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Effect of dietary addition of herbal and microbial supplements on growth performance, nutrient retention and immunity of commercial broiler chicken

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ABSTRACT

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This study evaluated the effect of dietary supplementation of herbal and microbial supplements on growth, immunity and nutrient retention in broiler chicken. The experiment was conducted using 420 day-old commercial broiler chicks, which were randomly divided into 7 dietary treatments, with 12 replicates having 5 chicks in each. Dietary treatments include T1: Basal diet (BD) alone, T2: BD + Nbiotic at 250 g/tonne of feed; T3: BD + Liqbiotic (in drinking water; per day/100 birds) 0-2 weeks: 2.5 mL; 2-4 weeks: 5.0 mL; 4-6 weeks: 10.0 mL; T4: BD + Salcochek Pro at 500 g/tonne of feed, T5: BD + AV/EGM/19 at 250 g/tonne of feed, T6: BD + AV/EGM/20 at 250 g/tonne of feed, T7: BD + Clostat (Bacillus subtilis) at 100 g/tonne of feed. The results revealed that significantly ($p \leq 0.05$) higher body weight gain (BWG) and better feed conversion ratio (FCR) were observed in Nbiotic (T2), Liqbiotic (T3), AV/EGF/20 (T6) and Clostat (T7) groups during finisher phase when compared to control (T1) and other treatment groups. Dietary treatments did not show any significant effect on feed intake during pre-starter and starter phases, however significantly higher feed intake was observed in Nbiotic (T2) group. The humoral immune response was significantly ($p \leq 0.05$) higher in Nbiotic (T2), Liqbiotic (T3) and Salcochek Pro (T4) groups when compared to control (T1) and other treatment groups. Dietary inclusion of herbal and microbial supplements, significantly ($p \leq 0.05$) improved the crude protein (CP) digestibility, whereas energy and dry matter digestibilities were not affected. Finally, it could be concluded that supplementation of Nbiotic (T2), Liqbiotic (T3), AV/EGF/20 (T6) and Clostat (T7) can be recommended to improve the finisher phase BWG and FCR of broiler chicken. The CP digestibility was improved in all the dietary supplemented groups when compared to control group, whereas improved immunity was noticed in Nbiotic (T2), Liqbiotic (T3) and Salcochek pro (T4) supplemented groups.

Keywords: Broilers, Herbal supplements, Microbial product, Growth, Nutrient retention, Immunity

INTRODUCTION

The increasing global demand for poultry products necessitates the exploration of alternative feed additives that can enhance the performance and health of broiler chickens. Traditional methods, primarily relying on antibiotic growth promoters (AGPs), have faced scrutiny due to concerns over antibiotic resistance and food safety. Consequently, there is a growing interest in herbal and microbial supplements as viable alternatives that can promote the health and productivity of poultry without any adverse effects associated with AGPs.

Herbal supplements, derived from various plants, have demonstrated significant potential in improving broiler performance. Herbs and spices have appetizing and stimulatory effects on the digestion process by increasing the production of digestive enzymes and juices, which stimulates digestion and peristaltic motion, thus improving feed efficiency (Rajeshwari and Andallu, 2011; Naeemasa et al., 2015) and also have immunity activation, antiviral, antioxidant and anthelmintic properties. Studies indicate that these natural additives can enhance feed efficiency, weight gain and overall health by modulating the gut microbiota and improving nutrient absorption (Omar et al., 2016). Furthermore, the combination of different herbs often yields synergistic effects that enhance their benefits, making them promising candidates for inclusion in poultry diets (Sun et al., 2024).

In addition to herbal additives, microbial supplements such as probiotics have gained attention in poultry nutrition. Probiotics are beneficial live microbes that provide health benefits to the host when taken in sufficient quantities. They play a crucial role in enhancing gut health by outcompeting pathogenic bacteria, thus improving FCR and overall growth performance (Krysiak et al., 2021). Research has shown that probiotics also stimulate immune responses, leading to better disease resistance among broiler chickens (Kumar et al., 2013).

