

# Effect of Pelleting Temperature, Probiotic and Wheat Grain on Growth Performance, Blood Biochemical Variables, Immune Responses and Mucin 2 Gene Expression of Broiler Chicks

Research Article

V. Ighani<sup>1</sup>, A.A. Sadeghi<sup>1\*</sup>, S.N. Mousavi<sup>2</sup>, P. Jafari<sup>3</sup> and M. Chamani<sup>1</sup>

<sup>1</sup>Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

<sup>2</sup>Department of Animal Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

<sup>3</sup>Department of Microbiology, Arak Branch, Islamic Azad University, Arak, Iran

Received on: 30 Nov 2018

Revised on: 14 Feb 2019

Accepted on: 16 Mar 2019

Online Published on: Sep 2020

\*Correspondence E-mail: [a.sadeghi@srbiau.ac.ir](mailto:a.sadeghi@srbiau.ac.ir)

© 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran

Online version is available on: [www.ijas.ir](http://www.ijas.ir)

## ABSTRACT

Conditioning is a major stage in the pelleting process for feeds. Probiotics are known to have the ability for improvement of the immune system and growth performance. Wheat can be used as a replacement for corn. Thus, the present study was conducted to investigate the effects of pelleting temperature, probiotics and wheat grain on growth performance, blood biochemical variables, immune responses and mucin 2 gene expression of broiler chicks. Eight-hundred 1-d-old broiler chicks were assigned into 8 treatments with 4 replicates (n=25 chicks). A completely randomized design in a 2 × 2 × 2 factorial arrangement was used with two levels of wheat (0 and 500 g/kg), two levels of probiotic (0 and 200 mg/kg) and two levels of conditioning temperature (70 or 85 °C). Growth performance, immune responses, blood biochemical variables, and mucin 2 gene expression were evaluated. Main and interaction effects were not significant on growth performance, immune responses and blood biochemical variables (P>0.05). Wheat and corn-based diets containing probiotic up-regulated mucin 2 gene expression (P<0.05). Wheat can be used as an alternative for corn.

**KEY WORDS** broiler chicks, cholesterol, immune responses, mucin 2.

## INTRODUCTION

It is well accepted that feed cost comprises a significant portion of poultry rearing costs. The cost of feed processing comprises a major part of feed costs (Behnke and Beyer, 2002). The physical form of feed is known as a key factor in broiler chicken production. Pelleting has commonly been used in order to accumulate the ingredient particles by mechanical process, along with moisture, pressure, and temperature (Muramatsu *et al.* 2015). Preparation of pelleted diets not only increases feed intake and weight gain but also improves feed conversion ratio (Freitas *et al.* 2008; Corzo

*et al.* 2011). Conditioning is a major stage where steam is used to prepare the pellet (Skoch *et al.* 1981). The different temperatures are used for preparing the pellet (Mc Cracken, 2002). Appropriate temperature is essential in preparing the pellet so that inappropriate temperature can negatively influence performance in broiler chicks (Ighani *et al.* 2017). Wheat is used in diet composition as an energy source in chicken diets due to significant levels of starch and crude protein (Gutierrez *et al.* 2008). It is also known to have components such as non-starch polysaccharides (NSPs) that enhance intestinal digestive viscosity (Agboola *et al.* 2015). High viscosity limits contact between digestive enzymes

and substrates. Mucin glycoproteins produced by goblet cells protect intestinal tract epithelium (Uni *et al.* 2003). Mucin is known to have protective effects on the intestine and also protect acidic chime and digestive enzymes (Horn *et al.* 2009).

Feed additives must be able to tolerate the high temperatures during processing. *Bacillus* species are known to have ability for resistance against temperature and also could live in the low gastric pH (Cutting, 2010; Lee *et al.* 2010a). Probiotics control the pathogenic bacteria, modulate immune responses, compete with toxin-producing bacteria in order to adhere to receptors in the gut epithelium (Cutting, 2010; Lee *et al.* 2010a) and change metabolism by enhancing digestive enzyme activity (Jin *et al.* 2000). Amerah *et al.* (2011) have reported that dietary inclusion of probiotics (*Bacillus subtilis* strains) improve feed conversion ratio in broiler chicks fed with maize/soy diets (Ameraha *et al.* 2013). They have also reported that chemical changes created by higher pelleting temperatures stimulated the immune response. To the best of our knowledge, no studies have been conducted to investigate the interaction effects of pelleting temperature, probiotic and wheat grain on growth performance, blood biochemical variables, immune responses and mucin 2 gene expression of broiler chicks. Thus, this study was conducted to evaluate the effects of pelleting temperature, probiotic and wheat grain on growth performance, blood biochemical variables, immune responses and mucin 2 gene expression of broiler chicks.

