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Utilisation of prebiotics and acidifiers in broiler chicks

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ABSTRACT

This study evaluated effect of prebiotics (fructo-oligosaccharides) and acidifiers as dietary supplementation. Experimental trial involved 120 one-day-old chicks fed different dietary treatments for the duration of 42-days. The birds were randomly divided into four groups (C, T_1, T_2, T_3; 30 birds each). Group C (control) received standard broiler mash, T_1 received mash with prebiotics (0.5% for 0-3 weeks, 0.25% for 4-6 weeks), T_2 received acidifiers at same supplementation levels and T_3 received a 1: 1 combination of prebiotics and acidifiers at same levels. There was improvement ($P<0.01$) in carcass traits (dressing percentage, eviscerated weight and giblet yield) with T_3 group outperforming others. Morphometric analysis showed increased villus height and width along with reduced crypt depth in T_3 group indicating improved intestinal health. Improvements in dry matter metabolizability and nitrogen balance were also higher ($P<0.05$) in T_3 group. Mortality rates were lower in supplemented groups. Furthermore, economic analysis highlighted higher net profit in T_3 group, emphasizing cost-effectiveness of combined supplementation.

Keywords: Acidifiers, Carcass characteristics, Fructo-oligosaccharides, Intestinal morphology, Prebiotics

The extensive use of antibiotics as growth promoters in poultry production has raised serious concerns regarding antibiotic resistance and the presence of drug residues in poultry products, posing potential risks to human health (Gadde *et al.* 2018). Consequently, the European Union prohibited the use of antibiotics for growth promotion in 2006, limiting their application to therapeutic purposes only. This restriction has accelerated the search for safe and sustainable alternatives, including probiotics, prebiotics, organic acids, phytobiotics, bee-derived products and enzymes (Jha *et al.* 2020). Among these alternatives, prebiotics and organic acidifiers have gained considerable attention because of their positive effects on gut health and production performance. Prebiotics are non-digestible feed ingredients that selectively stimulate beneficial intestinal microbiota, thereby, improving host health. Fructo-oligosaccharides (FOS) enhanced the proliferation of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, resulting in improved nutrient utilization and intestinal health (Yang *et al.* 2018). Organic acids, including acetic, propionic, formic, fumaric and lactic acids improved feed efficiency, nutrient digestibility and growth performance in poultry (Yang *et al.* 2018). Moreover, they suppress pathogenic bacteria such as *Salmonella* and *E. coli* and enhance immune responses by reducing gastrointestinal pH and promoting favorable conditions for enzymatic activity (Ebeid and Homidan, 2022). Short-chain fatty acids particularly butyric acid support intestinal epithelial regeneration, increase villus height and improve absorptive capacity (Bedford and Gong, 2018). Since gut morphology is closely associated with nutrient absorption and carcass yield in broilers (Abou-Ashour *et al.* 2021), the combined use of FOS and acidifiers may exert synergistic effects on intestinal health and carcass characteristics (Li *et al.,* 2008). Therefore, the present study evaluated the effects of dietary FOS and acidifier supplementation on gut morphology, carcass parameters and economics of parameters in broiler chicken.

MATERIALS AND METHODS

Ethics approval: The experimental procedures involving broilers were approved by the Institute's Animal Ethics Committee (IAEC) (Approved No. IAEC/RES/03/05) at College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur in compliance with the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

The experiment was conducted at the poultry unit of the College of Veterinary and Animal Science (C.V.A.S), Navania, Vallabhnagar, Udaipur, Rajasthan University

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of Veterinary and Animal Sciences, Bikaner, Rajasthan, India (24°35′ N latitude, 73°43′ E longitude and 582 m above mean sea level). During the experimental period (September-October, 2023), the ambient temperature ranged between 20 and 40°C and relative humidity varied from 40 to 70%. The experimental protocol involving broiler chicks (VENCOBB-430Y strain) was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur and the experiment was conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

