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Evaluating A Polyherbal Choline Substitute: Impacts On Performance, Liver Health, and Fat Content in Broilers

Bhagwat VG[1*], Santoshkumar T[1] , Varun Kumar K[1]

[1](*R&D Center, Himalaya Wellness Company, Makali, Bengaluru-562162, Karnataka, India)*

[*]For corresponding
Vishwanath G Bhagwat
Principal Scientist, R&D Center, Himalaya Wellness Company, Makali, Bengaluru-562162, Karnataka, India.

Abstract: Choline, an essential vitamin found in phospholipids, is gaining traction in poultry research. While synthetic choline chloride (CCL) has been the go-to source, its limitations have spurred the quest for natural alternatives. This study explored a polyherbal formulation (PHF) as a potential CCL substitute in broiler diets. Impact of PHF evaluated on performance, liver health, and abdominal and liver fat content in 200 Cobb 430 Y chicks. Five groups, each with 40 birds, received either a control diet or a control diet supplemented with CCL (1000 g/ton), a combination of CCL and Liv.52 Protec powder (LPP) (250 g/ton), or two different PHF inclusion levels (500 and 750 g/ton). Interestingly, birds fed diets containing either CCL or PHF displayed improved body weight (up to 7.30% increase) and feed conversion ratio (FCR), meaning they required less feed to gain 1 kg of weight gain compared to the control group. Notably, the 500 g/ton PHF group boasted the most impressive gains in carcass traits. Furthermore, both PHF inclusion levels yielded similar benefits for liver enzymes as the standard CCL supplementation. Based on these findings, PHF holds promise as a 500 g/ton replacement for synthetic CCL in broiler diets. This potentially unlocks an alternative that maintains performance and supports liver health.

Keywords:*Polyherbal formulation, Synthetic choline chloride, Growth performance, Liver health, Cobb 430 Y*

I. INTRODUCTION

Proper nutrition is essential for broiler performance, since it regulates energy balance and metabolic processes[1,2]. Balanced diets with essential nutrients such as protein, minerals, vitamins, and fatty acids are crucial for optimal broiler growth[3]. Choline, a rediscovered B4 vitamin primarily found in phospholipids, plays a vital role in poultry biology.

Choline is essential for the healthy growth of bones as well as the development of cell membranes and organelles (mitochondria and microsomes)[4]. In addition, it acts as a lipotropic factor, supporting cell structure and formation of the neurotransmitter acetylcholine[5]. Unlike mammals, birds are unable to synthesize enough choline, which causes deficiencies that manifest as growth impairment and bone deformities in chicks[6,7].

The source and bird-specific factors such as breed, age, diet, and methionine content affect the bioavailability of choline[8-10]. Dietary methionine or methyl donors, while important, cannot completely substitute for choline in avian diets. This is because birds have a limited ability to perform the initial step of choline biosynthesis[11]. This limited capacity contrasts with mammals like pigs and rats[12].

Therefore, synthetic choline chloride (SCC) supplementation has become a common practice to meet choline requirements and address these issues. Nevertheless, the search for natural alternatives has been stimulated by drawbacks such as hygroscopic nature, accelerated vitamin degradation, and trimethylamine (TMA) formation in the gut[13].

This study explores the potential of HimChol-P, a polyherbal formulation (PHF) as a feed supplement developed by the Himalaya Wellness Company (Bengaluru, India), as a substitute for synthetic choline chloride (SCC). We hypothesize that by providing a natural source of choline, PHF can replicate the positive effects of SCC on broiler performance and liver health. The study investigates the impact of PHF on these factors in commercial broilers, including performance metrics, liver enzyme activity, liver fat content, and carcass characteristics like ready-to-cook weight and abdominal fat.

II. Method

Test Product

PHF offers an alternative to synthetic choline chloride (SCC). It contains phosphatidylcholine, which plays a vital role in preventing fatty liver in broilers by promoting fat export from the liver and maintaining healthy cell membranes. PHF is also fortified with liver-supporting botanicals, namely:

- *Ocimum basilicum*
- *Azadirachta indica*
- *Cichorium intybus*
- *Curcuma longa*
- *Tephrosia purpurea*
- *Andrographis paniculata*

These botanicals have been traditionally used to support liver function and may enhance detoxification processes or promote bile production, further aiding liver health.

Ethical Approval

All protocols and ethical use of experimental animals were approved by the Institutional Animal Ethics Committee Protocol No. AHP/P/12/22.