## MATERIALS AND METHODS

### Chickens and experimental design

This study was performed in Animal Research Center (Karaj, Iran) in summer of 2017. All the procedures were approved by the Ethical Standard Committee, Science and Research Branch, Islamic Azad University (No. SRBIAU, 1110). All the chemical materials were purchased from Merck Company. A total number of eight hundred one-day-old broiler chicks (Cobb 500 strain), weighting  $44 \pm 2$  g, were purchased from a commercially local hatchery. The requirements of Cobb 500 broiler chickens were used in order to formulate the diet (Table 1). All the birds were randomly divided into 8 treatments of 4 replicates per treatment with 25 birds per replication. A  $2 \times 2 \times 2$  factorial arrangement in the completely randomized design was used with wheat inclusion (0 or 50%), probiotic supplementation (0 or 200 mg/kg) and conditioning temperature (70 or 85 °C) as the main factors. Experimental treatments were as follows:

1) wheat-based diets formulated without probiotic and prepared in 70 °C (Treatment 1)

2) wheat-based diets formulated without probiotic and prepared in 85 °C (Treatment 2)

3) wheat-based diets formulated with probiotic and prepared in 70 °C (Treatment 3)

4) wheat-based diets formulated with probiotic and prepared in 85 °C (Treatment 4)

5) corn-based diets formulated without probiotic and prepared in 70 °C (Treatment 5)

6) corn-based diets formulated without probiotic and prepared in 85 °C (Treatment 6)

7) corn-based diets formulated with probiotic and prepared in 70 °C (Treatment 7)

8) corn-based diets formulated with probiotic and prepared in 85 °C (Treatment 8)

Probiotic was prepared from TakGen Company (Tehran, Iran). It contained *Bacillus subtilis* (JQ61816). Broiler chicks were reared in floor pens. The lighting program and other rearing conditions were performed as recommended by Cobb 500 broiler guidelines. Feedstuffs were grounded, blended and conditioned at 70 or 85 °C for 45 s and finally pelleted by a ring die pellet set.

### Growth performance

Birds were weighed at the beginning and end of the trial in order to determine the body weight gain (BWG). In order to calculate the feed intake (FI), feed consumption was daily measured. It was calculated as the difference between the presented feed and residue feed. Mortality was daily recorded and feed conversion ratio (FCR) was calculated by dividing FI by BWG of live plus dead birds.

### Immunity variables

On 21 and 35 days of the experiment, 1 mL of 5% suspension of sheep red blood cells (SRBCs) was intravenously injected into wing of two birds per replicate. Blood samples were obtained 7 d after administration and centrifuged at 1000 g for 10 minutes. The sera were achieved and stored at -20 °C until analysis. Each serum sample was inactivated at 56 °C for 30 min and then analyzed for total anti-SRBC antibodies. Briefly, each inactivated serum sample was titrated for total and mercaptoethanol (ME)-resistant (IgG) anti-SRBC antibody titers. ME-sensitive (IgM) antibody titers were obtained through subtracting the level (titer) of IgG antibodies from that of the total antibodies evaluated. All the titer data were reported in term of log<sub>2</sub> (Hosseini *et al.* 2018).

At 21 and 35 days of the experiment, 0.25 mL of dinitrochlorobenzene (DNCB) was injected to 2 chicks from each pen. One area, by 10 cm<sup>2</sup>, was marked in order to administer the DNCB. Skin thickness was evaluated before sensitization.