A total of 120 day-old commercial broiler chicks (VENCOBB-430Y strain) were randomly distributed into four treatment groups following a completely randomized design (CRD). Each treatment consisted of three replicates having 10 chicks in each replicate. The birds were reared under standard managemental conditions on deep litter system for a period of 42 days. The basal diets were formulated according to BIS (2007) specifications to meet the nutrient requirements of broiler starter (1-21 d) and finisher (22-42 d) phases. Fructo-oligosaccharide (FOS) was used as prebiotic whereas, a blend of propionic acid, acetic acid and formic acid was used as acidifier supplement. Experimental diets were prepared by mixing the respective additives in broiler mash feed. The treatments included- Four experimental diets were formulated for the study. The basal control diet was prepared without the inclusion of any feed additives and served as the control group (C). Birds in treatment group T_1 were offered a standard broiler mash supplemented with prebiotic at the rate of 0.5% of feed during the starter phase (0-3 weeks) and 0.25% during the finisher phase (4-6 weeks). Treatment group T_2 received standard broiler mash supplemented with acidifier at 0.5% during 0-3 weeks and 0.25% during 4-6 weeks. Birds in treatment group T_3 were fed a combination of prebiotic and acidifier in a 1:1 ratio at an inclusion level of 0.5% during the starter phase and 0.25% during the finisher phase.

Feed and fresh drinking water were provided ad libitum throughout the experimental period. Chicks were vaccinated against Marek's disease at hatchery level. Newcastle disease vaccination was administered through intraocular route on 7th d of age while Infectious Bursal Disease vaccination was carried out on 14th day of age. At the end of the feeding trial, two birds from each replicate having body weight close to the replicate mean were selected for carcass evaluation. The selected birds were fasted for 12 h with free access to water prior to slaughter. Birds were weighed individually, slaughtered by standard Jiang *et al.* (2018). The carcasses were scalded in hot water (80°C) for approximately 2 min and manually defeathered, therefore evisceration was performed by removing the gastrointestinal tract along with head, neck, shank and abdominal fat as described by Jiang *et al.* (2018). Dressing percentage, eviscerated yield and organ weights including heart, liver and gizzard were recorded and expressed as percentage of live body weight.

Table 1. Proximate composition of broiler and finisher ration (% DM basis)

Proximate principle	Starter	Finisher
Dry matter	91.19	92.24
Crude protein	21.50	20.18
Ether extract	04.72	05.08
Crude fiber	04.65	05.00
Total ash	08.53	08.10
Nitrogen free extract	60.60	61.64

For intestinal histomorphological studies, samples were collected from six birds per treatment group. Approximately 3 cm sections were excised from the midpoint of duodenum, jejunum and ileum and immediately fixed in 10% neutral buffered formalin. The tissues were processed according to the method described by Lillie (1965). A metabolism trial was conducted during the 6th week of experiment using six birds from each treatment group maintained in individual metabolic cages. The trial consisted of 2 days adaptation period followed by 5 days collection period. Feed offered and residual feed were recorded daily to calculate feed intake. Total excreta voided were collected daily using polythene-lined trays placed beneath each cage. Representative samples of feed and excreta were collected for proximate analysis. Excreta samples meant for nitrogen estimation were preserved with 25% sulphuric acid while samples for DM estimation were dried in a hot air oven at 85°C for 24 h until constant weight was achieved. Nitrogen content in feed and excreta samples was determined by Kjeldahl method following AOAC (2016) procedures.

Economic efficiency of different dietary treatments was calculated at the end of the experiment. Total expenditure included chick cost, feed cost, additive cost, vaccination and miscellaneous management costs. The selling price of live broilers was calculated at the rate of ₹100 per kg live body weight. Net return per broiler was estimated by subtracting total expenditure from gross income obtained from sale of birds.

The experimental data were statistically analyzed using Statistical Package for Social Sciences (SPSS) software version 20.0. Data were subjected to one-way analysis of variance (ANOVA) following completely randomized design. Differences among treatment means were compared using Duncan's multiple range tests as modified by Kramer (1956).

