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IMPACT OF DIETARY SUPPLEMENTATION OF PREBIOTICS ON THE GROWTH PERFORMANCE AND IMMUNITY IN BROILERS FED LOW PROTEIN DIETS

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ABSTRACT

The current study was conducted to find out the influence of feeding broilers on low protein diets supplemented with prebiotic. Growth performance, carcass traits, muscle cholesterol, triglycerides, chemical composition, blood parameters and immune response of broilers were evaluated. A total number of 68 birds' one- day old broiler chicks (Ross 308) randomly distributed into 4 equal groups each of 17 chicks. The first group (control) was fed the basal (100%NRC CP) diet free from prebiotic. The other three groups (T2, T3 and T4) were fed on low protein diets (95, 90 & 85% of NRC requirements, respectively) and supplemented with prebiotic at level of 0.1%. The results showed that, birds in the second group had significantly higher live body weight, feed conversion, and dressing percentages. Supplementation of prebiotic had no significant effects on relative weight of liver, gizzard and immune organs. Reduction in the relative weight of abdominal fat pad in all prebiotic treated groups. There were significant reduction ($P<0.05$) in the meat cholesterol, triglycerides and fat mass of broiler breast and thigh, while protein content was significantly ($P<0.05$) increased in all prebiotic treated groups. A numerical increased in antibody titre in birds in the second and third groups. Birds in the second group had the best economic feed efficiency. It could be concluded that adding prebiotic to broiler diets low in protein (95, 90 and 85% of NRC) has a beneficial effect on growth parameters, carcass traits, economic value in addition to healthy and nutritious poultry products for consumers.

Key words: Prebiotic, Low protein diet, Growth Performance, Immunity, and Broiler.

INTRODUCTION

The issue that poultry scientists and industry face is to provide an acceptable amount of poultry meat in the most efficient manner possible (Yadav and Jha, 2019), which can be accomplished in part by using certain feed additives. Antibiotic have been utilized in chicken feed for a long time as a growth stimulant to help balance the gut microbial flora, enhance overall performance, and stop certain intestinal pathologies (Khan and Iqbal 2016). However, due to the emergence of antibiotic-resistant microorganisms the European Commission (EC) decided to restrict the advertising, commercialization, and usage of

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antibiotics as growth stimulants in diet as of January 1, 2006 (Gadde *et al.,* 2017). Prebiotics, probiotics, essential oils, and plant extracts are now being researched by the poultry industry in the hopes of keeping chicken intestines healthy and promoting immune system and performance (Wang *et al.,* 2016).

Prebiotics are well-defined as non-digestible food ingredient that have a favourable effect on the host by encouraging the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestines, thus having a beneficial impact in the host's microbial balance (Fallah *et al.,* 2013). In general, Prebiotics can be fermented in the intestines by health-promoting bacteria to produce lactic acid, short-chain fatty acids, or antibacterial compounds like bacteriocine against harmful bacteria (Bogusławska-Tryk *et al.,* 2012). Pathogen colonization could be reduced by oligosaccharides and monosaccharides inhibiting pathogen receptor sites on epithelial cell surfaces (Pourabedin and Zhao, 2015).

Mannan oligosaccharides (MOS), a prebiotic produced from yeast cell walls, is one of these alternatives (Chacher *et al.,* 2017). This indigestible sugar performs a wide range of tasks which includes selectively enhance the development and proliferation of native Bifidobacteria and Lactobacilli in the hindgut, suppressing the action of putrefactive or dangerous bacteria and resulting in reduced levels of harmful fermentation products in the GIT (Samanta *et al.,* 2013), increased villus height and decreased crypt depth (Yang *et al.,* 2009), modulated immune response (Khalaji *et al.,* 2011), and improved broiler’s growth performance in terms of weight gain and feed conversion (Žikic *et al.,* 2011, kamran *et al.,* 2013, Hussein *et al.,* 2020 and Rehman *et al.,* 2020).

Another commercial prebiotic active component, beta-glucan, has been shown to improve innate immunity and body growth in broilers (Chae *et al.,* 2006, Huff *et al.,* 2006 and Shendare *et al.,* 2008). The usage of β-glucans or MOS to improve broiler performance has been well documented and has been linked to increased innate immune function (Wang *et al.,* 2016).

Ingredients in feed, especially protein and energy sources, are the most expensive aspects of poultry nutrition (Wijtten *et al.,* 2004). Poultry require a lot of protein in their diet, and this nutrient has a significant impact on their performance. Due to excessive nitrogen excretion and the high expense of dietary protein sources, the use of low protein diets (LPD) in chicken nutrition has gained considerable interest in recent years (Ravangard *et al.,* 2017). So, the aim of this research is to see how prebiotic supplementation affect protein utilization of broilers fed low protein diets and to mitigate the detrimental effects of these diets.

MATERIALS AND METHODS

The current work was carried out at the Applied Nutrition Research Unit (ANRU), Teaching Veterinary Hospital, Faculty of Veterinary Medicine, Assuit University.

