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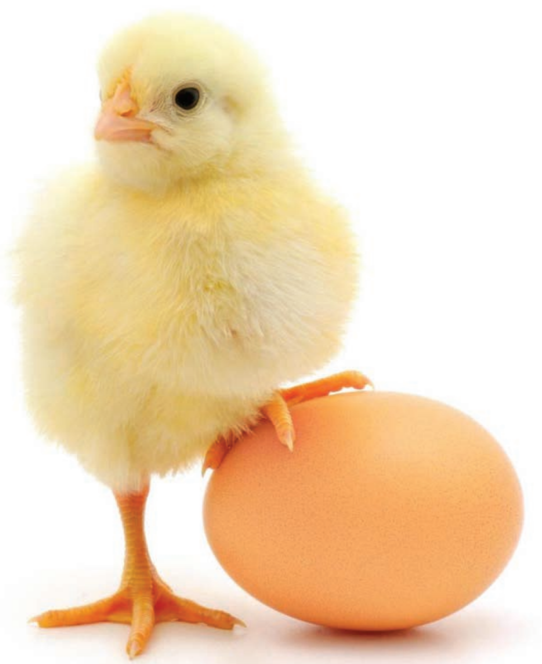

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Research Article
Effects of Proprietary Hepatoprotective Additives (Cadliv™ liq.) Supplementation on the Growth Performance and Hepatic Histological Architecture of Commercial Broiler Chickens

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Abstract

Background and Objective: The effects of proprietary hepatoprotective additives supplementation (Cadliv™ liq., CDLV)on the growth performance and hepatic histological architecture of broiler chickens were evaluated through a field experiment which lasted for 29 days involving 3016 mixed sex flock of Vencobb chickens. **Materials and Methods:** The chicks having an initial mean body weight (BW) of 42.5±0.5 g were randomly housed into two groups (n = 1508 chicks/group) and were fed with a basal diet (negative control, NC) and the basal diet supplemented with CDLV via drinking water at 0.1 mL bird^{-1} day^{-1} for three days a week during 1-29 day. Growth performance viz. BW, average daily body weight gain (ADG) and feed conversion ratio (FCR) were recorded weekly and histopathological scoring of livers (n = 10 birds/group) were done before slaughtering on the 29th day. **Results:** CDLV supplementation significantly improved ($p\leq 0.01$) BW, ADG and FCR in experimental broiler chickens as compared to the NC group. These findings were corroborated by the data on histology of liver where CDLV supplementation in broiler chickens significantly improved ($p = 0.024$) histopathological scores as compared to the NC group. Data on the farm production economics indicated that CDLV supplementation in broiler chickens resulted in savings of 0.91 per kg BW due to significant improvement ($p\leq 0.01$) in growth performance. **Conclusion:** The combined supplementation of hepatoprotective additives (CDLV) significantly improved the growth performance by exerting substantial beneficial effects on the hepatic histological architecture of commercial broiler chickens.

Key words: Broiler chickens, silymarin, growth performance, histology, liver, Cadliv™ Liq.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Modern-day broiler breeds are the results of the development and successful application of quantitative genetics in the selection of desired traits[1]. These genetic selection programs have primarily focused on growth rate and feed utilization efficiency since the 1950s[2,3]. Consequently, there has been an over fivefold increase in the growth performance of broiler chickens when the growth characteristics of the genetically representative birds of those years are compared in identical environment[4]. Herein, the liver plays a key role in supporting the growth and development of highly efficient genetic lines of broiler chickens[5]. Intensive production conditions for a rapid growth rate has greater stress on the liver which increase various disease vulnerabilities[6,7].

Various hepatoprotective supplements are in commercial use for poultry, of which sorbitol, L-carnitine and choline chloride have been used separately to promote liver function and growth performance of broiler chickens[8–11]. Silymarin, a pharmacologically active compound obtained from the seeds of milk thistle (*Silybum marianum*), is known to have strong hepatoprotective effects and improve the growth performance of broiler chickens[12–15]. In these studies, the dietary inclusion levels of 500- and 1000-ppm silymarin (SLM) has shown the desired beneficial effects in the presence of acute toxicity. However, they were not economically viable compared with the other dietary treatment in an unchallenged (no toxicity) environment[14].