The studies on the pharmacological actions of individual herbal extracts in animals are available very well, but the studies on the combination of different herbal products in comparison to probiotics (microbial supplements) in poultry are scanty. Hence, the present research was conducted to investigate the effect of different herbal and microbial supplements on growth, immunity and nutrient retention in commercial broiler chicken.

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MATERIALS AND METHODS

To conduct the proposed study, 420 day-old broiler chicks were weighed individually and wing banded. The birds were randomly distributed into 7 dietary treatment groups with 12 replicates of five birds each. T1: Basal diet (BD); T2: BD + Nbiotic (Garlic, Ginger, Thymus vulgaris and MOS) at 250 g/tonne of feed; T3: BD + Liqbiotic (Contain essential oils of eugenol, thymol and cineole, supplied in drinking water; per day/100 birds) 0-2 wks: 2.5 mL; 2-4 wks: 5.0 mL; 4-6 wks: 10.0 mL; T4: BD + Salcochek Pro (a mixture of extracts from plants Woodfordia fruticose, Acacia catechu, Holarrhena antidysentrica, Plantago ovate, Berberis aristata and Aegle marmelos) at 500 g/tonne of feed; T5: BD + AV/EGM/19 (Ajwain and Tulasi oil) at 250 g/tonne of feed; T6: BD + AV/EGM/20 (Garlic essential oil) at 250 g/tonne of feed; T7: BD + Clostat (Bacillus subtilis PB6) at 100 g/tonne of feed.

The chicks were reared in battery brooders in an open-sided house under standard managemental conditions and fed with iso caloric and iso nitrogenous corn soya-based diets (as per Vencobb recommendations) from day old to 42 days of age. Body weights were recorded for individual birds, while cumulative feed consumption was recorded for each replicate group every week. In the event of mortality, the feed for that particular group was weighed back to ensure accurate feed consumption data. The feed conversion ratio (FCR) was calculated using the data on feed consumption and body weight gain. Mortality among the birds was recorded throughout the experimental period and the causes were determined. Blood samples were collected on the 3rd and 6th week of age from the wing vein of broilers (1 bird/ replicate) to estimate New Castle disease (ND) titers. Haemagglutination inhibition (HI) activity of serum was estimated and the antibody titers (log_2) were measured following the standard procedure (Wegmann and Smithies, 1966). During the last 3 days of the trial, the metabolic trial was conducted on 8 birds/treatment. Before the start of metabolic trial, the birds were fasted for 12 hours to ensure their gastrointestinal tracts were empty, marking the start of the feces collection process. The feces collected were oven-dried for a period of 18 hours at a temperature of about 105^0C and weighed daily. At the end of the collection period, the fecal samples collected from each treatment per day were pooled, ground and thoroughly mixed to obtain a homogenous mixture. Samples of feces were subjected to proximate analysis according to the standard methods outlined (AOAC, 2012) and the results obtained were used to calculate the apparent digestibility using the following formula.

$$\text{Apparent digestibility coefficient} = \frac{\text{Nutrient in feed} - \text{Nutrient in feces}}{\text{Nutrient in feed}} \times 100$$

Total nitrogen in feeds and excreta was determined using the Kjeldahl method (AOAC, 2012) and the nitrogen utilization was calculated as follows:

$$\text{N utilization (\%)} = \frac{\text{(N intake} - \text{N excretion)}}{\text{N intake}} \times 100$$

The data were subjected to one-way ANOVA using the general linear model process of the statistical package for social sciences (SPSS) 20th version.

