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Effects of Dairy Waste Supplement, Galipro Probiotic, Technomos Prebiotic in the Diet on Growth Performance, Immune Response, Microbial Flora and Intestinal Morphometry in Broiler Chickens

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Abstract: This study was conducted to evaluate the effects of dairy waste supplement, Galipro probiotic, TechnoMos prebiotic in the diet on growth performance, immune response, microbial flora and intestinal morphometry in broiler chickens. This study was conducted in completely randomized design with 8 treatments and 5 replications. A total number of 1200 male broiler chickens (Ross 308) with similar body weight (42.0±1.0 g) were used. Diets contained Galipro probiotic, TechnoMos prebiotic and Dairy Waste (DW) supplement in the different level (2.5, 5, 7.5 and 10 kg ton^{-1}). In this study, some parameters including growth performance, cellular and humoral immunities, cecal microbial population and intestinal morphology in broiler chicks were investigated. Results showed that birds in synbiotic group consumed more feed intake in comparison to other groups in finisher period ($p<0.05$). Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) were not influenced by experimental treatments in starter and grower periods ($p>0.05$) while BWG and FCR were significantly higher and lower in 7.5 and 10% DW in comparison to control group in finisher period ($p<0.05$). The obtained results showed that levels of Immunoglobulin (IgG), Immunoglobulin (IgM), Immunoglobulin (IgT), intestinal villus height and thickness and becterial population of beneficial bacteria in the both periods were significantly higher in broiler chicks fed the 5, 7.5 and 10% DW+synbiotic in comparison to control group ($p<0.05$). In conclusion, the use of higher levels of DW

(7.5 and 10%) and synbiotic in broiler chicks diets could be advised to improve immune system and growth performance that act by modulation in intestinal morphology and beneficial microbial population.

INTRODUCTION

The use of feed additives in poultry diets is considered as solution for efficient application of feed by poultry. The use of antibiotics can residue these substances in animal products such as meat and egg that increase pathogens transformation to human body and also cause antibiotic resistance. Such happens cause that antibiotic could not efficiently in when diseases[1]. Additives such as probiotics and prebiotics may be appropriate alternatives for antibiotics. Dairy wastes supplements are economic that can be used in poultry diet and increase gastrointestinal microbial population. This supplement contain proteins, non-protein nitrogenous substances, high amounts of metabolizable energy, lactic acid, vitamins, riboflavin and pantothenic acid. It contains major amounts of methionine, lysine and theronine but it contains lower levels of sulphor and glycine[2]. Dairy wastes supplement contain significant amounts of lactose and on the other hand, broiler chicks did not have lactase enzyme and lactose, therefore cannot be absorbed, fermented and converted lactic acid and volatile fatty acids which increase lactobacillus population in poultry intestine and finally, cause problems in digestive system. Effects of probiotics and prebiotics on performance, immune response and microbial flora in broiler chicks have been investigated. Bozkurt *et al*.[3] showed that broiler chicks fed with probiotic showed higher body weight in comparison to control group. They also showed that dietary inclusion of prebiotic increased weight gain and improved feed conversion ratio. Salim *et al*.[4] and Bai *et al*.[5] reported that dietary inclusion of probiotic improved weight gain and feed conversion ratio. Application of probiotics and prebiotics can beneficially influence immune system in broiler chicks[6]. Salim *et al*.[4] showed that broiler hcikcs fed with probiotic had higher white blood cells, monocytes and immunogloubines in comparison to control group. Yange *et al*.[7] showed that the blood concentration of immunoglubins of IgA, IgG and IgM was significantly higher in broiler chicks fed with probiotic. Yang *et al*.[7] reported that dietary inclusion of probiotic decreased harmful bacterial population and increased beneficial bacterial population. Kim *et al*.[8] observed decreased clostridium and *E. coli* population and increased lactobacillus in broiler chicks receiving the prebiotics. Probiotics are defined as live microorganisms that are used balancing intestinal microbial population and fighting against pathogenic microbes in digestive system[9].

