Int.J.Curr.Res.Aca.Rev.2026; 14(10): 25-31

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 14 Number 10 (October-2026)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2026.1410.003

Dietary Supplementation of Liver Tonic in CCl4 challenged Broilers: Effects on Growth Performance and Nutrient Digestibility

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Abstract

A 5-week study evaluated the efficacy of a hepatic product on growth, nutrient retention and carcass traits of 240-day-old Vencobb-430Y broiler chicks under hepatic toxin induced conditions. Birds were randomly allotted to four treatments (4 replicates/15 birds each): A (Control; basal diet), B (Negative control; CCL_4 liver challenger @1ml/kg body weight orally at every 3rd day from 15th to 28th days of trial), C (As B + test liver tonic @ 2 ml/L water), and D (As B + standard liver tonic @ 5,10 and 20 ml/100 birds at pre-starter, starter and finisher phases, respectively). All birds were floor-reared with ad libitum basal feed (BIS 2007) and routine vaccinations. Oral CCL_4 challenge significantly ($p<0.05$) depressed final body weight (2033.13 g) and overall weight gain (1989.26 g) in group B compared to control group A (2233.06 g and 2189.26 g). Supplementation of test liver product (Group C) effectively counteracted hepatotoxicity, restoring final weight (2158.87 g) and total gain (2115.54 g) to levels comparable ($p>0.05$) to control A and numerically superior to standard liver tonic group D (2082.50 g). Feed consumption remained non-significant across groups, but overall FCR significantly ($p<0.05$) worsened in group B (1.62) relative to A (1.51). Test liver tonic supplementation (Group C) improved FCR (1.54), outperforming group D (1.56) numerically. Furthermore, the test liver tonic restored CCl_4 induced numerical drops in dry matter digestibility (75.6%), nitrogen retention (70.34%), crude fibre (48.74%), and ether extract digestibility (90.54%) back toward baseline levels. However, the digestibility difference of different nutrients in different groups remained non-significant ($p>0.05$). In conclusion, the test liver tonic @ 2 ml/L drinking water successfully protected against toxin based (CCl_4-induced) hepatic damage, preserving growth performance, feed efficiency, and nutrient digestibility in broilers.

Article Info

Received: 15 August 2026
Accepted: 28 September 2026
Available Online:20 October 2026

Keywords

CCL_4, Growth performance, Nutrient-digestibility

Introduction

The modern broiler industry relies heavily on rapid growth rates, high feed conversion efficiency, and short production cycles to satisfy the rising global demand for poultry meat. However, intensive genetic selection for fast growth renders commercial broilers highly susceptible to metabolic disorders, oxidative stress, and exogenous toxicity. Central to the bird's overall health and physiological productivity is the liver, a vital organ responsible for nutrient metabolism, protein synthesis, lipid digestion via bile production, and xenobiotic detoxification. Any compromise in hepatic integrity directly impairs nutrient utilization, depresses growth performance, and leads to substantial economic losses in commercial operations. Under commercial farming conditions, broilers are frequently exposed to a number of hepatotoxic substances, including dietary mycotoxins,

chemical pollutants, heavy metals, and therapeutic drugs. Exposure to liver toxins induces severe hepatocellular injury, mitochondrial damage, and systemic oxidative stress through the excessive generation of reactive oxygen species (ROS). This toxicity induced hepatic impairment redirects metabolic energy away from tissue accretion toward physiological repair and detoxifying processes, resulting in reduced feed intake, poor feed conversion ratios (FCR), and suppressed body weight gain (Oke *et al.,* 2024). To mitigate toxicity-induced hepatic damage and restore physiological efficiency, liver tonic formulations have gained significant scientific and commercial interest. Bioactive compounds with lipotropic, antioxidative, and hepatoprotective properties support liver health by stabilizing hepatocyte membranes, promoting cell regeneration, facilitating fatty acid transport, stimulating digestive enzyme secretions, and enhancing end-product metabolic utilization. Various hepato-protective supplements are used commercially in poultry out of which l-carnitine, betaine, choline chloride and sorbitol have been used individually to promote liver function and growth performance of broiler chicken (Takahashi *et al.,* 2010; Wang *et al.,* 2013; Selvam *et al.,* 2018). This study involves the use of one such L-carnitine, betaine, choline chloride, sorbitol and magnesium-sulphate based liver tonic formulation designed to support hepatic function, alleviate metabolic stress, and optimize feed conversion efficiency during periods of toxicity or physiological challenge. While individual ingredient compounds have demonstrated protective effects against liver injury, there remains a need to systematically evaluate the combined efficacy of these hepato-protective compounds on the growth performance and nutrient utilization in broilers under hepatotoxic challenge conditions.

