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Effect of Dietary Supplementation of Chicory Root Powder and Avian Specific *Lactobacillus spp* on the Hematology, Blood Biochemical, Lipid, Enzyme and Antioxidant Profile of Broiler Birds

David Lalthlamuana*, A. K. Samanta, L. Hmar, P. Behera, R. Buragohain, M. Ayub Ali and V. P. Vaisakh

Department of Animal Nutrition, College of Veterinary Sciences and A.H., Selesih, Mizoram, 796 014, India

**Corresponding author*

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A B S T R A C T

An experiment was conducted to assess the effect of dietary supplementation of chicory root powder and avian specific *Lactobacillus spp* on blood biochemical and anti-oxidant profile of broiler birds. 250 day old commercial (Ven Cobb) broiler chicks were selected randomly and were distributed into five treatments groups. Duration of experiment was 42 days. Each treatment comprises of five replicates with 10 chicks in each replicate. Dietary treatment consists of: Group1 - control (basal diet as per BIS, 2007), Group 2 - (basal feed + BMD 0.5g/kg), Group 3 - (basal feed + 1% chicory root powder), Group 4 - (basal feed + *Lactobacillus spp)*, Group 5 - (basal feed + 1% chicory root powder + *Lactobacillus spp).* Haematological parameters were not affected due to treatment imposed in the study except PCV which was higher ($P<0.05$) in CRP+LB group. Serum SGPT was found to be significantly decreased ($P<0.01$) in CRP & LB group at day 28, and average level in serum SGOT was also observed to be significantly decreased ($P<0.05$) for all treatment groups as compared to AGP group. Total protein value showed significant increased ($P<0.05$) in CRP+LB group at day 42. Globulin level was observed to be significantly increased ($P<0.05$) in CRP & CRP+LB group at day 42. Serum triglyceride level was significantly decreased ($P<0.01$) for all the treatment group as compared to AGP group. Serum LDL concentration was found to be significantly decreased ($P<0.05$) in CRP and CRP+LB group as compared to control, AGP & LB group on day 42, and serum HDL level was significantly increased ($P<0.01$) for all treatment group as compared to AGP group. The anti-oxidant profile as indicated by FRAP value showed significant increase ($P>0.01$) in CRP+LB group as compared to other treatment groups. Thus, the result from the study suggests that chicory root powder and avian specific *lactobacillus spp* are both beneficial feed supplement which can substitute antibiotic in the diet of broiler.

Introduction

Poultry production has undergone an enormous expansion during the past decades throughout the world (Praveen *et al.*, 2017). India's poultry industry has shown to grow at a steady rate of around 7-8% per year (Soundararajan, 2017). The poultry industry

benefitted from the inclusions of antibiotic growth promoters (AGP) in feed which enhance the performance and health of broiler birds (Awad *et al.,* 2009).

Current trends in poultry production aim at reducing the repeated use of antibiotic growth promoters, and the use of alternatives to antibiotic growth promoters because of development of antimicrobial resistance, and antibiotic residue in meat (Jackson *et al.,* 2004). Potential alternatives to antibiotics in broilers are prebiotics and probiotics. Probiotic lactic acid bacteria and oligosaccharide have been showing positive effect in the digestive tract of birds.(Spring *et al.,* 2000).

Chicory root (*Cichorium intybus*) is a source of inulin & fructo-oligosaccharides (FOS) considered as prebiotic. Research showed that the addition of chicory root fructans either inulin or oligo fructose to broiler feed improved body weight gain, feed conversion, carcass yield and increased the small intestine length of female broilers (Yusrizal and Chen, 2003). Another feed additive is probiotics, which are live microorganisms that have a positive effect on the host by improving the balance of pathogenic to beneficial bacteria in the gut (Simon *et al.,* 2001). The benefits of probiotics are based on two main functions, stimulating the growth of beneficial microflora and suppressing the growth of pathogenic bacteria.

