EFFECT OF DIETARY SUPPLEMENTATION OF SYNBIOTICS ON HEMATOLOGY AND IMMUNE PARAMETERS IN VENNCOBB BROILERS

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(Received : 20 May 2025; Accepted : 26 June 2025)

SUMMARY

An experiment was conducted to evaluate the effect of dietary supplementation of synbiotics in broiler production. A day old, 160 commercial Ven Cobb broiler chicks were randomly distributed into five treatments having four replicates with eight birds per replication. The experiment was conducted in the poultry shed of Department of Animal Nutrition, LUVAS, Hisar. A standard soybean-maize based basal diet was formulated and fed to the chicks in three different growth phases *i.e.* pre-starter (0-7 d), starter (8-21 d) and finisher (22-42 d). Basal ration was formulated as per BIS (2007) to fulfill the metabolizable energy (ME) and crude protein requirements of birds. The first treatment group was kept as a negative control (T_1) and was given the basal diet without antibiotic, while second group (T_2) was fed basal diet with antibiotic (Control). Birds in third (T_3), fourth (T_4) and fifth (T_5) groups were fed basal diet of negative control supplemented with synbiotics at the rate of 2.0 g, 2.5 g and 3.0 g synbiotic/ kg of the feed, respectively. At the end of the trial, the birds from the metabolic cages were slaughtered for estimation of carcass traits/immune parameters. Hematological (Hb, TLC, TEC) parameters and mortality were also recorded.

Key words: Synbiotics, broiler, antibiotics, hematology, carcass traits/immune parameters

The broiler chicken industry in India has transformed from a traditional, small-scale farming practice into a highly organised and vertically integrated agri-business. This evolution has enabled even smallholders to participate in commercial poultry farming, significantly increasing productivity and profitability. Broiler chickens are a type of chicken specifically bred and raised for meat production. Rearing of chickens comes under poultry farming. According to Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) production data (2020), India ranks 3rd in egg production and 8th in meat production in the world Meat production in the country has increased from 6.69 million tonnes in 2014-15 to 9.29 million tonnes in 2021-22. Broiler meat production in the country is estimated at around 5 million tonnes (MT) annually. The poultry sector in India has shown impressive growth, with poultry meat production increasing at an average annual rate of 8%, and egg production rising by 7.45% between 2014-15 and 2021-22. In large scale rearing facilities, where poultry are continuously exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and result in serious economic losses. Prevention and control of diseases have led to substantial increase in use of antibiotics during recent decades. Overuse of antibiotics in poultry industry has led to the emergence of the problem of the development of antimicrobial resistance (AMR). Residues of antibiotics in meat and eggs can pose human health risks. Regulatory bodies (like the European Union and many countries) have banned or restricted AGPs (full form) in animal feed. Also, growing consumer demand for antibiotic-free poultry products has left the researchers to look for some safe natural growth promoters as alternatives to the antibiotics. In poultry production, several options have been considered, such as prebiotics, probiotics, synbiotics, organic acids, essential oils, enzymes, and emerging novel compounds. Probiotics, prebiotics, and their combination (synbiotics) have become increasingly significant substitutes (Hassan *et al.*, 2018). One such natural growth promoter which has been tested and found safe are synbiotics. Synbiotics are combinations of probiotics and prebiotics that work synergistically to promote gut health. Probiotics are live beneficial microorganisms (e.g., *Lactobacillus*,

Bacillus, *Bifidobacterium*) that colonize the gut and inhibit pathogens. While, prebiotics are non-digestible food components (e.g., fructo oligosaccharides, inulin) that stimulate the growth and activity of beneficial gut bacteria. Synbiotics are essential to support probiotic growth because they provide the substrate required for fermentation (Hassanpour *et al*., 2013) and create a synergistic effect (Basturk *et al*., 2016). The addition of synbiotic to broiler diets enhanced body weight, growth, feed efficiency, and carcass yield by improving the condition of the intestinal tract, thereby increasing villi height (VH), crypt depth (CD), and the overall absorptive area. 0.1% and 0.15% synbiotic supplementation to broilers' diets from day 1 to day 42 enhanced their body weight gain and feed conversion ratio (Karimi *et al*., 2010). Broilers' body weight and hemato-biochemical profile have been improved by probiotics and synbiotics (Islam *et al*., 2021). A synbiotic, rather than a probiotic, is more effective in enhancing growth and health in broilers (Al-sultan *et al*., 2016). Thus, keeping in mind the above mentioned benefits of synbiotics in the broiler chicken we have conducted this study on Venn Cobb broiler whose diet were supplemented with three levels of symbiotic and assess the effect of supplementation of synbiotic on the carcass traits and haematological parameters in the chicken broilers, without the involvement of health risks associated with antibiotics.