AGRIMOS Prebiotics is a feed ingredient produced from a high quality saccharomyces cervices yeast cell wall. AGRIMOS is a high source of Mannan-Oligosaccharides (MOS) and β-Glucans). LALLEMAND SAS Co. Provided the commercial product AGRIMOS®, which was supplied by Egavet Co., Egypt.

Birds, housing and feeding:
A total number of 68 birds one- day old unsexed broiler chicks (Ross 308) were obtained from local commercial source, weighed and randomly distributed to 4 equal groups each of 17 chicks. The initial average weight of the experimental chicks was (40.29±0.45g). Birds in all groups were housed in floor pens and kept under the same management system and environmental conditions. Birds were fed

according to two phases feeding program: starter diet (0 – 21 days) and grower-finisher diet (22–42 days). The first group was provided a prebiotic-free basal diet (100 percent NRC CP) as a control group. The other three groups were fed on low protein diets (95, 90 & 85% of NRC requirements) enriched with 0.1% prebiotic. Birds were fed ad-libitum on the respective diets (Table 1) and given free access to fresh water throughout the experimental period.

Feed ingredients analysis:
The dietary ingredients were chemically analyzed to determine dry matter (DM), ether extract (EE), crude protein (CP), and ash, while nitrogen free extract was computed using the Association of Official Analytical Chemists' procedures (AOAC, 2011). Metabolizable energy content of the feed ingredients and experimental diets were calculated based on chemical composition cited by **NRC (1994).**

Data Collection and Sampling:
Performance indicators:
Performance parameters including body weight, body weight gain and feed intake were recorded weekly.

Carcass Traits:
At the end of the experimental period, three birds were randomly taken from each group weighed and slaughtered to complete bleeding after a night fasting. The weight of dressed carcass (the weight of slaughtered birds after removal feathers, head, feet and viscera but including all the edible offal's) was recorded. The absolute weights of some internal organs including (liver, gizzard and heart), abdominal fat pad and immune organs (bursa, spleen and thymus) were recorded. Immune organs and abdominal fat pad weights were expressed as relative weight of live body weight.

Meat parameters:

1. **Muscle cholesterol and triglycerides:**
Using reagent kits that are commercially available (Wako pure chemical industries, Ltd., Tokyo, japan), total cholesterol and triglyceride values in breast and thigh meat samples were measured enzymatically as described previously (Bligh and Dyer .1959, Naeemi *et al.,* 1995 and Afrose *et al.,* 2009).

2. **Meat chemical composition:**
Meat samples from breast and thigh of the slaughtered birds in all the experimental groups were prepared (carefully minced and homogenized) and chemically analyzed for moisture, crude protein, ether extract and ash following AOAC (2011) official methods.

Blood collection and analysis:
At day 42, three birds were chosen at random from each group and blood samples were taken from the wing vein. The blood samples were centrifuged for 15 minutes at 4000 rpm and the sera were transferred into aseptically vials and saved at -20 °C until further analysis. As previously published, the log NDV serum antibody titer was measured using the Haemagglutination Inhibition (HI) assay. (OIE, 2013). Using a spectrophotometer and commercial test kits (Spectrum, Cairo, Egypt), total protein, albumin, globulin, triglycerides, and cholesterol levels were measured.

Economical evaluation:
Total feed cost, total production cost, body weight price, net income, and economic feed efficiency were computed at the end of the experiment, according to Hassan and El Shoukary (2019) and Omar *et al.* (2019).

Statistical analysis:
Using SPSS 20 statistical software (SPSS Inc., Chicago, IL, USA), all data have been analyzed using one-way analysis of variances (ANOVA) followed by Duncan's test, www.spss.com

Table 1: Composition and energy value of the experimental diets.

Ingredient (%)	Starter diets (0-21d)				Grower Finisher diets (22-42 d)			
	100% NRC	95% NRC	90% NRC	85% NRC	100% NRC	95% NRC	90% NRC	85% NRC
Yellow corn, ground	48.95	53.37	57.83	62.21	60.94	64.77	68.64	72.37
Soybean meal	39.85	36.02	32.18	28.40	30.31	26.98	23.66	20.40
Sunflower oil	7.25	6.50	5.74	5.00	5.20	4.57	3.89	3.30
Limestone, ground	1.71	1.75	1.75	1.76	1.68	1.70	1.70	1.72
Mono calcium phosphate	1.38	1.39	1.42	1.45	1.00	1.01	1.05	1.07
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.16	0.18	0.20	0.21	0.08	0.10	0.11	0.12
L-lysine	0.10	0.19	0.28	0.37	0.19	0.27	0.35	0.42
Total	100	100	100	100	100	100	100	100
Calculated chemical composition								
ME (Kcal/Kg)	3191.47	3191.51	3191.76	3192.10	3197.09	3198.85	3197.31	3200.05
Crude protein (%)	23.00	21.85	20.70	19.55	20.00	19.00	18.00	17.00
Calcium %	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Available phosphorus%	0.45	0.45	0.45	0,45	0.35	0.35	0.35	0.35
Lysine %	1.30	1.30	1,30	1,30	1.16	1.16	1.16	1.16
Methionine %	0.50	0.50	0.50	0.50	0.38	0.38	0.38	0.38
EE %	8.80	8.13	7.45	6.79	6.97	6.41	5.80	5.28
CF %	2.83	2.75	2.67	2.60	2.66	2.59	2.52	2.45

