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Modulating broiler gut microbial population: The prebiotic effect of date pit powder in wheat-based diets

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Abstract This study examined the prebiotic potential of date pit powder incorporated into a wheat-based diet, on the performance in broiler chickens. In this experiment 240 broiler chicks were used in a completely randomized design with six treatments and five replicates. The experimental treatments consisted of: 1- positive control (PC, based on the corn-soybean meal), 2-negative control (NC, based on the wheat-soybean meal), 3- NCP; NC with prebiotic, 4- NCE; NC with enzyme, 5- 1.5NCDP; NC with 1.5 % date pit powder and 6- 3NCDP; NC with 3 % date pit powder. The results showed that during the starter period, the PC group had the lowest feed intake and the best feed conversion ratio among the groups (P<0.05). The *Escherichia coli* population in cecal contents of the NCE and 3NCDP groups were lower significantly than in PC. The ileal content pH in NC was higher than that in PC, NCE, and 1.5NCDP groups. By adding prebiotics and enzymes to wheat-based broiler diets, the primary and secondary antibody titers against SRBC were increased compared to NC group. In conclusion, supplementing wheat-based broiler diets with 1.5% date pit powder enhanced birds gut health.

Keywords: broiler, cecal microbial population, date pit, performance, wheat

Introduction

Corn and soybean meal are the primary feed ingredients in the poultry industry, which constitute 70-75% of the production costs. Corn is the primary energy source in poultry diets. However, due to its various applications, including the production of ethanol, biofuels, etc. the use of local indigenous resources such as barley and wheat is increasing. Wheat is considered a suitable substitute for corn, and in some countries, it is one of the best energy-producing cereal ingredients in the poultry industry. However, the inclusion of high amounts of wheat in poultry diets is limited due to its anti-nutritional factors, such as non-starch polysaccharides (NSP) which increase the viscosity of digesta and negatively affect feed consumption by interfering with the digestion and absorption of lipid, protein and starch (Matthiesen et al., 2021). The use of feed additives such as enzymes, antibiotics, prebiotics, and probiotics in poultry diets has shown promising results in improving performance and reducing the NSP anti-nutritional effects (Hübener et al., 2002; Svihus et al., 2013; Kim et al., 2020).

Prebiotics, non-digestible feed compounds, are used in small amounts and can improve birds performance by selectively stimulating the growth or activity of beneficial bacteria such as *Bifidobacteria* and *Lactobacillus* in the digestive tract (Teng and Kim, 2018), and or inhibiting pathogenic ones by preventing these bacteria from binding the intestinal epithelium. Mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and galacto-oligosaccharid-es (GOS) have been used as prebiotics in poultry diets (Kim et al., 2019).

Date pits (also called stones, seeds, or kernels), which constitute approximately 10-20% (average 15%) of the fruit's weight, are byproducts of date processing contributes to environmental problems (Attia et al., 2021). In 2021, Iran produced 1,491,528 metric tons of dates (yielding approximately 223,729 metric tons of date pits), with an average yield of 6,478 kilograms per hectare (Pourghayoumi et al., 2024). Date pits contain valuable nutrients such as protein, fat, minerals, fiber, and unknown growth compounds that can be used in animal and even human nutrition (Nahirat et al., 2018; Attia et al., 2021; Pourghayoumi et al., 2024). Studies have shown that using 5-10% of date pits in broiler chicken feed maintains performance at the level of grain-based diets while reducing costs (Attia et al., 2021; Sholichatunnisa et al., 2022). Shahrami et al. (2012) concluded that palm kernel meal has high insoluble fiber that can be used as a prebiotic to improve chicken health. Mannan-oligosaccharides are the main components of date pits NSP (Attia et al., 2021). Polysaccharides isolated from date pits demonstrated resistance to digestion that was comparable to, and in some cases even superior to inulin (a commercial prebiotic). They enhanced the viability of probiotic bacteria, demonstrating behavior similar to that of inulin in this regard (Tadayoni et al., 2014). Given the well-documented beneficial effects of prebiotics on gut microbial balance, the present study aimed to investigate the prebiotic potential of date pit powder and compare its efficacy with commercial prebiotics and specific enzymes in wheat-based broiler diets.