Among different probiotics, Ghareeb *et al.,* (2012) reported that the administration of the probiotic containing avian- specific *Lactobacillus* spp to broiler chickens can help reduced *C. jejuni* by altering the gut microbiota that is beneficial for the intestine and provide protection against invasion from harmful pathogens. The potential health benefits associated with using a probiotics include improved digestion, stimulation of gastrointestinal immunity and increased natural resistance to enteric disease (Tellez *et al.,* 2001).

When probiotic & prebiotic are used together in the diet, they can help improve the microbial count and viability of beneficial microorganisms, since they can use prebiotics as a substrate for fermentation in the GI tract (Bengmark, 2001).

Therefore, this study was conducted with the hypothesis that avian specific *Lactobacillus spp* and chicory root inulin would have beneficial effect on the blood biochemical parameters and antioxidant profile of the broiler birds.

Materials and Methods

Experimental design and diets

A total of 250 day old broiler chicks having similar body weight from a single hatch were purchased and distributed randomly into 5 treatment groups, with 50 birds kept in each group following completely randomized design. Each group consists of 5 replicate with 10 birds in each replicate. All the chicks were reared under the same management and condition. Due attention was adopted to maintain the biosecurity and avoid stress inside and outside the farm. Three types of broiler diets were formulated and prepared i.e. broiler pre-starter (1-7d), broiler starter (8-21d) feed and broiler finisher (22-42d) feed as per specified by BIS (2007). Group 1 (CON) was fed standard basal diet without any additional supplements. Birds in group 2 (AB) was fed basal diet with Antibiotic Growth Promoters i.e. Bacitracin methylene disalicylate (BMD) @ 0.5g/kg of feed. Group 3(CRP) was fed basal diet with chicory root powder @ 1% of feed. Group 4 (LB) was fed basal diet with avian specific *Lactobacillus spp* at prescribed dose rate @10^6/g diet. Group

5 (CRP+LB) was fed the basal diet with combination of chicory root powder and avian specific *Lactobacillus spp*. Chicory root powder was purchased commercially from Earth Expo Company, Gujarat, India and it was incorporated in the diet at the rate of 1% diet. *Lactobacillus spp* was isolated from healthy broiler birds following standard procedures and used as probiotic in the diet of experimental birds. Birds were reared under deep litter system of management. The experimental birds were offered *ad libitum* feed everyday using a clean feeder. Vaccination against New Castle Disease (NDV) & Infectious Bursal Disease (IBD) was given on day 7 and day 14 respectively.

Hematological examination

Blood was collected by sample vial (EDTA) at day 42**.** Estimation of haematological parameters like packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), was done with the help of automated haematology cell counter (Model: MS4e) following the standard procedures as per the manufacturer's protocol.

Biochemical indices examination

2ml of blood sample was collected in a vial from all replicate of the treatment group from the wing vein at day 28 and day 42. The samples were left stand to clot for 30 min, and then centrifuged at 3000 rpm for 10 mins to separate the serum. The blood biochemical, enzyme and lipid profile was determined using Fujifilm clinical chemistry analyser as per manufacturer's protocol.

Anti – oxidant profile

Blood was collected in sample vial on day 28 and day 42, serum was collected by centrifugation at 3000rpm for 10 mins. Anti – oxidant profile for each treatment group were determined by using ferric reducing anti-oxidant power (FRAP) assay (Benzie & Strain, 1996). The serum samples was then run in U.V - Spectrophotometer. The value obtained was expressed as Trolox equivalent (10µgTE/10µl).

Statistical analysis

The statistical analysis of the recorded or estimated data was done using standard analytical procedure (Snedecor and Cochran, 1994). Differences among means were separated using Duncan's multiple range test (Duncan, 1955). Probability values less than 0.05 is considered to be statistically significant and values $P \leq 0.01$ was declared a trend.

