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# IMPACT OF PHYTOGENIC FORMULATION ON PERFORMANCE AND FATTY LIVER DISEASE OF BROILER CHICKENS

Arigesavan KANINATHAN, Suresh SUBRAMANIYAM, Saravanakumar MARIMUTHU, and Prashanth D'SOUZA

*R&D Centre, Natural Remedies Private Limited, Veerasandra Industrial Area, Electronic City (PO), Hosur Road, Bangalore 560100, Karnataka, India*

Email: saravana.k@naturalremedy.com

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**ABSTRACT**: This study was conducted to evaluate the effect of polyherbal (phytogenic) formulation (PHF: containing *Acacia nilotica* and *Curcuma longa*) on performance parameters, liver histopathology and prevention of fatty liver in broilers. 700 day-old chicks were randomly distributed to seven groups (10 replicates / group; 10 birds each), namely positive control (T1) fed with basal diet + choline chloride (CCL) 60% (1000g), negative control (T2) fed with high energy (5% increment), low protein (24% reduction), high cholesterol (2% increment) diet, T3 (T2 + PHF; 1000g-full cycle), T4 (T2 + PHF; 2000g-full cycle), T5 (T2 + CCL 60% (1000g-full cycle)), T6 (T5 + PHF; 1000g-grower and finisher stage), T7 (T5 + PHF; 2000g-finisher stage). Average daily gain (ADG; g), average daily feed intake (ADFI; g) and feed conversion ratio (FCR) were calculated at 1-14 days, 15-28 days, 29-42 days, and 1-42 days. Serum triglycerides analysis, gross and histopathological observations of liver morphology were performed for the samples of control and experimental groups on day 42. The performance parameters; ADG, ADFI, FCR, and liveability were found to be improved in all the groups as compared to the negative control group. However, better performance was observed in PHF (2000g) top-up group (during the finisher stage) as compared to the negative control group. Serum triglyceride levels were increased non-significantly as compared to the negative control indicating that more fat is mobilized from liver to serum. In addition, PHF supplementation at 2000g during the finisher phase had restored the liver tissue architecture as well as improved the liver score when compared to the negative control group. It is concluded that PHF (2000g/ton) during the finisher stage can be used as a top-up to improve the performance parameters as well as to prevent the fatty liver condition in broiler chickens.

**Keywords:** Basal diet, Broiler chicken, Choline chloride, Herbal formulation, Triglycerides.

**Abbreviation:** ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio; CCL: Choline chloride; PHF: Polyherbal formulation; HELP: High energy low protein

## INTRODUCTION

Domesticated chickens are the most important source of protein to be used for the human consumption worldwide, and consequently it would fetch an enormous economic added value in near future (Padhi, 2016). Generally, modern broilers would grow very fast and gain excessive weight due to the genetic modification and the standardized diet composition (Petracci et al., 2015). Owing to the rapid development of the meat-type chickens in a very short time would result in a high level of production performance but with some welfare issues that include skeletal leg deformity leading to defective movement (Julian, 1998; Çapar Akyüz and Onbaşılar, 2020). Therefore, society is mostly interested in animal–welfare friendly farmed meat-type chicken that would be reared using diet with high energy and low protein specifications (Bona et al., 2018). However, when the birds with restricted activity (occurs mostly in caged birds) are allowed to consume high energy diet, exhibits a disturbance in the metabolism of fat especially in the liver tissues, which results in accumulation of fat in the hepatocytes and thus causes a sudden death (Lin et al., 2021). The fatty liver syndrome is a non-infectious metabolic disorder mostly encountered in the poultry sector, as the 90% of *de novo* fatty acid is synthesized in the liver and its mobilization towards non-hepatic tissues depends upon the availability of lipoproteins in the liver. However, high energy and low protein diet increased the hepatic fatty acid without affecting the lipoprotein synthesis. As a result, the lipids cannot be completely mobilized which leads to the deposition of excess lipids in the hepatocytes (Zhang et al., 2017). The pathophysiology of the fatty liver associated haemorrhage syndrome is yet to be clearly identified. Nevertheless, the haemorrhage may result from the rupture of hepatic reticulin and capsule due to the abnormal liver swelling caused by deposition of fat (Trott et al., 2014). Hence, the better understanding of metabolic process and dietary factors that can interfere with the liver function is crucial to raise the commercial broiler flocks with exceptional zootechnical performance (Zaefarian et al., 2019).