Therefore, the objective of the present study was to evaluate and compare the growth performance, feed efficiency, and nutrient digestibility of broiler chickens supplemented with the test liver tonic in drinking water following an experimental toxicity challenge.

Materials and Methods

Ethical approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (CPCSEA), Dated 17.10.2021, with Res. no. IAEC 89/21 in the Department of Poultry Science, College of veterinary and animal sciences, MAFSU, Parbhani, Maharashtra

Experimental Design

This trial was conducted on 240, day old Vencobb-430 broiler birds for 5 weeks at broiler unit (deep litter system) of Department of Poultry Science, College of Veterinary & Animal Sciences, MAFSU, Parbhani, Maharashtra to assess the effect of a test liver tonic (Dozliv-Forte P; M/s. Carus Laboratories Pvt. Ltd., Sector 3, HSIIDC, Karnal, Haryana, India) on the growth performance and nutrient metabolizability of broiler birds. The birds were randomly allotted to four treatments (4 replicates/15 birds each): A (Control; basal diet), B (Negative control; CCL_4 liver challenger @1ml/kg body weight orally at every 3rd day from 15th to 28th days of trial), C (As B + Dozliv-Forte P @ 2 ml/L water), and D (As B + standard liver tonic @ 5,10 and 20 ml/100 birds at pre-starter, starter and finisher phases, respectively). All the birds were provided with standard and uniform managemental conditions throughout the experimental period. All the birds were vaccinated as per routine vaccination schedule. The ingredients and their composition for the basal diet which was formulated as per Bureau of Indian Standards (BIS 2007) are presented in Table 1.

The live body weight and feed intake were recorded weekly for calculating weekly weight gain and feed conversion ratio. For calculating percent digestibility of different nutrients six birds from each treatment group were randomly taken and subjected to two days adaptation period in cages, followed by a metabolic trial period of three days. During this metabolic trial, recordings and representative samples of the feed offered, feed leftovers, and excreta were collected daily for the dry matter, nitrogen and other nutrients digestibility estimation. The data obtained for different parameters was statistically analysed as per methods of Snedecor and Cochran (1994). The treatment means were compared by critical differences (CD) and analysis of variance.

Results and Discussion

Body growth parameters

The weekly live body weight, weight gain, feed consumption, and feed conversion ratio (FCR) of broilers across various treatment groups are presented in Table 2. Initial day-old body weights did not differ significantly among the experimental groups. During the first two weeks of age, no significant differences ($P>0.05$) were recorded in any growth parameters taken for studying.

Significant effects on growth performance emerged from the 3rd week onward following toxicity challenge. At the end of the 4th week, broilers in the control group (Group A) exhibited significantly higher (P<0.01) live body weight (1569.89 ± 15.81 g) compared to the toxin-challenged control groups. Supplementation with test liver tonic (group C) and standard liver tonic (group D) in challenged groups significantly restored body weight, achieving 1515.22±18.46 g and 1475.39±14.34 g respectively, in comparison to negative control group B (1421.60±19.21). By the end of the 5th week, final cumulative body weight remained significantly higher (P<0.01) in Group A (2233.06±37.97 g) and Group C (2158.87±35.00 g) compared to Group B (2033.13±2.66 g).