Probiotics enhance epithelial barrier, adhesion to intestinal mucosa, antimicrobial production and modulate in immune system[10]. Dietary inclusion of probiotics helps to decrease of the colonization of pathogenic bacteria in the gastrointestinal tract[11]. Probiotics prevent adhesion pathogenic bacteria to intestinal villi and provide inappropriate conditions for bacteria growth by secertion of some substances, also produce antimicrobial substances and help to regulate the immune system[10]. Diets containing probiotics help to decrease pathogenic bacteria in gastrointestinal system. Prebiotics are non-digestible compounds which can selectively stimulate growth and activity in one or number of beneficial bacteria in gastrointestinal system and can have beneficial effects on animals[12].

Prebiotics are known to have some characteristics including lack of absorption in gastrointestinal tract, resistant to acidic pH, promoting the growth of profit bacteria and modulating in host defense system[13]. Prebiotics can supply food source for host beneficial bacteria including Lactobacillus and Bifidobacteria in the lower gastrointestinal tract[9]. It has been shown that dietary inclusion of probiotic to laying hens diet increased Lactobacillus and Bifidobacteria in intestine. Dietary inclusion of prebiotics increased antibody titers of IgM and IgG, cecum IgA levels, mucin mRNA expression and intestinal immune activities[14]. Regarding to production of dairy wastes in dairy companies, this study aimed to evaluate the effects of dietary supplementation of probiotics, prebiotics and dairy wastes in broiler chicks. Thus, this study aimed to evaluate the effects of prebiotci and probiotic and a mixture of the both additives and dairy wastes supplement on growth performance, carcass traits, immune responses, blood biochemical parameters, microbial flora and intestinal morphometery in broiler chicks.

MATERIALS AND METHODS

Ethical standards: All the protocols used in this study were approved by the Institutional Animal Care and Use Committee of Science and Research Branch, Islamic Azad University, Tehran, Iran (Tehran-Iran). We tried to minimize the pain or discomfort of the birds at all times.

Animals and dietary: This study was conducted in a commercial poultry farm placed in Animal Science Research Institute of Iran, Karaj, Iran. A total number of 1200 male broiler chickens (Ross 308) with similar body weight (42.0±1.0 g) were randomly assigned to 8 treatments with 5 replicate cages and 30 birds per floor

Table 1: Composition of the broiler chicken diets (g kg^{-1})

Periods	Starter (0-14 days)	Grower 1 (15-28 days)	Grower 2 (29-42 days)	Finisher (43-56 days)
Corn (%)	57.90	62.50	64.200	65.160
Spybean meal (%)	33.00	28.50	25.600	26.100
Fish meal (%)	2.50	1.80	2.000	0.000
Soda (%)	0.20	0.10	0.080	0.150
Salt (%)	0.30	0.30	0.275	0.275
Dicalcium phosphate (%)	0.10	1.32	1.230	1.375
Mineral and vitaminized supplement (%)[a]	0.50	0.50	0.500	0.500
DL-methionine	0.19	0.21	0.210	0.220
L-lysine	0.06	0.1	0.100	0.180
Soy bean oil (%)	2.79	3.31	4.475	4.600
Oystershell	1.56	1.45	1.330	1.440
Chemical analysis				
Metabolizable energy (kcal kg^{-1})	2988.00	3083.00	3175.00	3176.00
Crude protein (%)	21.00	19.00	18.00	17.00
Lysine (%)	1.20	1.10	1.04	1.00
Methionine (%)	0.54	0.52	0.51	0.49
Methionine+Cystein (%)	0.89	0.84	0.82	0.78
Calcium (%)	1.00	0.96	0.90	0.90
Available phosphorous (%)	0.50	0.48	0.45	0.45
Sodium (%)	0.20	0.17	0.16	0.16

[a]Mineral-Vitamin premix provided the following per kilogram of diet: Vitamin A, 9000 IU; Vitamin D3, 2100 IU; Vitamin E, 30 mg; nicotinic acid, 30 mg; Vitamin B 12, 0.12 mg; calcium pantothenate, 10 mg; Vitamin K3, 5 mg; thiamin, 1.1 mg; riboflavin, 4.5 mg; Vitamin 6, 2.0 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 50 mg; Cu, 10 mg; Mn, 70 mg; Zn, 50 mg; Co, 0.2 mg; I, 1.0 mg; Se, 0.3 mg; Butylated Hydroxy Toluene (BHT), 150 mg; monensin, 100 mg

pen. Boiler chicks were reared on floor for 56 days. Experimental groups consisted of synbiotic, dairy wastes containing high lactose in the different level (2.5, 5, 7.5 and 10 kg ton^{-1}). Experimental treatments were as follows:

Broiler chicks fed basal diet without additive (Control). Broiler chicks fed basal diet containing synbiotic (Synbiotic). Broiler chicks fed basal diet containing lowest or 2.5% dairy wastes (Low DW). Broiler chicks fed basal diet containing highest or 10% dairy wastes (High DW).

Broiler chicks fed basal diet containing 2.5% dairy wastes+synbiotic (2.5% DW). Broiler chicks fed basal diet containing 5.0% dairy wastes+synbiotic (5.0% DW). Broiler chicks fed basal diet containing 7.5% dairy wastes+synbiotic (7.5% DW). Broiler chicks fed basal diet containing 10.0% dairy wastes+synbiotic (10.0% DW).

In this study, we used Galipro probiotic that contained Lactobacillus subtilis (4×109 CFU g^{-1}) and it also contained TechnoMos prebiotic that was extracted product from *Saccharomyces* cerevisiae. Techno Mos prebiotic contained high amounts of Mannan-Oligosaccharides (MOS) and ß-1&3 glucan. The additives were added into basal diets. The experimental diets were formoulated based on Ross[15] into 4 phase including starter (0-14 days), grower 1 (15-28 days), grower 2 (29-42 days) and finisher (43-56 days). The iso-nitrogenous and iso-caloric diets were formulated on basis UFFDA software. The different levels of dairy wastes, prebiotic and probiotic were included into diets.

Table 2: Chemical analysis of dairy waste supplement

Analysis	Percentage
DM	90.70
Moisture	9.30
Lactose	15.00
CP	18.37
CF	0.73
EE	8.60
ASH	4.00
Mg	0.15
K	1.00
Calcium	0.95
Phosphorous	0.59
Vitamins and minerals	41.31

Treatments included 4 levels of dairy wastes that included into diet in the levels of 0, 2.5, 5.0, 7.5 and 10 kg ton^{-1}. All the diets were prepared in mash form (Table 1). Dairy wastes supplement was prepared from Soroush Sabz Alborz Engineering Company. Dairy waste supplement was analysed by Horwitz[16] and the data were presented in Table 2.

Growth performance: To evaluate the growth performance in broiler chicks, following consumption of dairy wastes and synbiotics, Feed Intake (FI), Body Weight Gain (BWG), Feed Conversion Ratio (FCR) were measured in end of the period as well was calculated. Mortality was daily recorded.

Humoral immunity: To evaluate the humoral immunity, 3 mL of 5% suspension of SRBCs was intravenously injected to four birds in each replicate. In 35 days, blood samples were collected and centrifuged at 2500 g for

10 min. Sera samples were stored at -20°C and each serum sample was inactivated at 56°C for 30 min. Sera samples were analysed for anti-SRBC antibodies as described by Delhanty and Solomon[17]. The specified kits were used to evaluate the immunoglubins. On 21 and 42 days of age, the serum concentrations of complement component 3 (C3) and complement component 4 (C4) were assessed by the chicken-specific ELISA kits as recommended by producer company (Jiancheng Biological Engineering Institute, Nanjing, China).

Cellular immunity: In 22 and 42 days of age, 0.1 mL of DNCB and PHA was administrated to 1 birds each replication. Acetone and olive oil in 4:1 ratio was used. In order to administrate the DNCB, one area (10 cm^2) was considered and skin thickness was evaluated before sensitization. A mean of 3 parts was considered as skin thickness in 24 and 48 h after challenge. For this purpose, 0.1 mL PHA (10 mg mL^{-1} acetone and olive oil in 4:1 ratio) was intradermal injected between the third and fourth digits of the right foot and the area thickness and skin thickness was assessed by a constant tension micrometer (Global Sources, Shanghai, China).

Intestinal morphometry: In end of experiment (42 day), one broiler chicks in per replicate were killed and intestinal middle segments (jejunoum) were separated.