*Vitamines and minerals mixture (Multi Vita Co.): Each 3 kg contains: Vit. A, 12000000 IU; Vit. D3, 4000000 IU; Vit. E, 50000 mg; Vit. k3, 4000 mg; Vit. B1, 5000 mg; Vit.B2, 8000 mg; Vit. B6, 5000 mg; Vit. B12, 35 mg; Vit. C, 450 mg; Niacin, 70000 mg; Methionine, 3000 mg; Pantothenic acid , 20000 mg ; Folic acid 1000 mg; Biotin, 250 mg; Magnésium, 100000 mg; Copper, 15000 mg; Iron, 50000 mg; Zinc, 50000 mg; Cobalt, 250 mg ; iodine 1500 mg and Selenium 250 mg.

RESULT

Growth performance:

Inclusion of prebiotic to low protein broilers diet (95% of NRC) (T2) increased the body weight and body weight gain significantly (P<0.05) compared with control (T1). While the addition of prebiotic to low protein broilers diets (90 and 85% of NRC) had no significant (P>0.05) effect on body weight and body weight gain in comparison with the control (Tables 2 and 3).

Concerning cumulative feed intake during starter period (0-3 w), feed consumption of chicks fed diets containing (95, 90 and 85% protein of NRC (T2, T3 and T4) was nearly the same as showed in table (4). While the feed intake numerically decreased among all prebiotic treated groups during growing period (4-6w). In comparison with control group, adding prebiotics decreased the feed conversion ratio by 9.66 % in T2 and 3.41% in T3. Conversely, inclusion of prebiotic to low protein diet containing 85% of NRC (T4) slightly increased the feed conversion ratio by 1.14% compared with the control one as presented in Table (4).

Carcass traits:

Table (5) summarizes the dressing percentages and relative organ weights for the various experimental groups. Inclusion of prebiotic to diet have 95% and 90% of

NRC protein improved hot carcass%, eviscerated carcass % and dressing percent. However, adding of prebiotic to 85% NRC protein diet (T4) not significantly (P>0.05) affected the measured parameters. Prebiotic addition had no significant effect on relative weight of liver and gizzard. There were no significant effect on the relative weight of heart in broilers in the second and third groups, while the broiler feed 85% NRC protein showed an increased in the relative weight of heart. Reduction in the relative weight of abdominal fat pad in all treated groups compared with the control was observed. Concerning the effect of prebiotic on the relative weights of immune organs of broilers fed different protein levels, there were no significant (P>0.05) effect on spleen, thymus and bursa relative weights were observed.

Meat parameters:

Results in table (6) revealed the addition of prebiotic to 95, 90 and 85% protein of NRC requirements diets decreased significantly (P<0.05) the cholesterol and triglyceride contents of broiler breast and thigh meat compared with the control (T1).The cholesterol content decreased by 6.24%, 11.58% and 15.82% of breast meat and by 11.35%, 12.71% and 17.79% of thigh meat of broiler fed 95%, 90% and 85% NRC protein diets, respectively than the control. Triglycerides content was decreased by 13.39%, 19.95% and 25.55% of breast meat and by15.71%, 18.47% and 23.15% of thigh meat of broiler fed 95%, 90% and 85% NRC protein diet, respectively than the control.

Data in Table (7) indicated that supplementation of prebiotic to 95%, 90% and 85% NRC protein diets showed no significant (P>0.05) differences in dry matter and ash contents of broiler breast and thigh meat among all the treatment groups including the control one. However, the fat content was significantly (P<0.05) decreased, while protein content of broiler breast and thigh meat significantly (P<0.05) increased. The fat content was significantly decreased by 20.4%, 28.57% and 34.69% of breast meat and by 16.42%, 19.40% and 26.86% of thigh meat for broiler fed 95, 90 and 85% NRC protein , respectively than control one.

Blood parameters:

Supplementation of prebiotic showed no significant differences in the level of serum total protein, albumin, globulin and A/G ratio among all prebiotic treated groups and control one. However, serum cholesterol content and triglyceride numerically decreased in all treated groups.

The obtained data in Table (9) showed no significant differences in the antibody titre of Newcastle between different experimental groups at day 42 of age.

Economic analysis:

Regarding to economical evaluation, addition of prebiotic to diets containing 95, 90 and 85% of NRC protein requirements lowered the feed and total production costs in comparison with control as shown in table (10).

Broilers fed a 95 % of NRC protein diet had the highest average economic efficiency value of 86.01 %, followed by birds fed a 90 % NRC supplemented diet (76.41 %) and 85 % NRC supplemented diet (74.22). According to the income-outcome analysis, the birds fed prebiotic with 95 % NRC protein diet had the highest relative economic feed efficiency, followed by birds fed on prebiotic with 90% and 85% NRC protein diet, respectively.

Table 2: Weekly body weight development (g) of broilers fed different experimental diets.