RESULTS AND DISCUSSION

The effects of dietary supplementation with prebiotic and acidifier powders on broiler carcass characteristics are presented in Table 2. The T_3 group showed the highest dressed and eviscerated weight percentages while the control group exhibited the lowest values. Waqas *et al.* (2019) also reported a significant ($P<0.05$) improvement in dressing percentage following supplementation of

Table 2. Supplemental effect of prebiotic and acidifier single or combination on carcass traits of broilers

Parameter (% of body weight)	Treatment			
	C	T_1	T_2	T_3
Dressing	71.12^{a}±0.01	72.21^{b}±0.02	72.27^{b}±0.01	73.00^{c}±0.10
Eviscerated	62.35^{a}±0.01	63.39^{ab}±0.29	63.88^{b}±0.30	65.33^{c}±0.65
Heart	0.43^{a}±0.01	0.45^{b}±0.01	0.47^{bc}±0.01	0.48^{c}±0.01
Liver	1.62^{a}±0.01	1.71^{b}±0.03	1.72^{b}±0.03	1.80^{c}±0.01
Gizzard)	1.38^{a}±0.04	1.48^{b}±0.01	1.49^{b}±0.01	1.52^{b}±0.01
Giblet	3.50^{a}±0.01	3.57^{b}±0.01	3.60^{c}±0.01	3.64^{d}±0.01

a,b,c Mean bearing different superscript in a column differ significantly (P<0.05)

MOS 0.6 g/kg in broilers. Similarly, Palupi *et al.* (2022) observed an increase (P<0.05) in carcass percentage with supplementation of propionic acid at 0.5 and 0.75%. Abou-Ashour *et al.* (2021) also reported improvement (P<0.05) in dressing percentage with dietary inclusion of 1% acetic acid. Prebiotic supplementation has been reported to improve carcass traits by enhancing protein deposition and reducing fat accumulation in broilers (Șara *et al.* 2016). Prebiotic supplementation in broiler diets improves growth performance, carcass yield and eviscerated yield by enhancing gut microbial balance, nutrient utilization and feed efficiency (Askri *et al.* 2020). In addition, dietary additives may reduce intestinal length and weight, indirectly improving carcass traits (Lutfullah *et al.* 2011). There was no significant effect of prebiotic, acidifiers or their combinations supplementation on carcass yield and eviscerated weight percentage (Khemalapure *et al.* 2022).

The group T_3 showed the highest (P<0.01) heart, liver, gizzard and giblet percentages, whereas the control group recorded the lowest values (Table 2). Moilwa *et al.* (2021) also reported significant (P<0.05) effects on liver, heart and gizzard percentages following prebiotic supplementation at 2.5 and 5%. Abou-Ashour *et al.* (2021) also observed significant changes in liver, gizzard and giblet percentages with 1% acetic acid supplementation. A significant improvement in visceral organ traits with acidifier and combined feed additive supplementation has been reported Makled *et al.* (2023). On the other hand, Khemalapure *et al.* (2022) found no significant effect on visceral organ weights in broilers supplemented with prebiotics or acidifiers. The improvement in carcass and organ traits may be associated with enhanced nutrient digestibility and better enzyme activity. Propionic acid reduces gastrointestinal pH and activates pepsinogen, thereby, improving protein digestion and nutrient absorption. Organic acids also stimulate digestive enzyme secretion and improve utilization of nutrients such as protein and fiber (Capuano, 2017).

Statistical analysis revealed highly significant effects (P<0.01) on villus height, villus width and crypt depth in the duodenum and ileum, as well as villus height and crypt depth in the jejunum. The T_3 group exhibited greater villus height in all intestinal segments whereas the control group showed the lowest values (Table 3). Similarly, villus width in the duodenum and ileum was significantly higher in T_3 compared to the control group. In contrast, crypt depth was highest in the control group and lowest in T_3. Increased villus height and width indicate enhanced absorptive surface area and improved intestinal health, while reduced crypt depth suggests lower epithelial turnover and better gut integrity. Helal *et al.* (2015) observed improvement (P<0.05) in villus height and ileal crypt depth in broilers supplemented with prebiotics at 0.5 g/kg feed. Sayrafi

