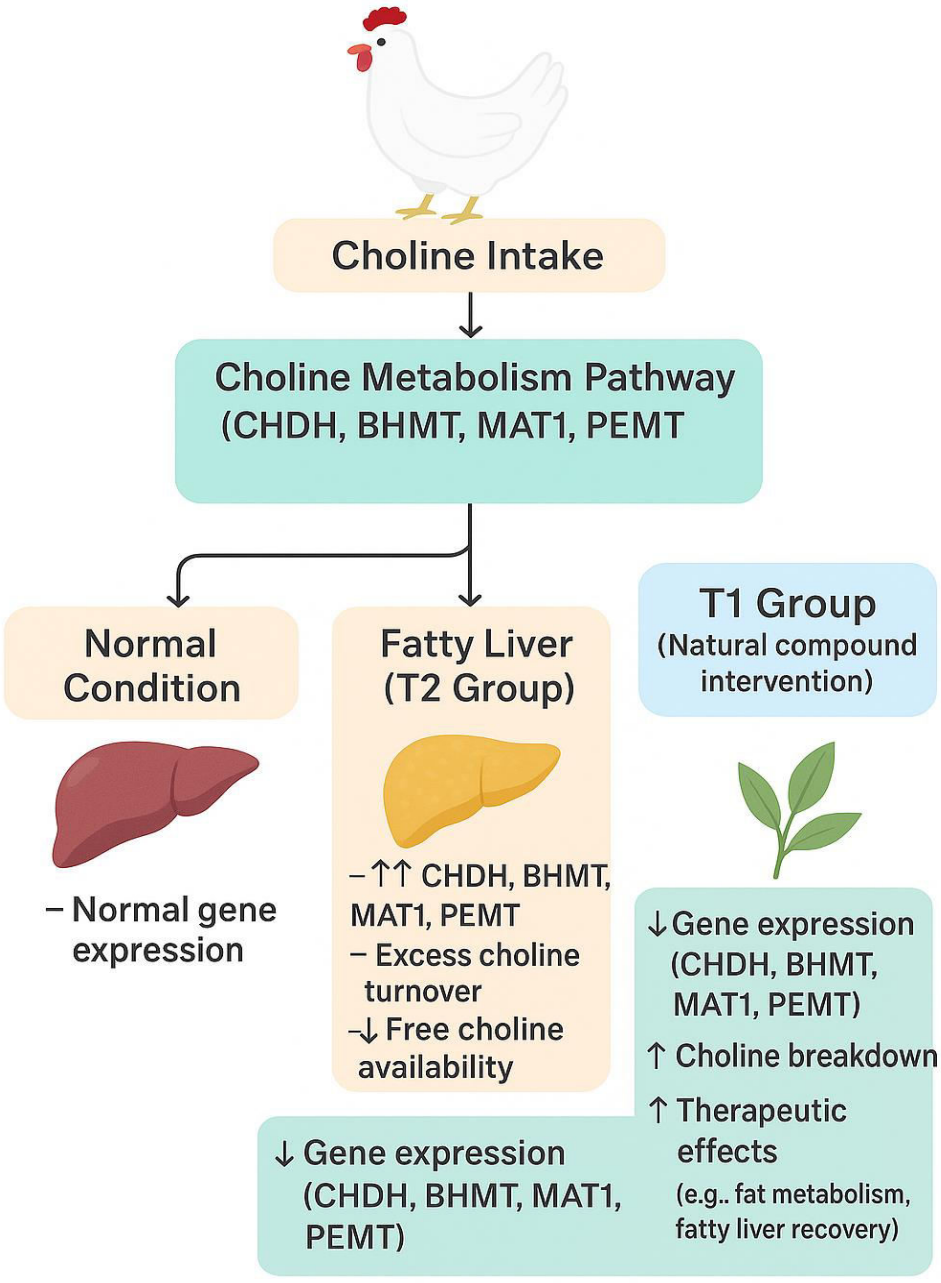


**Figure.3** This text shows the hypothetical relationship between choline metabolism and fatty liver conditions, illustrating how this influences the gene expression of CHDH, BHMT, MAT-1, and PEMT. It also demonstrates that the natural choline compound, Vincholine, used in this study, alters gene expression by promoting choline breakdown and increasing its bioavailability.