**Table 1** The experimental diet used in the starter (1-10 days), grower (11-22 days) and finisher (23-42 days) in broiler chickens

Ingredient (g)	Corn-based			Wheat-based		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Corn	562.80	620.30	651.70	116.00	167.80	203.50
Soybean meal (44%)	385.50	330.00	297.00	322.60	272.00	235.50
Wheat	0.00	0.00	0.00	500.00	500.00	500.00
Vegetable oil	9.50	10.00	15.00	17.80	19.20	23.50
Mineral oyster shell	8.20	7.80	7.4	8.40	8.00	7.50
Bicarbonate	1.50	1.50	1.50	1.50	1.50	1.50
Dicalcium phosphate	20.90	19.50	17.20	20.70	19.30	17.00
NaCl	2.50	2.50	2.50	2.10	2.10	2.10
Vitamin and premix <sup>1</sup>	2.50	2.50	2.50	2.50	2.50	2.50
Enzyme	-	-	-	-	-	-
DL-methionine	3.00	2.50	2.20	2.90	2.40	2.10
Choline chloride	1.50	1.50	1.50	1.50	1.50	1.50
Threonine	0.80	0.70	0.60	1.20	1.10	1.00
Lysine	1.30	1.20	0.90	2.80	2.60	2.30
<b>Nutrient composition</b>						
Metabolizable energy (kcal/kg)	2828.00	2900.00	2978.00	2828.00	2900.00	2978.00
Crude protein (%)	21.78	19.75	18.49	21.64	19.76	18.39
Calcium (%)	0.96	0.90	0.82	0.96	0.90	0.82
Av. phosphorus (%)	0.50	0.47	0.42	0.50	0.47	0.42
Crude fiber (%)	3.76	3.49	3.32	3.97	3.72	3.53
Potassium (%)	0.94	0.85	0.79	0.89	0.80	0.74
Chloride (%)	0.22	0.22	0.22	0.23	0.23	0.22
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16
Methionine (%)	0.59	0.52	0.48	0.56	0.49	0.45
Arginine (%)	1.36	1.21	1.12	1.25	1.12	1.02
Lysine (%)	1.18	1.05	0.95	1.18	1.05	0.95

<sup>1</sup> Vitamin and mineral premix supplied (content per kg): vitamin A: 1800000 IU; vitamin D<sub>3</sub>: 400000 IU; vitamin E: 3600 IU; vitamin K<sub>3</sub>: 400 mg; Thiamine: 360 mg; Riboflavin: 1320 mg; Niacin: 6000 mg; vitamin B<sub>6</sub>: 600 mg; vitamin B<sub>5</sub>: 2000; vitamin B<sub>12</sub>: 3 mg; Folic acid: 200 mg; Biotin: 20 mg; Choline: 80 g; Zinc: 17 g; Iron: 10 g; Copper: 2 g; Manganese: 20 g; Selenium: 40 mg and Iodine: 200 mg.

The broiler chicks were sensitized with DNCB at a dose of 0.1 mL per cm<sup>2</sup> area. Skin thickness was assessed 24 and 48 h after challenge (Hosseini *et al.* 2018).

#### Blood biochemical variables

On 42 days of the experiment, 3 mL of blood was collected from two birds from each replicate and centrifuged at 2500 × g for 15 minutes and the serum samples were obtained. Then, the levels of triglyceride, glucose, albumin, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and cholesterol were measured by commercial kits of Pars Azmun (Tehran-Iran) according to the kit manufacturer's guidelines (Akbari and Torki, 2014).

#### Mucin 2 mRNA gene expression

On 42 days, 12 broiler chicks per replicate (3 chicks per replicate) were randomly killed and intestinal segments

were removed. The jejunum part was separated, washed, transferred into the lab and RNA was extracted as recommended by Hosseini *et al.* (2018). GAPDH was used as control and the primers were prepared from Cynagen Company and were as follows: Forward for GAPDH: 5'GGTGGTGCTAAGCGTGTAT3' Reverse for GAPDH: 5' ACCTCTGTCATCTCTCCACA 3' Forward for Mucin 2: 5'-TCACCCTGCATGGATACTTGCTCA-3' Reverse for Mucin 2: 5'-TGTCATCTGCCTGAATGACAGGT-3'.

