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RESEARCH ARTICLE

Impact of Stored Wheat-based Feed on Gut Morphology, Digesta Viscosity and Blood Metabolites of Broiler Chickens

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A B S T R A C T

The aim of current experiment was to evaluate the effect of stored wheat based diet on intestinal morphology, digesta viscosity digesta viscosity and blood metabolites of the broiler chicken birds. For this purpose, 560-day old male broiler chicks were used with 8 replicates per treatment and 10 birds per replicate. Corn-soybean based diet was considered as control diet and the other six dietary treatments were prepared by replacing corn of the corn soybean diet with 50% and 100% replacement of fresh, 1.5 year stored and 2.5 year stored wheat. Results of intestinal morphology explored that villus height and villus surface area were higher (P<0.05) in broilers reared on corn soybean based diet and stored wheat diet as compared to the fresh wheat based diets. Results of plasma metabolites represented that serum cholesterol and urea nitrogen was high in diets contained new wheat as comparison to the other experimental treatments (P>0.05). However, blood glucose was high in birds fed stored wheat based diets and corn soy based diet (P<0.05). Replacement of corn with fresh wheat 50% and 100% in the diet of broiler significantly enhanced digesta viscosity (P<0.05). Based on results, it is concluded that stored wheat successfully replace corn and new wheat in the diet of broilers and have positive impact on digesta viscosity, intestinal morphology and blood metabolites. Therefore, it is recommended that corn of corn soybean diet should be replaced with stored wheat when stored wheat is available at lower price.

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INTRODUCTION

Pakistan Agricultural Storage and Services Corporation (PASSCO) store wheat every year for food security especially during scarcity period. After one year or couple of year, stored wheat is not considered safe for human consumption and stored wheat in excess is sold to the Pakistan Poultry Association at lower rates to produce high quality protein and to fulfill the protein requirement of growing population of country.

Wheat is the most important cereal grains used in poultry diets (Bautil and Courtin, 2019). In comparison with the maize, wheat usually have a lower energy value and higher percentage of dietary fibers (Knudsen, 2014). However, mechanism of action of dietary fiber in the body is complex but generally dietary fiber is defined as the one of the fractions of plant material that is prone to resistant to hydrolysis by body enzymes of the gastrointestinal tract (Choct, 2015). These non-digestible dietary fibers are called as non-starch polysaccharides (NSPs) that exhibit anti nutritional properties for poultry birds. Usually antinational properties of feed is reduced by supplementing exogenous enzymes in diet (Arshad *et al.*, 2020; Shahid *et al.*, 2020; Shahid *et al.*, 2021). It is well known that storage of grain reduces the antinutritional factors especially NSPs and improve the nutritional profile of the stored ingredients.

Non-starch polysaccharides are responsible for the formation of thick viscous solution when they are dissolved in water, and it has been reported that NSPs can absorb water up to 10 times of their weight (Choct, 1997). Previous study has explored that thick viscous solutions formed by NSPs can lower the rate of diffusion of substrates and endogenous and exogenous digestive enzymes and ultimately result in low digestibility of feed (Ikegami *et al.*, 1990). Furthermore, it has also been reported that higher digesta viscosity also lower the rate of digesta passage that results in less feed intake (Anwar *et al.*, 2023). Less feed intake due to lower passage rate could be due to longer stay duration of digesta in gastrointestinal tract and changes in gut microflora balance which forms a stable environment for microbial proliferation in the gut (Van der Klis *et al.*, 1993; Choct and Hughes, 1997). Therefore, stored wheat inclusion with lower

NSPs may have positive impact on digesta viscosity and better nutrient absorption in birds (Choct, 1997; Ravindran *et al.*, 2001).

Better nutrient absorption in broiler could easily be explained by intestinal histomorphology that main include villus length, crypt depth and their ratio. It has previously been reported that higher villi and reduced crypt depth are major indicators of better nutrient absorption. Crypt is known to be the major factory responsible for the synthesis of villus (Apperson and Cherian, 2017). However, there is limited information on the link between the gut morphology, blood metabolites and digesta viscosity of broiler birds and feed that contained stored wheat. Present work was designed particularly to determine the influence of stored wheat on the intestinal morphology (villus height (VH), villus width (VW), crypt depth (CD), ratio of villus height / villus width (VH/VW), ratio of villus height / crypt depth (VH/CD) and villus surface area), digesta viscosity and blood metabolites of broiler chickens.