Material and methods

Ethics statement

This experiment was conducted in strict accordance with the ethical guidelines approved by the Animal Science Committee at the Agricultural Sciences and Natural Resources University of Khuzestan, ensuring the humane treatment and welfare of all birds involved. All procedures involving the birds adhered to relevant local laws and regulations, maintaining the highest standards of care throughout the study

Birds and Housing

Two hundred forty 1-d-old Ross 308 strain broiler chickens (mixed sex) were used in a completely randomized design with six treatments and five replicates for 42 days. The experimental treatments consisted of: 1- PC (positive control, based on the corn-soybean meal), 2- NC (negative control, based on the wheat-soybean meal, the wheat cultivar was Chamran), 3- NCP; NC supplemented with 1g kg^{-1} prebiotic as on top (Safmannan®, yeast-derived manna-oligosaccharide), 4- NCE; NC supplemented with 0.5 g kg^{-1} enzyme as on top (Rovabio®, Adisseo, contains a blend of xylanases, cellulases, glucanases, and other fibrolytic enzymes), 5- 1.5NCDP; NC with 1.5 % date pit powder and 6- 3NCDP; NC with 3 % date pit powder. The analysis of date pit was according to the pervious study (Zaghari et al., 2010; Ghorbani et al., 2020). Isoenergetic and isonitrogenous diets (Table 1) were prepared to meet the nutrient requirements of broilers according to Ross 308 strain (2014). The rearing period was divided into starter (1-10 days), grower (11-24 days), and finisher (25-42 days) stages. The body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and European production efficiency factor (EPEF) were measured weekly and calculated.

To determine the humoral immunity of broilers, two chicks were randomly selected from each pen at 21 and 35 days of age. A 1 mL injection of 25% sheep red blood cells (SRBC) was administered into the breast muscle. Seven days after each injection, blood samples were collected from the brachial vein, and serum was assayed for antibody titer against SRBC using the hemagglutination (HA) test.

For determination of serum biochemical parameters in, one chick per pen was randomly selected on day 42. Blood samples were collected, and serum was obtained by centrifuging the tubes at 3,000 rpm (HK 36, Hermle, Wehingen, Germany) for 15 minutes. Blood triglyceride, total cholesterol, high-density lipoprotein (HDL-C), and blood sugar levels were measured calorimetrically using commercial kits (Pars Azmoon, Iran) in accordance with the manufacturer's protocols (Autoanalyzer, Alison 300, Abbott, USA). Low-density lipoprotein (LDL-C) was calculated by subtracting TC from HDL-C+TG/5.

For measuring the relative weight of the carcass and carcass components (based on live weight) at day 42, one chick from each replicate was randomly selected, weighed, and then slaughtered. During this step, digesta samples were collected from the ileum, and the pH of the samples was measured using a standard pH meter, as previously described (Izat et al., 1990).

For the determination of the cecal microbial population at the end of the study, one bird (these birds had not been injected with SRBC) per pen was randomly selected and euthanized aseptically and immediately each birds ceca were separated, tied and transported to the laboratory in a sterile Petri dish placed on ice.

One gram of the each cecal content was mixed with 9 mL of normal saline solution vigorously and then diluted serially to 10^{-6}. *Lactobacillus* sp., *Escherichia coli* (*E. coli*), and coliforms were grown on Rogosa SL agar, eosin methylene blue agar, and MacConkey agar, respectively. The *Lactobacillu*s medium agar plates incubated anaerobically at 37°C for 48 h. Coliforms and *E. coli* medium agar plates were incubated aerobically at 37°C for 48h. The plates were counted 48 h after inoculation, and the results were presented as log10-transformed data (Ghorbani et al., 2014).

Statistical analysis

Data were analyzed using the PROC GLM (SAS, 2002). Differences between treatment means were determined using the Duncan's multiple comparison test. Statistical significance was declared at P<0.05.