Results and Discussion

Hematological parameters

Average values of blood WBC, RBC, PCV, Hb level in different treatment groups at different age are presented in table 1. Statistical analysis revealed that there were no significant ($P>0.05$) difference in the WBC & RBC count and Hb level among the different treatment groups at day 42. However, the packed cell volume (PCV) was significantly ($P<0.05$) increased at 42 days in CRP and LB group as compared with control, AGP and CRP+LB group. Similar finding have been reported by Akoy (2015) who observed increased level of PCV in treatment groups diet supplemented with inulin and probiotic. On contrary to our findings, Beski and Al-Sardary (2015) reported that supplementation of fructooligosaccharide (FOS) and probiotics (strain of Lactic Acid Bacteria) did not have significant ($P>0.05$) effect on the PCV of broiler birds. Hashem and Mohamed (2009) also observed no significant ($P>0.05$) difference in the PCV of broiler birds fed inulin (5%diet) and probiotics (protexin 0.5g/L) in the diets of broiler birds. The

increased PCV value in LB group could be due to the probiotics of *Lactobacillus spp* which might have reduced the nutritional stress and maintaining a healthy gut status in broiler birds (Karoglu and Drudag, 2005).

Blood biochemical parameters

Blood biochemical parameters like glucose, total protein, albumin and globulin concentration on serum of different experimental groups are presented in Table 2. Glucose level showed no significant difference (P>0.05) between the treatment groups at both day 28 and day 42. Total protein level was observed to be significantly (P<0.05) different, with highest protein value observed in CRP+LB groups at day 42.

The present finding is in consistency with the findings of Mousa *et al.,* (2017) who reported significant increased in protein level on supplementation with *Cichorium intybus* and/or *Moringa oleifera*. However, the present study is in conflict with Koksal *et al.,* (2011) could not observe any significant difference (P>0.05) in total protein due to supplemental inulin in the diet of broiler birds. Globulin concentration was observed to be significantly (P<0.05) increased in CRP & CRP+LB group as compared to other groups.

The observed difference in globulin is comparable with the findings of Ashayerizadeh *et al.,* (2009), Hashem and Mohamed (2009) who observed significant difference in the level of protein between the treatment and control group.

However, the present findings is in disagreement with the findings of, Yenge *et al.,* (2018) and Kowalczuk-Vasilev *et al.,* (2017) who reported no significant difference (P>0.05) in the level of globulin on supplementation of inulin in the diet of broiler birds. This change in total protein level in serum indicates better metabolism of proteins in the body by providing feed additives (like prebiotic MOS, FOS) in the diet (Burkhardt, 2000).

Lipid profile

The effects of chicory root powder and avian specific *lactobacillus spp* treatments on serum lipid profile of broiler birds during day 28 and day 42 are presented in Table 3. Cholesterol level showed no significant difference (P>0.05) between all the treatment groups.

Triglyceride level was observed to be significantly (P<0.05) decreased in CRP, LB & CRP+LB group as compared to control and AB group. The observed reduction is in agreement with Yusrizal and Chen, (2003), Ashayerizadeh *et al.,* (2009) and Elrayeh *et al.,* (2011) who observed significant decreased (P<0.01) in the level of triglyceride of broiler birds supplemented with inulin and probiotic.

LDL and HDL showed significant decreased (P<0.05) in CRP, LB &CRP+LB groups as compared to control and AB group at day 42.. The observed significant difference (P<0.05) in the level of LDL was in line with the findings of Beski and Al-Sardary (2015) and Kalavathy *et al.,* (2010). The reduction in the serum LDL might be due to the fact that, large amount of LDL is made up of cholesteryl esters and free cholesterol with little triglycerides (Mc Eneny *et al.,* 2002), and treatment of both prebiotic and probiotic have the ability to reduce the cholesteryl esters level in LDL (Min-Tze Liong *et al.,* 2007)

Serum enzyme

The effects of chicory root powder and avian specific *lactobacillus spp* treatments on serum enzyme of broiler birds during day 28 and day 42 are presented in Table 4.