The protocol used was as follows: primary denaturation (1 cycle at 95 °C for 10 min), denaturation (1 cycle at 95 °C for 15 min), annealing (40 cycles at 60 °C for 30 min) and final extension (40 cycles at 72 °C for 30 min). The  $\Delta\Delta Ct$  method was used to evaluate the gene expression as mentioned:

$\Delta\Delta Ct = \Delta Ct$  of each treatment -  $\Delta Ct$  control

### Statistical analysis

The Kolmogorov-Smirnov test was used to check the normality of data distribution. The generalized linear model (GLM) procedure of SAS software was used in order to evaluate the data (SAS, 2004). A  $2 \times 2 \times 2$  factorial arrangement in the completely randomized design was used with conditioning temperature, prebiotic sand wheat as the main factors and their respective interactions. Means were separated by a Tukey test at  $P \leq 0.05$ . All the parameters measured were analyzed as follows:

$$Y_{ijklm} = \mu + A_j + B_k + C_l + (A \times B)_{jk} + [A \times C]_{jl} + (B \times C)_{kl} + (A \times B \times C)_{jkl} + e_{ijklm}$$

Where:

$Y_{ijklm}$ : characteristic measured.

$\mu$ : overall mean.

$A_j$ : main effect of the probiotic.

$B_k$ : main effect of temperature.

$C_l$ : main effect of wheat.

$(A \times B)_{jk}$ : interaction between the probiotic and temperature.

$(A \times C)_{jl}$ : interaction between the probiotic and wheat.

$(B \times C)_{kl}$ : interaction between the temperature and wheat.

$(A \times B \times C)_{jkl}$ : interaction among the probiotic, temperature and wheat.

$e_{ijklm}$ : residual error.

## RESULTS AND DISCUSSION

Effects of experimental treatments on growth performance are shown in Table 2. Growth performance was not influenced by experimental treatments ( $P > 0.05$ ). There were no significant two-way and three-way interactions among wheat, probiotic and temperature on growth performance ( $P > 0.05$ ).

The data for immunity variables are presented in Tables 3 and 4. As results indicate, humoral immunity (Table 3) and cellular immunity (Table 4) were not influenced by experimental treatments. It was not seen as a significant interaction among treatments ( $P > 0.05$ ).

Our findings showed that blood biochemical variables were not affected by different levels of wheat, probiotic and temperature (Table 5). In addition, significant interactions were not observed among treatments for blood parameters ( $P > 0.05$ ).

There was three-way significant interaction among probiotic, wheat and temperature (Figure 1) so that birds fed with diets pelleted in both temperatures and containing probiotic and wheat showed higher up-regulation in comparison to other groups.

In the current study, probiotic supplementation and pelleting temperature did not have significant effects on growth performance. Ameraha *et al.* (2013) have reported that broiler chicks fed pelleted diets prepared at 75 and 90 °C had higher BWG than birds receiving 85 °C diets. Abdollahi *et al.* (2010a) and Abdollahi *et al.* (2010b) have shown that conditioning temperature at 60 and 90 °C could improve BWG in comparison to birds fed a diet conditioned at 75 °C.

However, our findings did not show significant effects of probiotic and pelleting temperature on growth performance in the wheat and corn-based diets. Differences between our findings and others can be explained by a lubricating effect which could reduce adverse effects of the friction-created heat. Ameraha *et al.* (2013) reported that dietary inclusion of probiotic (three *Bacillus subtilis* strains) decreased food consumption and improved FCR but had no effect on BWG. Amerah *et al.* (2011), Amerah and Gracia, (2011) have reported that *B. subtilis* supplementing improved performance in broiler chicks fed probiotic (*B. subtilis*) in maize and wheat based diets. Differences in the type of probiotic, dose, procedure for preparation, type of diet, sanitary condition of the animals, age, etc could be reasons for conflict in our findings and others. With regards to inclusion of wheat, Shekari *et al.* (2013) reported lack of significant differences in growth performance of broiler chicks fed with corn based diets in comparison to birds fed with wheat-based diets. Agboola *et al.* (2015) have reported that probiotic supplementation into wheat-based diets in 35-day-old broilers could improve feed intake, dry matter intake and FCR.

Wheat is known to have anti-nutrient compounds (NSPs) which can negatively influence performance. It seems that dietary inclusion of wheat in pelleted diets did not have negative effects on performance compared with wheat-based diet. Thus, wheat can be included in a 50% diet in the pelleted diets in temperatures 70 and 85 °C. As results show, mortality was not affected by experimental treatments; suggesting that treatments have no negative effects on mortality.

