**Original Research Article**

**Efficacy of PhyCholine as a dietary replacement of synthetic choline chloride in commercial broiler production**

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**ABSTRACT**

This study was conducted to evaluate the efficacy of PhyCholine as a dietary replacement of synthetic choline chloride on production performance, serum biochemical profile, carcass characteristics and meat quality in commercial broiler chickens. A trial on the VenCobb 430 Y breed of commercial broiler (n=420, 4 replicates/group, each comprising 35 birds) was conducted for 42 days. The study was performed in 3 groups viz. T1 (basal diet fed), T2 (basal diet + synthetic choline chloride (60%) at 1.0 kg/MT feed, fed.) and T3 (basal diet + PhyCholine at 0.40 kg/MT feed, fed). The parameters such as live body weight, feed consumption, feed conversion ratio (FCR), European Production Efficiency Factor (EPEF), carcass characteristics, meat quality and serum biochemistry were assessed. It was concluded that the PhyCholine supplemented at dose level of 0.40 kg/MT was found to be equally effective to improve the overall performance when replaced synthetic choline chloride (1.0 kg/MT) in broiler diets. Moreover, the supplementation of PhyCholine was more beneficial in terms of survivability, carcass traits, meat quality and serum biochemical profile as compared to synthetic choline chloride.

**Key words:** Broiler, Carcass traits, Herbal Choline, PhyCholine, Synthetic choline chloride,

**INTRODUCTION**

Choline is a beta-hydroxy ethyl trimethyl ammonium hydroxide. Choline is essential for the formation of acetylcholine, which is a substance that is released at the termination of parasympathetic nerves for transmission of nerve impulses from presynaptic to postsynaptic fibers of the sympathetic and parasympathetic nervous systems. In addition, choline plays an essential role in fat metabolism in the liver. It prevents abnormal accumulation of fat (fatty liver) by promoting its transport as lecithin or by increasing the utilization of fatty acids in the liver itself (Xu *et al*., 2010).

Choline is thus referred as a potent “lipotropic” factor due to its function of acting on fat metabolism by hastening removal or decreasing deposition of fat in liver. Choline also acts as a methyl group donor, after oxidizing to betaine. In poultry diet, betaine can also be incorporated which helps to convert homocysteine to methionine in the transmethylation pathway.

Biotin also plays an important role in fat metabolism and is absolutely necessary in the prevention of fatty liver syndrome. Biotin deficiency in poultry affects the specific activities of the biotin-dependent enzymes such as pyruvate carboxylase and acetyl Co-A carboxylase. Pyruvate carboxylase controls the conversion of excess energy into glucose in the gluconeogenesis pathway instead of lipid through liponeogenesis. It has been reported that fatty liver syndrome occurs in birds with impaired hepatic gluconeogenic capacity as a result of dietary insufficiency of biotin. The important fact that remains unnoticed is the interdependence of choline and biotin for fat metabolism. It has been found out in various studies that fatty liver syndrome due to biotin deficiency is more aggravated by dietary supplementation of choline. Addition of supplemental choline to a biotin-deficient diet decreases the biotin status of chicks and increases mortality due to fatty liver syndrome (Kumar, P., & Sharma, S., 2014).

The use of high energy diets aims at shortening the rearing period may increase metabolic disorders such as fatty liver syndrome (FLS) in broiler chickens (Leeson *et al*., 1995). Increased abdominal fat pad (Corduk *et al*., 2007), incidences of leg problems (Van Emous *et al*., 2015) and hypertension (Gopi *et al*., 2014) are some other detrimental responses associated with high energy diets (Buyse *et al*., 2001). Choline is a rediscovered critical amino acid for poultry and usually added to poultry diets in the form of synthetic choline chloride to address the issues related to fatty liver. However, synthetic choline chloride has several drawbacks. Approximately 70% of choline chloride is not absorbed in the gut, and instead, intestinal bacteria convert it to trimethylamine (TMA) (Hoyles *et al*., 2018), a toxic compound (Fallah *et al*., 2016; Landfald *et al*., 2017). TMA is absorbed in small intestine and, through the bloodstream, reaches the body tissues, causing deleterious effects (Zeisel *et al*., 2003; Craciun & Balskus, 2012). Another occurring problem with choline chloride usage is its highly hygroscopic characteristic, and its presence in vitamin premixes and rations accelerates the destruction of other vitamins present in feed. In addition, choline chloride has obnoxious odour which is a matter of practical concern in processing units and feed mills. Currently, there are natural products, produced from selected plants, with content of choline in esterifies form and with high bioavailability, which may be an effective alternative to synthetic choline chloride.