The increase in CHDH, BHMT, MAT-1, and PEMT in fatty liver (T2) compared to normal liver suggests that the liver activates both choline oxidation and methylation pathways as a response to excess fat. CHDH converts choline into betaine, which supports BHMT's role in remethylating homocysteine into methionine (13). Methionine is then transformed by MAT-1 into SAM, boosting the methyl pool needed for PEMT activity and other SAM-dependent processes (14). Studies show that in chickens, BHMT levels rise in conditions that cause

fatty liver, like hypothyroidism, coinciding with higher plasma lipids (15). PEMT offers an alternative route for phosphatidylcholine (PC) production by methylating phosphatidylethanolamine (PE), a process vital for VLDL assembly and triglyceride export (16). In mouse liver cells lacking PEMT, VLDL-TG and apoB100 secretion drops significantly, leading to fat accumulation in the liver (17). The notable reduction in these genes in the treatment group (T1), compared to the control (no synthetic choline chloride), indicates that dietary Vincholine may provide sufficient free choline and methyl donors. This helps the liver avoid upregulating its internal synthetic and remethylation pathways, saving energy and reducing metabolic stress. Lowering BHMT and MAT-1 expression could also limit excessive methyl flux, prevent depletion of homocysteine and methionine stores, and maintain methionine for protein synthesis (18).

In summary, our data support a model where fatty liver stimulates an increase in choline oxidation (CHDH), which leads to the regeneration of methyl donors (BHMT, MAT-1), and subsequently supplies PC (via PEMT) to aid lipid export through VLDL. Vincholine supplementation reduces this demand by providing external choline, which helps regulate gene expression and maintain the choline/methyl donor balance (Figure 3). The study suggests that dietary choline supplementation can decrease fatty liver, improve lipid metabolism, and boost methylation capacity. This implies enhanced choline availability and potential liver-protective benefits. Vincholine may be a natural and effective alternative to synthetic choline sources in commercial poultry production.

## Author Contributions

Jaiguru Kadam, Conceived the original idea and designed the model. Neerad Khuje, Designed the model, and the work was executed. Akanksha V Dhende, Laboratory work done. Hitesh N. Pawar, Analyzed the data and manuscript writing.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

## References


1. Ramalho de Lima M, Kaneko IN, de Lima AV, de Melo LN, de Lima MC, de Brito ANEF, Costa FGP, Boas ADCV, Toledo AL, Ferrer SL, Marimuthu S. Choline supplementation: Impact on broiler chicken performance, steatosis, and economic viability from 1 to 42 days. PLoS One. 2024 19; 19(3): e0295488. https://doi.org/10.1371/journal.pone.0295488.
2. Nasir Rajput, Naeem Muhammad, Rui Yan, Xiang Zhong and Tian Wang. Effect of Dietary Supplementation of Curcumin on Growth Performance, Intestinal Morphology and Nutrients Utilization of Broiler Chicks. 2013. The Journal of Poultry Science, 50 (1), 44-52. https://doi.org/10.2141/jpsa.0120065.
3. Ramalho de Lima M, Kaneko IN, de Lima AV, de Melo LN, de Lima MC, de Brito ANEF, Costa FGP, Boas ADCV, Toledo AL, Ferrer SL, Marimuthu S. Choline supplementation: Impact on broiler chicken performance, steatosis, and economic viability from from 1 to 42 days. PLoS One. 2024 Mar 19; 19(3): e0295488. https://doi.org/10.1371/journal.pone.0295488.
4. Gangane, G. R., Gaikwad, N. Z., Ravikanth, K., & Maini, S. (2010). The Comparative effects of synthetic choline and herbal choline on hepatic lipid metabolism in broilers. *Veterinary World* 3(7): 318-320
5. Suryawanshi DS, Jaiguru Kadam, Neerad Khuje, Priyanka Chaudhari, Ankita Pawar and Pavan Pawar. “Efficacy of Vincholine (A Herbal Choline) in Improving the Performance of Commercial Broilers". Acta Scientific Veterinary Sciences 6.9 (2024): 33-45 https://doi.org/10.31080/ASVS.2024.06.0918
6. Zeisel SH. Metabolic crosstalk: BHMT as methyl donor pathway; CHDH, PEMT roles in choline metabolism. (2013). *Clin Chem Lab Med* 1; 51(3): 467-475 https://doi.org/10.1515/cclm-2012-0518.
7. St Germain M, Iraji R, Bakovic M. Phosphatidylethanolamine homeostasis under conditions of impaired CDP-ethanolamine pathway or phosphatidylserine decarboxylation. Front Nutr. 2023 Jan 5(9): 1094273. https://doi.org/10.3389/fnut.2022.1094273.
8. Abd El-Ghany WA, Babazadeh D. Betaine: A Potential Nutritional Metabolite in the Poultry Industry. Animals (Basel). 2022 Sep 30; 12(19): 2624. https://doi.org/10.3390/ani12192624.
9. Felix TMS, Souza CS, Santos SCL, Campos DB, Aggrey SE, Guerra RR, Silva JHV. Methionine