MATERIALS AND METHODS

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC), 1669/GO/ReBiBt-S/Re-L/12/CPCSEA06/12/2012 in the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences.

Birds, Experiment Design, and Management

A total of 160 one day old commercial Ven Cobb broiler chicks were randomly distributed into five treatments having four replicates with eight birds per replication. The experiment was conducted in the poultry shed of Department of Animal Nutrition, LUVAS, Hisar. A standard soybean-maize based basal diet was formulated and fed to the chicks in three different growth phases i.e. pre-starter (0-7 d), starter (8-21 d) and finisher (22-42 d). Basal ration was formulated as per BIS (2007) to fulfill the metabolizable energy (ME) and crude protein requirements of birds. The first treatment group was kept as a negative control (T_1) and was given the basal diet without antibiotic, while second group (T_2) was fed basal diet with antibiotic (Control). Birds in third (T_3), fourth (T_4) and fifth (T_5) groups were fed basal diet of negative control supplemented with synbiotic at the rate of 2.0 g, 2.5 g and 3.0 g symbiotic/ kg of the feed, respectively. Floor litter system was followed where the chicks were kept hygienically in separate pens. All the birds were reared adopting uniform management conditions. The chicks were brooded at 35°C during the first week and thereafter the temperature was reduced by 3°C every week until the temperature reached 25±1°C. The birds were vaccinated against prevailing diseases adopting a standard protocol. Individual body weight of chicks was recorded at 0-day age and thereafter weekly. At the end of the experiment, one bird from each replicate was slaughtered ethically by mechanical stunning followed by exsanguinations. Different carcass parameters like percentage of abdominal fat, spleen, thymus, bursa, thymus, thigh and breast weight percentage of the experimental birds under different dietary treatments were measured.

Blood collection and Analysis

At the end of the feeding trial (6^{th} week) blood samples were collected from one broiler per replicate, making four samples per treatment and thus a total of 20 samples were analyzed. About 2 ml of blood was collected from each bird via brachial wing vein puncture using sterilized syringes and 5 ml scalp vein needle set into tubes containing EDTA (vacutainer) for blood analysis. Plasma was prepared by centrifuging the blood at 3000 rpm for 10 min. The plasma was then transferred into a microcentrifuge tube using a Pasteur pipette and stored at "20°C until further analysis.

Statistical Analysis

Data were analyzed by one-way ANOVA as a completely randomized design using the GLM procedure of SAS Institute. Differences among means were tested by the least significant difference method, and $P<0.05$ was considered to be statistically significant group.

RESULTS AND DISCUSSION

The experiment was planned following Completely Randomized Design (CRD) at uniform and standard management practices. All the feed ingredients were procured in one lot before the start of the experiment. The feed ingredients, feed additives and supplements used were maize, soybean meal,fish meal, vegetable oil, mineral mixture, common salt, vitamins, synbiotic. The sources, composition and mixing rate of feed additives/supplements used in ration formulations are presented in Table 1.

TABLE 1
Ingredient Composition of Experimental Diets

Ingredient (Kg/100 Kg of feed)	Pre-starter (0-7 d)	Starter (8-21 d)	Finisher (22-42 d)
Maize	53.9	54.8	59.2
Soybean Meal	34.6	32.2	26.8
Fish Meal	6.0	6.0	6.0
Vegetable oil	3.75	4.95	5.82
Mineral mixture	2.0	2.0	2.0
Feed Additives (g/100 Kg feed)			
Spectromix Regular	10	10	10
Spectromix BE	20	20	20
Veldot	63	63	63
Choline chloride	50	50	50
Lysine	50	-	-
DL-Methionine	140	150	140