Table 1. The ingredients and chemical composition of basal diets for broiler chickens (%)

	Starter (1-10 days)				Grower (11-24 days)				Finisher (25-42 days)			
Item (%)	corn	wheat	1.5pit	3pit	corn	wheat	1.5 pit	3 pit	corn	wheat	1.5 pit	3 pit
Corn (8.5% CP)	54.00	0.00	0.00	0.00	59.20	0.00	0.00	0.00	64.20	0.00	0.00	0.00
Soybean meal(44% CP)	35.25	29.80	29.00	29.00	30.58	23.40	22.90	22.52	28.40	21.63	22.13	22.60
Gluten meal (62 %CP)	3.10	3.10	3.82	4.10	2.45	3.00	3.52	4.00	0.00	0.00	0.00	0.00
Wheat (11.5% CP)	0.00	58.94	57.11	54.78	0.00	66.06	64.10	61.98	0.00	71.15	68.47	65.90
Date pit	0.00	0.00	1.5	3.00	0.00	0.00	1.50	3.00	0.00	0.00	1.50	3.00
Sunflower oil	2.20	3.10	3.50	4.05	2.20	2.76	3.20	3.70	1.90	2.67	3.35	4.00
Dicalcium phosphate	1.80	1.75	1.75	1.75	1.60	1.60	1.58	1.60	1.48	1.45	1.48	1.45
L-lysine HCl	0.42	0.53	0.55	0.55	0.39	0.54	0.55	0.57	0.35	0.49	0.48	0.47
Dl-Methionine	0.35	0.40	0.39	0.39	0.32	0.36	0.36	0.36	0.35	0.41	0.41	0.42
L-Threonine	0.15	0.21	0.21	0.21	0.13	0.21	0.21	0.21	0.14	0.21	0.21	0.20
Salt	0.25	0.24	0.24	0.24	0.25	0.24	0.24	0.24	0.25	0.24	0.24	0.24
Oyster shell	1.20	1.23	1.23	1.23	1.08	1.13	1.14	1.12	1.00	1.05	1.03	1.03
NaHCO3	0.28	0.20	0.20	0.20	0.28	0.20	0.20	0.20	0.28	0.20	0.20	0.19
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Filler(grit)	0.50	0.00	0.00	0.00	1.02	0.00	0.00	0.00	1.15	0.00	0.00	0.00
Nutrient composition												
ME[3] (kcal kg^{-1})	2949.7	2949.7	2949.4	2948.2	2992.5	2993.1	2991.6	2992.3	2992.2	2992.1	2992.3	2992.4
Crude protein	22.84	22.84	22.84	22.84	20.76	20.76	20.75	20.76	18.68	18.68	18.68	18.68
Methionine and cysteine	1.08	1.08	1.08	1.08	0.99	0.99	0.99	0.99	0.94	0.94	0.94	0.94
Lysine	1.43	1.43	1.43	1.43	1.29	1.29	1.29	1.29	1.19	1.19	1.19	1.19
Threonine	0.97	0.97	0.97	0.97	0.88	0.88	0.88	0.88	0.81	0.81	0.81	0.81
Calcium	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.81	0.81	0.81	0.81
Available phosphorus	0.48	0.48	0.48	0.48	0.44	0.44	0.44	0.44	0.41	0.41	0.41	0.41
Crude fiber	3.70	3.54	3.89	4.27	3.47	3.26	3.62	3.99	3.40	3.22	3.63	4.04

[1,2]Vitamin and mineral premix supplied the following per kilogram of diet: retinyl acetate, 1.5 mg; cholecalciferol, 0.025 mg; α-tocopheryl acetate, 20 mg; menadione , 2 mg; thiamine, 3 mg; riboflavin , 6 mg; cyanocobalamin , 0.016 mg; niacin , 15 mg; folic acid, 1.75 mg; pantothenic acid, 15mg; choline chloride, 250 mg; Mn, 120 mg; Zn, 100 mg; Cu, 16 mg; Se, 0.3 mg; and I, 1.25 mg
[3]Metabolizable energy