Groups / Period (week)	Control group	Prebiotic groups		
	T1	**T2**	**T3**	**T4**
Initial	39.28±1.05	40.43±0.86	40.61±0.94	40.89±0.77
1	137.10±2.67	141.03±3.14	139.62±2.20	135.32±4.16
2	367.47± 7.26 ^{b}	402.15±10.81 ^{a}	357.69±8.94 ^{b}	369.13±13.01 ^{b}
3	755.60±14.62 ^{b}	852.69±24.59 ^{a}	734.46±20.54 ^{b}	735.53±25.31 ^{b}
4	1225.40±25.64 ^{b}	1376.69±45.27 ^{a}	1151.85±35.11 ^{b}	1190.93±41.44 ^{b}
5	1805.00±30.52 ^{b}	2052.69±66.78 ^{a}	1789.31±52.22 ^{b}	1790.87±67.93 ^{b}
6	2411.67±48.39 ^{ab}	2603.85±84.74 ^{a}	2374.62±76.89 ^{b}	2350.05±88.09 ^{b}

Means within the same row with different superscripts are significantly different ($P < 0.05$).
T1 (100% NRC CP, no additives), **T2** (95% NRC CP + prebiotic), **T3** (90% NRC CP + prebiotic) and **T4** (85% NRC CP + prebiotic).

Table 3: Weekly and cumulative weight gain (g) of broilers during different experimental periods

Groups / Exp. Period (week)	Control group	Prebiotic groups		
	T1	**T2**	**T3**	**T4**
0-1	97.82±1.67	100.6±2.32	99.01±1.29	94.43±3.46
1-2	230.37±4.65 ^{b}	261.12±7.82 ^{a}	218.07±6.90 ^{b}	233.81±9.02 ^{b}
2-3	388.13±8.35 ^{b}	450.54±14.13 ^{a}	376.77±11.97 ^{b}	366.40±13.72 ^{b}
3-4	469.80±13.00 ^{b}	524.01±21.44 ^{a}	417.39±15.67 ^{cd}	455.40±17.14 ^{bc}
4-5	579.60±11.78 ^{b}	676.00±26.82 ^{a}	637.46±20.20 ^{ab}	599.94±30.86 ^{b}
5-6	606.67±20.58	551.16±28.28	585.31±27.93	559.18±27.01
0-3	716.32±13.72 ^{b}	812.27±23.76 ^{a}	693.85±19.63 ^{b}	694.65±24.57 ^{b}
4-6	1656.07±36.60	1751.15±62.18	1640.16±56.98	1614.52±63.69
0-6	2372.39±47.39 ^{ab}	2563.42±83.98 ^{a}	2334.01±75.97 ^{b}	2309.17±87.38 ^{b}

Means within the same row with different superscripts are significantly different ($P < 0.05$).

Table 4: Feed consumption (g / chick) and feed conversion indices of broilers during different experimental period intervals.

Group / week	Control group	Prebiotic groups		
	T1	**T2**	**T3**	**T4**
Feed consumption (g / chick)				
(0-3)	1044.17	1077.23	1010.78	1003.49
(4-6)	3121.37	2989.84	2955.49	3117.90
(0-6)	4165.54	4067.06	3966.27	4121.39
Feed conversion				
(0-3)	1.46	1.33	1.46	1.44
(4-6)	1.88	1.71	1.80	1.93
(0-6)	1.76	1.59	1.70	1.78

Table 5: Carcass traits, abdominal fat pad and relative weight of immune organs of broilers fed different experimental diets

Item \ group	Control group	Prebiotic groups		
	T1	T2	T3	T4
Pre-slaughter weight, g	2418.33± 20.28[ab]	2545.00±25.66[a]	2361.67±66.73[b]	2311.67±48.68[b]
Hot carcass, %	86.70± 0.29[ab]	87.43±0.34[a]	87.57±0.10[a]	85.46±0.85[b]
Eviscerated carcass, %	70.59±1.31[b]	74.32±1.22[a]	71.42±0.14[ab]	70.18±1.18[b]
Dressed carcass, %	74.58±1.32[ab]	77.97±1.21[a]	75.65±0.24[ab]	74.23±1.16[b]
Liver, %	2.14±0.07	1.95±0.05	2.23±0.13	2.25±0.16
Heart, %	0.49±0.04[b]	0.45±0.01[b]	0.51±0.01[b]	0.60±0.02[a]
Gizzard, %	1.38±0.04[ab]	1.31±0.04[ab]	1.49±0.09[a]	1.23±0.05[b]
Abdominal Fat, %	1.52±0.03[a]	1.40±0.16[a]	1.01±0.08[b]	1.23±0.10[ab]
Spleen, %	0.10±0.02	0.10±0.01	0.12±0.00	0.10±0.01
Thymus, %	0.42±0.02	0.45±0.03	0.41±0.04	0.42±0.06
Bursa, %	0.09±0.02	0.11±0.02	0.09±0.01	0.09±0.01

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 6: Meat cholesterol and triglycerides content (mg/100g) of broilers fed different experimental diets.