Almost 1.5 cm of intestine was sampled and after washing with 85% saline was positioned for 24 h and then samples were positioned into formaline. After preparation the samples, light microscope equipped with computer was used and crypt height, villus width and crypt depth were measured and the data were reported as μm.

Caecal bacterial populations: In d 42, to investigate the caecal bacterial populations, 1 g of caecal content for each bird was transferred to 9 mL of sterile physiological salt solution and bacterial populations were measured as described by Mookiah *et al*.[18]. Bacterial populations were expressed as $\log_{10}$ Colony Forming Units (CFU) g^{-1} caecal content. All bacterial enumerations were performed in duplicate.

Statistical analysis: The data were analyzed using the ANOVA procedure of SAS[19]. Means were subsequently compared using Duncan's least significance multiple-range test. Results are expressed as means± Standard Error (SE). Differences were considered as significant if $p<0.05$. In order to evaluate the antibody titer, the $\log_2$ transformations were performed for antibody titer before statistical analysis.

RESULTS AND DISCUSSION

Growth performance: Effects of experimental treatments on growth performance are presented in Table 3. Results showed that feed intake was significantly higher in higher dairy wastes treatments in comparison to 5.0% DW group in starter period ($p<0.05$). Significant difference was not observed among other groups in same period ($p>0.05$). Statistically, birds in synbiotic group consumed more feed intake in comparison to other groups in finisher period ($p<0.05$). Results also showed that body weight and feed conversion ratio were not influenced by experimental treatments in starter and grower periods ($p<0.05$). Body weight and feed conversion ratio were significantly higher and lower in 7.5 and 10% DW in comparison to control group in finisher period ($p<0.05$).

Humoral immunity: Effects of experimental treatments on humoral immunity are shown in Table 4. Levels of IgG, IgM, IgT in the both periods and C3 in 21 days were significantly higher in 5, 7.5 and 10% DW in comparison to control group ($p<0.05$). The levels of C4 in 21 days and C3 and C4 in 42 days were not influenced by experimental treatments ($p>0.05$).

Effects of experimental treatments on cellular immunity are shown in Table 5. Results indicated that experimental treatments did not have significant effect on cellular immunity ($p>0.05$).

Intestinal morphology: Effects of experimental treatments on intestinal morphology are presented in Table 6. Results showed crypt depth was not influenced by experimental treatments ($p>0.05$). Villus height and villus depth were significantly higher in broiler chicks fed 5, 7.5 and 10% DW in comparison to other groups ($p<0.05$).

Effects of experimental treatments on cecal microbial population are shown in Table 7. Lactobacillus and Bifidobacteria populations were significantly higher in broiler chicks in 7.5 and 10% DW in comparison to control group ($p<0.05$). *E. coli* population was significantly lower in high-DW, 5, 7.5 and 10% DW in comparison to control group ($p<0.05$).

Growth performance is known as the general criteria in poultry due to direct effects of feed utilization and overall effectiveness in broiler chicks production[20]. In this study, birds in High-DW consumed more feed in comparison to 5.0% DW in starter period.

However, significant difference was not observed between groups in comparison to control group. Birds in synbiotic group consumed more feed intake in comparison to other groups in finisher period. In contrast to our findings, Huang *et al*.[14] showed that dietary inclusion of inulin prebiotic in levels of 5-10 g kg^{-1}