Dietary inclusion of wheat, probiotic and pelleting temperature had no significant effects on cellular and humoral immunities. Ameraha *et al.* (2013) have reported dietary inclusion of probiotic lowered the levels of IgA and IgM in birds supplemented with diets containing probiotic and prepared in 90 °C compared with those fed diets pelleted in 75 °C or 85 °C. Probiotics improved the immune system through altering mucosal lymphocyte populations and inducing cytokines interleukin-2, interleukin-4, interleukin-6 and interleukin-10 from intra-epithelial lymphocytes which are related to IgA class in B-cells (Lee *et al.* 2010b; Lee *et al.* 2011).

**Table 2** Effects of experimental treatments on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broiler chicks from 1 to 42 days

	Treatments		FI (g)	BWG (g)	FCR	Mortality %
	Probiotic	Temperature				
Wheat						
0	0	70	4436.96	2319.66	1.91	13.33
0	0	85	4374.97	2346.42	1.86	15.00
0	200	70	4378.57	2286.45	1.91	11.00
0	200	85	4502.30	2318.25	1.94	7.00
500	0	70	4459.27	2316.82	1.92	10.00
500	0	85	4599.15	2311.52	1.99	11.00
500	200	70	4444.20	2261.50	1.96	9.00
500	200	85	4515.87	2409.07	1.89	9.00
SEM			38.14	29.21	0.014	1.21
P-value			8.77	0.972	0.465	0.836
Wheat			0.325	0.912	0.234	0.512
Probiotic			0.936	0.941	0.858	0.210
Temperature			0.393	0.429	0.828	0.896
Wheat			0.575	0.701	0.252	0.468
Wheat × probiotic			0.684	0.750	0.926	0.719
Wheat × temperature			0.747	0.532	0.537	0.537
Wheat × probiotic × temperature			0.448	0.573	0.0723	0.658

SEM: standard error of the means.

**Table 3** Effects of experimental treatments on humoral immunity (log<sub>2</sub>): immunoglobulin G1 (IgG1) and (IgM1) in 28 days and IgG2 and IgM2 at 42 days

	Treatments		IgG1	IgM1	IgG2	IgM2
	Probiotic	Temperature				
Wheat						
0	0	70	4.00	1.00	4.33	1.00
0	0	85	4.25	1.50	4.50	1.00
0	200	70	4.25	1.25	4.75	1.50
0	200	85	4.50	0.75	4.75	1.00
500	0	70	4.50	0.75	5.00	1.50
500	0	85	4.50	1.00	4.50	1.50
500	200	70	4.25	1.25	4.75	1.00
500	200	85	4.25	1.25	4.50	1.25
SEM			0.12	0.13	0.13	0.15
P-value			0.986	0.627	0.969	0.956
Wheat			0.702	0.349	0.772	0.595
Probiotic			0.951	0.614	0.764	0.827
Temperature			0.694	0.602	0.586	0.822
Wheat			0.391	0.508	0.479	0.632
Wheat × probiotic			0.660	0.699	0.458	0.560
Wheat × temperature			1.00	0.0780	0.934	0.870
Wheat × probiotic × temperature			1.00	1.00	0.731	0.580

SEM: standard error of the means.

Indeed, probiotic influences antibody production by increasing local immune defenses (Kabir *et al.* 2004) and production of modulatory immune molecules (Zhang *et al.* 2016).

Immune responses were not different in broiler chicks fed with corn and wheat-based diets. It means that inclusion of wheat by 50% diet did not have adverse effects on the immune system.

Biochemical variables were not influenced by experimental treatments. We did not find any study conducted to investigate the effects of pelleting temperature on blood biochemical variables.

Dietary inclusion of probiotics could decrease serum triglycerides, cholesterol and / or LDL-C and increased HDL-C levels (Mohan *et al.* 1995; Mohan *et al.* 1996; Kalavathy *et al.* 2003). Serum lipid is one important parameter used in order to evaluate the results related to animal health and meat quality (Fletcher, 2002). Probiotics decrease serum cholesterol by modulating cholesterol absorption in the gut by deconjugation of the bile salts or by assimilating cholesterol (Ooi and Liong, 2010). However, our findings showed that probiotics had no significant effect on lipid profile, which is due to dose and probiotic strain.