MATERIALS AND METHODS

Experimental animals, design and treatments: One month before the arrival of chicks, shed was cleaned thoroughly and fumigated. A total of 560-day old male broiler chicks (Ross-308) were provided by Arslan Chicks (Pvt Ltd) Islamabad. Experimental diets were formulated as, control diet=corn-soybean based diet, no wheat, 50%-new wheat (50% NW)=50% replacement of control diet corn with new wheat, 100%-NW=100% replacement of control diet corn with new wheat, 50% 1.5 year stored wheat (50%-1.5YOW)= 50% replacement of control diet corn with 1.5 year old wheat, 100%-1.5YOW= 100% replacement of control diet corn with 1.5 year old wheat, 50%-2.5YOW = 50% replacement of control diet corn with 2.5 year old wheat and 100%-2.5YOW = 100% replacement of control diet corn with 2.5 year old wheat. The ingredient chemical composition and nutrient profile data presented in adjacent paper and considering Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2011; Anwar *et al.,* 2023). Nutrient composition of diet was determined as described in recent studies (Rehman *et al.*, 2019; Bajwa *et al.*, 2020; Hussain *et al.*, 2021). In the current experiment, seven dietary treatments were randomly assigned to the 56 pens, each pen had 10 birds. Pens were covered with three-inch wood shavings for chicks bedding. During the experiment, chicks was reared for 35 days keeping the same environmental conditions for all treatments. Fresh and clean water was offered round the clock. Birds was vaccinated according to local vaccination program. A circular bottom feeder was provided for each pen, and nipple drinking system allowed for continuous water availability.

Measurements, sampling and analyses

Intestinal morphology: On day 35, three birds were randomly selected and slaughtered from each experimental treatment for collecting ileum specimens. By using an image analysis software (Top View 3.7) the following parameters were measured:

a) VH
b) VW
c) CD
d) VH/VW
e) Ratio of villus height to crypt depth (VH/CD)

Ilium specimens (after slaughtering of birds) were obtained from the experimental birds. After collecting of samples, Ilium specimens were fixed in 10% neutral buffered formalin solution for approximately 24 h, then embedded in paraffin and sectioned at 4 µm. By using an image analysis software (ToupView 3.7), VH, VW, CD (Govil *et al.*, 2017) were measured and ratios of VH/VW, ratios of VH/CD, villus surface area (mm2) were calculated by multiplying 2π x VH x VW/2 (Sakamoto *et al.*, 2000).

Determination of viscosity: Two broiler birds per treatment were slaughtered at day 30 for the purpose of collecting digesta in order to determine the viscosity of sample. Digesta contents off the gastrointestinal tract (GIT) (except for the pro-ventriculus) were collected gently by finger, stripping each GIT segment and finally frozen at −20°C for future analysis.

The sample of digesta were pooled in a falcon tube per replicate. The viscosity of digesta samples were determined by using a Brookfield DV-E viscometer (Brookfield Engg., Middleboro, Mass., USA). The tubes were centrifuged for 5 min at 3000 rpm and then the supernatant was centrifuged for 5 min at 12,500 rpm. The viscometer was preheated at 40 °C then digesta supernatant was introduced in the viscometer. The average shear rate used for measuring viscosity ranged between 45.0 s−1 and 22.5 s−1.

Blood metabolites parameters: Blood serology was done on 35^{th} day of the experiment. For collection of blood, a total of two birds were slaughtered from each replicate and blood was collected in Bolton gel & clot tube. Prior to determination of blood metabolites, blood cells and serum were separated by centrifugation. After centrifugation, serum from blood was collected and preserved in Eppendorf tubes. Blood serum glucose, urea nitrogen and cholesterol were determined by commercial kits BioMed-Urea, Bio-MedGlucose LS and Bio-MedCholesterol LS kits.