Composition, sources and rate of mixing of feed additives/supplements

1. Spectromix: Powder (Ranbaxy Animal Health, New Delhi). Each gm. contained VitaminA-82,500 IU, Vit D3-12000 IU, Vit B2-50 mg and Vit.K-10mg.Mixing rate: 10 g/100Kg of feed.
2. Spectromix BE: Powder (Ranbaxy Animal Health, New Delhi). Each gm. Contained Vit.B1- 8mg, Vit.B6- 16mg, Vit.B12- 80mg, niacin-120mg, calcium pentothenate-80mg, Vit. E-160 mg, Lysine hydrochloride-10 mg, DL-methionine-10 mg and calcium 260 mg. Mixing rate: 20g/100kg of feed.
3. Veldot: Venkeys- Dinitro-O-Toluamide (Coccidiostat). Mixing rate: 50g/100kg of feed.
4. Choline chloride: Contain 60 percent choline. Mixing rate: 50g/100kg of feed.
5. Lysine: Contained 98% lysine. Mixing rate: 50g/100kg of feed.
6. DL-methionine: Contained 98% methionine. Mixing rate: 150g/100kg of feed.

Experimental treatments:

The basal diet of broiler chicken were formulated as per BIS (2007) standards. The experimental diets and treatments used in the experiment were as below:

T_1 = Basal diet without antibiotics (negative control)
T_2 = Basal diet with antibiotics (positive control)
T_3 = Basal Diet + synbiotic @ 2.0g/Kg of feed
T_4 = Basal Diet + synbiotic @ 2.5g/Kg of feed
T_5 = Basal Diet + synbiotic @ 3.0g/Kg of feed

Feed additives and supplements were premixed and then mixed with weighed quantity of feed ingredients to make a homogenous mixture of rations.

Carcass traits:

The results of the present study on the effects of increasing levels of synbiotics on the carcass traits of broilers revealed that, groups supplemented with higher levels of synbiotic showed significant ($P<0.05$) increment in giblet percentage and highest giblet percentage was found in T_5 group. Abdominal fat percentage under different treatment ranged from 0.92% (T_1) to 0.85% (T_5) as presented in Table 2. As the level of synbiotic in diet of experimental birds

TABLE 2
Percent mean values of abdominal fat, spleen, thymus, bursa, thymus, thigh and breast weight percentage of the experimental birds under different dietary treatments

Treatments	Abdominal fat %	Spleen %	Bursa %	Thymus %	Thigh %	Breast %
T_1	$0.92^{a}\pm0.01$	$0.13^{a}\pm0.01$	$0.14^{a}\pm0.01$	$0.31^{a}\pm0.01$	$9.91^{a}\pm0.20$	$26.21^{a}\pm0.17$
T_2	$0.87^{a}\pm0.02$	$0.15^{abc}\pm0.00$	$0.16^{abc}\pm0.01$	$0.34^{abc}\pm0.01$	$10.19^{ab}\pm0.03$	$27.67^{b}\pm0.20$
T_3	$0.89^{a}\pm0.01$	$0.14^{ab}\pm0.01$	$0.15^{ab}\pm0.01$	$0.33^{ab}\pm0.01$	$10.06^{ab}\pm0.09$	$26.09^{a}\pm0.22$
T_4	$0.86^{b}\pm0.02$	$0.16^{bc}\pm0.00$	$0.18^{bc}\pm0.00$	$0.35^{bc}\pm0.01$	$10.21^{ab}\pm0.04$	$28.33^{c}\pm0.16$
T_5	$0.85^{b}\pm0.01$	$0.17^{c}\pm0.01$	$0.19^{c}\pm0.01$	$0.37^{c}\pm0.01$	$10.28^{b}\pm0.01$	$27.56^{b}\pm0.30$

The mean values in same column with different superscripts differ significantly ($P< 0.05$).

increased, abdominal fat percentage reduced. A significant (P<0.05) reduction was observed in T_4 and T_5 groups as compared to groups supplemented with 2g/kg of feed of synbiotic, antibiotic and basal diet, respectively. Also, dietary probiotics and prebiotics can exhibit lipid-lowering effects on the lipid metabolism of animals, as summarized by Ooi and Liong (2010). Recently, Ghasemi *et al.* (2016) observed that the supplementation of synbiotic (a combination of the probiotic strain *Enterococcus faecium* and prebiotic fructo-oligosaccharides) reduced circulating cholesterol and low-density lipoprotein cholesterol in broilers. Ashayerizadeh *et al.* (2009) have reported that dietary synbiotic inclusion decreased abdominal fat yield in chickens.