Table 3: Effects of experimental treatments on growth performance

Groups	FI (starter)	FI (grower)	FI (finisher)	BW (starter)	BW (grower)	BW (finisher)	FCR (starter)	FCR (grower)	FCR (finisher)
Control	256.40±18.45^{ab}	908.62±40.56^{b}	2606.52±121.30^{b}	276.10±18.45	652.82±27.42	1616.82±58.43^{c}	0.931±0.065	1.39±0.076	0.062^{b}±1.61
Synbiotic	246.80±8.71^{ab}	921.93±16.41^{ab}	2901.13±25.87^{a}	267.30±8.71	645.83±8.62	1638.37±45.32^{c}	0.929±0.050	0.026±1.42	0.053^{a}±1.77
Low-DW	263.83±13.98^{ab}	928.03±39.56^{ab}	2637.56±36.10^{b}	275.73±13.98	647.17±34.66	1672.07±69.94^{abc}	0.958±0.049	0.049±1.43	0.066^{bc}±1.57
High-DW	265.86±17.18^{a}	963.77±32.35^{ab}	2624.00±140.80^{b}	291.83±17.18	667.27±25.11	1672.00±74.87^{abc}	0.910±0.059	0.017±1.44	0.030^{bcd}±1.56
2.5 DW	255.56±8.86^{ab}	930.80±32.94^{ab}	2622.17±149.60^{b}	279.30±8.86	645.37±35.89	1664.37±103.90^{bc}	0.915±0.018	0.067±1.44	0.010^{bc}±1.57
5.0 DW	237.53±11.16^{b}	968.33±42.78^{a}	2559.13±30.03^{b}	278.06±11.16	662.57±20.48	1712.00±29.84^{abc}	0.856±0.052	0.059±1.46	0.03^{bcd}±1.49
7.5 DW	254.20±15.06^{ab}	921.46±43.24^{ab}	2576.47±61.62^{b}	267.96±15.06	645.36±21.48	1798.20±76.39^{a}	0.952±0.085	0.040±1.42	0.03^{d}±1.43
10.0 DW	244.50±20.55^{ab}	915.93±19.79^{ab}	2578.00±134.60^{b}	269.00±20.55	630.20±27.04	1788.40±38.36^{ab}	0.909±0.088	0.051±1.45	0.05^{d}±1.44
p-values	0.185	0.0118	0.0001	0.185	0.512	0.0002	0.186	0.719	0.0001
SEM	2.38	5.13	16.85	2.38	4.22	10.51	0.009	0.008	0.01

Table 4: Effects of experimental treatments on humoral immunity

Groups	IgG35 (log_2)	IgM35 (log_2)	IgT35 (log_2)	IgG42 (log_2)	IgM42 (log_2)	IgT42 (log_2)	C3-21 (mg mL^{-1})	C4-21 (mg mL^{-1})	C3-42) (mg mL^{-1})	C4-42 (mg mL^{-1})
Control	3.12±0.08^{d}	0.90±0.07^{b}	4.02±0.10^{d}	3.92±0.10^{b}	0.84±0.089^{b}	4.76±0.05^{b}	0.248±0.008^{c}	0.067±0.001	0.008±0.198	0.020±0.092
Synbiotic	3.12±0.04^{d}	0.96±0.07^{b}	4.08±0.04^{cd}	3.90±0.07^{b}	0.94±0.054^{b}	4.84±0.08^{b}	0.250±0.010^{bc}	0.001±0.068	0.008±0.198	0.004±0.112
Low-DW	3.06±0.11^{d}	0.94±0.05^{b}	4.00±0.14^{d}	3.96±0.09^{b}	0.96±0.054^{b}	4.92±0.08^{b}	0.250±0.010^{bc}	0.001±0.069	0.008±0.192	0.001±0.113
High-DW	3.38±0.04^{bc}	1.02±0.13^{b}	4.40±0.14^{b}	3.98±0.08^{b}	0.94±0.054^{b}	4.92±0.10^{b}	0.248±0.008^{c}	0.001±0.069	0.008±0.198	0.008±0.112
2.5 DW	3.32±0.08^{c}	0.95±0.03^{b}	4.27±0.05^{bc}	4.02±0.08^{b}	0.94±0.041^{b}	4.96±0.11^{b}	0.250±0.010^{bc}	0.001±0.068	0.005±0.196	0.001±0.113
5.0 DW	3.56±0.11^{a}	1.22±0.08^{a}	4.78±0.13^{a}	4.24±0.11^{a}	1.14±0.054^{a}	5.38±0.10^{a}	0.276±0.011^{a}	0.001±0.068	0.010±0.202	0.001±0.113
7.5 DW	3.52±0.08^{ab}	1.22±0.08^{a}	4.74±0.13^{a}	4.26±0.11^{a}	1.20±0.07^{a}	5.46±0.15^{a}	0.270±0.012^{ab}	0.001±0.067	0.013±0.202	0.008±0.113
10.0 DW	3.68±0.08^{a}	1.28±0.08^{a}	4.96±0.08^{a}	4.28±0.08^{a}	1.16±0.054^{a}	5.44±0.11^{a}	0.272±0.013^{a}	0.001±0.068	0.888±0.196	0.001±0.111
p-values	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.114	0.723	0.413
SEM	0.036	0.025	0.058	0.027	0.021	0.046	0.002	0.0002	0.004	0.002