Results

The effect of different treatments on FI, WG, FCR, and EPEF are shown in Table 2. During the starter period, FI, FCR, and EPEF were significantly affected by dietary treatments. The PC group exhibited the lowest FI and the best FCR and EPEF compared to the other groups (P<0.05). Among the birds that fed diets based on wheat, the 1.5NCDP group had the lowest FI and the best FCR and EPEF. During this period, there were no significant differences in EPEF between the PC and 1.5NCDP groups. During the grower, finisher, and overall experimental periods, FI, WG, FCR, and EPEF were not significantly affected by the dietary treatments (P>0.05). The effects of experimental treatments on the carcass characteristics of broiler chickens are shown in Table 3. Except for the relative weight of the liver, the relative weight of the carcass and its components were not affected by experimental treatments. Compared with PC, the use of wheat-based diets increased relative liver weights, with the highest value observed in the 1.5NCDP group. Blood biochemical parameters were not influenced by the experimental treatments (Table 4). On day 28, the birds in NCP group exhibited the highest antibody titer compared to the NC, 1.5NCDP and 3NCDP groups (Table 5). The secondary antibody titer was highest in the NCE group (6.25), with this group showing 33, 42, and 44%, higher titers compared to the PC, NC, and 3NCDP groups, respectively. No significant differences were observed between NCE, NCP, and 1.5NCDP groups in secondary antibody titer against SRBC. The relative weight of immune organs was not affected by dietary treatments. The pH of ileal contents increased in the NC group compared to the PC, NCE, and 1.5NCDP groups (Table 6). The experimental treatments did not affect the coliform and *lactobacillus* bacteria (Table 6). The PC groups had the highest population of *E. coli*, and the NCE and 3NCDP had the lowest one (P<0.05).

Table 2. Effect of experimental diets on performance of broiler chickens in different rearing periods

Treatment[1]	PC	NC	NCP	NCE	1.5NCDP	3NCDP	SEM[2]	P-value
Starter (1-10 d)								
FI[3] (g)	210.0^{c}	295.3^{a}	264.7^{a}	260.2^{a}	241.8^{b}	260.8^{a}	3.63	0.01
WG[4] (g)	197.5	208.5	208.8	211.5	203.8	201.5	1.90	0.27
FCR[5]	1.06^{c}	1.25^{ab}	1.27^{ab}	1.23^{ab}	1.19^{b}	1.29^{a}	0.01	0.01
EPEF[6]	185.9^{a}	168.0^{b}	160.5^{b}	167.4^{b}	172.0^{ab}	155.8^{b}	2.76	0.02
Grower (11-24 d)								
FI(g)	1086.6	1024.7	1030.5	1043.9	965.0	1033.2	12.91	0.16
WG(g)	691.4	650.2	668.3	656.1	643.6	681.4	9.50	0.70
FCR	1.57	1.58	1.56	1.59	1.50	1.53	0.19	0.75
EPEF	284.0	287.4	297.2	288.0	291.3	290.5	7.84	0.99
Finisher (25-42 d)								
FI(g)	2915.8	2613.0	2786.5	2676.3	2800.9	2595.1	41.75	0.18
WG(g)	1590.5	1627.7	1659.4	1721.2	1738.1	1654.0	21.57	0.36
FCR	1.84	1.61	1.69	1.55	1.62	1.57	0.03	0.07
EPEF	440.7	522.5	526.6	569.7	529.3	575.3	16.21	0.19
Overall period (1-42 d)								
FI(g)	4212.5	3897.0	4081.7	3980.4	4007.6	3889.1	46.45	0.34
WG(g)	2479.5	2486.5	2536.6	2588.9	2585.7	2537.0	21.75	0.60
FCR	1.70	1.57	1.61	1.53	1.55	1.53	0.02	0.07
EPEF	285.4	341.1	331.0	351.9	330.3	346.8	8.70	0.28

[1] PC =Positive control (based on corn-soybean meal); NC= Negative control (based on wheat-soybean meal); NCP= Negative control supplemented with prebiotic; NCE= Negative control supplemented with enzyme; 1.5NCDP= Negative control with 1.5% date pit powder; 3NCDP = Negative control with 3% date pit powder

[2] Standard error of the mean. ^{a,b}: Within the row, means with a common superscript(s) do not differ (P>0.05)

[3] FI: Feed intake (g)

[4] WG: Weight gain

[5] FCR: Feed conversion ratio (g of feed: g of gain)