Table 1: Ingredient (kg/quintal) and nutrient composition (%) of broiler diets

Ingredient	Pre-starter	Starter	Finisher
Maize	55.5	58.5	59.5
Oil	2.8	4	5
Soybean meal	37.8	33	30
Stone grit	1.4	1.45	1.1
Dicalcium Phosphate	1.75	1.75	2.17
Salt	0.4	0.42	0.42
Threonine	-	-	0.001
DL-Methionine	0.17	0.2	0.19
Lysine	0.14	0.16	0.17
Trace mineral mixture	0.1	0.1	0.1
Vitamin AB2D3K	0.02	0.02	0.02
B-complex vitamins	0.025	0.025	0.025
Choline	0.1	0.1	0.1
Toxin binder (herbal)	0.1	0.1	0.1
Nutrient composition (Calculated values)			
Metabolizable Energy (Kcal/kg)	3020	3105	3150
Crude protein (%)	23.05	20.98	19.61
Lysine (%)	1.29	1.21	1.12
Methionine (%)	0.50	0.49	0.48
Calcium (%)	0.90	0.90	0.98
Available Phosphorus (%)	0.43	0.42	0.48

*AB_2D_3K provided per kg diet: Vitamin A 20000 IU, Vitamin B_2 25 mg, Vitamin D_3 3000 IU, Vitamin K 2 mg.
** Vitamin B-Complex provided per kg diet: Riboflavin 25 mg, Vitamin B_1 1mg, Vitamin B_6 2 mg, Vitamin B_{12} 40 mg, and Niacin 15 mg.

***Trace mineral provided per kg diet: Manganese 120 mg, Zinc 80 mg, Iron 25 mg, Copper 10 mg, Iodine 1 mg

RESULTS AND DISCUSSION

Growth

The data on BWG, feed intake and FCR of broilers in different dietary treatment groups were presented phase-wise in Table 2.

During the pre-starter and starter phases, the BWG of broilers was not affected by the dietary treatments. However, during the finisher phase, significantly ($p \leq 0.05$)

Table-2

Treatment		Pre starter phase (0-2 weeks)			Starter phase (3-4 weeks)			Finisher phase (5-6 weeks)		
		BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR
T_1	Control	352.80	442.60	1.26^{c}	825.00	1300.80	1.58^{b}	1167.00^{b}	2115.20^{bc}	1.81^{c}
T_2	Nbiotic	382.80	445.60	1.14^{a}	853.02	1290.80	1.51^{a}	1236.80^{a}	2187.60^{a}	1.77^{ab}
T_3	Liqbiotic	385.00	443.60	1.15^{a}	855.05	1310.40	1.53^{ab}	1218.60^{a}	2144.00^{b}	1.76^{ab}
T_4	Salcochek Pro	356.00	446.00	1.25^{c}	820.40	1330.00	1.62^{c}	1170.60^{b}	2123.00^{bc}	1.82^{c}
T_5	AV/EGM/19	368.40	439.40	1.19^{b}	829.80	1325.00	1.60^{bc}	1175.20^{b}	2135.00^{b}	1.82^{c}
T_6	AV/EGF/20	380.00	440.60	1.16^{a}	856.20	1285.40	1.50^{a}	1235.00^{a}	2121.00^{bc}	1.72^{a}
T_7	Clostat	379.20	444.00	1.17^{ab}	861.00	1299.80	1.51^{a}	1220.60^{a}	2090.00^{c}	1.71^{a}
	SEM	2.39	1.41	0.08	4.81	7.48	0.08	1.19	7.43	0.09
	P-value	0.62	0.24	0.01	0.14	0.78	0.02	0.03	0.04	0.01