Experimental Birds, Diets, and Management

This study includes 200, 1-day-old chicks that were divided into five dietary groups with 40 chicks per group. A corn-soybean meal-based diet was formulated to meet the National Research Council nutrient requirements for Cobb 430 Y commercial broilers.[8] The basal diet's nutrient composition is shown in Table 1.

The chicks were housed in semiclosed housing, which is divided into pens with 60 sq ft of floor space each. The measurement of each pen is approximately 6 ft by 10 ft by 5 ft (length × width × height). Individual pens were equipped with brooders, drinkers, and feeders. A minimum of one sq ft of floor space is given to each chick. PVC sheets were used to adjust pen size and floor space according to the number of housed chicks. Dust-free rice husk was used as litter material, which is topped up weekly or as needed. To prevent rice husk from accumulating in feeders and drinkers during the first 5 days of acclimation, a thin layer of newspaper was used as a cover. Chicks had unrestricted access to fresh, chlorinated drinking water throughout the study.

Three mash feed lines (pre-starter, starter, and finisher) were produced at Kavi Feed Mill, Nelamangala, Bengaluru. Required quantities of additives were mixed with each feed in a horizontal, 500 kg capacity Toshniwal ribbon mixer. Pre-starter feed was provided for days 1–14, followed by starter feed (days 15–25), and finisher feed (days 26–35). On days 7 and 22, Newcastle disease (ND) vaccinations were administered via eye drops. The day 7 strain was live Lentogenic from VHL, while the day 22 strain was live I.P. VH from Abic. Vaccination against infectious bursal disease (IBD) was carried out on day 15 using B2K, the invasive intermediate strain from Indovax. Ambient temperature, lighting, and humidity were maintained according to standard farm management practices. During the first week, lighting was provided for 24 hours and then for 20 hours until the trial's end. The experimental birds had ad libitum access to the experimental diets.

Table 1: Nutrient composition of basal diet

Particulars	**Pre-starter**	**Starter**	**Finisher**
Protein (%)	22.10	21.15	19.39
Metabolizable energy (kcal/Kg)	2867	2876	3061
Crude fiber (%)	4.02	4.35	3.85
Ether extract (%)	3.42	3.45	6.30
Calcium (%)	1.07	1.06	0.82
Available phosphorus (%)	0.42	0.41	0.36
Methionine (%)	0.62	0.61	0.52
Lysine (%)	1.33	1.34	1.13
M + C (%)	0.92	0.95	0.80
Threonine (%)	0.83	0.81	0.79
Sodium (%)	0.19	0.17	0.17
Chloride (%)	0.26	0.27	0.25
Potassium (%)	0.92	0.91	0.80
DEB (MEq./Kg)	234	236	213

Study Design

Two hundred, one-day-old Cobb 430 Y broiler chicks were obtained from Sriya Farms and Feeds Pvt. Ltd., Bangalore, and randomly assigned to five groups (T1, T2, T3, T4, and T5) of 40 birds each on the first day of the experiment. The T1 (control) group is raised on a standard corn–soybean meal-based diet to serve as the standard basal diet. Those in the T2 group are raised on the standard diet and additionally received a supplement of SCC at 1000 g/ton of feed. Those in the T3 group are raised on the standard diet and further provided with a combination of synthetic CCL at 1000 g/ton and Liv.52 Protec powder (250 g/ton). The T4 group is raised on the standard diet and supplemented with PHF (500 g/ton). Lastly, those in the T5 group are raised on the standard diet and supplemented with PHF (750 g/ton).

Table 2: Study design

Group	Supplement	Duration of Supplement	Number of chicks/group
T1 – control	Basal diet	35 days	40
T2 – choline chloride (CCL) synthetic	Basal diet + 1000 g/ton	35 days	40
T3 – CCL + LPP	Basal diet + 1000 g/ton + 250 g/ton	35 days	40
T4 – PHF	Basal diet + 500 g/ton	35 days	40
T5 – PHF	Basal diet + 750 g/ton	35 days	40

Assessment Parameters

Growth Performance Parameters

Chicks were monitored daily for mortality throughout the experiment. On the first day, their body weight was measured, and weight and feed intake were subsequently recorded at regular intervals: day 7, day 14, day 28, and day 35. Gain in body weight, cumulative mortality, FCR, and Europeaon production efficiency factor (EPEF) were evaluated and calculated by using these measurements. These data provided valuable insights into the impact of HimChol-P on Cobb 430 Y broiler chickens growth performance.