The immune organ index, the ratio of immune organs to live body weight, indicates the animal's immune functional status. The relative weight of the spleen, thymus, and bursa are closely connected to the immune response (Kabir *et al.*, 2005). Increased weight of lymphoid organs (bursa, spleen, and thymus) also enhances the immune response, both specific and non-specific, by activating macrophages, boosting cytokine production by intraepithelial lymphocytes, and raising immunoglobulin levels (Huang *et al.*, 2004). The growth, development, and division of immune cells may contribute to the increase in the weight of immune organs in broilers (Wang *et al.*, 2021). The current study established that supplementation with increasing levels of synbiotics leads to a significant increase in the relative weight of the spleen, bursa, and thymus. The data pertaining to the percent weight of the lymphoid organs viz. thymus, bursa and spleenof the present study are presented in Table 2. Spleen percentage also differed significantly (P<0.05) between the control group and other treatment groups. Spleen percentage ranged from 0.13% (T_1) to 0.17% (T_6) among different dietary treatments and the highest was recorded in T_5.. Under different dietary treatments bursa percentage ranged between 0.14% (T_1) to 0.19% (T_5). Highest bursa percentage was observed in T_5 (3g/kg of feed) was significantly (P<0.05) higher than antibiotic (T_2) supplemented and control (T_1) groups. Thymus percentage ranged from 0.31% (T_1) to 0.37% (T_6) under different dietary treatments. The values differed significantly (P<0.05) among the supplemented and control group. A tri-strain probiotics comprised of *Clostridium butyricum*, *Bacillus subtilis*, and *Lactobacillus acidophilus* significantly increased the bursal index in broilers (Hossain *et al*., 2015). Furthermore, the probiotic and coriander combination also led to increased bursa and spleen weight (Gurram *et al.*, 2022). However, Zou *et al.* (2022) also reported a significantly increased spleen index and a non-significant increase in thymus and bursa index after feeding male broilers with a multi-strain probiotic. Taken together with those of other studies, the findings of this study suggest that symbiotic inclusion at 2.5g/Kg of feed could help enhance the immunity of broilers and work as an alternative to antibiotics.

The results of the current research work related to the percentage of breast and thigh meat is presented in the Table 2. Thigh percentage ranged from 9.91% (T_1) to 10.28% (T_5) and breast percentage ranged from 26.21(T_1) to 27.56 (T_5) under different dietary treatments. The highest (P<0.05) thigh percentage was found in 3g/Kg of feed of synbiotic (T_5) treatment group. While, highest (P<0.05) breast percentage was found in 2.5g/Kg of feed of synbiotic in T_4 treatment group. The breast muscle percentage and thigh leg muscle percentages are essential measures of broilers' performance and serve as a valuable assessment of carcass characteristics.

Furthermore, compared to the control, the treatment group had significantly higher thigh muscle and breast muscle percentages and significantly reduced abdominal fat percentages. Abdel-Raheem and Sherief Abd-Allah (2011) reported a similar finding where the broilers fed synbiotics had better carcass yield than those fed single probiotics and the control. However, Zou *et al.* (2022) reported no significant differences in carcass traits between the control groups and the groups fed multi-strain probiotics. The differences may be attributed to differences in basal diets fed to these birds. In consonance with our research findings, Poorghasemi *et al*., (2018), demonstrated that, breast and thigh percentages in broiler affected by a diet which was containing different dietary oil and probiotic, but carcass percentage and abdominal fat amounts were not affected by this ratio. Similarly, Pourakbari *et al* (2016), indicated that adding a different percent (0.005%, 0.01%, 0.015%, and 0.02) of probiotics in broilers diet has a significant effect on wings, abdominal fat, left caecum, and thymus (but does not have any significant effect on breast, drumsticks, liver, and bile, spleen and bursa of fabricious. In agreement with our test results, Ashayerzadeh *et al.* (2009) also indicated that supplementation broiler diets with 650 g t-1 antibiotic, 900 gt-1 probiotics, and 2000g t-1 prebiotic have a significant effect on the carcass, thigh, breast, and abdominal fat weights.

Hematological parameters:

The data related to hematological parameters of the experimental birds under different dietary regimes present in the study are presented in Table 3.

The study results unveiled that mean values of Hb (g/dl) ranged from 10.26 g/dl (T_1) to 10.82 g/dl (T_5) and significantly (P<0.05) higher values were observed in T_3, T_4 and T_5. Mean values of TEC (total erythrocytic count) were found significantly (P<0.05) higher in 2.5g synbiotic/Kg of feed (T_4) and 3.0g synbiotic/Kg of feed (T_5) as compared to other treatments, antibiotic supplemented group and control group. While, the mean values of the TLC (total leucocyte count) showed a numerical decrease but there is no significant difference among the different treatment groups. The beneficial effect of synbiotics on growth performance appears to be related to their involvement in the modulation of intestinal microbial composition, regulation of immune system, and maintenance of intestinal integrity and barrier function (Tuohy *et al.*, 2003; Saad *et al.*, 2013). In corroboration with our research findings Khalil *et al.*, 2021, found that broilers' body weight and hemato-biochemical profile have been improved by probiotics and synbiotics.