Fatty liver is induced by various factors such as nutritional, hormonal, environmental and metabolic changes (Juanola et al., 2021). Many nutrients such as choline, phospholipids, vitamin B and vitamin E, demonstrate a vital role in maintaining the lipid homeostasis in the liver. Lack of these nutrients in the diet causes a disturbance in the metabolic functions such as lipid absorption, β-oxidation and lipoprotein synthesis that leads to excessive fat accumulation in the liver (Zeisel and Da Costa, 2009). More specifically, choline, a vitamin like water-soluble micronutrient and a lipotropic



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agent, plays a preventive role by enabling the fat utilization and transportation from the hepatic to the extrahepatic tissues (Biswas and Giri, 2015). It was reported that a diet with low choline content causes fatty liver syndrome due to low availability of carrier lipoproteins (Fon Tacer and Rozman, 2011), growth retardation and perosis in fast-growing broiler strains. To avoid the detrimental responses associated with choline deficiency, synthetic choline chloride (CCL) has been supplemented to the poultry diet to improve the growth performance and carcass quality (Gregg et al., 2022). However, due to its hygroscopicity, it would accelerate the oxidative loss of other vitamins which is already present in the feed when it is included in the feed formulation (Rath et al., 2017). To counteract the negative impacts, an herbal feed additive, Kolin Plus™, is used in the place of synthetic choline chloride in broiler diet. It is already reported to have choline like function in broilers (Selvam et al., 2018). On the other hand, a recent finding suggested that a high energy and low protein along with a high cholesterol diet caused the fat accumulation in the liver which leads to fatty liver disorder in chickens (Lin et al., 2021).

Hence in the current study, we have employed high energy (5% increment), low protein (24% reduction), high Cholesterol (2% increment) formulated diet to induce fatty liver model in broiler chicken. Besides, we have evaluated the effect of PHF alone or in combination with CCL on prevention of fatty liver associated problems in broiler chickens.

## MATERIAL AND METHODS

### Polyherbal formulation (PHF)

Kolin Plus™ is a PHF developed by M/s. Natural Remedies Pvt. Ltd., Bengaluru, India, containing *Acacia nilotica* and *Curcuma longa* plant parts.

### Ethical approval

The study was performed by authorized, qualified and trained veterinarians, scientists, and technicians, in compliance with the guidelines of the Institutional Animal Ethics Committee (IAEC).

### Study design

A total of 700 one-day-old Ross AP95 broiler chicks weighing between 30 and 60 g were selected for the study. The experiment was performed at Agrivet Research & Advisory Pvt. Ltd., Kolkata, India for 42 days. Chicks were randomly assigned to seven groups with ten replicates having 10 birds each, namely positive control (PC; Treatment-T1) fed on basal diet plus CCL 60% (1000g), negative control (NC; T2) fed with diet containing high energy (5% increment), low protein (24% reduction of crude protein), high Cholesterol (2% increment), T3 (T2 plus 1000g of PHF for full cycle), T4 (T2 plus 2000g of PHF for full cycle), T5 (T2 plus 1000g of CCL 60% for full cycle), T6 (T5 plus 1000g of PHF for grower and finisher stage), T7 (T5 plus 2000g of PHF for finisher stage). The birds were fed *ad libitum* with a starter (1-10 d), a grower (11-24 d) and a finisher (25-42 d) diet; the composition and nutritive values of the diet were presented in Table 1.

### Productive performance

The body weight (BW) of the birds was recorded pen-wise at weekly intervals. A measured quantity of the feed was offered to each pen and the cumulative feed intake (FI) was calculated during the periods of 1-14 d, 15-28 d and 29-42 d by subtracting the quantity of feed left in each pen from the total quantity offered during the respective period. Average daily gain (ADG) and average daily feed intake (ADFI) were calculated by the total weight gain or total weight of feed consumed (g) / total number of days in each respective period. The feed conversion ratio (FCR) was calculated as total feed consumption divided by total body weight gain. Mortality was recorded as and when it happened, and the weight of the dead birds was recorded to adjust the FCR data accordingly. Overall liveability was calculated for the cumulative period of 1-42 d.