$^{(a-b)}$ show significant differences in each row ($p<0.05$); SEM = Standard Error of Means

Table 5: Effects of experimental treatments on cellular immunity

Treatments	DNCB-24 (μm)	DNCB-48 (μm)	PHA-24 (μm)	PHA-48 (μm)
Control	0.743±0.020	0.424±0.013	0.721±0.019	0.416±0.015
Synbiotic	0.729±0.015	0.437±0.011	0.685±0.029	0.416±0.005
Low-DW	0.731±0.011	0.430±0.009	0.712±0.014	0.414±0.011
High-DW	0.747±0.014	0.427±0.001	0.699±0.023	0.412±0.008
2.5 DW	0.728±0.018	0.426±0.012	0.705±0.032	0.416±0.005
5.0 DW	0.739±0.001	0.437±0.011	0.705±0.027	0.414±0.011
7.5 DW	0.731±0.011	0.430±0.009	0.696±0.029	0.406±0.011
10.0 DW	0.722±0.010	0.421±0.007	0.701±0.024	0.410±0.012
p-values	0.107	0.173	0.549	0.783
SEM	0.002	0.001	0.004	0.001

SEM = Standard Error of Means

increased feed intake in starter period (0-21 d) but did not have any effect on feed intake in 42 days in broiler chicks. The reason for increased feed intake is known[21]. The different levels of dairy wastes supplements did not have any significant effects on feed intake. Increased body weight and feed conversion ratio in 7.5 and 10% groups dairy wastes were significantly lower in 7.5 and 10% groups in comparison to control group. Several studies have reported positive effects of single or multi-species probiotics in order to improve the growth performance in broiler chicks[22, 18, 6]. In one study, dietary inclusion of prebiotics into broiler chicks diets increased body weight gain and feed conversion ratio[23, 24]. Probiotics are known to have effects in increasing feed intake[21]. Improved in body weight gain and feed conversion ratio in 7.50 and 10% DW is thus attributed to synergism interaction between prebiotic, probiotic and high levels of dairy wastes. Prebiotics modulates in gut microbiota through increasing beneficial bacteria[25] and also decreasing pathogenic bacteria colonization. Prebiotics also produces Short Chain Fatty Acids (SCFA) that regulates the metabolic activities[26]. On the other words, probiotics keep the dynamic equilibrium for the microbiota[27] and prebiotic activities promote the growth and function of colonic beneficial bacteria[28]. Mookiah *et al*.[18] believed that probiotic and prebiotic improve weight gain and feed conversion by modulation in intestinal microbiota. The exact for dairy wastes are not known but 7.50 and 10% DW groups showed lower counts for harmful bacteria and higher population for beneficial bacteria. In addition, villus width and height were significantly higher in same groups that help to more absorption of nutrients. In sum, it can be stated that prebiotic, probiotic and higher levels of DW synergistically improve weight gain and FCR by modulating in intestinal system.

In investigation of humoral immune responses, levels of IgG, IgM, IgT in the both periods and C3 in 21 days were significantly higher in 5, 7.5 and 10% DW in comparison to control group but cellular immunity was not influenced by experimental treatments. Previous studies have shown that dietary inclusion of prebiotics into diet increased levels of IgM and IgG, cecum IgA, mucin mRNA expression and intestinal immune activities[29, 14]. Improved immune function by prebiotic can be attributed to preferential colonization of beneficial