[6] EPEF: European Production Efficiency Factor ([Viability (%) x BW (kg)] / [FCR (kg feed per kg gain) x age (day)] 100)

Table 3. Effect of experimental diets on live weight (g) and carcass characteristics (%) in broiler chickens

Treatment[1]	PC	NC	NCP	NCE	1.5NCDP	3NCDP	SEM[2]	P-value
Live weight	2704.8	2544.4	2761.6	2761.0	2902.6	2830.6	54.20	0.55
Carcass	64.7	65.2	64.9	64.1	63.0	65.0	0.36	0.56
Breast	26.0	25.3	24.7	25.1	24.9	24.4	0.24	0.55
Thigh	18.9	19.4	19.4	18.1	18.5	19.3	0.19	0.24
Liver	1.98^{c}	2.37^{b}	2.29^{b}	2.27^{b}	2.66^{a}	2.41^{ab}	0.04	0.01
Abdominal fat	1.16	0.89	0.74	1.05	0.99	1.08	0.06	0.53
Pancreas	0.20	0.22	0.19	0.21	0.22	0.19	0.01	0.58
Gizzard	2.00	2.40	2.24	1.92	2.59	2.01	0.08	0.08
Proventriculus	0.51	0.49	0.48	0.48	0.55	0.49	0.02	0.95

[1] PC =Positive control (based on corn-soybean meal); NC= Negative control (based on wheat-soybean meal); NCP= Negative control supplemented with prebiotic; NCE= Negative control supplemented with enzyme; 1.5NCDP= Negative control with 1.5% date pit powder; 3NCDP = Negative control with 3% date pit powder

[2] Standard error of the mean. ^{a,b}: Within the row, means with a common superscript(s) do not differ (P>0.05)

Table 4. Effect of experimental diets on blood biochemical parameters (mg dL^{-1}) in broiler chickens

Treatment[1]	PC	NC	NCP	NCE	1.5NCDP	3NCDP	SEM[2]	P-value
Glucose	178.8	203.6	193.8	200.8	193.4	197.4	4.11	0.61
Triglyceride	39.6	56.0	56.8	47.0	56.0	49.2	2.95	0.50
Cholesterol	92.8	101.6	108.8	89.8	110.6	110.4	3.15	0.20
HDL-C[3]	48.2	53.6	61.0	49.8	59.4	62.0	1.85	0.11
LDL-C[4]	35.8	42.2	37.3	33.6	40.2	38.2	1.50	0.64

[1] PC =Positive control (based on corn-soybean meal); NC= Negative control (based on wheat-soybean meal); NCP= Negative control supplemented with prebiotic; NCE= Negative control supplemented with enzyme; 1.5NCDP= Negative control with 1.5% date pit powder; 3NCDP = Negative control with 3% date pit powder

[2] Standard error of the mean

[3] LDL-C: low-density lipoproteins

[4] HDL-C: high-density lipoproteins

Discussions

The broiler performance indicators were affected by different experimental treatments only in the starter period. During this period, the PC treatment, which utilized a corn-based diet, resulted in the lowest FI, the best FCR, and the highest EPEF. All groups fed wheat-based diets exhibited higher FI. Among the birds fed wheat-based diets, only the group receiving 1.5% date pit showed a significant reduction in FI. Although, in the present experiment the diets were isonitrogenous and isoenergetic (Table 1), their digestibility were not identical. It has been demonstrated that the FI in birds is regulated by the dietary energy concentration (Lesson, 2012). In the present study, the PC group, due to the high digestibility and availability of corn carbohydrates (compared to wheat) for young chickens-whose digestive systems are not yet fully developed, obtained the required energy from a lower amount of feed. During this period, wheat-based diets increased FI and FCR while decreasing the EPEF compared to the positive control. The tested feed additives – except for date pits at the 1.5% inclusion level – showed no positive effects on performance parameters. This suggests that the 1.5% date pit supplementation may have improved intestinal conditions (Table 6), thereby enhancing nutrient digestion and absorption. Consequently, birds were able to meet their energy requirements from a reduced feed quantity. However, at the 3% inclusion level, the date pit did not positively affect the gastrointestinal pH, and birds on these diluted diets consumed more feed to compensate for their energy requirements. As the chickens grew up and their digestive systems developed, the adverse effects of wheat on performance parameters diminished. Consequently, no significant differences were observed between the treatments in the grower, finisher, and overall rearing periods.