Table 3. Effect of dietary supplementation of prebiotic and acidifier powder on villus height, villus width and crypt depth of broilers

Parameter	Treatment			
	C	T_1	T_2	T_3
Villus height of duodenum	1465.38^{a}±78.76	1540.39^{ab}±39.93	1606.15^{ab}±32.96	1638.64^{b}±15.90
Villus height of jejunum	1165.45^{a}±14.43	1187.08^{ab}±3.37	1193.07^{ab}±1.31	1211.09^{b}±6.01
Villus height of ileum	1020.27^{a}±6.68	1076.94^{b}±2.13	1072.15^{b}±8.67	1124.73^{c} ±19.92
Villus width of duodenum	195.42^{a}±10.59	213.33^{ab}±18.92	220.70^{ab}±2.92	240.62^{b}±11.30
Villus width of jejunum	211.28±6.54	236.81±10.43	231.28±10.50	237.75±1.53
Villus width of ileum	176.14^{a}±1.69	195.69^{ab}±11.54	201.76^{ab}±15.27	224.84^{b}±11.92
Crypt depth of duodenum	270.02^{c}±4.00	259.59^{bc}±10.96	245.57^{ab}±4.03	229.15^{a}±2.47
Crypt depth of jejunum	226.17^{c}±11.03	215.39^{bc}±6.53	201.38^{ab}±5.76	188.37^{a}±1.52
Crypt depth of ileum	199.66^{c}±4.11	180.21^{bc}±2.39	171.74^{ab}±11.72	155.67^{a}±0.80

a,b,c Mean bearing different superscript in a column differ significantly (P<0.05)

Table 4. Effect of dietary supplementation of prebiotic and acidifier powder on dry matter metabolizability and nitrogen balance (g/d) in broilers

Parameter	C	T_1	T_2	T_3
Dry matter intake (g)	109.90^a±0.13	117.33^b±0.34	117.34^b±0.26	117.31^b±0.54
Dry matter voided (g)	42.51^c±0.32	29.73^b±0.93	29.52^b±0.42	27.31^a±0.26
Dry matter metabolizability (%)	61.32^a±0.25	74.66^b±0.86	74.84^b±0.42	76.71^c±0.33
N intake (g/d)	4.14^a±0.02	4.45^b±0.03	4.48^b±0.06	4.57^b±0.05
N voided (g/d)	1.25^c±0.03	0.88^b±0.02	0.85^b±0.01	0.78^a±0.02
N balance (g/d)	2.89^a±0.04	3.57^b±0.05	3.64^{bc}±0.05	3.79^c±0.06

a,b,c Mean bearing different superscript in a column differ significantly (P<0.05)

et al. (2011) also reported improved villus height and width with dietary prebiotic supplementation. The improvement in intestinal morphology was attributed to the use of acidifiers in the diet (Supriya *et al.* 2022). The T_3 group possessed the highest DM metabolizability (P<0.01) whereas the control group showed the lowest value (Table 4). Cengiz *et al.* (2012) observed significant improvement in DM digestibility with supplementation of the prebiotic "Agrimos" at 0.1%. However, Emami *et al.* (2012) reported non-significant effects of FOS and formic acid on DM digestibility. The improvement in DM metabolizability may be attributed to modulation of intestinal microbiota, characterized by increased populations of beneficial bacteria such as *Lactobacilli* and reduced counts of pathogenic bacteria including *Salmonella typhimurium* and *Escherichia coli*. Prebiotics may also stimulate secretion of digestive enzymes from the pancreas and intestinal mucosa, thereby improving nutrient digestion and absorption while reducing intestinal inflammation (Cengiz *et al.* 2012).