Cadliv™ liq. (CDLV), a proprietary liver tonic developed by Zydus Animal Health (a div. of Cadila Healthcare Ltd.), Ahmedabad, India, is a combination of silymarin (SLM), choline chloride (CC), tricholine citrate (TC), sorbitol (SL) and L-carnitine (LC), which are primarily used in commercial broiler chickens as drinking water supplements for supporting liver functions. Several reports on hepatoprotective, antioxidant and lipotropic properties are available individually on these supplements[8-15]. Nonetheless, no scientific data demonstrate their combined effects on the growth performance of broiler chickens.

With this background, the present field study was conducted to evaluate the combined effects of these hepatoprotective additives (CDLV) via. drinking water on the growth performance and hepatic histological architecture of broiler chickens.

MATERIALS AND METHODS

Liver tonic: Cadliv™ liq. (CDLV) is a proprietary liver tonic developed by Zydus Animal Health (a div. of Cadila Healthcare Ltd.), Ahmedabad, India, which is primarily used to promote liver functions in commercial broiler chickens. Cadliv™ liq. (CDLV) contains SLM, TC, CC, SL, LC and carrier (q.s.). In this study, the liver tonic was administered in the experimental birds at 0.1 mL bird^{-1} day^{-1}, which is equivalent to 0.1 mg SLM, 10 mg TC, 1 mg CC, 2 mg SL and 0.25 mg LC.

General bird husbandry, diets and treatments: The field trial was conducted on a commercial production farm in Alibag, Maharashtra for a period of 29 day (as per the norms of the production company). A total of 3016 one-day-old Vencobb broiler chicks (initial mean body weight = 42.5$\pm$0.5 g) of mixed sex were randomly distributed in paired broiler houses (n = 1508/house) of the same design (7.5 by 28.8 m), had the same types of equipment, used feed from the same lots and operated under similar management practices. One of the houses was randomly selected as the negative control (NC) group where broiler birds were fed a starter diet (from 1-12 day), grower (from 13-24 day) and finisher diet (25-29 day of age). All feeds used in this study met the nutritional requirements of the breed. The second house was the treatment group (CDLV) where birds were given a basal diet supplemented with CDLV at 0.1 mL bird^{-1} day^{-1} via. drinking water for three days in a week throughout the trial period. Drinking water and feed were provided *ad libitum.*

The birds were raised on litter composed of paddy straws and the space was allocated according to the industry standard of approximately 0.14 m^2 per bird. The birds were vaccinated at 7 day against Newcastle disease (V.H., Phibro Animal Health Corp., NJ, USA) and infectious bursal disease (Bursa B2K, Haryana, India) at 12 day of age. Incandescent lighting was used throughout the trial period and the lighting schedule involved 24 h light during the first week and 20 h light up to the end of the trial period. The test farm facilities and birds were observed twice daily for general flock condition, lighting, water, feed, ventilation and unanticipated events and records were maintained whenever any bird was found dead, culled, or sacrificed due to any reason. All the mortalities were subjected to necropsy to determine the probable cause of death.

Growth performance parameters: The body weight (BW) of birds were recorded weekly and the average daily body weight gain (ADG) was calculated during 1-14, 15-29 and 1-29 day. Body weight was assessed as the average of the randomly selected 20 birds per group during the 1st, 2nd and 3rd weeks of the trial, while the final BW at 29 day was assessed by dividing the total weight per trial group by the number of birds alive before slaughtering. The feed consumption for each trial was recorded weekly on a flock basis and the average feed consumption per bird per week was calculated, which was used to find average daily feed intake (ADFI) during 1-14, 15-29 and 1-29 day. The feed conversion ratio (FCR) was calculated as a ratio between feed intake over body weight during corresponding growth periods as detailed above. Mortality, if any, was recorded as it occurred and the data were used to adjust subsequent measurements. The European performance efficiency factor (EPEF) and the European broiler index (EBI) were calculated using the following formula[16,17]:

$$EPEF = \frac{BW\ (kg) \times \%\ liveability \times 100}{FCR \times trial\ duration\ (day)}$$