Moreover, it is very compatible with short time high temperature processing for pelleting/extrusion which is common in feed manufacturing plants. It is very clear from various studies that plant-based choline can prevent fatty liver syndrome. Experimental studies also support facts that the complete replacement of choline chloride from poultry feed by plant-based choline (Chatterjee and Misra, 2004; Muthukumarasamy *et al*., 2004; Gangane *et al*., 2010)is very safe and effective along with optimum performance of the birds.

PhyCholine is a natural poly herbal animal feed supplement manufactured by PhyGeno a division of Avitech Nutrition Pvt. Ltd., Haryana, India. It is comprised of plant bioactive compounds like phosphatidylcholine, betaine, biotin and other phytogenic compounds. These compounds are rich in natural choline as well as conjugated choline in the stable and highly bioavailable form. Phytogenic compounds in PhyCholine also contain small quantities of betaine and biotin. These small quantities of betaine and biotin supply readily available methyl groups for required methylation reactions in the system.

The objective of the present study was to evaluate the comparative efficacy of PhyCholine and synthetic choline chloride on performance parameters, carcass characteristics, meat quality and serum biochemistry in commercial broilers.

Figure: Advantages of PhyCholine over synthetic choline chloride

**MATERIALS AND METHODS**

**Feed supplements**

PhyCholine was added in the broilers’ diet at a dose rate of 0.40 kg/MT of feed along with basal diet. Whereas synthetic choline chloride was added to the broiler’s diet at a dose rate of 1.0 kg/MT of feed. The control group was provided with the basal diet only. PhyCholine is a polyherbal feed supplement of PhyGeno a division of Avitech Nutrition Pvt. Ltd., Haryana, India. It mainly contains phosphatidyl choline, betaine, biotin and other phytogenic compounds.

**Experimental design**

The poultry trial of PhyCholine was conducted in VenCobb 430 Y breed of commercial broilers (n=420). The study was conducted in 3 groups viz control (basal diet fed), T2 (basal diet + synthetic choline chloride at 1.0 kg/MT feed, fed.) and T3 (basal diet + PhyCholine at 0.40 kg/MT feed, fed). The trial was designed in 4 replicates per group, where each replicate comprised 35 birds. The study was carried out for a period of 42 days. The biological experiment was conducted at “Sree Sai Poultry Solutions” (SSPS) Nildoh (Pannase) Mangrul Road, Ta. Hingna Dist. Nagpur which is recognized broiler research facility of Nagpur Veterinary College, MAFSU, Nagpur.

**Feeding and watering**

The feeding trial was continued up to 42 days of age of broiler chicken in three growth phases i.e., Pre-starter (0-12d), Starter (13-26 d) and Finisher (27-42 d). All the experimental birds were maintained on similar diets (iso-caloric and iso-nitrogenous) to meet the nutrient requirement of commercial broiler strain. Replicate wise body weight of birds (in group) and feed intake were recorded weekly to calculate growth, feed consumption and FCR. The replicate wise feed refusal was collected phase wise. The birds were vaccinated as practiced in commercial rearing of broilers. The chicks were vaccinated against Ranikhet disease (LaSota strain) and Infectious Bursal disease (intermediate strain) at 7th and 14thday of age, respectively, and with further booster of Raniket disease (LaSota strain) vaccine on 22nd day of age. However, all the birds received similar vaccination and medication program if any.