Items \ Groups	Control group	Prebiotic groups		
	T1	T2	T3	T4
The breast meat				
Cholesterol	79.98±0.29[a]	74.99±0.30[b]	70.72±0.29[c]	67.33±0.20[d]
Triglyceride	95.60±0.20[a]	82.80±0.24[b]	76.53±0.25[c]	71.17±0.11[d]
The thigh meat				
Cholesterol	108.19±0.20[a]	95.91±0.11[b]	94.44±0.18[c]	88.94±0.47[d]
Triglyceride	112.26±0.17[a]	94.62±0.21[b]	91.52±0.23[c]	86.27±0.19[d]

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 7: Meat composition (%) of broilers in the different experimental groups.

Items \ Groups	Control group	Prebiotic groups		
	T1	T2	T3	T4
Chemical composition (%) of the breast meat:				
DM	26.19±1.21	28.43±1.00	27.81±1.30	26.72±0.58
CP (%DM)	76.00±1.73[b]	81.80±2.00[a]	84.50±2.07[a]	86.00±3.18[a]
EE (%DM)	4.90±0.17[a]	3.90±0.12[b]	3.50±0.12[bc]	3.20±0.12[c]
Ash (%DM)	3.27±0.57	4.58±0.38	3.90±0.23	3.63±0.37
Chemical composition (%) of the thigh meat:				
DM	26.34±1.15	26.38±1.5	27.97±1.15	29.47±2.6
CP (%DM)	74.90±1.73[b]	80.20±2.3[a]	82.60±2.05[a]	84.70±1.73[a]
EE (%DM)	6.70±0.06[a]	5.60±0.12[b]	5.40±0.12[b]	4.90±0.17[c]
Ash (%DM)	4.15±0.58	4.10±0.53	4.27±0.58	4.98±0.29

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 8: Blood biochemical parameters of broilers in the different experimental groups.

Item \ Groups	Control group	Prebiotic groups		
	T1	T2	T3	T4
Total protein (g/dl)	2.47±0.33	2.20±0.00	2.43±0.12	2.40±0.20
Albumin (g/dl)	1.13±0.07	1.17±0.00	1.17±0.09	1.10±0.06
Globulin (g/dl)	1.33±0.33	1.10±0.00	1.27±0.07	1.30±0.15
A/G ratio	0.83±0.07	1.00±0.00	0.93±0.09	0.83±0.09
Cholesterol (mg/dl)	89.25±0.55	83.90±7.33	81.50±0.86	80.80±3.12
Triglycerides (mg/dl)	77.11±6.46[a]	64.46±3.42[ab]	67.06±5.64[ab]	60.83±0.50[b]

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 9: Humeral antibody titers post Newcastle vaccination of broiler in the different experimental groups

day \ Groups	Control group	Prebiotic groups		
	T1	T2	T3	T4
42day	5.00±0.00	6.00±1.15	6.00±0.00	4.67±0.33

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 10: Economical evaluation of the experimental diets.

Items \ Groups	Control group	Prebiotic groups		
	T1	T2	T3	T4
Total feed cost (L.E)*	28.20	27.50	25.88	25.97
Total production cost (L.E)	42.70	42.00	40.38	40.47
Body weight (Kg/bird)	2.41	2.60	2.37	2.35
Total revenue	72.35	78.12	71.24	70.50
Net revenue	29.65	36.12	30.86	30.03
Economic feed efficiency (%)	69.43	86.01	76.41	74.22
Relative economic feed efficiency	100	123.87	110.05	106.89

* LE= Egyptian pound according to price at the experimental time.

DISCUSSION

Growth performance:

Results of growth performance parameters supported by the findings of kamran *et al.* (2013) and Rehman *et al.* (2020) who found that, supplementation of diets with MOS increased broiler live body weight and gain. In addition, Chae *et al.* (2006) and Huff *et al.* (2006) recorded that, the significant improvement in body weight and weight gain of broilers fed on diets supplemented with B-glucan may be due to the enhancement in innate immune response (Bozkurt *et al*. 2012). Similar result found by Hooge *et al.* (2013) and Abdel-Hafeez *et al.* (2017) who found a significant improvement in body weight and gain of broilers fed on prebiotic supplemented diets. On the other hand, Midilli *et al.* (2008), Corrigan *et al.* (2011) and Salehimanesh *et al.* (2016) suggested that, the dietary supplementation of MOS and B-glucan did not affect body weight and gain of broiler. The addition of MOS & B-glucan had no significant effect on duck body weight and gain (Mahmoud *et al.*, 2020). The inclusion of prebiotic improved the low level protein diet which gave the same effect of 100% NRC protein diet (control) on body weight and body weight gain. The positive effect of dietary inclusion of prebiotic might be attributed to the health of gut lining, which may be due to short chain fatty acids production, which provide the energy for intestinal epithelial cells (Ferket *et al.,* 2005) and increased the partition of nutrients into other tissues of body (Ajuwon, 2016), enhance enzyme activity or provide a larger surface area for efficient nutrients absorption (Yang *et al.*, 2009).