$$EBI = ADG\ (g\ bird^{-1}\ day^{-1}) \times \%\ liveability \times 0.1/FCR$$

Histology of the liver: For histopathological analysis, the sections of the liver were collected from ten birds from each experimental flock at 29 day and fixed by immersion in 10% buffered formalin for 24 h, followed by dehydration in increasing concentrations of ethanol, diaphonization in xylol and embedding in paraffin. Sections of 5 μm were stained with hematoxylin and eosin (H and E) for histopathological analysis under an optical microscope coupled to a camera in 100 and 400× magnification. Moreover, the lesions were assessed according to intensity and given a score: 0 = no damage, 1 = mild damage, 2 = moderate damage and 3 = severe damage[18]. Table 1 presents the categorization scheme for histopathological scores.

Statistical analysis: The data were analysed using one-way analyses of variance. All the results were expressed as Means±standard error of means. The differences between the treatment groups were also evaluated using two-tailed Student's t-test assuming unequal variances. All the statistical Differences among the groups were considered significant at $p<0.05$.

RESULTS

Growth performance: Table 2 presents the data (Mean±SEM) on the BW and ADG of the birds in experimental flocks. The birds fed diet supplemented with CDLV had significantly greater ($p = 0.01$) BW (1405.2 g vs. 1392.2 g) and ADG (46.99 g vs. 46.54 g) compared to the NC group of birds at 29 day. The CDLV-supplemented birds had higher ($p<0.05$) BW and ADG than that of the NC group. However, the difference in ADG between the experimental flocks was non-significant

Table 1: The categorization scheme for histopathological score of liver[1]

Score	Description
0	Absence of focal congestion of vascular tissue with no cellular swelling in the hepatic parenchyma. Normal histomorphology hepatocytes with intact cellular details. Absence of any marked pathological cellular changes in the liver tissue.
1	Presence of focal congestion of vascular tissue in the hepatic parenchyma along with focal and minimal cellular swelling of hepatocytes
2	Moderately higher congestion of vascular tissue in the hepatic parenchyma along with presence of micro and macro vacuoles in hepatocytes indicating diffuse lipidosis
3	Significantly higher congestion of vascular tissue in the hepatic parenchyma along with presence of micro and macro vacuoles in hepatocytes indicating multi-focal lipidosis

[1]The histopathological score of liver is divided into four grades (0-3), according to the intensity of damage and levels of changes associated with lipidosis

Table 2: Body weight (BW) and average daily body weight gain (ADG) at different periods of the experiment

	Treatments			
Items	NC[1]	CDLV	SEM	p-value[2]
BW (g)				
14 day	450.2[a]	470.3[b]	1.86	<0.001
21 day	780.0[a]	820.3[b]	3.74	<0.001
29 day	1392.2[a]	1405.2[b]	2.56	0.010
ADG (g bird^{-1} day^{-1})				
1-14 day	29.12[a]	30.55[b]	0.133	<0.001
15-29 day	62.80	62.32	0.163	0.148
1-29 day	46.54[a]	46.99[b]	0.090	0.010

[1]NC: Negative control, birds fed basal diet only; CDLV: NC+0.1 mL bird^{-1} day^{-1} Cadliv[TM] liq. supplement (equivalent to 0.1-mg SLM, 10 mg TC, 1 mg CC, 2 mg SL and 0.25 mg LC) via., drinking water for three days a week during 1-29 day. [2]Means bearing different superscripts within a row differ significantly

Table 3: Feed conversion ratio (FCR) at different periods of the experiment, EPEF and EBI at 29 day

	Treatment			
Items	NC[1]	CDLV	SEM	p-value[2]
FCR (g of feed g^{-1} of gain)				
1-14 day	1.113[a]	1.087[b]	0.003	<0.001
15-29 day	1.668[a]	1.647[b]	0.005	0.020
1-29 day	1.386[a]	1.413[b]	0.003	<0.001
EPEF	339.8.00[a]	349.500[b]	1.370	<0.001
EBI	329.5.00[a]	338.900[b]	1.350	<0.001

[1]NC: Negative control, birds fed basal diet only; CDLV: NC+0.1 mL bird^{-1} day^{-1} Cadliv[TM] liq. supplement (equivalent to 0.1 mg SLM, 10 mg TC, 1 mg CC, 2 mg SL and 0.25 mg LC) via. drinking water for three days a week during 1-29 day. [2]Means bearing different superscripts within a row differ significantly.