Carcass Evaluation (Day 35)

At the experiment's conclusion, six birds from each group, chosen for their weight similarity to the average, were assessed for carcass characteristics. The birds were euthanized humanely through cervical dislocation following the official animal welfare guidelines. Feathers were then removed by using a hot water (51°C–55°C for 120 seconds) and hand-pinning for complete removal. Cleaned carcasses (excluding head, neck, and feet) and liver samples were collected. Abdominal fat was then weighed and recorded.

Blood Collection and Analysis (Day 35)

On day 35, approximately 2 ml of blood was drawn from the brachial vein of eight birds per group. The blood was collected in plain centrifuge tubes and centrifuged at 2000 rpm for 5 min to separate the serum. This serum was stored at −20°C for later analysis, which included the following:

- **Biochemical parameters:** By using standardized kit-based assays on an automated analyzer. eight birds per group were evaluated for various blood chemistry markers (total protein, albumin, globulin, A/G ratio, SGPT, SGOT, TGL, and total cholesterol).
- **Antibody titers:** Standardized ELISA tests were carried out on serum samples from eight birds per group to measure antibody titers for ND and IBD, following the manufacturer's instructions.
- **Liver fat content:** The crude fat content of liver samples (eight per group) was determined using established AOAC methods.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). To compare the control group with the supplemented groups, statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. A p-value of less than 0.05 was considered statistically significant. The software used for analysis was the Statistical Package for Social Sciences version 20.

III. Results and Discussion

Growth Performance Parameters

By day 35, birds in groups T2, T3, T4, and T5 displayed increases in body weight compared to the control group (T1). These increases were 4.00%, 4.72%, 5.11%, and 7.30%, respectively. This suggests that the incorporation of the supplements into their diet has a positive influence on their growth. The results also revealed an improved FCR in the supplemented groups. Birds in the T2, T3, T4, and T5 group consumed 14g, 9g, 11g, and 13g less feed per kilo gram of body weight gain, respectively, compared to the control group. This indicates a more efficient use of feed for growth in these groups. Furthermore, the European production efficiency factor (EPEF) increased in all the supplemented groups compared to the control. T2, T3, T4, and T5 group showed increases of 13.22%, 16.25%, 12.67%, and 16.53%, respectively. This suggests that protein utilization for growth and development has improved in birds fed supplemented diets. There were no significant differences in mortality rates observed between groups T4 and T5, which received different PHF supplementation. However, there was a notable decrease in mortality in T3 group compared to the control group (T1) (Table 3). These findings suggest that supplementing the diet with PHF at a level of 750 g/ton (group T5) had the most positive impact on growth performance parameters.

Table 3: Effect of PHF on growth performance parameters in Cobb 430 Y broiler chickens

Parameters	Group	Day 14	Day 28	Day 35
Body weight, g	T1 – control	200.95	1536.05	2171.11
	T2 – CCL	217.37	1584.00	2257.89
	T3 – CCL + LPP	214.25	1636.32	2273.53
	T4 – PHF-500	202.24	1636.76	2282.08
	T5 – PHF-750	208.08	1639.67	2329.71
FCR	T1 – control	1.13	1.41	1.63
	T2 – CCL	1.05	1.30	1.49
	T3 – CCL + LPP	1.03	1.24	1.54
	T4 – PHF-500	1.07	1.33	1.52
	T5 – PHF-750	1.20	1.62	1.50
EPEF	T1 – control			363
	T2 – CCL			411
	T3 – CCL + LPP			422
	T4 – PHF-500			409
	T5 – PHF-750			423
Cumulative mortality, %	T1 – control			4.76
	T2 – CCL			4.76
	T3 – CCL + LPP			0.00
	T4 – PHF-500			4.88
	T5 – PHF-750			4.76

Values are expressed as mean.
$p > 0.05$ as compared to T1 based on one-way ANOVA followed by Dunnett's multiple comparison post-hoc test.

Carcass traits: Table 4 presents the results of the effect of PHF supplementation on carcass traits.

- **Rel. RTC (%):** There are marginal increases in the relative weight of the entire carcass as compared to control group birds, which suggests that dietary supplementation has an impact the overall growth of chickens.
- **Rel. liver wt. (%):** Compared to the control group (T1), all supplemented groups (T2, T3, T4, and T5) have slightly lower relative liver weights. This could indicate improved liver health or function in the supplemented birds.
- **Rel. ab. fat (%):** There are marginal differences in the relative weight of abdominal fat pads between the groups. This suggests that the supplements have an impact on abdominal fat deposition.
- **Crude fat (%):** All supplemented groups (except T3) have lower crude fat content compared to the control group. The T4 group (500 g/ton PHF) have the biggest reduction in fat content. This might indicate that PHF supplementation, especially at the 500 and 750g/ton supplementation, may have an impact on the overall body fat content in chickens.