**Parameters studied**

Phase wise (0-12 d pre-starter, 13-26 d starter and 27-42d finisher) live body weight changes, feed consumption, feed conversion ratio, European Broiler Index (EBI) or European Production Efficiency Factor (EPEF) were recorded. Mortality and weight of dead birds were recorded as and when occurred to calculate mortality corrected feed conversion ratio. EBI or EPEF were calculated taking into account of feed conversion, mortality and average daily gain in live weight using the following formula: EBI or EPEF = (Average grams gained/day X livability %) / (FCR X Age of slaughter). At the end of each feeding phase (Pre starter; Starter; Finisher) blood samples were collected aseptically (4 birds per group) to study haematological profile. The cholesterol, LDL, HDL, glucose, total protein, albumin and globulin were estimated. 4 birds from each dietary group were sacrificed on 42nd day of age. At the end of the experiment different meat quality parameters like extract releasing volume, general acceptability, pH, colour and smell were studied. The carcass traits viz. dressed weight and cut-up parts yields were also recorded.

**Statistical analysis**

The data were subjected to statistical analysis using SPSS in a completely randomized design. One-way ANOVA was followed to all the parameters. Each replicate was used as a single observation for body weight gain, feed intake and feed conversion ratio and EPEF.

**RESULTS AND DISCUSSION**

**Growth performance**

Cumulative feed intake and live body weight (LBW) (Table 2 &3, respectively) remained more or less similar (non-significant) among the group during pre-starter growth phase. The FCR (Table 4) did not differ statistically during pre-starter growth phase. During starter growth phase no major difference existed among the group for live body weight, body weight gain, feed intake and feed conversion ratio. However, during finishing phase (27-42 d of age), live body weight and feed intake was lower with poor FCR (Feed: LBW) in T1 (control) group maintained without any source of choline. Increased live body weights (2388 and 2407 g respectively, Table 3) is recorded in synthetic choline fed (T2 group) birds and PhyCholine fed birds (T3group). The live body weights at 42 days of age (P<0.01)differed statistically in dietary treatment group as compared to control group. In treatment group having either source of choline had significantly higher live body weight or body weight gain than the diet without any supplemental choline in broiler chickens.

Similar to present finding, Kathirvelan *et al*. (2013) reported that the body weight of control group receiving diet without choline was significantly (p<0.05) less as compared to birds receiving diet with choline. They also concluded that supplementation of natural choline @1.0 kg/MT in broiler diet may be replaced synthetic choline (1.0 kg/MT of feed) without affecting body weight gain. The data found agree with those of Calderano *et al*. (2015) and Farina *et al*. (2017), who found similar results on performance of broilers when evaluating the substitution of choline chloride with vegetable choline. Feed intake did not differ statistically during the growth phase. However, there were numerical differences in FCR calculated on live weight as well as on weight gain basis during 0-42 and 27-42days of age respectively (Table 4). Choline supplemented diets had significantly improved FCR than control diet. As per values depicted, (Table 4) FCR at 2.0 kg body weight ranged from 1.40 (T2-synthetic choline chloride) or 1.41(T3-PhyCholine) to 1.76 (T1-control) and improved significantly (P<0.01) in all the diets containing either source of choline in comparison to control group. The marginal improvement in feed intake observed in PhyCholine group (Table 2) could be due to its plant ingredients containing a broad spectrum of vitamins, acids and alkaloids among many active compounds act by increasing bile flow, thereby improving feed intake, and consequently, increasing weight gain without change in feed intake in the supplemented group suggests that lipotropic agents (especially vegetable choline and choline chloride) may improve energy use in the feed.

In corroboration with present finding, Sharma *et al*. (2015) reported that the feed intake in birds fed diets supplemented with herbal choline at 500 gm per MT of feed was higher than birds fed diets supplemented with synthetic choline chloride (60%) at 1.0 kg /MT of feed for a period of 42 days. The vegetal source of choline acts as a replacement of choline chloride in the diet of broilers (Calderano *et al*., 2015). This was consistent with the findings of (Kumar 2009 ; Demattê Filho *et al*., 2015) that weight gain, feed intake, feed conversion and livability of broiler chickens were similar when replacing the choline chloride by a vegetal source of choline in the diets. This result was similar to those verified by Chatterjee & Misra (2004) and Muthukumarasamy *et al*. (2004) reported that the performance of broiler chickens was similar when replacing the choline chloride by a vegetal source of choline in the diets. Although, some studies reported an improvement in weight gain and FCR of birds supplemented with vegetal source of (Yu, 2009 ; Pompeu *et al*., 2011).