(p = 0.148) during 15-29 day where birds in the NC group had higher ADG (62.80 g vs. 62,32 g) compared to the CDLV-supplemented group.

Table 3 depicts the data (Mean± SEM) on FCR, EPEF and EBI. The feed conversion ratio (FCR) in CDLV-supplemented birds was significantly better (p<0.05) than that of the NC group at different periods of experiment. The EPEF and EBI in CDLV-supplemented group were found significantly higher (p<0.001) (349.5 and 338.9, respectively) than that of the NC group (339.8 and 329.5, respectively).

The mortality rates were the same and of non-specific nature in the experimental flocks (data not shown). Hence, liveability was not affected by CDLV supplementation.

Histopathological scoring of liver: On the basis of the categorization scheme of the histopathological scores of the liver (Table 1), the effect of CDLV supplementation on hepatic histological architecture in broiler chicken is presented in Fig. 1. The histopathological scores of the liver were significantly lower (p = 0.024) in the CDLV-supplemented birds compared to the NC group. In the NC group, six out of

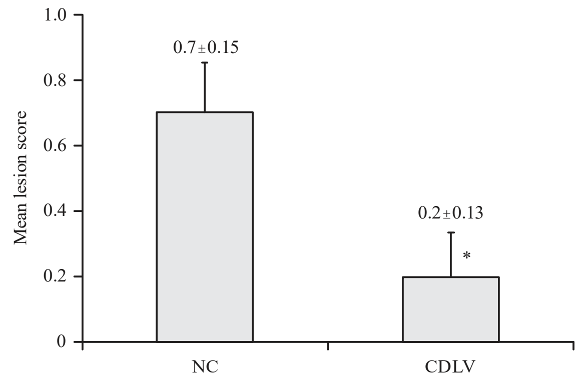

Fig. 1: Histopathological score of liver of broiler chickens in experimental flocks at 29 day

NC: Negative control, birds fed basal diet only; CDLV: NC +0.1 mL bird^{-1} day^{-1} Cadliv[TM] liq. supplement (equivalent to 0.1 mg SLM, 10 mg TC, 1 mg CC, 2 mg SL and 0.25 mg LC) via. drinking water for three days a week during 1-29 day. *indicates statistical significance at p = 0.024, data represent the Mean±SEM (n =10 birds per experimental flock)

Table 4: Economics of production in experimental flocks

Item	NC[1]	CDLV
Cost of chick, INR	28.000	28.000
Feed consumption per bird (kg)	1.967	1.948
Feed cost[2] per bird, INR	59.010	58.440
Misc. cost[3] per bird, INR	15.000	15.000
Amount of liver tonic supplementation per bird (mL)	--	1.200
Cost of supplementation, INR	--	0.240
Mean BW per bird (kg)	1.392	1.405
Total investment, INR per kg BW	73.280	72.370
Savings vis-à-vis the control, per kg BW	--	0.910

[1]NC: Negative control, birds fed basal diet only; CDLV: NC + 0.1 mL bird^{-1} day^{-1} CadlivTM liq. supplement (equivalent to 0.1-mg SLM, 10-mg TC, 1-mg CC, 2-mg SL and 0.25-mg LC) via drinking water for three days a week during 1-29 d. [2]Feed cost per kg = INR 30. [3]Expenses related to vaccination, water, electricity, litter, labour and transportation of feed.

ten birds presented the liver with lesions in the 'mild damage' (score = 1) category where the major finding was the presence of the congestion of vascular tissues in hepatic parenchyma along with the focal swelling of hepatocytes (Fig. 2). By contrast, eight out of the ten birds scored '0' according to the scheme categorization (Table 1), while two birds presented the liver with lesions in the 'mild damage' (score = 1) category in the CDLV-supplemented broiler chickens (Fig. 2).

Economics of production in experimental flocks: Table 4 illustrates the data on the economics of production in experimental flocks. The data indicate that CDLV supplementation in broiler chickens resulted in savings of 0.91 per kg BW compared to the NC group.