Table.1 Effect of chicory root powder and avian specific *Lactobacillus spp* on hematological parameter

Attributes	Treatment					P value
	Group-1 (C)	Group- 2 (AGP)	Group- 3 (CRP)	Group- 4 (LB)	Group- 5 (CRP+LB)	
WBC	6.26±4.80	6.93±8.35	6.96±3.92	6.83±3.92	6.93±2.90	0.85^{NS}
RBC	2.33±0.26	2.53±0.17	2.74±0.32	2.60±0.23	2.56±0.08	0.45^{NS}
PCV	29.20 ± 0.43^{b}	31.03 ± 1.23^{ab}	33.40 ± 0.51^{a}	31.93 ± 0.37^{a}	31.40 ± 0.70^{b}	0.02*
Hb	14.40±0.66	15.30±1.60	15.66±0.86	15.20±0.41	15.90±1.15	0.86^{NS}

CON- Control; AB- Antibiotic growth promoter; CRP- Chicory Root Powder; LB- *Lactobacillus spp*; CRP+LB- Chicory Root Powder & *Lactobacillus spp*; *Calculated value; ^{a,b,c} means with different superscripts in a row differ significantly; NS= Non significant; * means (P<0.05); ** means (P<0.01); Avg- Average

Table.2 Effect of chicory root powder and avian specific *Lactobacillus spp* on serum enzyme SGPT (U/I) & SGOT (U/I)

Attributes	Treatment					P value
	Group-1 (C)	Group- 2 (AGP)	Group- 3 (CRP)	Group- 4 (LB)	Group- 5 (CRP+LB)	
SGPT						
d 28	7.00 ± 0.40^{a}	5.25 ± 0.62^{b}	4.77 ± 0.25^{b}	4.25 ± 0.47^{b}	5.50 ± 0.64^{ab}	0.01**
d 42	6.25±0.47	4.75±0.62	3.75±0.47	5.50±1.19	6.00±0.57	0.14^{NS}
Average	6.62 ± 0.44^{a}	5.00 ± 0.62^{bc}	4.26 ± 0.34^{c}	4.87 ± 0.81^{bc}	5.75 ± 0.60^{ab}	0.03*
SGOT						
d 28	164.25 ± 11.6^{a}	204.50 ± 9.36^{b}	167.00 ± 8.87^{a}	171.00 ± 0.57^{a}	170.50 ± 7.96^{a}	0.02*
d 42	176.25±6.70	195.50±19.1	148.75±7.08	167.00±25.4	157.50±6.61	0.27^{NS}
Average	170.25 ± 8.68^{a}	200.00 ± 16.1^{b}	157.87 ± 7.02^{a}	169.00 ± 11.8^{a}	164.00 ± 1.98^{a}	0.01^{**}

CON- Control; AB- Antibiotic growth promoter; CRP- Chicory Root Powder; LB- *Lactobacillus spp*; CRP+LB- Chicory Root Powder & *Lactobacillus spp*; *Calculated value; ^{a,b,c} means with different superscripts in a row differ significantly; NS= Non significant; * means (P<0.05); ** means (P<0.01); Avg- Average

Table.3 Effect of chicory root powder and avian specific *Lactobacillus spp* on serum Glucose (mg/dl), Total protein (g/dl), Albumin (g/dl) and Globulin (g/dl) in broiler chickens