**European broiler index and survivability**

Highest value of European Broiler Index (EBI) or European Production Efficiency Factor (EPEF) (Table 5) during 0-42 days of age was observed in T3 diet with PhyCholine @ 0.4 kg per MT of feed, followed by in the T2 diet fed synthetic choline chloride 60% @ 1.0 kg per MT of feed. However, lowest value of EPEF (240.93) was recorded in the diet devoid of any form of choline. Mortality (Table 5) ranged from 5.71 to 7.14 % in different groups. Highest mortality (7.19%) was observed in the group without any form of choline. Survivability of birds was better on inclusion of choline, either synthetic or herbal one.

**Carcass characteristics**

The dressing yields (% of live weight) did not differ significantly (P>0.05) among the dietary treatment groups (Table 6). Similarly, the cut-up parts did not differ statistically except the wing yield. However, higher values of dressing yields were observed in PhyCholine group (72.02 %).

**Meat quality parameters**

Meat quality parameters in different groups are presented in Table 7. Colour, smell and general acceptability of meat did not differ among the dietary treatments. The pH values of breast meat samples were recorded shortly after slaughtering (15 min of post-mortem). Extract release volume of meat was also differed significantly (P<0.05) among the groups. In the present study the pH values were between 6.45 and 6.70 and thus in normal range. Highly accelerated pH (pH<5.6) is related to low meat colour and poor juice retention.

**Serum biochemical profile**

Serum biochemical parameters are presented in Table 8. Cholesterol, HDL and glucose did not differ among the dietary treatment groups. Although, numerical improvement in glucose level in T3 (PhyCholine) was recorded than that of T1 (control, without any choline) and T2 (with synthetic choline chloride). It could be due to fact that biotin dependent enzyme Pyruvate carboxylase controls the conversion of excess energy into glucose in the gluconeogenesis pathway instead of lipid through liponeogenesis.

However, LDL cholesterol differed significantly (P<0.01) among dietary treatment groups. LDL cholesterols were higher in dietary control group (without any choline) and T2group (with synthetic choline chloride) while lower in T3(PhyCholine) group.

The results indicated that the choline chloride @1.0 kg per MT of feed and PhyCholine @ 0.40 kg per MT of feed supplementation in broiler diet was safe and did not alter the haematological parameters at 42nd day of age.

**CONCLUSION**

It was concluded that the PhyCholine supplemented at dose level of 0.40 kg/MT was found to be equally effective to improve the overall performance when replaced synthetic choline chloride (1.0 kg/MT) in broiler diets. Moreover, the supplementation of PhyCholine was more beneficial in terms of survivability, carcass traits, meat quality and serum biochemical profile. On the basis of results, the use of PhyCholine at dose rate of 0.40 kg/MT can replace the use of synthetic choline chloride (60%) in commercial broiler birds.

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| **Table 2: Feed consumption at different growth phases** | | | | |
| Groups | Feed Intake | | | |
| 0-12 d | 13-26 d | 27-42 d | 0-42 d |
| T1 | 435.07±05.74 | 1118.98±39.40 | 2248.50b±66.28 | 3802.55±84.81 |
| T2 | 436.86±12.86 | 1079.99±58.95 | 2475.36a±78.16 | 3992.21±137.00 |
| T3 | 445.21±07.03 | 1115.38±26.46 | 2489.00a±38.04 | 4050.60±59.38 |
| SEM | 4.93 | 23.43 | 46.88 | 60.83 |
| P-Value | (NS) | (NS) | (P<0.05) | (NS) |

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| **Table 1:** Experimental design | | | | | |
| Groups | Groups details | Replicate/treatment | Birds/replicate | Birds/treatment | |
| T1 | Basal Diet | 4 | 35 | 140 | |
| T2 | Basal Diet + Synthetic choline chloride @ 1.0 kg/MT feed | 4 | 35 | 140 | |
| T3 | Basal Diet + PhyCholine @ 0.40 kg/MT feed | 4 | 35 | 140 | |
| Total no. of birds | | | | | 420 |
| Trial Period | | | | | 42 days |