Regarding to feed intake, results were in line with the findings of Salianeh *et al.* (2011) who demonstrated decreased feed intake in broiler chickens as a result of dietary inclusion of prebiotic. In addition, Salehimanesh *et al.* (2016) demonstrated that, prebiotic at 0.9 g/kg numerically decreased the feed intake of broiler chicks. Broilers fed the prebiotic were more effective at converting feed to body mass during the raising period. In general, improvements in feed conversion were attributed to stimulated growth of the beneficial microflora in the GIT induced by dietary supplementation of prebiotic as demonstrated by Cinar *et al.* (2009). These results agreed with the finding of Salianeh *et al.* (2011) who observed the addition of prebiotic decreased feed conversion ration significantly. In agreement with our results, a series of scientific reports (Žikic *et al.*, 2011; Hooge *et al.*, 2013 and Hussein *et al.*, 2020) demonstrated that addition of prebiotics resulted in either a numerical or significant improvement in the feed efficiency. On the other hand, Baurhoo *et al.* (2009) and Salehimanesh *et al.* (2016) stated that, addition of prebiotic did not affect significantly the feed conversion ratio. Regarding the effect of crude protein levels (90% and 85% NRC CP diet), deleterious effects of low protein diet on FCR have been showed in some previous studies (Zeng *et al.*, 2015; Ravangard *et al.*, 2017 and Xie *et al.*, 2017). In addition, Ospina-Rojas *et al.* (2014) demonstrated that, birds fed on low protein diets recorded significantly worse feed conversion than those fed on control diets during starter and grower periods. Such effect on feed conversion ratio was not observed in the present study, this means that the addition of prebiotic improved the adverse effect of low protein diet.

Carcass traits:

The current data matched with the findings recorded by Park and Park (2011) and Sojoudi *et al.* (2012) who found significantly increased in dressing percentage and empty carcass percent. Also Abdel-Fattah and Fararh, (2009) demonstrated a slight improvement in dressing percentage in bird fed diets supplemented with MOS. While other studies Salehimanesh *et al.* (2016); Abdel-Hafeez *et al.* (2017) and Rehman *et al.* (2020)) reported that, prebiotic seemed to have no significant impact on carcass parameters of broilers. As well as, non-significant effect in carcass characteristics due to the use of different protein levels have been reported in some previous studies (Gheisari *et al.* (2015); Xie *et al.* (2017) and Shao *et al.* (2018)). Similarly, Abdel-Raheem and Abd-Allah (2011) and Odefemi (2016) recorded that adding prebiotic to broiler had no significant effect on gizzard and liver relative weight. However, Sojoudi *et al.* (2012) and Abdel-Hafeez *et al.* (2017) reported that, the relative weight of liver and gizzard significantly increased by feeding prebiotic. There were no significant effect in the relative weight of heart in broilers fed 95% and 90%NRC, while the broiler feed 85% NRC protein showed an increased in the relative weight of heart. Awad *et al.* (2014) explained the greater relative heart weight of broilers fed low protein diet by their worse body weight compared to other groups.

Results of immune organ weights came in accordance with those reported by Cox *et al.* (2010) who observed that, supplementation of β-glucan did not significantly affect relative immune organ weights. Also Sojoudi *et al.* (2012) demonstrated that broiler fed prebiotic (0.1%, 0.15%, 0.2%) showed no significant difference between treatments in thymus weight, thymus percent, bursa fabricius weight and bursa fabricius percent ($P>0.05$), but there was a significant difference in spleen weight and spleen percent. Li *et al.* (2016) found that, yeast cell wall feeding had no effect on the relative weights of the thymus or the spleen (P > 0.05). In contrast, Abdel-Raheem and Abd-Allah (2011) demonstrated that, there was a significant increase in the bursa and thymus absolute weights due to MOS supplementation of broilers.

The numerical decrease in the relative weight of abdominal fat pad is extremely fascinating and good for the customers. The positive effect of prebiotic has been showed to reduce the abdominal fat level as reported by Abdel-Hafeez *et al.* (*2*017) and Ilham *et al.* (2019). In terms of the impact of various crude protein levels on abdominal fat, the results showed that abdominal fat was significantly increased in low level crude protein diet (Zeng *et al.*, 2015, Dehghani-Tafti and Jahanian, 2016 and Xie *et al.*, 2017). Feeding with low protein diet stimulated lipogenesis in the birds' livers, causing in increased liver weight and, as a result, increased fat deposition in the abdomen (Swennen *et al.*, 2006). However, in the current investigation, no such effect was reported, this means that, the addition of prebiotic improved the adverse effect of low protein diet.

Meat parameters:

There were a significant reduction (P<0.05) in the meat cholesterol, triglyceride and fat mass content of broiler breast and thigh meat of the prebiotics treated groups. Similar results were reported by Sutama *et al.* (2010), Ilham *et al.* (2019) and Okrathok and Khempaka (2020) who found that probiotic and prebiotic feed additives are able to decline the fat and cholesterol levels in the animals. The decreased in meat fat mass was consistent with some previous reports (Ooi and Liong (2010) and Weitkunat *et al.* (2015)).