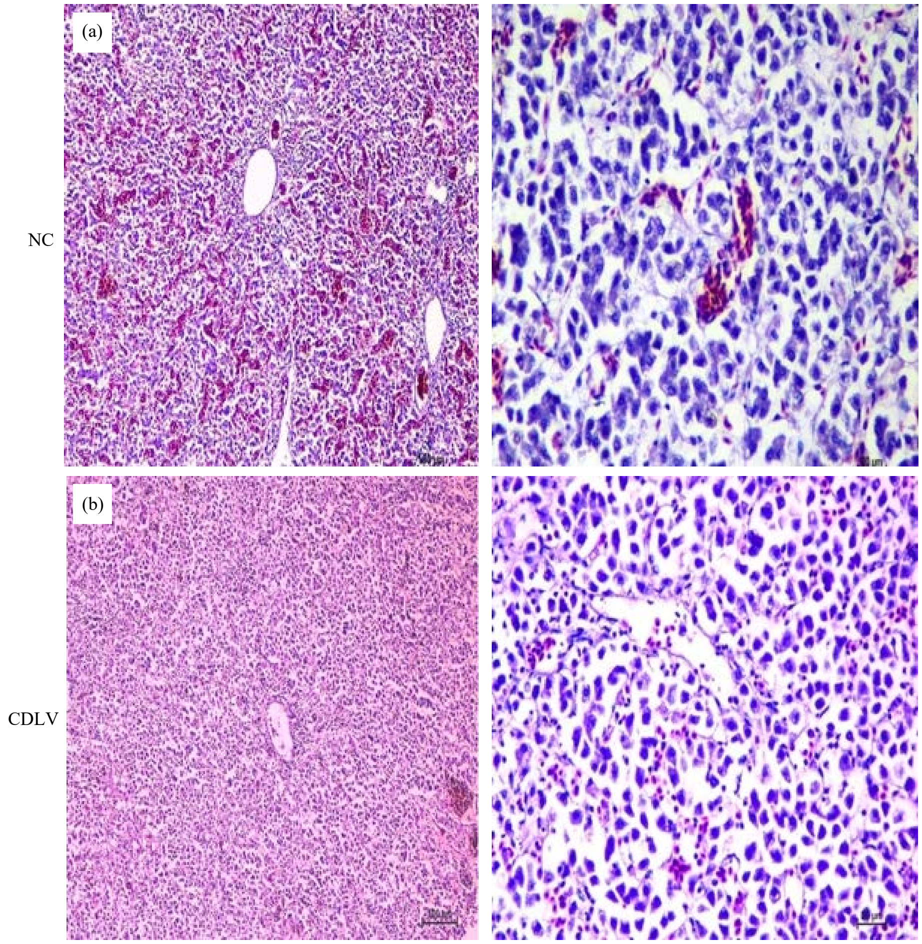

Fig. 2: Broiler chicken livers were stained with HE, viewed under a microscope (100× and 400×) and assigned pathological scores according to categorization scheme presented in Table 1. Normal histological architecture of liver observed in broiler chickens supplemented with Cadliv™ liq. (CDLV) (b); congestion of vascular tissue in the hepatic parenchyma along with focal swelling of hepatocytes observed in the untreated control, NC (a)

DISCUSSION

Silymarin (SLM) is a pharmacologically active compound derived from the seeds of milk thistle (*Silybum marianum*) and is known to have strong hepatoprotective activities[19]. The hepatoprotective activities of silymarin are due to immunomodulation; the inhibition of free radicals; the restoration of the function of antioxidative enzymes (e.g., glutathione concentrations); the decrease of oxidative stress, anti-fibrotic and anti-inflammatory effects; and the generation of cell membrane stabilization[20,21]. Earlier studies related to the hepatoprotective and growth-promoting effects of SLM in broiler chickens had considered higher inclusion levels, which seemed to be economically non-viable and, in most cases, responded toonly acute toxicity[12-15].