higher BWG was observed in Nbiotic (T2), Liqbiotic (T3), AV/EGF/20 (T6) and Clostat (T7) groups when compared to control and other treatment groups. The present findings in the T2 group are in accordance with Darwish et al. (2021) with supplementation of 1 % ginger powder, Eltazi (2014) with 1.75 % mixture powder (1.5 % garlic + 0.25 % ginger), Giannenas et al. (2019) and Swaroop et al. (2022) with herbal feed additive (Nbiotic) in broilers, reported significant improvement in BWG of broilers. Similar to T3 findings in this study, Xing et al. (2019) found higher average daily gain in broilers supplemented with thymol essential oil @ 15 g/ton and cinnamaldehyde @ 5 g/ton in the diet. In support of T6 findings, Elbaz et al. (2022a) reported that dietary supplementation of garlic essential oil @ 200 mg/kg in broilers improved the BWG. Garlic and thymol essential oils improve body weight gain in broilers primarily through their antioxidant properties and enhanced feed conversion efficiency. Garlic oil has been shown to lower serum cholesterol levels and improve metabolic rates, while thymol promotes gut health by enhancing digestive enzyme activity and reducing pathogenic bacteria, leading to better nutrient absorption and overall growth performance in poultry (Moustafa et al., 2020).

The T7 group findings are in accordance with Sen et al. (2012), Sikandar et al. (2020) and Zhang et al. (2021) with dietary supplementation of Bacillus subtilis PB6 (CLOSTAT^{TM}), found significant ($P \leq 0.05$) improvement in BWG of broilers. Probiotic (Bacillus) supplementation in broilers enhances body weight gain primarily by improving gut health, which leads to better nutrient absorption and feed efficiency. Additionally, Bacillus probiotics help control harmful pathogens in the gut, further promoting overall growth and health in broiler chickens (Ogbuewu et al., 2022).

During the pre-starter and starter phases, no significant difference in the feed intake of broilers among the treatment groups. However, in the finisher phase, the feed intake was significantly ($P \leq 0.05$) higher in the Nbiotic (T2) group when compared to the control and other treatment groups, whereas other treatment groups had comparable feed intake to the control group. The present findings are in accordance with report of Ozek et al. (2011) with essential oil mixture (containing carvacrol, thymol, 1:8-cineole, p-cymene and limonene) supplementation in layers, Xing et al. (2019) with thymol essential oil, Kirkpinar et al. (2011) and Elbaz et al. (2022) with garlic essential oil @ 300 mg/kg and 200 mg/kg, respectively in broilers, also reported the non-significant effect on feed intake.

During the pre-starter, starter and finisher phases, a significant ($P \leq 0.01$) improvement in FCR of broilers in Nbiotic (T2), Liqbiotic (T3), AV/EGF/20 (T6) and Clostat (T7) groups when compared to control and other treatment groups. The present findings of the T2 group were in accordance with reports of Darwish et al. (2021) with the supplementation of ginger or garlic, Eltazi (2014) with 1.75 % mixture powder (1.5 % garlic 0.25 % ginger) and Giannenas et al. (2019) with herbal feed additive (Nbiotic) also reported a significant improvement in FCR in broilers. Similar to T3 findings, Ghanima et al. (2020) found thymol, carvacrol & eugenol supplementation @ 300 mg/kg in the diet of layers significantly improved the FCR from 28 to 78 weeks of age. In support of T6 findings, Elbaz et al. (2022) reported that diet supplemented with 200 mg/kg of garlic essential oil in broilers, significantly improved the FCR.

The improved growth performance with herbal supplements might be due to improved digestibility of nutrients by stimulating the digestive enzyme secretion and activity and by increasing the bile synthesis (Rajeshwari and Andallu, 2011).

Similar to the present findings in case of T7 group was reported by Melegy et al. (2011), Sikandar et al. (2020) and Zhang et al. (2021) with Bacillus subtilis PB6 (CLOSTAT^{TM}) supplementation in broilers. The positive impact of dietary supplementation with CLOSTAT^{TM} on broiler performance could be associated with improved

Table 3: Effect of herbal and microbial supplements on digestibility of chemical constituents (%)