### Gross observation

At 42 d of age, the whole liver was removed carefully and kept on wooden sheet for gross anatomical observation. The severity of fatty liver was assessed by liver color and scored, as modified based on a study conducted by Zhu et al. (2020). Briefly, liver color was scored as follows: Score-1, dark red and no haemorrhage; Score-2, mild yellow and haemorrhages (mild case); Score-3, light yellowish red and haemorrhages (moderate case); Score-4, large and massive haemorrhages with putty-coloured livers (extreme case).

### Serum biochemical parameters

At 42 d of age, after the final BW was recorded, 10 birds (1 bird / pen) were selected randomly in each dietary group, and whole blood was collected from the right brachial vein in vacutainer tubes without any anticoagulant and allowed to clot at room temperature for 2 hrs. Then the separated serum was harvested and analysed photometrically for the estimation of triacylglycerol using commercially available biochemical kits (Delta Lab®, Mumbai, India).

### Histopathological examination

At 42 d of age, the whole liver was cut into small pieces of approximately 4-5 mm width and was preserved in 10% buffered formal saline solution. Paraffin embedded tissue was stained with standard Haematoxylin and Eosin method, mounted using DPX mounting medium (S.D. fine-chem Ltd., Bengaluru, India) and was observed under a microscope



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(Olympus Corporation, Tokyo, Japan) connected with the camera (DP20) for histopathological examination to evaluate the hepatic changes due to dietary treatments.

### Statistical analysis

Data were analysed using the pen averages as the experimental units for the performance traits (ADG, feed intake, ADFI, FCR and liveability). For histological observations and serum biochemical assay, individual observations were considered as the experimental units. The data were analysed in the general linear model of SPSS (V. 26.0) using multivariate ANOVA and expressed in terms of mean and pooled standard error of the mean. Probability values of $p<0.05$ were described as statistically significant; whenever found significant, the means were separated by Tukey's B test.

**Table 1** - Composition and calculated nutritive values (g/kg as fed basis unless stated otherwise) of the positive control diets

| Ingredients (g/kg diet) | Starter (1-10 d) | Grower (11-24 d) | Finisher (25-42 d) |
|---|---|---|---|
| Maize | 578.13 | 616.24 | 644.1 |
| Soybean meal Hi Pro | 322.43 | 279.7 | 234.1 |
| Palm oil | 24.1 | 28.23 | 38.84 |
| Cholesterol-91% | 0 | 0 | 0 |
| Maize gluten meal | 34.7 | 40 | 49.2 |
| Dicalcium phosphate | 15.61 | 13.23 | 11.24 |
| Limestone powder | 9.5 | 8.86 | 8.8 |
| Salt | 2.72 | 2.22 | 2.2 |
| Sodium bi carbonate | 2 | 2 | 2 |
| DL-methionine | 2.54 | 2 | 1.82 |
| L-lysine HCl | 2.64 | 2.3 | 2.57 |
| L-threonine | 0.93 | 0.52 | 0.43 |
| Vitamin premix+ | 1 | 1 | 1 |
| Trace mineral premix++ | 0.5 | 0.5 | 0.5 |
| NSPase enzyme | 0.1 | 0.1 | 0.1 |
| E. coli phytase 5000 | 0.1 | 0.1 | 0.1 |
| Choline chloride | 1 | 1 | 1 |
| Filler (rice husk)+++ | 2 | 2 | 2 |
| **Nutrients** | | | |
| AME kcal/kg | 3000 | 3100 | 3200 |
| Crude protein | 23 | 21.5 | 20 |
| **SID amino acids %** | | | |
| Lysine | 1.28 | 1.15 | 1.06 |
| Methionine | 0.6 | 0.54 | 0.52 |
| Met + Cys | 0.95 | 0.87 | 0.66 |
| Threonine | 0.86 | 0.77 | 0.71 |
| Tryptophan | 0.25 | 0.22 | 0.2 |
| Arginine | 1.37 | 1.23 | 1.13 |
| Isoleucine | 0.91 | 0.85 | 0.76 |
| Valine | 0.98 | 0.93 | 0.85 |
| Crude fibre | 2.68 | 2.6 | 2.56 |
| Crude fat | 5.38 | 5.73 | 7.11 |
| Calcium | 0.96 | 0.87 | 0.81 |
| Available P | 0.48 | 0.44 | 0.41 |
| Sodium | 0.22 | 0.2 | 0.2 |
| Potassium | 0.94 | 0.84 | 0.79 |
| Chloride | 0.22 | 0.18 | 0.18 |
| Choline ppm | 1700 | 1600 | 1500 |