L-carnitine (LC) plays a key metabolic role in the transportation of long-chain fatty acids to the mitochondria for b-oxidation and energy production. Herein, endogenous LC synthesis together with its dietary intake is sufficient for normal function. However, in fast-growing broiler chickens, energy demands are high, requiring the exogenous supplementation of LC and are a critical factor for oxidative metabolism[22]. The performance responses of broiler chickens to LC supplementation alone are inconsistent. Previous studies have shown that the dietary supplementation of LC had no effect on the performance of broiler chickens[23-25]. In contrast, Khoshkhoo *et al*.[26] argued that LC significantly improved body weight gain during 35 to 49 d of age but no effect in earlier ages. Hossininezhad *et al*.[27] also reported that dietary LC supplementation significantly decreased the feed conversion ratio.

Broiler chickens fed corn-soybean meal diets exceed the NRC requirement of choline[28]. However, the poor bioavailability of choline in feed ingredients necessitates the exogenous supplementation of choline[29,30]. Furthermore, significant variation in the bioavailability of native choline in feed ingredients accentuates the requirement of exogenous supplementation[31]. Diets supplemented with choline chloride improved the growth performance of broiler chickens[32,33]. TC has lipotropic action because it remove excess fat from the liver and is a critical constituent of liver tonic[34].

Sorbitol (SL) maintain the growth performance of broiler chickens during stress probably by exerting anti-inflammatory effects[9]. In addition, SL enhanced bile secretion and positively influenced the growth performance of broiler chickens through improved fat digestion[8].

The present study demonstrated that 0.1 mL CDLV supplementation per bird containing 0.1 mg SLM, 10 mg TC, 1 mg CC, 2 mg SL and 0.25 mg LC improved BW, ADG and FCR compared to the untreated control group (NC). Interestingly, ADG during 15-29 day was numerically greater ($p = 0.148$) in the NC group of birds compared to the CDLV-supplemented birds. It showed the greater effects of CDLV supplementation on growth performance during 1-14 day in broiler chickens. It might be due to the higher dosage regimes (mg kg^{-1} BW) of additives (SLM, TC, CC, SL and LC) during 1-14 days as compared to 15-29 days in CDLV-supplemented birds. The present study agrees with the findings of Deniz *et al*.[35] who confirmed that the supplementation of 0.05 mg LC, 0.4 mg SL and 0.15 mg CC per ml in drinking water during the first three days and then for two days in every feed change period significantly improved the growth performance of broiler chickens. In the present study, the CDLV supplemented concentration of SLM was lower than that of the previous studies. No comparable scientific document could be found related to the usage of SLM in lower concentrations in combination with other additives in broiler chickens. Given the paucity of the literature, extensive works are warranted for segregating the individual effects of SLM in lower concentrations from other additives.

In this study, improvements noted in the performance parameters of broiler chickens in the CDLV-supplemented birds might be related to the combined positive effects on the lipid metabolism of LC, TC, CC and SL coupled with the antioxidant activity of SLM. Gropp and Schweigert[36] reported that dietary LC supplementation could improve fatty acid and energy utilization. Therefore, ADG and FCR may be improved in poultry. Choline may have a carnitine-like effect by increasing fatty acid utilization in the liver[37]. Moreover, lipid digestion may be increased by SL as it is considered to have choleretic effects[8]. The hepatoprotective activity was well correlated with the improvements of hepatic histopathological scores in the CDLV-supplemented birds compared to the birds in the NC group. Finally, these findings verify that improvement in growth performance parameters (BW, ADG and FCR) and the liver histopathology of the CDLV-supplemented broiler chickens could be attributed to the role of additives in lipid metabolism and hepatoprotective activities.

CONCLUSION

The combined supplementation of hepatoprotective additives (SLM, TC, CC, SL and LC) improved the growth performance (BW, ADG and feed efficiency) by exerting substantial beneficial effects on the hepatic histological architecture of commercial broiler chickens.

SIGNIFICANCE STATEMENT

This study discovered that the combined supplementation of hepatoprotective additives caused the significant improvement of growth performance in commercial broiler chickens by exerting beneficial effects on hepatic histological architecture. This study will help researchers uncover critical information on the potential beneficial effects of these additives on commercial broiler chickens in combination than alone that has not been explored previously. Thus, the findings of the study may set the trend for researchers to design new models for studying the effects of these hepatoprotective additives in combination at dosing levels justifying substantial economic return to farmers.

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