**Table 4** Effects of experimental treatments on dinitrochlorobenzene (DNCB) at 21 and 35 days after 24 and 48 h

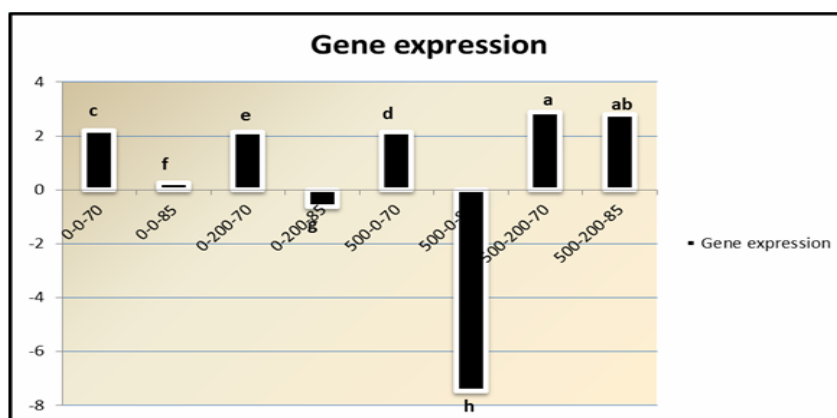
Treatments	21 days		35 days			
	24 h	48 h	24 h	48 h		
Wheat	Probiotic	Temperature				
0	0	70	0.640	0.406	0.601	0.763
0	0	85	0.647	0.500	0.701	0.475
0	200	70	0.730	0.490	0.701	0.692
0	200	85	0.675	0.525	0.501	0.792
500	0	70	0.745	0.455	0.901	0.685
500	0	85	0.710	0.575	0.401	0.790
500	200	70	0.722	0.512	0.190	0.820
500	200	85	0.690	0.507	0.320	0.762
SEM			0.017	0.025	0.750	0.019
P-value			0.815	0.974	0.054	0.721
Wheat			0.275	0.764	0.285	0.182
Probiotic			0.650	0.801	0.344	0.147
Temperature			0.406	0.406	0.208	0.676
Wheat			0.310	0.763	0.0589	0.503
Wheat × probiotic			0.919	0.870	0.132	0.436
Wheat × temperature			0.704	0.540	0.212	0.441
Wheat × probiotic × temperature			0.667	0.612	0.300	0.054

SEM: standard error of the means.

**Table 5** Effects of experimental treatments on blood biochemical variables (mg/dL) at 42 days of age

Treatments	Glucose	Albumin	HDL	LDL	Cholesterol	Triglycerides		
Wheat	Probiotic	Temperature						
0	0	70	171.18	1.71	52.43	160.14	176.66	139.93
0	0	85	180.20	1.69	51.97	165.75	176.25	138.30
0	200	70	176.86	1.75	52.62	165.18	176.75	136.98
0	200	85	174.71	1.65	51.37	169.19	182.00	140.88
500	0	70	175.55	1.63	50.72	160.87	183.00	133.56
500	0	85	176.56	1.64	52.17	166.90	179.75	135.62
500	200	70	172.36	1.65	51.10	165.11	178.50	135.92
500	200	85	175.40	1.73	50.67	164.12	175.50	134.94
SEM			1.17	0.012	0.23	1.29	1.56	1.08
P-value			0.904	0.124	0.198	0.860	0.926	0.751
Wheat			0.848	0.073	0.056	0.745	0.930	0.097
Probiotic			0.759	0.163	0.398	0.644	0.807	0.861
Temperature			0.449	0.865	0.704	0.248	0.888	0.686
Wheat			0.558	0.341	0.666	0.801	0.295	0.865
Wheat × probiotic			0.996	0.052	0.121	0.769	0.407	0.861
Wheat × temperature			0.529	0.956	0.133	0.277	0.680	0.817
Wheat × probiotic × temperature			0.209	0.181	0.540	0.877	0.695	0.359

SEM: standard error of the means.



**Figure 1** Effects of experimental treatments on mucin 2 gene expression at 42 days of age



Mucin 2 gene expression was up-regulated in birds fed with pelleted diets containing probiotics in both temperatures. Probiotics join to intestinal mucin 2 through competition between pathogenic and beneficial bacteria (Craven and Williams, 1998; Hosseini *et al.* 2018). Smirnov *et al.* (2005) have reported that dietary inclusion of probiotic significantly increased mucin2 gene expression. So far, no study has been conducted to evaluate the effects of wheat and pelleting temperature on mucin 2 gene expression. Future studies will be required to investigate the effects of additives on mucin 2 gene expression.