Treatment	Diets	Dry matter	Organic matter	Crude protein	Crude fibre	Ether extract	NFE	Calcium	Phosphorous
T_1	Control	66.02	68.03	65.36^{c}	52.32	70.21	71.12	65.09	41.05
T_2	Nbiotic	67.75	69.99	69.88^{a}	53.89	71.21	70.21	66.01	42.01
T_3	Liqbiotic	68.02	72.03	67.98^{b}	54.03	70.32	71.05	66.05	40.12
T_4	Salcochek Pro	69.32	72.03	66.99^{b}	54.12	70.39	70.14	67.23	41.23
T_5	AV/EGM/19	68.95	71.28	68.79^{ab}	53.39	71.11	72.32	66.08	41.69
T_6	AV/EGF/20	66.00	71.36	66.89^{b}	54.00	71.21	73.65	65.12	41.32
T_7	Clostat	71.02	71.23	69.08^{a}	55.36	70.12	71.23	67.08	40.06
SEM		0.890	0.623	0.239	1.02	1.26	0.998	2.32	1.01
N		8	8	8	8	8	8	8	8
P-value		0.856	0.411	0.003	0.112	0.236	0.659	0.325	0.421

gut health and is thought to be caused by better absorption of different nutrients by the birds. Contrary results in comparison to the present findings in the case of T7 were reported by Rodjan et al. (2018) with Bacillus subtilis supplementation.

Nutrient digestibility

Dry matter (DM %), organic matter (%), crude fiber (%), Ether extract (EE %), Nitrogen free extract (NFE %), Calcium (%) and Phosphorus (%) digestibility were not influenced by the dietary treatments (Table 3). However, the crude protein (CP) digestibility was significantly (P≤0.01) improved in all the dietary treatment groups (T2 to T7). Supplementation of Nbiotic (T2) and Clostat (T7) showed significantly (P≤0.01) higher crude protein digestibility when compared to other treatment groups. However, the CP digestibility in other treatment groups was intermediate and significantly (P≤0.01) higher than the control group. The present findings agree with reports of Bai and Li (2022) who found that dietary supplementation of Chinese herbal medicine @ 8 g/kg feed in broilers significantly improved the CP digestibility, however did not show any effect on DM digestibility. Similarly, Guler et al. (2006) with Coriander supplementation in layer Japanese quails and Singh and Kumar (2018) with dietary black cumin (Nigella sativa) in broilers reported significant (p≤0.05) improvement in CP retention. Aswal et al. (2017) found Garlic powder @ 2 g/kg and amla powder @ 5 g/kg addition to White Leghorn hen diet, improved the retention of DM, CP and EE. The improved crude protein digestibility in broilers by using herbal additives might be due to modulating gut microbiota, increasing digestive enzyme activity, and reducing anti-nutritional factors. These effects lead to improved nutrient absorption and overall feed efficiency, contributing to better growth performance in the birds (Vlaicu et al., 2021).

The present findings in the T7 group are in accordance with Sen et al. (2012) who reported that apparent retention of CP was higher in birds that are supplemented with B. subtilis when compared to the control group. Probiotic (Bacillus) supplementation improves crude protein digestibility by enhancing gut health, increasing the secretion of digestive enzymes, and reducing intestinal inflammation, collectively leading to better nutrient absorption. Studies have shown significant increases in protein digestibility when broiler diets are

Table 4: Effect of herbal and microbial supplements on NDV titers (log_2) and Immune organ weights (% live weight) of broiler chicken

Treatment	Diets	3rd week (HI titer to ND vaccine)	6th week (HI titer to ND vaccine)	Bursa	Spleen	Thymus
T_1	Control	3.03^{c}	3.16^{b}	0.072	0.14	0.23^{b}
T_2	Nbiotic	3.35^{a}	3.40^{a}	0.132	0.18	0.52^{a}
T_3	Liqbiotic	3.31^{a}	3.31^{a}	0.910	0.16	0.39^{ab}
T_4	Salcochek Pro	3.26^{ab}	3.29^{a}	0.110	0.16	0.30^{b}
T_5	AV/EGM/19	3.09^{bc}	3.15^{b}	0.720	0.19	0.32^{b}
T_6	AV/EGF/20	3.10^{bc}	3.10^{b}	0.730	0.15	0.30^{b}
T_7	Clostat	3.09^{bc}	3.15^{b}	0.104	0.18	0.26^{b}
SEM		0.024	0.20	0.006	0.006	0.021
N		12	12	12	12	12
P-value		0.001	0.001	0.097	0.245	0.030

supplemented with Bacillus probiotics, indicating their beneficial impact on overall feed efficiency and growth performance (Bao et al., 2022).