+: each kg contained vitamin A 13.5 MIU, vitamin D3 4.5 MIU, vitamin E 60 g, vitamin K3 3.5 g, vitamin B1 3.5 g, vitamin B2 8.0 g, vitamin B6 3.5 g, vitamin B 12 0.02 g, biotin 0.145 g, pantothenic acid 14.5 g, folic acid 2.25 g, niacin 60 g); ++: protein chelates of manganese 60 mg, iron 30 mg, zinc 50 mg, copper 10 mg, selenium 0.5 mg, chromium 0.4 mg, iodine 4.0 mg (as potassium iodide). +++: The test material was added by replacing an equivalent amount of the filler substance.

## RESULTS

### Effect of PHF on the performance trait

High energy and low protein along with high cholesterol diet suppressed the production performance of birds in the T2 when compared to the T1. However, all the groups showed an improvement in the ADG, ADFI, feed intake and FCR as compared to T2 ($P\leq0.01$). Supplementation of PHF (2000g/ton) as a top-up (along with CCL-60%) in during the finisher stage showed better performance as compared to other supplemented groups (Table 2).



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**Effect of PHF on triglycerides and liver color score**

Table 3 showed the effect of PHF on serum triglycerides and liver color score. The birds in T2 showed low serum triglycerides and a higher color score of liver (gross observation) as compared to T1 (Figure 1). On the other hand, product supplemented groups showed a significant increase the serum triglycerides level (P≤0.01) and numerical increment in the liver color score (P>0.05) as compared to T2. This indicates that more fat is mobilized from liver to non-hepatic tissues in broilers supplemented with PHF and CCL. Supplementation of PHF (2000g/ton) as a top-up (along with CCL-60%) in T7 during the finisher stage showed higher serum triglycerides level and better color score as compared to other supplemented groups.

**Effect of PHF on liveability (%)**

Table 2 showed the effect of PHF on liveability %. When compared with T2 group, liveability % was improved in all the supplemented groups.

**Effect of PHF on histopathological alteration**

Table 4 showed the effect of PHF on histopathological alteration. Hepatic vacuolization along with necrosis was predominantly observed in T2 which was fed with high energy and low protein diet devoid of choline and the presence of cholesterol (Figure 2). This hepatic vacuolization along with hepatocellular necrosis is subject to fat deposition in hepatic tissue. Moreover, changes have become mild to moderate in different groups with the least pathologic changes observed in T7 (PHF 2000 g along with CCL 60% 1000 g- during finisher stage) followed by T5 (CCL 60% 1000 g) and T4 (PHF 2000 g).