Table 5. Effect of experimental diets on immunity responses in broiler chickens

Treatment[1]	Response to SRBC[2] (log2)		Lymphoid organs (% of live weight)	
	Primary (d 28)	Secondary(d 42)	Spleen	Bursa of Fabricius
PC	3.14[abc]	4.16[bc]	0.10	0.17
NC	2.12[c]	3.62[c]	0.10	0.16
NCP	3.71[a]	5.57[ab]	0.12	0.19
NCE	3.40[ab]	6.25[a]	0.10	0.19
1.5NCDP	2.28[bc]	4.85[abc]	0.11	0.17
3NCDP	2.00[c]	3.50[c]	0.10	0.15
SEM[3]	0.17	0.26	0.01	0.01
P-value	0.01	0.01	0.99	0.86

[1] PC =Positive control (based on corn-soybean meal); NC= Negative control (based on wheat-soybean meal); NCP= Negative control supplemented with prebiotic; NCE= Negative control supplemented with enzyme; 1.5NCDP= Negative control with 1.5% date pit powder; 3NCDP = Negative control with 3% date pit powder

[2] SRBC: Sheep red blood cell

[3] Standard error of the mean. [a,b]: Within the column, means with a common superscript(s) do not differ (P>0.05)

Table 6. Effect of experimental diets on cecal microbial population (log CFU[3] g^{-1}) and ileal digesta pH in broiler chickens

Treatment[1]	PC	NC	NCP	NCE	1.5NCDP	3NCDP	SEM[2]	P-value
Lactobacillus	6.93	7.21	7.25	7.24	7.66	7.00	0.08	0.24
Coliform	8.38	8.27	8.31	7.79	8.20	8.22	0.10	0.73
E. coli	8.82[a]	8.52[ab]	8.21[abc]	7.66[c]	8.29[ab]	8.03[bc]	0.10	0.02
Ileal pH	6.13[b]	7.22[a]	6.43[ab]	6.12[b]	6.04[b]	6.48[ab]	0.12	0.03

[1] PC =Positive control (based on corn-soybean meal); NC= Negative control (based on wheat-soybean meal); NCP= Negative control supplemented with prebiotic; NCE= Negative control supplemented with enzyme; 1.5NCDP= Negative control with 1.5% date pit powder; 3NCDP = Negative control with 3% date pit powder

[2] Standard error of the mean. [a,b]: Within the row, means with a common superscript(s) do not differ (P>0.05)

[3] CFU: colony forming units

It was assumed that using feed additives such as enzyme and prebiotic in wheat-based diets could mitigate the adverse effect of wheat's NSPs and make the performance of birds comparable to that of bird fed a corn-based diet (PC). However, except during the starter period, none of the performance parameters were affected by the experimental treatments. The results of the present study are consistent with those of Mohammadi et al. (2014) who demonstrated that during the starter period, chickens fed with wheat-based diets exhibited lower weight gain compared to those fed corn-based diets. They believed this adverse effect on body weight is attributed to the insufficient synthesis of endogenous enzymes, such as amylase and lipase, which are essential for the digestion of carbohydrates and fats, respectively. In the latter periods, chickens fed wheat-based diets could utilize the diet more effectively (Mohammadi et al., 2014).

The present study demonstrated that supplementing broiler diets with NSP-degrading enzyme did not have a significant effect on broiler BWG when comparing the NC with NCE. These results are consistent with those Seyedoshohadaei et al. (2024), who observed no significant impact of wheat cultivars (with or without enzymes) on broiler body weight or FCR over 0-39 days. Craig et al. (2019) demonstrated that supplementing NSP-degrading enzymes or prebiotic oligosaccharides did not significantly affect the growth performance of broiler chickens fed nutrient-adequate diets based on either wheat or barley. In this regard, it was reported that environmental condition (Choct et al., 1999) and wheat cultivar (Gutierrez del Alamo et al., 2008; Seyedoshohadaei et al., 2024) influence wheat arabinoxylan levels and consequently, its nutritive value. Mohammadi et al. (2014) suggested that the lack of significant differences in growth performance parameters between wheat- and corn-based diets could be attributed to the low NSP content of wheat or the appropriate proportion of nitrogen-free extract in wheat (768.8 g/kg dry matter).