Immunity

The data on humoral immune response was evaluated in terms of antibody response to the New Castle disease (ND) vaccine at 21 and 42 d of age using serum Hemagglutination inhibition (HI) and expressed in mean $\log_2$ titer values (Table 4). Significantly (P≤0.05) higher ND titers were recorded in Nbiotic (T2) and Liqbiotic (T3) and Salcochekpro (T4) groups when compared to control and other treatments during 3rd and 6th week of age, whereas the NDV titers in other treatment groups were comparable to the control group.

The present findings of the T2 group were in accordance with Saleh et al. (2014) who reported antibody titers against NDV were significantly higher in birds that are supplemented with thyme essential oil @ 200 mg/kg and garlic essential oil @ 100 mg/kg. Similar to the present findings, Reema (2011) reported increased immunoglobulin (Ig) G levels in mice and pigs which are fed on a diet supplemented with thyme and oregano as compared to the control diet. El-Hack and Alagawany (2015) conveyed that dietary addition of Thymol powder @ 6 g/kg in layers, significantly improved the IgG levels in serum. Di et al. (2022) found that dietary supplementation of 20-30 mg/kg of 1,8-cineole, increased serum IgA, IgG and IgM levels at 21 and 42 days of age (P<0.05). Elbaz et al. (2022b) revealed that Clove essential oil supplementation in the diet of broilers significantly increased (P≤0.05) serum IgA level.

Dietary treatments did not show any significant effect on immune organ weights (Bursa and Spleen) during 6th week of age. However, significantly (P≤0.05) higher thymus weight was observed in the Nbiotic group (T2) compared to control and other treatment groups. The non-significant effect on immune organ weights of the spleen and bursa was in accordance with Rahimi et al. (2011) with essential oils supplementation and Melegy et al. (2011) with Bacillus subtilis supplementation.

Mortality

The mortality rate among the experimental diets is shown in Table 5. It was observed that all the mortality occurred during the pre-starter phase (0-14 d), which was primarily due to unabsorbed yolk condition. However, the mortality rate was within the acceptable range and no specific disease outbreak was recorded.

General health and appearance

None of the groups exhibited any negative symptoms or changes in behavior throughout the study. All the birds were active and showed normal appetite during the period of study.

CONCLUSION

Finally, it could be concluded that dietary addition of Nbiotic (T2), Liqbiotic (T3), AV/EGF/20 (T6) and Clostat (T7) can be recommended to improve the BWG and FCR of broilers during finisher phase. Nbiotic (T2), Liqbiotic (T3) and Salcochek pro supplementation, improved the immunity in broilers chicken. The CP digestibility was improved in all the dietary supplemented groups when compared to control group.

Ethical approval and consent statement

The experiment was approved by the Institutional Animal Ethics Committee (IEAC) of the College of Veterinary Science, Rajendra Nagar.

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Conflict of interest

The authors report no conflict of interest.

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Table 5: Effect of herbal and microbial supplements on phase-wise mortality of broiler chicken

Treatment	Diets	No. of birds housed	No. of birds died	Mortality %		
				Pre-starter(0-14 d)	Starter(15-28 d)	Finisher(29-42 d)
T_1	Control	60	1	1.66	-	-
T_2	Nbiotic	60	2	3.33	-	-
T_3	Liqbiotic	60	1	1.66	-	-
T_4	Salcochek Pro	60	2	3.33	-	-
T_5	AV/EGM/19	60	1	1.66	-	-
T_6	AV/EGF/20	60	2	3.33	-	-
T_7	Clostat	60	1	1.66	-	-

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