The T_3 group showed the highest nitrogen balance (P<0.01) followed by T_2, whereas the control group recorded the lowest value (Table 4). Thorat *et al.* (2015) also reported significant improvement in nitrogen balance due to use of prebiotics, synbiotics and organic acids. Padihari *et al.* (2015) observed non-significant effects of MOS supplementation on nitrogen balance. Prebiotics improve nitrogen utilization by modulating gut microbiota, increasing volatile fatty acid production and enhancing nutrient digestibility, thereby reducing nitrogen excretion and ammonia emission (Such *et al.* 2023). Organic acids improve protein digestibility by lowering endogenous nitrogen losses and reducing ammonia production while stimulation of intestinal cell proliferation and nutrient absorption (Samanata *et al.* 2010). The acidic intestinal environment created by organic acids further enhances nutrient availability and utilization (Boling *et al.* 2001). In the present study, improved villus height and width likely increased absorptive surface area, contributing to enhanced

Table 5. Effect ofdietary supplementation of prebiotic and acidifier powder on percent mortality at different weeks in broilers

Treatment	No. Initial	Mortality No. in different weeks							Mortality (%)
		1	2	3	4	5	6	0-6	
C	30	0	0	0	2	0	0	2	6.66
T_1	30	0	0	0	0	0	0	0	0.00
T_2	30	0	0	0	1	0	0	1	3.33
T_3	30	0	0	0	1	0	0	1	3.33
Total	120	0	0	0	4	0	0	4	3.33

Table 6. Effect of dietary supplementation of prebiotic and acidifier powder on comparative economics of broilers

Cost (₹)	C	T_1	T_2	T_3
Total feed cost/chick	180.56^d ± 0.38	199.75^b ± 0.48	197.13^c ± 0.39	206.42^a ± 0.51
Chick cost	45.00 ± 0.00	45.00 ± 0.00	45.00 ± 0.00	45.00 ± 0.00
Miscellaneous cost	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Total expenditure	228.56^d ± 0.38	247.75^b ± 0.48	245.13^c ± 0.39	254.42^a ± 0.51
Final weight in kg/broiler	2.30^c ± 0.02	2.65^b ± 0.02	2.69^b ± 0.02	2.79^a ± 0.02
Sell cost 100 /kg	230.00^c ± 1.73	265.00^b ± 1.73	269.00^b ± 1.73	279.00^a ± 1.73
Net profit (return/broiler)	1.44^c ± 0.39	17.25^b ± 0.42	23.88^a ± 0.45	24.58^a ± 0.45

a,b,c,d Mean bearing different superscript in a column differ significantly (P<0.05)

DM metabolizability and nitrogen retention.

Out of 120 chicks, four mortalities were recorded during the experimental period, resulting in an overall mortality rate of 3.33% (Table 5). The present findings are supported by Cao *et al.* (2005) observed a significant reduction (P<0.05) in mortality with fructo-oligosaccharide supplementation. Ebeid and Homidan (2021) also reported reduced mortality following acidifier supplementation. Organic acids and prebiotics improve gut health by promoting beneficial microbiota, suppressing pathogenic bacteria and enhancing immune response. Praxedes-Campagnoni *et al.* (2021) reported that probiotics enhance gut barrier integrity and reduce endotoxin levels, thereby lowering mortality. Organic acids also improve humoral immunity and disease resistance in broilers (Ali *et al.* 2024). Abdullahi *et al.* (2020) further suggested that organic acids enhance protective immune responses by increasing CD4 T-cell concentration and cytokine expression. However, Dizaji *et al.* (2012) reported non-significant effects of FOS and acidifiers on mortality.

The feed cost was the highest in group T_3 whereas the control group recorded the lowest values (Table 6). However, selling price and net return per bird was higher (P<0.05) in group T_3 indicating superior economic efficiency. Waqas *et al.* (2019) reported improved net profit following prebiotic supplementation. The economic returns due supplementation of organic acids and combinations of prebiotics and acidifiers was higher (Abou-Ashour *et al.* 2021, Khemalapure *et al.* 2022). Dietary supplementation of fructo-oligosaccharides and acidifiers improved (P<0.05) intestinal morphology, nutrient utilization, carcass characteristics and economic returns in broiler chicken. Combined prebiotic and acidifier supplementation signified synergistic effects between prebiotics and acidifiers. The findings support the use of these feed additives as sustainable alternatives to antibiotic growth promoters in poultry production systems.

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