In the present study, date pit powder was used as a source of prebiotic and compared with a commercial prebiotic. Linear mannans are the main sugar polymers of the NSP in palm kernel meal (Dusterhöft et al., 1992), and can also have prebiotic properties with beneficial effects (Yusrizal et al., 2013). In the starter phase, FI decreased significantly only with 1.5% inclusion date pit, while in the other periods performance indicators were not significantly affected by the fed additives. Similar to the results of the present study for the grower, finisher and total periods, Biggs et al. (2007) in 3 experiments showed that oligosaccharides (such as inulin, MOS, oligofructose (OF), trans-GOS and scFOS) at both low (4 g kg^{-1}) and high (8 g kg^{-1}) concentrations did not affect FI, BW and FCR in broiler chickens. Also, Waldrop et al. (2003) showed that adding 0.2% of MOS to the diet of broilers had no effect on WG and FCR. Mehrabadi and Jamshidi, (2019) reported that using prebiotics in diets containing 20% barley, improved FCR in overall period without affecting FI and BW of broiler chickens. The discrepancies in the obtained results is likely due to variations in the type of the basal diets, wheat variety, grain quality, age and breed of birds, as well as differing environmental conditions.

The increase in liver relative weight observed in wheat-based diets in the present study is consistent with findings from other studies. Nahirat et al. (2018) observed an increase liver relative weight following the addition of 3% date pit powders. Mohammadi et al. (2014) suggested that certain NSPs can bind to bile salts, lipids, and cholesterol, rendering them unavailable. This process may put pressure on the liver and increase its weight as it work to resynthesize bile acids from cholesterol, aiming to maintain the levels of these metabolites in the bloodstream.

Based on the presented results, the blood biochemical parameters were not influenced by the experimental treatments. The results of the present study are in line with the findings of Mohammadi et al. (2014) who did not observe any significant difference in blood biochemical parameters between the corn-based and wheat-based diets.

An increase in blood immunoglobulins plays vital role in enhancing the birds' immune response against of various infections. There was no difference in the primary antibody titer against SRBC between the PC and NC groups, which is in line with the results of Mohammadi et al. (2014). Among the wheat-based diet, the highest titer was observed in the diet containing enzymes and prebiotics (3.40 and 3.71, respectively). It has been shown that prebiotics improve the function of the immune system of broilers by different mechanisms, such as the increase of macrophages in the different parts of the intestine, the increase of gamma globulins, leukocytes, and lymphocytes in the intestinal mucus, as well as the increase of the number of heterophils and basophils (Ebrahimi et al., 2016). It was reported that during the hydrolysis of NSP by NSP-degrading enzymes, some oligosaccharides with prebiotic action may be released (Kim et al., 2020). These oligosaccharides, are selectively utilized by suitable gut bacteria, particularly *Bifidobacteria* and *Lactobacillus* (Svihus et al., 2013) with volatile fatty acids (VFA) as the end products (Kim et al., 2020). This process can enhance the birds' immunity system. Craig et al. (2019) demonstrated that supplementing the NSP-degrading enzymes or prebiotic oligosaccharides could modify the development of immune organs in broilers fed nutrient-adequate diets based on either wheat or barley.