Ilham *et al.* (2019) suggested that the decreasing of meat fat and cholesterol level in prebiotic supplemented birds may be related to the absorption process of bile acids and cholesterol from digestive tracts which depending on the food crude fiber content. Crude fiber of prebiotic binding with bile acids resulted in hindering the absorption of fat. Therefore, feces excretion with bile elements such as cholesterol was increased, and hence reducing abdomen fat (Akhadiarto, 2010). Wiryawan *et al.* (2005) stated that lactic acids bacteria produce organic acids which hinder the absorption of the bile acids and enhance their excretion from the birds body. When the level of the bile acids reduced, the body will depend on the cholesterol which came from bloods and tissues system in the creations of the bile acids. This cycle will lower the levels of cholesterol in the blood and meat.

An increase in CP content of broiler muscle may be attributed to low protein diet rather than prebiotic feeding. Gheisari *et al.* (2015) found the percentage of protein in female carcasses was significantly (P<0.05) lower in chicks fed on higher protein diets than other groups. Hai and Bláha (2000) and Kamran *et al.* (2008) also recorded lowering of dietary CP level had no adverse effect on carcass proteins. In contrast, Marcu *et al.* (2009) found that broiler fed high protein and energy levels, resulted in higher water and protein content and fat content was lower.

Blood parameters:

Our result confirming the earlier findings of Li *et al.* (2016), Abdel-Hafeez *et al.* (2017) and Mahmoud *et al.* (2017) who found that serum total protein, albumin and globulin of broilers did not significantly affected by prebiotic supplementation. Similar findings were reported by Mahmoud *et al.* (2020) who found the addition of prebiotic had no prominent effect on total protein, albumin and globulin. In addition, other studies (Taherpour *et al.*, 2009, Abdel-Raheem and Abd-Allah, 2011 and Okrathok and Khempaka, 2020) demonstrated low serum cholesterol level as a result of prebiotic feeding. On the other hand, Abdel-Hafeez *et al.* (2017) and Hazrati *et al.* (2020) reported no significant (P<0.05) effect on serum cholesterol level. In agreement with our result, Yalçinkaya *et al.* (2008), Jahanian and Ashnagar (2015) and Hazrati *et al.* (2020) recorded that feeding prebiotic decreased serum triglycerides. In contrast, Kamran *et al.* (2010); Zeng *et al.* (2015) and Dehghani-Tafti and Jahanian (2016) found an increased in serum triglycerides level due to low protein diet. Birds fed a low-protein diet utilized carbohydrates as a source of energy rather than free fatty acids, resulting in an increase in plasma triglyceride content. Swennen *et al.* (2005) and Kamran *et al.* (2010).

The inclusion of prebiotic improved the post vaccination NDV antibody titers especially during the weeks when titers were decreasing. These findings are consistent with previous researchs (Zakeri and Kashefi, 2011, Mehdi and Hasan, 2012 and Salehimanesh *et al.*, 2016) who observed an elevation in antibody titers against NDV as a result of MOS supplementation. Gao *et al.* (2008) found that YCW has a positive effect on the humeral immunity of broiler chicks, which is consistent with this study. Much of the mechanism underlying the immunomodulation caused by the MOS is yet unknown. One theory is that, the presence of microorganisms is recognized by immune cells in the gut-associated lymphoid tissue (GALT) by detecting molecules that are specific to pathogens named Pathogen Associated Molecular Patterns (PAMP). The yeast cell walls (MOS and B-glucan) considered as Pathogen Associated Molecular Patterns (Ballou, 1970), they bind to pattern-recognition receptors on a variety of GALT defense cells, triggering immunological responses (Mehdi and Hasan 2012). Huang *et al.* (2007) found that, inclusion of oligochitosan in the diet increased immunological response, as indicated by increased serum post vaccination NDV antibody titers, they came to the conclusion that prebiotics could improve bird immunity through a various mechanisms, including prevent pathogenic microbes from colonizing so these pathogens are still allowed to be given to immune cells as attenuated antigens, the active group(s) had a direct promoting effects on the immune system, in addition, they compete with pathogenic organisms for nutrient. In contrast, Houshmand *et al.* (2012) noticed no significant differences ($P<0.05$) with dietary addition of the prebiotic.

Regarding the effect of low protein diet on antibody titre against ND virus, Zeng *et al.* (2015) reported that, broilers fed a feed that included 16.81% or lesser CP had significantly lower ND antibody titer ($P<0.05$). Therefore, the minimum dietary CP requirement of broilers from 22 to 42 days of age was suggested as 17.63%. In contrast Rao *et al.* (2011) demonstrated that, antibody titre against ND virus were not affected ($P<0.05$) by reduction in CP.

CONCLUSION

The addition of 0.1% prebiotic to broilers fed low protein diets (95, 90 and 85% of NRC protein requirements) has a beneficial effect on growth parameters, carcass traits and economic value without adverse effect on broiler immunity. It is both fascinating and healthy to the consumer, addition of prebiotic decreased ($P<0.05$) cholesterol, triglyceride and fat content of broiler meat (breast and thigh), while protein content of broiler breast and thigh meat significantly ($P<0.05$) increased.