Attributes	Treatment					P value
	Group-1 (C)	Group- 2 (AGP)	Group- 3 (CRP)	Group- 4 (LB)	Group- 5 (CRP+LB)	
Glucose						
d 28	228.00±9.81	262.75±26.9	202.75±10.0	228.00±14.38	201.25±10.00	0.08^{NS}
d 42	242.75±14.0	295.25±21.4	235.25±8.27	272.25±31.96	257.75±3.68	0.21^{NS}
Average	235.37±10.4^{b}	279.00±22.4^{a}	219.00±8.90^{b}	250.12±20.1^{ab}	238.75±7.54^{b}	0.03*
Total Protein						
d 28	3.17±0.17	3.02±0.08	3.12±0.17	3.12±0.11	3.17±0.08	0.92^{NS}
d 42	3.05±0.09^{c}	3.37±0.06^{bc}	3.52±0.26^{ab}	3.32±0.10^{bc}	3.85±0.09^{a}	0.02*
Average	3.11±.09^{b}	3.20±0.06^{b}	3.32±0.14^{ab}	3.22±0.07^{ab}	3.51±0.07^{a}	0.01**
Albumin						
d 28	1.05±0.02	1.00±0.04	1.12±0.07	1.10±0.05	1.02±0.04	0.44^{NS}
d 42	1.02±0.09	1.07±0.04	1.05±0.09	1.05±0.05	1.25±0.05	0.21^{NS}
Average	1.03±0.04	1.03±0.03	1.08±0.06	1.07±0.05	1.13±0.02	0.27^{NS}
Globulin						
d 28	2.12±0.16	2.02±0.07	2.00±0.10	2.02±0.06	2.15±0.06	0.78^{NS}
d 42	2.02±0.13^{b}	2.30±0.07^{ab}	2.47±0.18^{a}	2.27±0.07^{ab}	2.60±0.05^{a}	0.03*
Average	2.07±0.07^{b}	2.16±0.05^{ab}	2.23±0.11^{ab}	2.15±0.05^{ab}	2.37±0.0 4^{a}	0.01**

CON- Control; AB- Antibiotic growth promoter; CRP- Chicory Root Powder; LB- *Lactobacillus spp*; CRP+LB- Chicory Root Powder & *Lactobacillus spp*; *Calculated value; ^{a,b,c} means with different superscripts in a row differ significantly; NS= Non significant; * means (P<0.05); ** means (P<0.01); Avg- Average

Table.4 Effect of chicory root powder and avian specific *Lactobacillus spp* on serum lipid : Triglyceride (mg/dl), Cholesterol (mg/dl), LDL (Mg/dl) and HDL (mg/dl) in broiler chickens

Attributes	Treatment					P value
	Group-1 (C)	Group- 2 (AGP)	Group- 3 (CRP)	Group- 4 (LB)	Group- 5 (CRP+LB)	
Triglyceride						
d 28	53.00±3.69^{b}	71.25±5.15^{a}	47.75±50.75^{b}	50.75±4.30^{b}	52.00±1.87^{b}	0.01**
d 42	48.50±5.83^{a}	65.75±3.63^{b}	40.50±1.84^{a}	43.00±1.47^{a}	42.75±3.40^{a}	0.01**
Average	50.75±2.19^{a}	68.50±2.10^{b}	44.12±10.12^{a}	46.87±2.71^{a}	47.37±2.11^{a}	0.01**
Cholesterol						
d 28	109.00±7.01	116.75±7.57	113.25±5.57	102.00±6.28	109.00±1.47	0.51^{NS}
d 42	109.25±6.22	110.25±10.94	102.50±3.27	100.75±0.47	104.25±0.85	0.72^{NS}
Average	109.12±5.06	113.50±8.05	108.62±4.11	101.62±4.31	106.63±3.37	0.63^{NS}
LDL						
d 28	17.35±3.92	14.30±2.60	14.00±0.40	13.50±0.64	12.75±0.62	0.62^{NS}
d 42	18.75±2.39^{a}	18.80±0.71^{a}	12.00±0.40^{b}	15.75±1.49^{ab}	11.75±0.47^{b}	0.02*
Average	18.05±1.26	16.55±1.23	13.00±0.36	14.62±1.02	12.25±0.36	0.053^{NS}
HDL						
d 28	83.00±5.11	90.75±2.17	93.75±5.15	87.50±5.67	89.00±9.32	0.77^{NS}
d 42	84.75±4.53^{a}	72.50±3.86^{b}	94.75±1.54^{a}	92.25±5.02^{a}	87.50±0.64^{a}	0.01**
Average	83.87±3.46	81.62±1.78	94.25±2.73	89.87±4.51	88.25±2.34	0.11^{NS}