Statistical analysis: Collected data was analyzed using General Linear Model of Minitab Statistical s**o**ftware 17 (Minitab Inc. 2010) under c**o**mpletely rand**o**mized design. Significant means will be tested by using Tukey's test.

RESULTS

Intestinal morphology: There was effect of stored wheat on the intestinal morphology parameters of broiler chickens (P<0.05) (Fig. 1 and Fig. 2). Villus height and villus surface area were higher (P<0.05) in birds fed control diet and 2.5 years old wheat diet as compared to the other dietary treatments. Crypt depth was higher (P<0.05) in birds fed 100% new wheat diet. VH:CD were higher (P<0.05) in birds fed control diet than 2.5-year-old wheat diet. VH: VW were higher (P<0.05) in birds fed 1.5-year-old wheat-based diet.

Blood metabolites: Results of stored wheat on blood cholesterol, glucose and urea at day 35 revealed that significant difference was observed in blood metabolites (P>0.05) (Table 1). Serum cholesterol and urea nitrogen was high in diets had 50% and 100% replacement of corn with new wheat as compared to the other dietary treatments (P>0.05).

Table 1: Effect of stored wheat on blood metabolites

	Dietary Treatments								
	Control	50% NW	100% NW	50% 1.5-YOW	100% 1.5 YOW	50% 2.5-YOW	100% 2.5 YOW	SEM	P-value
Cholesterol	146.7[c]	158.1[ab]	162.2[a]	151.2[b]	154.1[b]	152.7[b]	154.9[b]	1.99	0.039
Glucose	155.1[b]	151.4[c]	150.2[c]	168.7[a]	164.2[a]	162.1[a]	166.6[a]	8.74	0.025
Urea-nitrogen	3.11[b]	4.37[a]	4.76[a]	3.71[b]	3.66[b]	3.48[b]	3.39[b]	0.82	0.014

Control diet=no wheat, 50%-NW=Replacement of corn with new wheat (50%), 100%-NW=Replacement of corn with new wheat (100%), 50%-1.5YOW=Replacement of corn with 1.5-year-old wheat (50%), 100%-1.5YOW=Replacement of corn with 1.5-year-old wheat (100%), 50%-2.5YOW =Replacement of corn with 2.5-year-old wheat (50%), 100%-2.5YOW =Replacement of corn with 2.5-year-old wheat (100%),

Table 2: Digesta viscosity of broilers fed fresh or stored wheat.

	Dietary Treatments								
Viscosity (cps)	Control	50%-NW	100%-NW	50%-1.5YOW	100%-1.5YOW	50%-2.5YOW	100%-2.5YOW	SEM	P-Values
Viscosity (cps)	6.87[c]	11.32[b]	14.20[a]	7.67[c]	7.21[c]	7.61[c]	7.20[c]	0.31	0.001

Control diet=no wheat. 50%-NW=Replacement of corn with new wheat (50%), 100%-NW=Replacement of corn with new wheat (100%), 50%-1.5YOW=Replacement of corn with 1.5-year-old wheat (50%), 100%-1.5YOW=Replacement of corn with 1.5-year-old wheat (100%), 50%-2.5YOW