sources at different dietary levels alters the growth and expression of genes related to homocysteine remethylation in the jejunum of broilers. PLoS One. 2023 Nov 13; 18(11): e0291998. https://doi.org/10.1371/journal.pone.0291998.

10. Livak K J and Schmittgen T D (2001). Analysis of Relative Gene Expression Data Using Real- Time Quantitative PCR and the 22DD*C*T Method. *Methods* 25: 402–408. https://doi.org/10.1006/meth.2001.1262
11. Chiao-Wei Lin, Ting-Wei Huang, Yu-Ju Peng, Yuan-Yu Lin, Harry John Mersmann, Shih-Torng Ding, A novel chicken model of fatty liver disease induced by high cholesterol and low choline diets, Poultry Science, 100 (3), 2021, 100869, ISSN 0032-5791, https://doi.org/10.1016/j.psj.2020.11.046.
12. Guo, X., Zhou, Q., Jin, J. *et al.,* Hepatic steatosis is associated with dysregulated cholesterol metabolism and altered protein acetylation dynamics in chickens. J Animal Sci Biotechnol 14, 108 (2023). https://doi.org/10.1186/s40104-023-00910-8
13. Mahmoudi M, Azarfar A, Khosravinia H. Partial Replacement of Dietary Methionine with Betaine and Choline in Heat-Stressed Broiler Chickens. J Poult Sci. 2018; 55(1):28-37. doi: 10.2141/jpsa.0170087. Epub 2017 Aug 25. PMID: 32055153; PMCID: PMC6756378.
14. Lin CW, Huang TW, Peng YJ, Lin YY, Mersmann HJ, Ding ST. A novel chicken model of fatty liver disease induced by high cholesterol and low choline diets. Poult Sci. 2021 Mar; 100(3): 100869. https://doi.org/10.1016/j.psj.2020.11.046.
15. Shibata T, Akamine T, Nikki T, Yamashita H, Nobukuni K. Synthesis of betaine-homocysteine S-methyltransferase is continuously enhanced in fatty livers of thyroidectomized chickens. 2003. Poultry Science 82 (2) 207-213. https://doi.org/10.1093/ps/82.2.207
16. Sun C, Lan F, Zhou Q, Guo X, Jin J, Wen C, Guo Y, Hou Z, Zheng J, Wu G, Li G, Yan Y, Li J, Ma Q, Yang N. Mechanisms of hepatic steatosis in chickens: integrated analysis of the host genome, molecular phenomics and gut microbiome. Gigascience. 2024 Jan 2; 13:giae023. https://doi.org/10.1093/gigascience/giae023.
17. Li Z, Agellon LB, Vance DE. Phosphatidylethanolamine N-methyltransferase deficiency leads to steatosis and defective VLDL secretion. J Biol Chem. 2006; 281(10): 7517–7523. https://doi.org/10.1074/jbc.M511903200
18. Finkelstein JD. Metabolic regulatory properties of S-adenosylmethionine and S-adenosylhomocysteine. Clin Chem Lab Med. 2007; 45(12):1694–1702. https://doi.org/10.1515/CCLM.2007.341

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