CON- Control; AB- Antibiotic growth promoter; CRP- Chicory Root Powder; LB- *Lactobacillus spp*; CRP+LB- Chicory Root Powder & *Lactobacillus spp*; *Calculated value; ^{a,b,c} means with different superscripts in a row differ significantly; NS= Non significant; * means (P<0.05); ** means (P<0.01); Avg- Average

Table.5 Effect of chicory root powder and avian specific *Lactobacillus spp* on FRAP(10TE/10µl) value in blood serum

Attributes	Treatment					P value
	Group-1 (C)	Group- 2 (AGP)	Group- 3 (CRP)	Group- 4 (LB)	Group- 5 (CRP+LB)	
d 28	1.14±0.07	1.39±0.12	1.20±0.07	1.06±0.08	2.08±0.54	0.10^{NS}
d 42	1.35±0.05	1.62±0.12	1.38±0.06	1.27±0.05	2.26±0.51	0.08^{NS}
Average	1.25±0.05^{b}	1.50±0.12^{b}	1.29±0.06^{b}	1.16±0.06^{b}	2.17±0.50^{a}	0.01**

CON- Control; AB- Antibiotic growth promoter; CRP- Chicory Root Powder; LB- *Lactobacillus spp*; CRP+LB- Chicory Root Powder & *Lactobacillus spp*; *Calculated value; ^{a,b,c} means with different superscripts in a row differ significantly; NS= Non significant; * means (P<0.05); ** means (P<0.01); Avg- Average

The blood serum lipid was significantly ($p \leq 0.05$) decreased in SGPT and SGOT level at day 28. Similar findings was observed by Khodadadi *et al.,* (2016) who observed reduction in SGPT level when supplemented with *Cichorium intybus L.* in the diet of broiler birds. Kanjilal *et al.,(*2014) observed significant decreased (P<0.05) in SGOT level of broiler birds fed with probiotic (Protexin®).

However, the findings are in contrast with the findings of Abdel Fatteh *et al.,* (2009), Hashem and Mohamed (2009) who observed no effect on SGPT and SGOT supplemented with probiotics (protexin 0.5g/L) and inulin (5%diet) in the diet of broiler birds. The observed SGPT and SGOT level maybe due to the effect of inulin and probiotic which consist of antioxidant and free radical scavenging property that are effective in regulating the serum liver enzyme like SGPT and SGOT (Hassan and Yousef, 2010)

Anti – oxidant profile

Based on perusal of table 5, serum FRAP value (10µgTE/10µl) was found to be non - significant (P<0.05) among the treatment groups at day 28 and day 42. However, FRAP value in the treatment group was tended to be increased on both 28th and 42nd day when compared with control group, highest value observed in CRP+LB group. Overall, the average FRAP value was significantly (P<0.01) higher in CRP+LB group when compared with control, AGP, & LB groups.

The present findings are in agreement with Sohail *et al.,* (2011) and Shen *et al.,* (2014) who observed increased level of antioxidant for treatment group as compared to control group when supplemented with probiotic mainly *Lactic acid bacteria*.

However, the present study disagree with the finding of Capcarova *et al.,* (2011) who observed significant difference (P<0.05) in the antioxidant level of broiler birds provided with Lactic acid probiotic.

The increased antioxidant value maybe due to chicory root inulin which can regulate glutathione metabolism to enhance the antioxidant defense, and regulate cellular metabolism, where its deficiency can result in oxidative stress (Wu and Luo, 2009).

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