While both enzyme supplementation and 1.5% date pit inclusion reduced the ileal pH in wheat-based diets, neither the 3% date pit nor the commercial prebiotic significantly affected the ileal pH. The date pits observation aligns with existing research demonstrating that the concentration of oligosaccharides influences their efficacy in the digestive tract. In this context, Biggs et al. (2007) reported that although oligosaccharide supplementation at 4 and 8 g kg^{-1} did not adversely affect the young chicks' growth performance, higher concentrations impaired the MEn and amino acid digestibility. It is known that fiber influences the diversity of species and the abundance of intestinal microflora by affecting the digestibility, viscosity, and fermentability of nutrients (Mahmood and Gio, 2020). Supplementing wheat-based broiler diets with exogenous enzymes reduced digesta viscosity and increased the cecal VFA production (Kim et al., 2020), consequently lowering the intestinal pH and protecting the host from infections (Wexler, 2007). This phenomenon demonstrates that the enzymes hydrolyzed the dietary NSP into more readily fermentable substrates for hindgut microbiota (Kim et al., 2020). Teng and Kim, (2018), in a review study, also reported that supplementation of MOS could alter the cecal microbial composition by increasing *lactobacillus* and *bifidobacterium*. These bacteria can efficiently ferment indigestible polysaccharides into short-chain fatty acids (SCFA) and consequently, reduce the intestinal pH. Alhomsi and Bayraktar, (2025) reported that the polysaccharides in dates exhibited prebiotic properties, supporting beneficial gut microbiota (including *Bifidobacterium* and *Lactobacillus*), which enhance intestinal health, improve barrier function, and increase the production of short-chain fatty acids.

In the present study, adding enzymes to wheat-based diets caused a 10% and 13% decrease in *E.coli* bacteria population compared to the NC and PC groups, respectively. In this regard, Hübener et al. (2002) showed that xylanase could modulate the intestinal bacterial population by reducing the intestinal viscosity and limiting the interference of bacteria with the absorption of nutrients. It has also been demonstrated that enzymes exert their positive effects by reducing the digesta viscosity through hydrolyzing certain chemical bonds, breaking down anti-nutritional factors, modulating the GIT microflora, and improving gut health (Kim et al., 2020).

Date pits have high amounts of MOSs, and in the 3NCDP group, the population of *E. coli* bacteria decreased compared to the positive control. Nahirat et al. (2018) had previously reported that adding date pit powder to the diet of broilers reduced pathogenic bacteria in the cecum. The MOSs and compounds containing mannose reduce the colonization rate in the intestine by binding to bacteria (Chacher et al., 2017).

Micciche et al. (2018), in a review study, reported that prebiotics can limit the colonization of pathogenic bacteria, such as *Salmonella*, through two mechanisms. These materials can change the microbial species and population by fermentation or, in the case of MOS, directly interfere with mannose-specific type 1 fimbriae attachment by pathogenic bacteria (Micciche et al., 2018). The MOSs bind to bacteria that have type-1 fimbriae, such as *E. coli* and *Salmonella* species (Spring et al., 2000), and can reduce the colonization of these intestinal pathogens by blocking bacterial lectins (Ebrahimi et al., 2016; Micciche et al., 2018). Teng and Kim (2018), in a review study, reported that supplementation of MOS could alter microbial composition by increasing total anaerobic bacteria, such as *Lactobacillus* and *Bifidobacterium*, while decreasing *Salmonella*, *E. coli*, and *Clostridium perfringens*, in the cecum. It was shown that the inclusion of fibrous feed, such as date pit, provided additional substrates for microbial composition in the gastrointestinal tract (Ricke, 2021). The microbial population was influenced by their fermentation through specific bacteria (Ricke, 2021). Ebrahimi et al. (2016) showed that adding prebiotics to the diet of broilers reduced the number of *Salmonella Typhimurium* bacteria in the ileum and cecum and increased the population of *lactobacillus* and *bifidobacterium* species in the cecum, which affects the bird's health.

Conclusions

Overall, the results of the present study indicated that: 1. Replacing corn with wheat in broiler diets did not negatively affect the performance during the rearing period. 2. Date pits, as an inexpensive byproduct of date processing industry, improved feed efficiency when included at 1.5% in starter diets. Furthermore, 3% inclusion throughout the entire rearing period enhanced both FCR and EPEF numerically. 3. Supplementation of wheat-based broiler diets with 1.5% date pit powder reduced the ileal digesta pH and enhanced gut health conditions. 4. It seems that date pit powder could be used as a cost-effective alternative to commercial prebiotics despite performance neutrality. 5. These findings suggested that while wheat substitution and date pit supplementation show promise, further research is needed.

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Conflict of interest

The authors declared no conflict of interest.

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