Table 4: Effect of PHF on carcass traits in Cobb 430 Y broiler chickens

Groups	Rel. RTC (%)	Rel. liver wt. (%)	Rel. ab. fat (%)	Crude fat (%)
T1 – control	62.93	2.71	1.18	4.00
T2 – CCL	66.22	2.19	0.99	3.21
T3 – CCL + LPP	67.04	2.30	1.10	3.75
T4 – PHF-500	65.74	2.25	1.12	2.54
T5 – PHF-750	67.88	2.36	1.08	2.60

Values are expressed as mean.
p > 0.05 as compared to T1 based on one-way ANOVA followed by Dunnett's multiple comparison post-hoc test.

Serum Biochemical Parameters

The effect of PHF supplementation at 500 and 750 g/ton was par with the synthetic CCL at 1000 g/ton in terms of improvement effects of liver enzymes (Table 5).

Compared to the higher supplementation level (1000 g/ton) of synthetic CCL (Table 5), PHF supplementation at lower supplementation levels (500 g/ton and 750 g/ton) achieved comparable results in improving liver enzymes.

Table 4: Effect of PHF on serum biochemical in Cobb 430 Y broiler chickens

Groups	**Total protein (g/dl)**	**Albumin (g/dl)**	**Globulin (g/dl)**	**A/G ratio (g/dl)**	**SGPT (IU/L)**	**SGOT (IU/L)**	**TGL (mg/dL)**	**Chol (mg/dL)**
T1 – control	2.85 ± 0.14	1.45 ± 0.09	1.40 ± 0.11	1.04 ± 0.11	14.68 ± 1.21	274.62 ± 57.62	30.88 ± 5.32	160.88 ± 13.27
T2 – CCL	2.68 ± 0.26	1.39 ± 0.13	1.29 ± 0.14	1.08 ± 0.05	12.32 ± 3.02	250.14 ± 21.40	26.35 ± 7.91	142.13 ± 17.55
T3 – CCL + LPP	2.87 ± 0.24	1.51 ± 0.10	1.36 ± 0.15	1.11 ± 0.10	10.50 ± 2.87	253.58 ± 29.41	*29.10 ± 10.50	150.43 ± 7.50
T4 – PHF-500	2.83 ± 0.26	1.49 ± 0.12	1.34 ± 0.17	1.12 ± 0.10	12.04 ± 1.67	245.15 ± 36.32	24.54 ± 6.31	152.50 ± 12.07
T5 – PHF-750	2.93 ± 0.11	1.54 ± 0.07	1.39 ± 0.06	1.11 ± 0.06	12.80 ± 1.14	220.75 ± 14.98	29.85 ± 1.73	158.38 ± 8.21

Values are expressed as mean ± SD; n=8.
*p > 0.05 as compared to T1 based on one-way ANOVA followed by Dunnett's multiple comparison test.

Serum ND and IBD Titer

The geometric mean (GM) of ND titer and vaccination index (VI) of IBD titer was increased in the T2, T3, T4, and T5 group as compared to the T1 group (Table 6). These results indicated that PHF supplementations at 500 g/ton and 750 g/ton were effective in better immune-modulatory effects as compared to synthetic CCL.

An increase in the geometric mean of the ND titer and the IBD VI was manifested in chickens fed diets supplemented with PHF at 500 g/ton (T4) and 750 g/ton (T5) compared to the control group (T1) (Table 6). This suggests that PHF supplementation, particularly at these levels, has a more potent immune-modulatory effect than synthetic CCL on these birds.

Table 6: Effect of PHF on serum ND and IBD titer in Cobb 430 Y broiler chickens

Groups	ND titer value Geometric mean (GM)	IBD titer value Vaccination index (VI)
T1 – control	4.49	96.24
T2 – CCL	4.88	105.01
T3 – CCL + LPP	5.19	113.59
T4 – PHF-500	5.04	113.85
T5 – PHF-750	4.90	114.52

Values are expressed as mean ± SD; n=8.
*p > 0.05 as compared to T1 based on one-way ANOVA followed by Dunnett's multiple comparison test.