**Table 2 -** Effect of PHF on the performance traits

| Day | (T1) PC | (T2) NC | (T3) T2+PHF 1000g FC | (T4) T2+PHF 2000g FC | (T5) T2+CCL60% 1000g FC | (T6) T5+PHF 1000g GF | (T7) T5+PHF 2000g F | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| **Average Daily Gain (ADG, g)** | | | | | | | | | |
| 1-14d | 37.14$^{b}$ | 31.14$^{a}$ | 30.43$^{a}$ | 30.49$^{a}$ | 31.74$^{a}$ | 30.78$^{a}$ | 31.25$^{a}$ | 0.29 | 0.0001 |
| 15-28d | 74.61$^{b}$ | 52.01$^{a}$ | 54.01$^{a}$ | 51.59$^{a}$ | 54.71$^{a}$ | 52.20$^{a}$ | 55.90$^{a}$ | 0.98 | 0.0001 |
| 29-42d | 85.83$^{b}$ | 71.62$^{a}$ | 74.23$^{a}$ | 76.23$^{a}$ | 74.95$^{a}$ | 75.40$^{a}$ | 76.62$^{a}$ | 0.73 | 0.0001 |
| 1-42d | 65.86$^{c}$ | 51.59$^{a}$ | 52.89$^{ab}$ | 52.77$^{ab}$ | 53.80$^{ab}$ | 52.79$^{ab}$ | 54.59$^{b}$ | 0.59 | 0.0001 |
| **Average Daily Feed Intake (ADFI, g)** | | | | | | | | | |
| 1-14d | 43.52$^{c}$ | 41.88$^{abc}$ | 41.55$^{ab}$ | 41.11$^{a}$ | 42.96$^{bc}$ | 41.82$^{ab}$ | 41.90$^{abc}$ | 0.17 | 0.001 |
| 15-28d | 111.99$^{b}$ | 95.01$^{a}$ | 95.56$^{a}$ | 94.76$^{a}$ | 99.21$^{a}$ | 96.43$^{a}$ | 100.67$^{a}$ | 0.94 | 0.0001 |
| 29-42d | 156.93 | 146.88 | 151.73 | 152.09 | 152.25 | 151.52 | 152.03 | 1.14 | 0.488 |
| 1-42d | 104.15$^{b}$ | 94.59$^{a}$ | 96.28$^{a}$ | 95.99$^{a}$ | 98.14$^{a}$ | 96.59$^{a}$ | 98.20$^{a}$ | 0.54 | 0.0001 |
| **Feed Conversion Ratio (FCR)** | | | | | | | | | |
| 1-14d | 1.172$^{a}$ | 1.346$^{b}$ | 1.366$^{b}$ | 1.349$^{b}$ | 1.354$^{b}$ | 1.359$^{b}$ | 1.341$^{b}$ | 0.008 | 0.0001 |
| 15-28d | 1.501$^{a}$ | 1.828$^{b}$ | 1.772$^{b}$ | 1.839$^{b}$ | 1.815$^{b}$ | 1.849$^{b}$ | 1.802$^{b}$ | 0.016 | 0.0001 |
| 29-42d | 1.829$^{a}$ | 2.056$^{b}$ | 2.042$^{b}$ | 1.998$^{b}$ | 2.033$^{b}$ | 2.010$^{b}$ | 1.985$^{b}$ | 0.013 | 0.0001 |
| 1-42d | 1.582$^{a}$ | 1.834$^{b}$ | 1.820$^{b}$ | 1.821$^{b}$ | 1.825$^{b}$ | 1.830$^{b}$ | 1.799$^{b}$ | 0.011 | 0.0001 |
| **Liveability (%)** | | | | | | | | | |
| 1-42d | 94 | 92 | 95 | 94 | 97 | 95 | 100 | 0.73 | 0.09 |

Means with dissimilar superscripts in a row varied significantly; PHF – Polyherbal formulation; CCL – Choline chloride; FC – Full cycle; GF – Grower & finisher; F – Finisher

**Table 3 -** Effect of PHF on triglycerides and liver color score

| Parameters | (T1) PC | (T2) NC | (T3) T2+PHF 1000g FC | (T4) T2+PHF 2000g FC | (T5) T2+CCL60% 1000g FC | (T6) T5+PHF 1000g GF | (T7) T5+PHF 2000g F | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| TG (mmol/L) | 1.336$^{a}$ | 2.595$^{b}$ | 3.061$^{b}$ | 2.671$^{b}$ | 2.614$^{b}$ | 2.875$^{b}$ | 2.812$^{b}$ | 0.084 | 0.0001 |
| Color | 0.3 | 0.8 | 0.7 | 0.6 | 0.6 | 0.45 | 0.45 | 0.067 | 0.484 |

Means with dissimilar superscripts in a row varied significantly; PHF – Polyherbal formulation; TG – Triglycerides; CCL – Choline chloride; FC – Full cycle; GF – Grower & finisher; F – Finisher; Lower the color score indicates less fat accumulation; Liver color score: 1 – dark red, without any haemorrhagic spots and firm in consistency, 2 – firm in consistency but slightly yellowish in colour with few haemorrhagic spots, 3 – softer in consistency and moderate area of haemorrhagic patches were observed, 4 – pale yellow in colour with large haemorrhagic patches