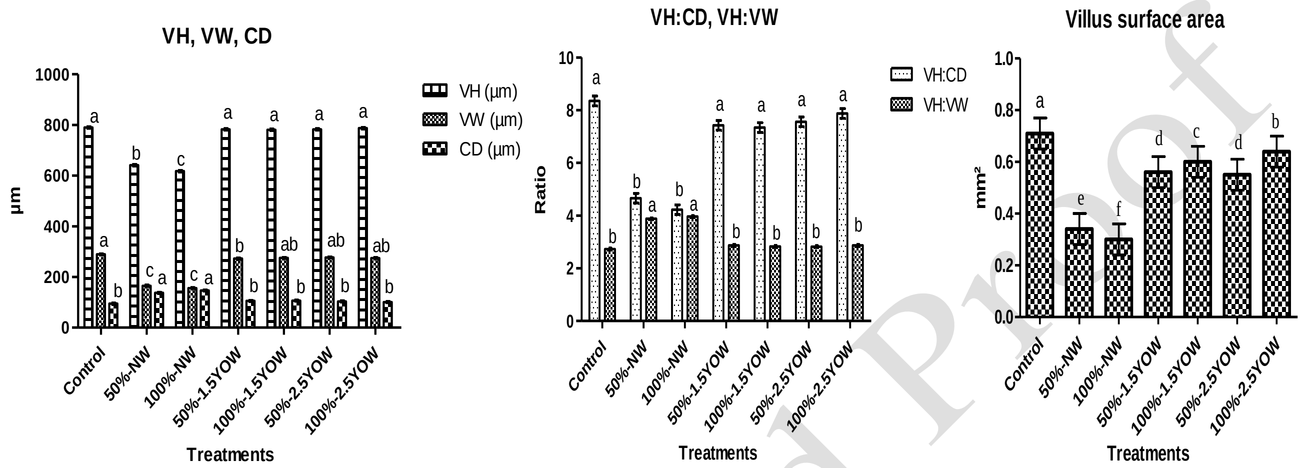

Fig. 1: Control diet=no wheat, 50%-NW=Replacement of corn with new wheat (50%), 100%-NW=Replacement of corn with new wheat (100%), 50%-1.5YOW=Replacement of corn with 1.5-year-old wheat (50%), 100%-1.5YOW=Replacement of corn with 1.5-year-old wheat (100%), 50%-2.5YOW =Replacement of corn with 2.5-year-old wheat (50%), 100%-2.5YOW =Replacement of corn with 2.5-year-old wheat (100%)

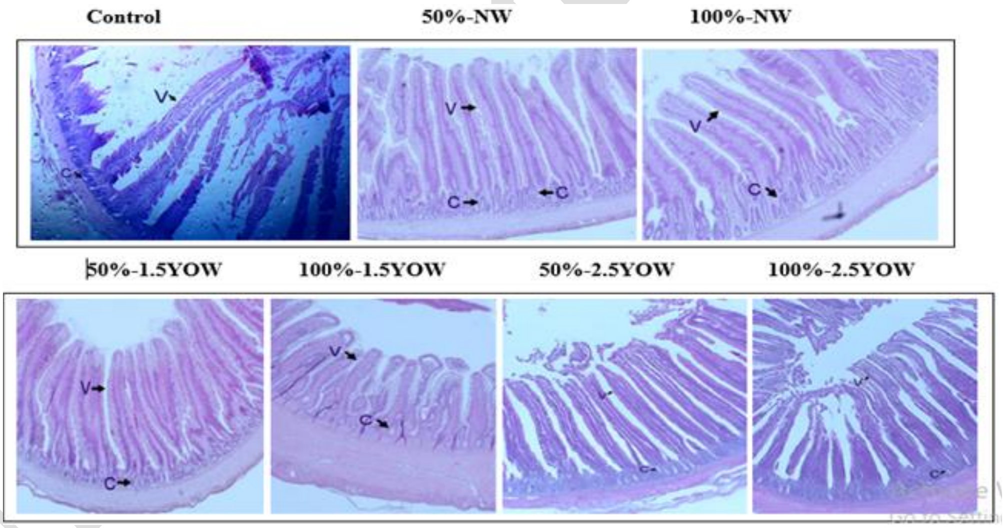

Fig. 2: Control diet=no wheat, 50%-NW=Replacement of corn with new wheat (50%), 100%-NW=Replacement of corn with new wheat (100%), 50%-1.5YOW=Replacement of corn with 1.5-year-old wheat (50%), 100%-1.5YOW=Replacement of corn with 1.5-year-old wheat (100%), 50%-2.5YOW =Replacement of corn with 2.5-year-old wheat (50%), 100%-2.5YOW =Replacement of corn with 2.5-year-old wheat (100%), [1]PBM=Poultry by product meal, [2]MCP= Mono calcium phosphate

Blood glucose was high in birds fed stored wheat based diets either 1.5-year-old wheat or 2.5-year-old wheat in 50% and 100% replacement with control diet corn (P<0.05).