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| **Table 3:** Live body weight at different stages of production (g/bird) | | | |
| Groups | Live body weight | | |
| 12d | 26d | 42d |
| T1 | 330.25±08.83 | 1047.80±15.40 | 2081.80b±34.23 |
| T2 | 322.93±10.05 | 1038.09±17.25 | 2388.33a±43.59 |
| T3 | 329.54±16.82 | 1066.80±27.45 | 2406.82a±79.59 |
| SEM | 6.56 | 11.40 | 53.62 |
| P-Value | NS | NS | (P<0.01) |

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| **Table 4:** Feed conversion ratio at different growth phase | | | | |
| Groups | Feed Conversion Ratio | | | |
| Live body weight (kg) | | | FCR 2.0 Kg. BW |
| 0-12 d | 0-26 d | 0-42 d |
| T1 | 1.31±0.02 | 1.48±0.04 | 1.83±0.06 | 1.76a±0.08 |
| T2 | 1.35±0.02 | 1.47±0.09 | 1.67±0.03 | 1.40b±0.02 |
| T3 | 1.35±0.05 | 1.47±0.04 | 1.69±0.04 | 1.41b±0.08 |
| SEM | 0.02 | 0.03 | 0.03 | 0.06 |
| P-Value | (NS) | (NS) | (NS) | (P<0.01) |

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| **Table 5:** EPEF and Survival % of the birds at the end of the experiment | | |
| Groups | EPEF | Survival % |
| T1 | 240.93 b±07.20 | 92.86±3.40 |
| T2 | 306.70 a ±09.60 | 93.57±2.44 |
| T3 | 309.58 a ±17.56 | 94.29±0.00 |
| SEM | 11.51 | 1.27 |
| P-Value | (P<0.01) | (NS) |

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| **Table 6:** Carcass characteristics (% of live weight) | | | | |
| **Carcass characteristics (% of live weight)** | | | | |
| Groups | Dress yield | Thigh yield | Back yield | Wing yield |
| T1 | 70.33 | 9.80 | 9.12 | 6.16b |
| T2 | 70.29 | 9.81 | 9.13 | 7.21a |
| T3 | 72.02 | 10.42 | 10.10 | 7.40a |
| SEM | 0.46 | 0.19 | 0.26 | 0.23 |
| P Value | (NS) | (NS) | (NS) | (P<0.05) |

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| **Table 7:** Meat quality parameters at the end of the experiment (42 d age) | | | | | |
| Meat quality parameters | | | | | |
| Groups | Colour | Smell | General Acceptability | pH | Extract release volume |
| T1 | 4.50 | 4.50 | 4.25 | 6.45b | 33.50 |
| T2 | 4.50 | 4.38 | 4.50 | 6.70a | 31.50 |
| T3 | 4.25 | 4.38 | 4.38 | 6.54b | 43.00 |
| SEM | 0.10 | 0.08 | 0.09 | 0.04 | 2.34 |
| P value | NS | NS | NS | (P<0.05) | NS |
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| **Table 8:**Overall serum biochemical profile of broiler chickens in different dietary groups | | | | | | | | |
| Overall serum biochemical profile of broiler chickens | | | | | | | | |
| Groups | Cholesterol  (mg/dl) | HDL  (mg/dl) | LDL  (mg/dl) | Glucose  (mg/dl) | Total protein  (g/dl) | Albumin  (g/dl) | Globulin  (g/dl) |
| T1 | 144.33 | 56.43 | 69.08a | 147.13 | 4.34 | 1.62 | 2.72 |
| T2 | 127.22 | 54.63 | 58.30a | 128.53 | 4.51 | 1.58 | 2.92 |
| T3 | 119.78 | 66.43 | 32.48b | 179.70 | 4.56 | 1.60 | 2.96 |
| SEM | 6.23 | 2.41 | 4.87 | 9.42 | 0.16 | 0.05 | 0.13 |
| P value | NS | NS | P<0.01 | NS | NS | NS | NS |
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