Weekly weight gain showed significant variation during the 3rd week (P<0.05), where Group A (496.52±14.21 g) and Group C (469.47±23.65 g) gained significantly more weight than Group B (406.60 ± 25.08 g). Overall cumulative weight gain (0-5 weeks) followed a similar trend, with Group A (2189.26±37.72 g) and Group C (2115.54±34.96 g) demonstrating superior gains over Group B (1989.26±2.85 g). Weekly feed consumption did not differ significantly (P > 0.05) among all treatments throughout the 5-week experimental period. Overall cumulative feed intake ranged from 3180.27 ± 59.34 g (Group D) to 3302.16 ± 41.01 g (Group A).

Feed conversion efficiency was significantly impaired by toxicity challenge during weeks 3 and 4. In the 3rd week, Group B exhibited a significantly poorer FCR (1.67 ± 0.08; P < 0.01) compared to Group A (1.33 ± 0.04), Group C (1.42 ± 0.07), and Group D (1.48 ± 0.01). Similarly, in the 4th week, FCR was significantly elevated in Group B (1.79 ± 0.04; P < 0.05) compared to all other groups. Over the entire 5-week trial, overall FCR was significantly improved (P < 0.01) in control group A (1.51 ± 0.01), group C (1.54±0.02) and group D (1.56±0.01) compared to the un-supplemented challenged group (Group B; 1.62 ± 0.02).

Nutrient Digestibility and Retention

The data pertaining to nutrient intake, excretion, digestibility coefficients, and nitrogen retention at the end of the experimental trial are summarized in Table 3. No statistically significant differences (p>0.05) were observed among the treatment groups for any of the nutrient digestibility parameters evaluated. Dry matter digestibility ranged between 72.55 ± 2.16% (Group B) and 77.62 ± 0.86% (Group A). Nitrogen retention values ranged from 66.32 ± 2.87% in Group B to 71.79 ± 1.04% in group A, with supplementation groups (C and D) exhibiting intermediate values of 70.34 ± 3.08% and 67.69 ± 2.85%, respectively. Similarly, the crude fibre digestibility (43.11 ± 4.81% to 48.74 ± 3.06%) and ether extract digestibility (88.20 ± 1.40% to 90.54 ± 1.15%) showed numerical improvements in test liver tonic (Group C) and standard liver tonic (Group D) supplemented groups compared to negative control (Group B), though variations remained statistically non-significant (p>0.05).

The evaluation of growth performance parameters clearly demonstrates the adverse impact of hepatotoxin (CCl_4) induced stress on broiler productivity, as well as the therapeutic potential of test liver tonic and standard liver tonic supplementation. During the initial two weeks of the trial, identical performance across all treatment groups confirmed uniform baseline physiological status prior to the challenge phase. The onset of toxicity challenge from the 3rd week onward resulted in a pronounced decline in live body weight, weekly weight gain, and feed conversion efficiency in the un-supplemented challenged group (Group B).

This depression in growth performance can be attributed to liver dysfunction, mitochondrial oxidative damage, and impaired systemic metabolic efficiency typically induced by hepatotoxic CCL_4. Hepatocellular injury compromises bile synthesis, essential enzyme pathways, and nutrient utilization, ultimately redirecting metabolic energy away from tissue accretion toward physiological repair and detoxifying mechanisms (Zaefarian *et al.,* 2019; Tokofai *et al.,* 2021; Carpio *et al.,* 2024).

Supplementation of test liver product effectively mitigated the growth depression caused by the CCl_4 challenge. The significant improvement in 5th week cumulative body weight and overall FCR in comparison to negative challenge group B underscores its protective efficacy. This protective response is directly linked to the synergistic metabolic roles of its active constituents. In line with our study, the similar effects of these ingredients have been documented previously by many authors (Aziz *et al.,* 2019; Azizi-Chekosari *et al.,* 2021; Ahmadipour *et al.,* 2025; Behboodi *et al.,* 2026). By maintaining hepatic integrity, test liver tonic preserved metabolic capacity, allowing birds in group C to maintain growth trajectories comparable to the non-challenged control (Group A).