While regular CCL gets quickly broken down and excreted by gut bacteria[15], phosphatidylcholine, another form of choline, survives this process much better. This enables it to play a role in the digestion of fat by indirectly aiding bile production and bile composition[16]. According to studies, phosphatidylcholine supplements such as herbal choline can improve broiler weight gain through better energy utilization (efficient carbohydrate and fat metabolism), without necessarily affecting feed intake or feed conversion ratio[17]. Other research[18], however, found a slight increase in feed intake with herbal choline supplementation; this may be because the supplement contains a variety of plant-based ingredients that enhance bile flow. Our study found that supplementing broiler chicken feed with PHF at a 750 g/ton significantly improved the FCR and overall growth performance. This aligns with previous research by Kumar and Dematte Filho et al. (2019, 2020), who observed similar positive effects on weight gain, feed intake, FCR, and chick survival when replacing CCL with a plant-based choline source[19,20]. Furthermore, another study investigating herbal choline, a potential alternative to CCL, demonstrated increases in FCR and reductions in feed intake, indicating improved dietary fat absorption in broilers[21]. These combined findings suggest that supplementing poultry diets with alternative choline sources, like PHF in our study, can be a promising strategy for improving broiler performance. Studies suggest that plant-based sources of choline can potentially replace CCL in the diets of broiler chickens[22]. This might be due to the lower absorption of plant-based methionine compared to its synthetic counterpart. Nonetheless, the effectiveness of supplementation of CCL on broiler performance appears to be a debated topic among researchers[23]. These variations could be linked to the level of methionine in the experimental diets. According to deSouzaReis et al., addition of choline might not be necessary when dietary methionine and cystine levels exceed 0.91%. This is due to the fact that methionine can donate methyl groups for the liver's endogenous choline synthesis[24].

Current choline recommendations for broilers have not been updated in decades. This is concerning since broiler genetics, diets, and overall performance have significantly improved during that time. Higher choline requirements are probably required as a result of these advancements, particularly faster lean muscle growth after the third week of age[25].

Our findings are consistent with a growing body of evidence indicating that plant-based choline sources can be beneficial for broilers. Supplementing with PHF at 500 g/ton improved carcass traits in our study, similar to the findings of Khose et al. with herbal choline at 500 kg/ton (improved liver protection and carcass traits)[26].

Moreover, Devegowda et al. reported that abdominal and liver fat had reduced in broilers fed with a vegetal choline source[23].

These findings are further supported by several studies demonstrating positive effects of other plant-based choline alternatives. According to Pande and Durape, FCR and reduced liver fat were improved in birds supplemented with the phytogenic product[27]. Marimuthu and D'Souza's field trial data suggest that herbal choline, a blend of *Acacia nilotica* and *Curcuma longa*, can be used effectively in commercial settings for commercial farm[28]. Microencapsulated basil oil at 500 ppm was shown to have a potential for enhancing intestinal integrity and nutrient utilization, leading to better broiler performance (improved gut health and performance)[29]. Based on the study by Dias et al., a plant-based choline source composed of different herbs maintained good health in broilers without any signs of nutrient deficiency[30]. Supplementation of chicory ethyl acetate extract at levels exceeding 250 mg/kg feed improved the growth performance and reduced blood lipids in heat-stressed broilers[31]. In addition, Kaninathan et al. concluded that PHF at 2000 g/ton during the finishing stage can improve the performance of broilers and prevent fatty liver in broilers[32].

The liver, a vital organ for bird metabolism, is sensitive to changes in diet. Serum levels of alanine transaminase (ALT) and AST enzymes are frequently used to measure liver health and function. When cells are damaged due to metabolic stress or high blood pressure, these enzymes leak into the bloodstream. Our study found that supplementing broiler feed with PHF at 500 and 750 g/ton was as effective as the supplementing standard (1000 g/ton) SCC in improving liver enzyme activity and reducing total serum cholesterol. It also helped maintain all measured blood chemistry parameters within healthy ranges. These findings suggest that PHF plays a role in protecting liver health. This is in line with the research by Khosravinia et al. who reported that supplementation of CCL prevented liver damage, as evidenced by lower serum levels of AST levels[34].

IV. Conclusions

Supplementing broiler chicken feed with PHF as feed supplement at either 500 or 750 g/ton improved the growth performance, carcass quality, and overall health parameters. These findings suggest that feed supplement PHF, at the 500 g/ton inclusion level, has the potential to replace SCC in broiler diets without compromising performance. This is further evidenced by the observed improvements in various performance metrics.

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