Viscosity parameters: The data of digesta viscosity of the current experiment is presented in Table 2. Results explored that replacement of corn with fresh wheat 50% and 100% in the diet of broiler significantly enhance the digesta viscosity (P>0.05).

DISCUSSION

Grains are major energy source for commercial poultry production. In poultry feed mills grains are procured from farmers and used in fresh basis but most commonly grains are stored to provide feed ingredients reserves during scarcity period. It has been reported that new season grains (Barley and wheat) are problematic for broiler production due to the high contents of soluble NSPs that are responsible for increasing the digesta viscosity (Pirgozliev *et al.*, 2006). However, storing grains for three to four months improve nutritive value and have positive impact on broiler chicken production (Choct, 1997; Ravindran *et al.,* 2001).

In the present study, special attention was given to the changes in the nutritive value of wheat and chemical composition of wheat during post-harvest storage and its impact on intestinal morphology. Better nutrient absorption in broiler could easily be explained by intestinal histomorphology (villi length, crypt depth and their ratio). In the current study, higher villus height and reduced crypt dept in birds fed corn-based diet and stored wheat diet. It has previously been reported that higher villi and reduced crypt depth are major indicators of better nutrient absorption (Apperson and Cherian, 2017). In the current study, new wheat-based diets had less villus surface area, and it has been reported previously that NSPs contents of new wheat are higher that resulted in higher viscosity of digesta that supposed to encapsulate nutrients thus inhibit significant contact between villi and nutrients and results in weak structure of intestine (Dworkin *et al.*, 1976). In new wheat-based diet less villus height and surface area could be responsible for poor nutrient absorption, hence the poor performance and feed conversion ratio in birds. While higher villus height and surface area is linked with better absorption of nutrients (Parsaie *et al.,*, 2007) and higher villus height and surface could ensure better feed conversion and performance in the corn based diets and stored wheat based diets.

Crypt depth was higher ($P<0.05$) in birds fed 100% new wheat diet. Villus height to crypt depth ratio (VH:CD) were higher ($P<0.05$) in birds fed corn-based diet and 1.5 and 2.5-year-old wheat diet. Villus height to villus width ratio (VH:VW) were higher ($P<0.05$) in birds fed new wheat-based diet. Villus surface area was less in birds fed new wheat diet either 50% or 100% while crypt depth were high. These results are because of the high intestinal viscosity caused by higher contents of new wheat-based diet that leads to the abrupt changings in the intestinal mucosa. It has been previously reported that close proximity of mucosal surface to the higher viscous digesta material in the intestinal leads to lower villus surface area and increase crypt depth (Saki *et al.*, 2011; Yaghobfar and Kalantar, 2017). Crypt is known to the major factory responsible for the synthesis of villus and as in current study, a higher crypt depth represents fast tissue turnover and higher demand of new tissues. Based on findings of the crypt depth, it could be supposed that new wheat with higher NSPs contents inclusion in the diet of broiler may result in deeper crypts with higher rate of cell proliferation and tissue renewal and hence negatively influence villus height. Stored wheat result in release of entrapped nutrients by lowering the content of non-starch polysaccharides.

Inclusion of stored wheat in the diet of broilers resulted in an increase in blood glucose level that could be explained by lesser phytate contents in the stored wheat as compared to fresh wheat because it has been previously reported that storage reduce the phytate contents in grains (Choct, 1997; Ravindran *et al.,* 2001). These finding are also supported by the results of previous researcher, Johnston *et al*. (2004) who reported that breaking of phytate by addition of exogenous phytase resulted in an increase in circulating blood glucose in pigs. Previous study have reported that intake of higher phytate resulted in higher endogenous Na loss from the intestines of broilers and this is thought to be associated with a physiological cascade that begins with phytate-induced protein precipitation and cross-linking in the gastric phase of digestion and ends with an increase in $NaHCO_3$ secretion and loss in the small intestine (Cowieson *et al.*, 2004). Therefore, it could be speculated that higher phytate contents in fresh wheat could interferes with Na partitioning in the small intestine and ion balance in general, and reduction of phytate contents by storing wheat before adding in the diet of broiler could enhance Na-dependent transport systems. It has been reported that 80% of dietary glucose is absorbed via Na-dependent active transport systems (Fuller and Reeds, 1998), and it could be assumed that lower phytate contents due to storage may increase blood glucose level in the current study indirectly via effects on Na. Our results are in accordance with the study of Khadem *et al*., (2016).