Table.1 Ingredient and nutrient composition of pre-starter, starter, and finisher feed

Sr. No.	Ingredients (%)	Pre-starter	Starter	Finisher
1	Maize	53.000	54.000	58.220
2	Soybean meal	40.200	37.800	32.600
3	Vegetable oil	2.770	4.150	5.100
4	Dicalcium phosphate (DCP)	1.800	1.800	1.900
5	Limestone powder (LSP)	1.200	1.200	1.200
6	Salt (NaCl)	0.250	0.250	0.250
7	Trace mineral premix*	0.100	0.100	0.100
8	Vitamin premix**	0.050	0.050	0.050
9	DL-Methionine	0.160	0.190	0.150
10	L-Lysine	0.070	0.060	0.030
11	Choline chloride 60%	0.100	0.100	0.100
12	Toxin binder	0.100	0.100	0.100
13	Coccidiostat	0.050	0.050	0.050
14	Sodium bicarbonate	0.150	0.150	0.150
Total		**100.00**	**100.00**	**100.00**
Nutrient Composition (Calculated basis)				
1	ME (kcal/kg)	3000.33	3101.54	3200.44
2	Crude Protein (%)	23.019	22.04	20.03
3	Ether Extract (%)	5.18	6.57	7.64
4	Crude Fiber (%)	4.20	4.08	3.87
5	Calcium (%)	1.01	1.00	1.01
6	Total Phosphorus (%)	0.70	0.69	0.68
7	Available Phosphorus (%)	0.42	0.42	0.43
8	Total Lysine (%)	1.30	1.23	1.08
9	Total Methionine (%)	0.50	0.52	0.45

* **Trace Mineral Mixture:** Each 1 kg contains: Copper – 15 g, Iron – 80 g, Manganese – 100 g, Zinc – 80 g, Iodine – 2 g, Cobalt – 0.5 g, Selenium – 0.5 g, Chromium – 10 mg, and Molybdenum – 50 mg.
Vitamin Premix: Each 500 g contains: Vit. A – 12.50 MIU, Vit. D3 – 2.50 MIU, Vit. E – 12 g, Vit. K – 1.50 g, Thiamine (B1) – 1.50 g, Riboflavin (B2) – 5 g, Pyridoxine (B6) – 2 g, Cyanocobalamin (B12) – 0.015 g, Niacin – 15 g, Cal D Pantothenate – 10 g, and Folic acid – 0.50 g.

Table.2 Weekly growth performance parameters of different treatment groups

Weekly live body weights (g/bird)						
Treatments	**Day-old**	**1st**	**2nd**	**3rd**	**4th**	**5th(Overall)**
A	43.80 ± 0.38	158.15 ± 3.26	478.00 ± 6.75	974.52 ± 8.30	1569.89^{a} ± 15.81	2233.06^{a} ± 37.97
B	43.87 ± 0.41	158.68 ± 5.37	472.75 ± 13.44	879.35 ± 38.02	1421.60^{c} ± 19.21	2033.13^{c} ± 2.66
C	43.33 ± 0.09	155.33 ± 1.64	468.08 ± 5.22	937.55 ± 21.36	1515.22^{b} ± 18.46	2158.87^{ab} ± 35.00
D	43.37 ± 0.34	153.45 ± 1.56	469.17 ± 4.52	902.50 ± 7.69	1475.39^{b} ± 14.34	2082.50^{bc} ± 37.26
Weekly weight gain (g/bird)						
Treatments	**1st**	**2nd**	**3rd**	**4th**	**5th**	**Overall**
A	114.35 ±	319.85 ±	496.52^{a} ±	595.37 ±	663.17 ±	2189.26^{a}±37.72