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**Table 4 -** Effect of PHF on histopathological alteration

| Parameters | (T1) PC | (T2) NC | (T3) T2+PHF 1000g FC | (T4) T2+PHF 2000g FC | (T5) T2+CCL60% 1000g FC | (T6) T5+PHF 1000g GF | (T7) T5+PHF 2000g F |
|---|---|---|---|---|---|---|---|
| Hepatic vacuolization | 0.3 (10) | 2.3 (80) | 2 (60) | 1.5 (50) | 1.5 (50) | 1.7 (70) | 1.2 (30) |
| Congestion | 0 | 1.1 | 0.5 | 1 | 0.3 | 0.1 | 0.1 |
| Haemorrhages | 0 | 1.4 | 0.4 | 1 | 0.2 | 0 | 0.2 |
| Inflammation | 0.1 | 0.6 | 0.4 | 1 | 0 | 0 | 0.1 |
| Hepatocellular necrosis | 0.2 (0) | 1.7 (40) | 1.6 (50) | 1.3 (40) | 1.2 (40) | 1 (20) | 1.1 (20) |

Values are expressed as Mean and Percentage in parentheses; n=10; PHF – Polyherbal formulation; CCL – Choline chloride; FC – Full cycle; GF – Grower & finisher; F – Finisher

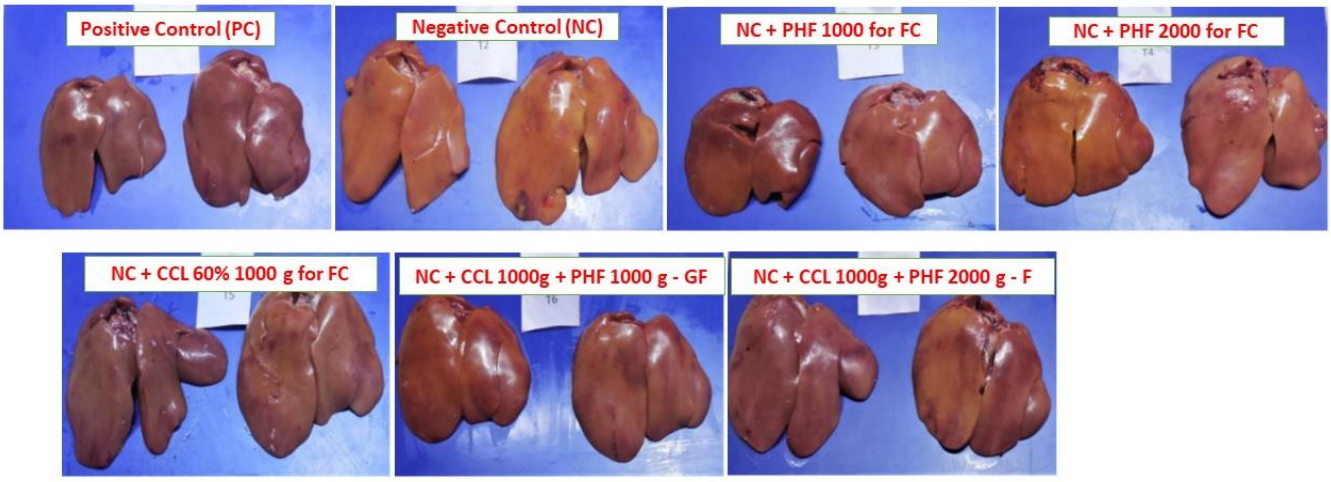


**Figure 1 - Effect of PHF on liver color score.** Liver color was scored as follows: 1: dark red, without any haemorrhagic spots and firm in consistency; 2: firm in consistency but slightly yellowish in color with few haemorrhagic spots; 3: softer in consistency and moderate area of haemorrhagic patches were observed; 4: pale yellow in colour with large haemorrhagic patches. Means with dissimilar superscripts in a row varied significantly; PHF: Polyherbal formulation; CCL: Choline chloride; FC: Full cycle; GF: Grower and finisher; F: Finisher