Further our current research trial unveiled that mean values of heterophil % ranged from 27.30% (T_5) to 28.62% (T_1) and lowest heterophil count was observed in T_5 group followed by T_4 and T_3 group. Although there is numerical decrease in the heterophil% but non-significant difference was observed among the treatments. Lymphocytes mean value ranged from 57.60% (T_1) to 59.82% (T_5) and highest lymphocyte % was observed in T_4 and T_5. Mean values for H: L ratio ranges between 0.457 (T_5) to 0.497 (T_1) and significantly (P<0.05) lowest ratio was found in T_4 and T_5 groups as compared to control group (T_1) and antibiotic supplemented group (T_2). Our results are in agreements with the finding of Kumar *et al.* (2014) reported that stress was indicated by increased percentage of heterophils and decreased percentage of lymphocytes and thus high H/L ratio (0.71). Also, several studies on broiler chicken showed that synbiotics have favorable effects on the hematological and serum biochemistry, which regulate chicken immunity (Ghasemi *et al.*, 2014; Khalil *et al.*, 2021).

Liveability in the broilers:

In the current research trial, we also observed the birds regularly for any abnormal behaviour and mortality, if any during the experimental period. Weekly and percent mortality of broilers under different dietary treatments recorded had been presented in Table 4.

All through the experimental period only 12% birds died. Highest mortality was recorded in the control group (T_1). During the whole experimental period highest mortality was recorded in 4th and 5th week. The increase in the liveability and decrease in

TABLE 3
Mean values of hematological parameters of birds under different dietary treatments

Treatments	Hemoglobin g/dl	TEC×10^6/µl	TLC×10^3/µl	Heterophil %	Lymphocyte %	H: L
T_1	10.26^a±0.03	1.89^a±0.05	27.35±0.06	28.62±0.56	57.60^a±0.42	0.497^b±0.013
T_2	10.33^{ab}±0.04	1.99^a±0.05	27.46±0.05	27.58±0.64	58.76^{abc}±0.30	0.469^{ab}±0.012
T_3	10.44^b±0.04	2.02^a±0.14	27.51±0.05	28.08±0.17	58.10^{ab}±0.53	0.483^{ab}±0.007
T_4	10.77^c±0.08	2.41^b±0.08	27.66±0.34	27.49±0.27	59.31^{bc}±0.55	0.464^a±0.009
T_5	10.82^c±0.04	2.47^b±0.08	27.70±0.26	27.30±0.21	59.82^c±0.37	0.457^a±0.006

The mean values in same column with different superscripts differ significantly (P< 0.05).

TABLE 4
Mortality of the experimental birds under different dietary treatments

Treatments	Chicks	1st Wk	2nd Wk	3rd Wk	4th Wk	5th Wk	6th Wk	Total	% Mortality
T_1	32	1	1	-	1	2	-	5	16
T_2	32	1	-	1	1	1	-	4	13
T_3	32	1	-	1	1	-	2	5	16
T_4	32	1	-	-	1	-	1	3	9
T_5	32	-	-	1	-	1	-	2	6
Total	160	4	1	3	4	4	3	19	12

the mortality in the symbiotic treated groups is because of their positive effect on the absorption and digestion of nutrients. Also, Mookiah *et al*. (2014) found that the term "synbiotic" describes the combination of probiotics and which involves maintaining intestinal integrity, controlling the spread of harmful microorganisms, controlling immunological function, and nutritional absorption.

CONCLUSION

In conclusion, this study demonstrated that synbiotic treatment had a positive impact on the hemato-biochemical parameters, immune response and overall liveability in Ven Cobb broiler chickens in India. These findings contributed to the potential benefits of synbiotic application in poultry health and production as suitable replacement candidate to the antibiotic.

ACKNOWLEGEMENT

The authors are thankful to The Head, Department of Animal Nutrition and Department of Animal Genetics & Breeding, LUVAS, Hisar for providing the required facilities used in the experiment.

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