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تأثير البريبيوتيك علي الاداء والاستجابة المناعية لبداري التسمين المغذاه علي علائق منخفضة في البروتين

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اجريت هذه الدراسة لمعرفة تأثيراضافة البريبيوتيك (أجريموز) على أداء النمو ، صفات الذبيحة ، كوليسترول العضلات ، الدهون الثلاثية والتركيب الكيميائي للعضلات ، الخواص الكيميائيه للدم والاستجابة المناعية لبداري التسمين المغذاه على نسب منخفضه من البروتين. تم استخدام عدد ٦٨ كتكوت من بداري التسمين عمر يوم واحد (روس ٣٠٨) من مصدر تجارى محلى ، تم وزنها وتوزيعها عشوائيا على ٤ مجموعات متساوية كل منها ١٧ كتكوت. تم تغذية الطيور على مرحلتين من برنامج التغذية: البادئ (٠-٢١ يومًا من العمر) والنامي- الناهى (٢٢-٤٢ يومًا من العمر) واعتبرت المجموعة الاولى مجموعة ضابطة حيث غذيت على عليقة ضابطة (٣٢٠٠ كيلو كالورى طاقة ممثلة/كجم عليقه والبروتين ١٠٠٪ من الاحتياجات الموصى بها فى الجداول القياسية من المجلس القومى للبحوث (١٩٩٤)) وباقى المجموعات (المجموعة الثانية والثالثة والرابعة) غذيت فيها الطيور على مستوى منخفض من البروتين (٩٥ ، ٩٠ ، ٨٥٪ من الاحتياجات الموصى بها من البروتين فى الجداول القياسية من المجلس القومى للبحوث (NRC,١٩٩٤) مضافا اليها البريبيوتيك بنسبة ٠,١٪. أظهرت النتائج أن الطيور التي تم تغذيتها على ٩٥٪ بروتين من الاحتياجات الموصى بها فى الجداول القياسية من المجلس القومى للبحوث (١٩٩٤) لبداري التسمين مدعم بالبريبايوتيك (T2) قد سجلت أفضل وزن حي للجسم ، زيادة في وزن الجسم ، معدل التحويل الغذائي ، نسبة الذبائح المحتوية على الاحشاء ، والذبائح منزوعة الاحشاء وكذلك نسبة التصافى مقارنة مع المعاملات الأخرى. لم يلاحظ اى فرق معنوية ذات دلالات احصائية بين المجموعات المختبرة والمجموعة الضابطة فى الوزن النسبى للاحشاء الداخلية (الكبد والقونصة) والوزن النسبي للطحال وغدة الفبريشيس والغدة التيموسية مقارنة بالمجموعة الضابطة بينما اظهرت المجموعه الرابعة المغذه على ٨٥٪ بروتين من الاحتياجات الموصى بها فى الجداول القياسية من المجلس القومى للبحوث (١٩٩٤) لبداري التسمين مدعم بالبريبايوتيك زيادة في الوزن النسبي للقلب. اظهرت النتائج انخفاض في محتوى الدهون في منطقة البطن وانخفاض معنوي في محتوى الكوليسترول والجليسريدات الثلاثية في لحم الذبيحه (الصدر والفخذ) المعالجة بالبريبيوتيك مقارنة مع المجموعة الضابطة. لا توجد فروق ذات دلالة إحصائية فى كل من المادة الجافة والرماد فى لحوم كل من الصدروالفخذ بين جميع المجموعات المعالجة بما في ذلك المجموعة الضابطة. ولكن لوحظ انخفض معنويا فى نسبة الدهون وارتفاع نسبة البروتين فى كل المجموعات المعالجه بالبربيوتيك مقارنة بالمجموعة الضابطة فى لحوم كل من الصدروالفخذ. لا يوجد فروق معنوية فى نسبة كل من البروتين الكلى، الالبيومين ، الجلوبيولين ، معدل الالبيومين الى الجلوبيولين والكوليستيرول فى مصل دم الطيور المغذاه على البريبيوتيك والمجموعة الضابطة. فى حين لوحظ انخفاض فى الجليسريدات الثلاثية بنسبه (١٦,٤١%، ١٣,٠٣% و ٢١,١١%) فى مصل دم طيورالمجموعات المغذاه على البريبيوتيك (الثانيه والثالثة والرابعة على التوالى). في اليوم ٤٢ من العمر ، وجدت زيادة عددية في عيار الأجسام المضادة بعد التطعيم ضد مرض النيوكاسل في المجموعه الثانيه والثالثه ولكن ليست هناك فروق معنويّه بين باقى المجموعات والمجموعة الضابطة. كانت الجدوى الاقتصادية النسبية افضل ما يمكن فى المجموعه الثانيه المغذاة على علائق تحتوى ٩٥ ٪ بروتين من الاحتياجات الموصى بها فى الجداول القياسية من المجلس القومى للبحوث (١٩٩٤) مضاف اليها البريبايوتيك. أن إضافة ٠,١٪ أجريموز بريبيوتيك إلى علائق بداري التسمين المنخفضه فى البروتين (٩٥ ، ٩٠ ، ٨٥٪ من احتياجات NRC) له اثر ايجابى على معايير النمو وصفات الذبيحة والقيمة الاقتصادية وتوفير لحوم ومنتجات صحية وذات قيمه غذائيه للمستهلكين.