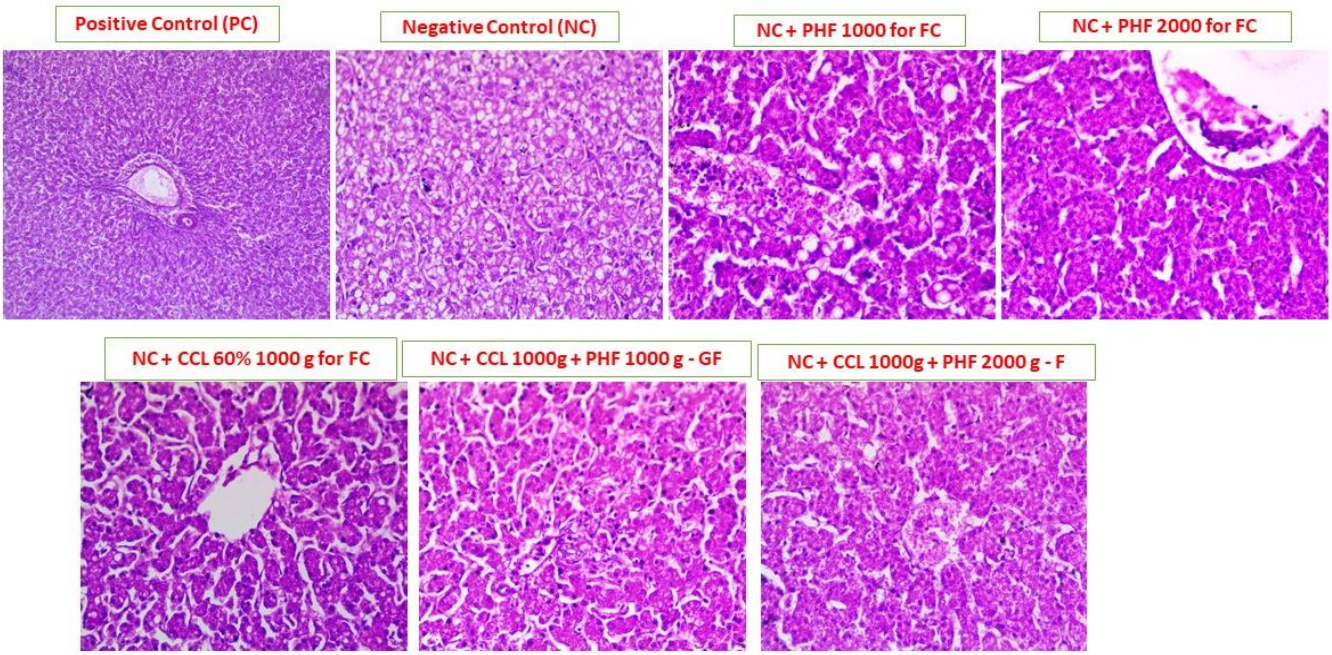


**Figure 2 - Effect of PHF on histopathological alteration.** A: normal hepatic parenchyma (Score 0); B: Severe hepatic vacuolization (Score 3); C: Moderate hepatic vacuolation (Score 2); D: No to rare hepatic vacuolization (Score 0); E: Mild hepatic vacuolization (Score 1); F: Mild hepatic vacuolization (Score 1); G: Mild hepatic vacuolization (Score 1); PHF: Polyherbal formulation; CCL: Choline chloride; FC: Full cycle; GF: Grower & finisher; F: Finisher



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## DISCUSSION

Fatty liver disease is a serious problem to poultry sectors in several parts of the world even in well-managed farms (Whitehead, 1979). Poultry liver injury, a clinically common disease, can lead to low performance and even death, and has created a huge economic loss in the poultry industry (Lin et al., 2021). At present, due to the lack of effective hepatoprotective agent in the market, herbal based supplement has gained a significant importance to prevent the fatty liver disease in broiler chickens. Hence in the current study, we have employed a high energy and low protein with high cholesterol diet to induce fatty liver associated problems in broiler chickens. Furthermore, we have evaluated the effect of PHF alone or in combination with CCL on the prevention of fatty liver associated problems in broiler chickens.