Table 6: Effects of experimental treatments on intestinal morphology

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)
Control	1271.00±17.64^{c}	189.40±1.14^{d}	181.40±0.89
Synbiotic	1243.80±42.86^{c}	194.00±0.71^{bc}	183.60±1.14
Low-DW	1295.60±74.46^{c}	192.40±1.14^{cd}	182.20±1.09
High-DW	1269.60±41.17^{bc}	192.40±1.14^{cd}	182.80±1.30
2.5 DW	1262.00±46.62^{c}	191.20±0.80^{cd}	182.00±1.22
5.0 DW	1397.60±7.92^{a}	198.40±4.33^{ab}	181.80±0.83
7.5 DW	1387.00±20.17^{a}	200.00±1.14^{a}	182.20±1.09
10.0 DW	1352.60±10.34^{bc}	198.00±3.67^{ab}	182.80±1.30
P-value	0.0001	0.212	0.104
SEM	10.6	0.658	0.191

Table 7: Effects of experimental treatments on cecal microbial population

Treatments	Lactobacillus (log CFU g^{-1} caecal digesta)	E. coli (log CFU g^{-1} caecal digesta)	Bifidobacteria (log CFU g^{-1} caecal digesta)
Control	8.38±0.18^{b}	7.52±0.08^{a}	7.18±0.23^{b}
Synbiotic	8.28±0.08^{b}	7.44±0.11^{ab}	7.28±0.19^{b}
Low-DW	8.40±0.10^{b}	7.40±0.15^{ab}	7.18±0.08^{b}
High-DW	8.34±0.17^{b}	7.26±0.08^{c}	7.18±0.08^{b}
2.5 DW	8.22±0.13^{b}	7.36±0.11a^{abc}	7.18±0.08^{b}
5.0 DW	8.34±0.11^{b}	7.14±0.16^{cd}	7.22±0.08^{b}
7.5 DW	8.64±0.11^{a}	7.00±0.15^{d}	7.78±0.39^{a}
10.0 DW	8.76±0.08^{a}	6.90±0.07^{d}	7.90±0.12^{a}
P-value	0.0001	0.0001	0.0001
SEM	0.03	0.037	0.052

$^{(a, b)}$ show significant differences in each row ($p<0.05$); SEM = Standard Error of Means

bacteria and microbial products that produces immune cells[28]. Pourabedin *et al*.[25] reported supplementation of prebiotics into diet promotes lactobacillus growth in the contents of chicken cecum. Increased antibody production in probiotic group is due to increased B-lymphocytes[30]. In addition, inclusion of probiotics into diet not only inhibits pathogenic bacteria but also increases maturation of the gut and its integrity[31]. It seems that prebiotic, probiotics and levels 7.5 up to 10% of DW improve immune function by modulating in intestine system.

Villus height and villus depth were significantly higher in broiler chicks fed 5, 7.5 and 10% DW in comparison to other groups. Agboola *et al*.[32] showed diet inclusion of probiotics and synbiotic to diets of turkey poults the increased villus height and crypt depth. Studies have shown that dietary inclusion of prebiotics increased villi height and crypt depth in broiler chicks intestine[33]. Inclusion of mannan oligosaccharides and lignin into poultry diet increased production of SCFA and subsequently increased villi height[23]. Exact mechanism for additives is not defined.

Lactobacillus and Bifidobacteria populations were significantly higher in broiler chicks in 7.5 and 10% DW in comparison to control group but *E. coli* population was significantly lower in high-DW, 5, 7.5 and 10% DW in comparison to control group. Inclusion of prebiotics into diet increased beneficial bacteria and also decreased pathogenic bacteria colonization[2]. Mookiah *et al*.[18] believed that probiotic and prebiotic decrease harmful population and increase beneficial population. Prebiotics are capable to adhere to mannose-specific lectin of gram-negative pathogens which express Type-1 fimbriae, i.e., *E. coli* and excrete them from the intestine[34]. On the other hand, prebiotics are able to compete with sugar receptors and inhibit adhesion of pathogens such as Salmonella and *E. coli*[35]. It can be thus stated that high levels of DW, prebiotic and probiotic synergistically compete increases Lactobasillus and Bifidobacteria population that compete with *E. coli*.

CONCLUSION

In conclusion, higher levels of DW and synbiotic synergistically improved humoral immunity, growth performance in finisher period, intestinal morphology and beneficial microbial population. In conclusion, the use of higher levels of DW (7.5 and 10%) and synbiotic improve immune system and growth performance by modulating in intestinal morphology and benificial microbial population. It can be stated these treatments to improve the immune responses and growth performance.

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