## CONCLUSION

In conclusion, main and interaction effects were not significant on growth performance, immune responses, and blood biochemical variables. Wheat and corn-based diets prepared to contain probioticup regulated mucin 2 gene expression. Wheat can be used instead of corn by 50%. Future studies would be needed for further investigations.

## ACKNOWLEDGEMENT

The authors thank the Science and Research Branch, Islamic Azad University for financial support of the current study.

## REFERENCES

- Abdollahi M.R., Ravindran V., Wester T.J., Ravindran G. and Thomas D.V. (2010a). Influence of conditioning temperature on the performance, nutrient utilization and digestive tract development of broilers fed on maize- and wheat-based diets. *British Poult. Sci.* **51**, 648-657.
- Abdollahi M.R., Ravindran V., Wester T.J., Ravindran G. and Thomas D.V. (2010b). Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed maize- and sorghum-based diets. *Anim. Feed Sci. Technol.* **162**, 106-115.
- Agboola A.F., Odu O., Omidwura B.R.O. and Iyayi E.A. (2015). Effect of probiotic, carbohydrase enzyme and their combination on the performance, histomorphology and gut microbiota in broilers fed wheat-based diets. *Am. J. Exp. Agric.* **8(5)**, 307-319.
- Akbari M. and Torki M. (2014). Effects of dietary chromium picolinate and peppermint essential oil on growth performance and blood biochemical parameters of broiler chicks reared under heat stress conditions. *Int. J. Biometeorol.* **58**, 1383-1391.
- Amerah A.M. and Gracia M.I. (2011). Influence of three *Bacillus subtilis* strains combination on the performance, intestinal morphology and blood parameters of broilers fed wheat-based diet. Pp. 38-46 in Proc. Int. Poult. Sci. Forum., Atlanta, Georgia.
- Amerah A.M., Jansen van Rensburg C. and Plumstead P.W. (2011). Effect of feeding diets containing a probiotic or antibiotic on broiler performance and litter water-soluble phosphorus. *Poult. Sci.* **90(1)**, 15-23.
- Ameraha A.M., Quilesb A., Medelc P., Snchez J., Lehtinend M.J. and Gracia M.I. (2013). Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. *Anim. Feed Sci. Technol.* **180**, 55-63.
- Behnke K.C. and Beyer R.S. (2002). Effect of feed processing on broiler performance. Pp. 56-62 in Proc. 8<sup>th</sup> Int. Sem. Poult. Prod. Pathol., Santiago, Chile.
- Corzo A., Mejia L. and Loar R.E. (2011). Effect of pellet quality on various broiler production parameters. *J. Appl. Poult. Res.* **20**, 68-74.
- Craven S.E. and Williams D.D. (1998). *In vitro* attachment of *Salmonella typhimurium* to chicken cecal mucus: Effect of cations and pretreatment with *Lactobacillus* spp. isolated from the intestinal tracts of chickens. *J. Food Prot.* **61**, 265-271.
- Cutting S.M. (2010). Bacillus probiotics. *Food Microbiol.* **28**, 214-220.
- Fletcher D.L. (2002). Poultry meat quality. *World's Poult. Sci. J.* **58**, 131-146.
- Freitas E.R., Sakomura N.K., Dahlke F., Santos F.R. and Barbosa N.A.A. (2008). Performance, efficiency of nutrient utilization and gastrointestinal structures of broiler chick fed in prestarter phase with ratios with different physical form. *Brazilian J. Anim. Sci.* **37(1)**, 73-38.
- Gutierrez A., Versteegen M.W., Den Hartog L.A., Perez P. and Villamide M.J. (2008). Effect of wheat cultivar and enzyme addition to broiler chicken diets on nutrient digestibility, performance and apparent metabolizable energy content. *Poult. Sci.* **87**, 759-767.
- Horn N.L., Donkin S.S., Applegate T.J. and Adeola O. (2009). Intestinal mucin dynamics: Response of broiler chicks and white Pekin ducklings to dietary threonine. *Poult. Sci.* **88**, 1906-1914.
- Hosseini S.M., Chamani M., Mousavi S.N., Hosseini S.A. and Sadeghi A.A. (2018). Growth performance, mucin 2 gene expression, morphology of small intestine and intestinal lactobacillus population of broiler chicks fed with triticale based diets. *Kafkas Univ. Vet. Fak. Dreg.* **24(2)**, 203-210.
- Ighani V., Sadeghi A.A., Mousavi S.N., Jafari P. and Chamani M. (2017). Effect of pelleting temperature, probiotic and wheat grain on intestinal pH, cecal microbial population and intestinal morphometry in broiler chickens. *J. Livest. Sci.* **8**, 210-215.
- Jin L.Z., Ho Y.W., Abdualah N. and Jalaludin S. (2000). Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* cultures. *Poult. Sci.* **79**, 886-891.
- Kabir S.L., Rahman M.M., Rahman M.B., Rahman M.M. and Ahmed S.U. (2004). The dynamics of probiotics on growth performance and immune response in broilers. *Int. J. Poult. Sci.* **3(5)**, 361-364.
- Kalavathy R., Abdullah N., Jalaludin S. and Ho Y.W. (2003). Effect of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipid and weight of organs of