High cholesterol with low protein and high energy diet suppressed the broiler's production performance, which was reversed by PHF alone or PHF plus CCL 60% in dose dependent manner. In addition, the current data revealed that supplementation of PHF (2000g/ton) as a top-up (along with CCL-60%) during the finisher stage showed better body weight gain (ADG), feed intake (ADFI) and FCR as compared to NC group. This agreed with the earlier reports who showed that PHF improved the production performance in broilers fed with choline deficient diet (Selvam et al., 2018). Similarly, this herbal supplement comprised of *A. nilotica* and *C. longa* was reported that it efficiently regulates the specific genes involved in liver protection and growth in broilers fed a choline-deficient diet (D'Souza et al., 2019; Marimuthu et al., 2022). Ncube et al., (2012) also reported that body weight gain was noticed in birds supplemented with *Acacia* supplemented diet. Hence, it indicates that the negative impact was reversed through the lipotropic action of herbal ingredients present in the PHF.

Lower serum triglycerides level and higher gross liver color score with histopathological alterations (hepatic vacuolization along with necrosis) reflect the fatty liver associated problems in broilers fed with fed high energy, low protein (HELP) diet devoid of choline and the presence of high cholesterol. The liver is the largest substantive vital metabolic organ, which has several complex physiological roles including metabolism, excretion, and detoxification (Alamri, 2018). It oxidizes the dietary fatty acids to produce the energy required for its own metabolism. Nevertheless, the major function of liver is to convert the surplus dietary fatty acids to triglycerides (TG) and mobilize it to the extrahepatic tissues. On the other hand, when the TG production is more than 5% of liver weight, it leads to the accumulation of fat in the hepatocyte that weakens the organ function (Heeren et al., 2021). Fat accumulation exceeds 5% to 10% of the liver's weight directly correlates with the presence of fatty vacuoles in the cytoplasm of hepatocytes (Nassir et al., 2015). Despite being useful energy sources, an excess of fat evokes excessive β-oxidation and eventually produces high ROS levels. These ROS mediated oxidative stress could result in the damage of hepatocellular membrane due to inactivation of antioxidant enzymes and lipid peroxidation in the liver (Bjørklund et al., 2017). Also, the current results were in accordance with Mei et al. (2020) report who demonstrated that the fatty liver condition induced hepatocytic vacuolation and hepatocyte necrosis in broilers. The above findings clearly demonstrated that high energy, low protein and high cholesterol diet without lipotropic-agents leads to the accumulation of excess TG in liver, which eventually diminish the production performance in broiler chickens. However, the negative impact such as high serum triglycerides and low liver color score with mild to moderate hepatic vacuolization was observed in all supplemented groups, especially the remarkable improvement which was detected in birds supplemented with CCL 60% plus PHF (during finisher stage) when compared with NC. Abdel-Razik et al. (2006) suggested that *Acacia nilotica* exerts hepatoprotective effects by providing maximum protection against $CCl_4$-induced liver injury. Furthermore, it was reported that *Acacia* species supplementation restored the normal architecture of hepatocytes in acetaminophen induced liver toxicity models (Kannan et al., 2013). Ikarashi et al. (2011) also suggested that polyphenol extracted from the bark of *Acacia* species lowered fat accumulation, indicating that *Acacia* polyphenols suppressed the fatty liver in mice fed with high-fat diet. Furthermore, Feng et al. (2012) reported that curcumin can effectively prevents the high fat diet induced liver steatosis in mice. This agreed with Xie et al. (2019), who reported that curcumin supplementation regulates the lipid metabolism and reduces the fatty liver in broiler chickens. The above points clearly showed that both *Acacia* and *Curcumin* were known to have hepatoprotective property in birds. Moreover, the current results demonstrated that supplementation of PHF with CCL 60% at finisher stage facilitates the fat mobilization in the broiler chickens fed with high energy, low protein and high cholesterol diet.

## CONCLUSION

It is concluded that polyherbal formulation (PHF; 2000g/ton) with choline chloride (CCL 60 %) during the finisher stage can be used as a top-up to improve the performance parameters and to prevent the fatty liver condition in broiler chickens.

## DECLARATIONS

**Corresponding author**
E-mail: saravana.k@naturalremedy.com

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**Authors' contribution**

All authors contributed equally to research work execution, analysing, interpreting the data and manuscript preparation.

**Conflict of interests**

The authors declare that they have no competing interests.

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