	3.26	7.34	14.21	11.25	24.35	
B	114.82 ± 5.19	314.07 ± 11.35	406.60^{c} ± 25.08	542.24 ± 42.78	611.53 ± 16.70	1989.26^{c}±2.85
C	112.00 ± 1.70	312.75 ± 4.98	469.47^{ab} ± 23.65	577.66 ± 12.22	643.65 ± 17.60	2115.54^{ab}±34.96
D	110.08 ± 1.72	315.72 ± 5.20	433.33^{bc} ± 5.86	572.89 ± 8.05	607.11 ± 27.15	2039.13^{bc}±37.52
Weekly feed consumption (g/bird)						
Treatments	**1st**	**2nd**	**3rd**	**4th**	**5th**	**Overall**
A	136.02 ± 3.74	419.97 ± 11.98	656.81 ± 3.15	977.93 ± 9.66	1111.43 ± 33.94	3302.16 ±41.01
B	137.13 ± 5.63	413.03 ± 7.29	674.77 ± 40.94	969.70 ± 79.30	1031.25 ± 20.06	3225.89 ±46.55
C	132.00 ± 2.34	406.62 ± 7.71	663.09 ± 9.77	970.93 ± 15.36	1073.63 ± 23.50	3246.27 ±34.08
D	130.78 ± 1.59	408.70 ± 2.27	639.83 ± 12.22	973.50 ± 6.89	1027.45 ± 46.17	3180.27 ±59.34
Weekly feed conversion ratio						
Treatments	**1st**	**2nd**	**3rd**	**4th**	**5th**	**Overall**
A	1.19 ± 0.01	1.31 ± 0.02	1.33^{b} ± 0.04	1.64^{b} ± 0.02	1.68 ± 0.04	1.51^{c}±0.01
B	1.20 ± 0.02	1.32 ± 0.03	1.67^{a} ± 0.08	1.79^{a} ± 0.04	1.69 ± 0.02	1.62^{a}±0.02
C	1.18 ± 0.01	1.30 ± 0.01	1.42^{b} ± 0.07	1.68^{b} ± 0.02	1.67 ± 0.02	1.54^{bc}±0.02
D	1.19 ± 0.01	1.30 ± 0.02	1.48^{b} ± 0.01	1.70^{b} ± 0.02	1.69 ± 0.02	1.56^{b}±0.01

*Means bearing different superscripts in a column differ significantly ($p < 0.05$).

Table.3 Nutrient digestibility and nitrogen retention in broilers of different treatment groups.

Sr. No	Parameters	A	B	C	D
1	Average Feed Intake (g/b)	167.22 ± 7.79	160.83 ± 5.56	169.17 ± 16.84	177.22 ± 5.78
2	Dry matter Intake (g/b)	158.36 ± 7.38	152.31 ± 41.92	160.20 ± 15.94	167.83 ± 5.48
3	Dry matter Excretion (g/b)	35.49 ± 2.35	41.92 ± 3.92	39.15 ± 5.52	44.38 ± 2.83
4	Dry matter digestibility (%)	77.62 ± 0.86	72.55 ± 2.16	75.63 ± 2.59	73.41 ± 1.97
5	Nitrogen Intake (g/b)	5.05 ± 0.24	4.85 ± 0.17	5.11 ± 0.51	5.35 ± 0.17
6	Nitrogen Excretion (g/b)	1.42 ± 0.07	1.64 ± 0.17	1.52 ± 0.21	1.72 ± 0.14
7	Nitrogen Retention (%)	71.79 ± 1.04	66.32 ± 2.87	70.34 ± 3.08	67.69 ± 2.85
8	Crude fibre digestibility (%)	47.72 ± 4.98	43.11 ± 4.81	48.74 ± 3.06	46.18 ± 3.44
9	Ether extract digestibility (%)	89.90 ± 0.87	88.20 ± 1.40	90.54 ± 1.15	90.29 ± 0.59

*Means bearing different superscripts in a column differ significantly ($p < 0.05$).

Although, weekly feed consumption remained statistically non-significant across all groups, the noticeable divergence in FCR during weeks 3 and 4 further highlights that the primary driver of growth depression in group B was impaired metabolic utilization due to CCl_4 toxicity rather than reduced feed intake.