- broiler chickens. *British Poult. Sci.* **44**, 139-144.
- Lee K.W., Lee S.H., Lillehoj H.S., Li G.X., Jang S.I., Babu U.S., Park M.S., Kim D.K., Lillehoj E.P., Neumann A.P., Rehberger T.G. and Siragusa G.R. (2010a). Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poult. Sci.* **89**, 203-216.
- Lee K.W., Lillehoj H.S. and Siragusa G.R. (2010b). Direct-fed microbials and their impact on the intestinal microflora and immune system of chickens. *J. Poult. Sci.* **47**, 106-114.
- Lee K.W., Lillehoj H.S., Lee S.H., Jang S.I., Babu U.S., Lillehoj E.P., Neumann A.P. and Siragusa G.R. (2011). *Bacillus subtilis*-based direct-fed microbials augment macrophage function in broiler chickens. *Res. Vet. Sci.* **91**, 87-91.
- Mc Cracken K.J. (2002). Effects of physical processing on the nutritive value of poultry diets Pp. 301-316 in Poultry Feeds, Supply, Composition and Nutritive Value. J.M. McNab and K.N. Boorman, Eds., CAB International, New York, United State.
- Mohan B., Kadirvel R., Bhaskaran M. and Natarajan A. (1995). Effect of probiotic supplementation on serum / yolk cholesterol and on egg shell thickness in layers. *British Poult. Sci.* **36**, 779-803.
- Mohan B., Kadirvel R., Natarajan A. and Bhaskaran M. (1996). Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. *British Poult. Sci.* **37**, 395-401.
- Muramatsu K., Massuquetto A., Dahlke F. and Maiorka A. (2015). Factors that affect pellet quality: A review. *J. Agric. Sci. Technol.* **10**, 717-722.
- Ooi L.G. and Liong M.T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: A review of *in vivo* and *in vitro* findings. *Int. J. Mol. Sci.* **11**, 2499-2522.
- SAS Institute. (2004). SAS<sup>®</sup>/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.
- Shekari M., Shahir M.H. and Abdi A. (2013). Effects of different levels of canola meal on growth performance of broiler chicks fed with corn or wheat based diets. *Anim. Sci Res. J.* **2**, 131-145.
- Skoch E.R., Behnke K.C., Deyoe C.W. and Binder S.F. (1981). The effect of steam-conditioning rate on the pelleting process. *Anim. Feed Sci. Technol.* **6**, 83-90.
- Smirnov A., Perez E., Amit Romach E., Sklan D. and Uni Z. (2005). Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. *J. Nutr.* **135**, 187-192.
- Uni Z., Smirnov A. and Sklan D. (2003). Pre- and posthatch development of goblet cells in the broiler small intestine: Effect of delayed access to feed. *Poult. Sci.* **82**, 320-333.
- Zhang L., Zhang L., Zhan X., Zeng X., Zhou L., Cao G., Chen A. and Yang C. (2016). Effects of dietary supplementation of probiotic, *Clostridium butyricum*, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with *Escherichia coli* K88. *J. Anim. Sci. Biotechnol.* **7**, 3-10.