The restoration of feed conversion efficiency in test liver tonic and standard liver tonic supplemented groups indicates enhanced nutrient partitioning and metabolic recovery in comparison to negative control group B. Regarding nutrient utilization, although statistical significance was not reached across treatments, test liver tonic and standard liver tonic supplemented groups displayed consistent numerical superiorities in dry matter digestibility, nitrogen retention, and ether extract digestibility compared to negatively challenged group B. The recovery in ether extract digestibility in group C and D in comparison to group B aligns logically with improved hepatic health, as optimal bile secretion is essential for emulsification and absorption of dietary lipids. Similarly, elevated nitrogen retention in tonic supplemented groups supports the observed recovery in muscle mass and overall body weight gain. However, these effects were numerically higher in test liver tonic supplemented group C proving its efficacy better than competitor standard liver tonic (Group D) taken for comparison.

These enhancements in metabolism and energy diversion towards tissue deposition in group C might be due to the combined synergistic metabolic, lipotropic, and mucosal protective effects of the supplemented compounds in test liver tonic. L-carnitine helps in oxidative stress and prevent excess fat buildup in the liver and abdomen by shuttling long-chain fatty acids directly into the mitochondria for β-oxidation. Concurrently, betaine and choline chloride served as principal methyl donors that spare dietary methionine for protein synthesis while promoting synthesis and export of very low-density lipoproteins (VLDL) to prevent fatty liver accumulation and optimize lipid metabolism. Betaine further functions as an organic osmolyte, preserving intestinal mucosal integrity and cell hydration under metabolic stress to maximize nutrient absorption capacity. Sorbitol stimulates bile flow and pancreatic enzyme secretion to enhance the emulsification and utilization of dietary fats and clear fat-soluble toxins from the body, while magnesium sulphate acts as an indispensable ion cofactor for ATP-dependent enzymes involved in carbohydrate and protein metabolism, alongside facilitating metabolic waste clearance (Eklund *et al.,* 2005; Rehman *et al.,* 2017; Saeed *et al.,* 2017; Abd El-Ghany and Babazadeh, 2022; Gregg *et al.,* 2022; Toson *et al.,* 2024).

Together, in synergistic combination, these hepatoprotective and lipotropic agents enhanced the energy production & partitioning, optimized lipid digestibility, and boosted overall feed utilization efficiency, thereby driving superior growth kinetics. Corroborating our results, Ray *et al.,* (2020) reported that a proprietary hepatoprotective additive sharing a similar combination of ingredients showed significant improvements in body weight gain and FCR of broiler chicken when supplemented at a dose rate of 0.1 ml/ bird/day for 3 days a week.

In conclusion, Collective demonstration of the physiological and performance data of this present study concludes that the test liver tonic (Dozliv-Forte P) supplementation counters toxicity induced growth depression by safeguarding hepatic function and restoring feed efficiency in broiler chickens.

Acknowledgement

This research trial was fully funded by Carus Laboratories Pvt. Ltd., Karnal, and all the research work was conducted at College of Veterinary and Animal Sciences, Prabhani, Maharashtra animal and Fishery Sciences University (MAFSU), Nagpur.

Conflict of interest

The corresponding author of this manuscript is employed as an Assistant Technical Manager by Carus Laboratories Pvt. Ltd., Karnal, and is primary writer of this manuscript. All experimental work and study oversight were conducted independently at MAFSU, Parbhani, Maharashtra, India by K.K. Khose, M.G. Nikam, G.R. Gangane, and S.M. Wankhede, who declare no competing interests.

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How to cite this article:

Khose, K. K., M. G. Nikam, G. R. Gangane, S. M. Wankhede and Shubhnish Bisla. 2026. Dietary Supplementation of Liver Tonic in CCl4 challenged Broilers: Effects on Growth Performance and Nutrient Digestibility. *Int.J.Curr.Res.Aca.Rev.* 14(10), 25-31. doi: https://doi.org/10.20546/ijcrar.2026.1410.003