In broiler birds, proteins are degraded to nitrogen and blood urea nitrogen may indicate the protein metabolism regulation in broilers. Previous studies have reported a negative link between plasma urea nitrogen levels and efficiency of protein retention (Swennen *et al*., 2005). In the current experiment, blood metabolites results explored that stored wheat-based diets reduced the level of blood urea nitrogen and results suggests that inclusion of stored wheat enhanced nutrient metabolism, especially protein anabolism in broilers, therefore, could be responsible for better growth performance in broilers.

Conclusion: Based on the findings of the current study, it is concluded that inclusion of stored wheat in the diet of broiler have positive impact on intestinal morphology, digesta viscosity and blood metabolites. Therefore, it is recommended that corn of corn soybean diet should be replaced with stored wheat when stored wheat is available at lower price.

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Authors contribution: RV and MAR conceived and designed the experiment. QB, MY and MA carried out experiment and lab analysis. MAR performed statistcal analysis. All authors write, revised and reviewed the manuscript.

REFERENCES

Anwar U, Riaz M, Farooq KM *et al.,* 2023. Impact of exogenous xylanase and phytase, individually or in combination, on performance, digesta viscosity and carcass characteristics in broiler birds fed wheat-based diets. Animals 13:278.

Arshad M, Bhatti S, Hassan I *et al.,* 2020. Effects of bile acids and lipase supplementation in low-energy diets on growth performance, fat digestibility and meat quality in broiler chickens. Braz J Poult Sci 22: 1-8.

Apperson KD and Cherian G, 2017. Effect of whole flax seed and carbohydrase enzymes on gastrointestinal morphology, muscle fatty acids, and production performance in broiler chickens. Poult Sci 96: 1228-34.

Bajwa MH, Mirza MA, Ahmad G *et al.,* 2020. Comparative efficancy of vitamin D sources on growth response and bone mineralization in broilers. Pak J Agric Sci 57: 255-261.

Bautil A and Courtin CM, 2019. Chapter 1. Fibres making up wheat cell walls in the context of broiler diets. In The value of fibre: Engaging the second brain for animal nutrition (pp. 148-152). Wageningen Academic Publishers.

Choct M, 1997. Feed non-starch polysaccharides: chemical structures and nutritional significance. Feed Milling International 191: 13-26.

Choct M, 2015. Feed non-starch polysaccharides for monogastric animals: classification and function. Anim Prod Sci 55: 1360-1366.

Choct M and Hughes R, 1997. The nutritive value of new season grains for poultry. Rec Adv Anim Nut Aust 11: 146-150.

Cowieson AJ, Acamovic T and Bedford MR, 2003. The effect of phytase and phytic acid on endogenous losses from broiler chickens. Brit Poult Sci 44: 23-24.

Dworkin LD, Levine GM, Farber NJ *et al.,* 1976. Small intestinal mass of the rat is partially determined by indirect effects of intraluminal nutrition. Gastroenterology 71: 626-630.

Fuller MF and Reeds PJ, 1998. Nitrogen cycling in the gut. Ann Rev Nut 18: 385

Govil KS, Nayak, Baghel R *et al.,* 2017. Performance of broiler chicken fed multicarbohydrases supplemented low energy diet. Vet World 10: 727.

Hussain K, Abbas R, Abbas A *et al.,* 2021. Anticoccidial and biochemical effects of artemisia brevifolia extract in broiler chickens. Braz J Poult Sci 23: 1-6.

Ikegami S, Tsuchihashi F, Harada H *et al.,* 1990. Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. J Nut 120: 353-360.

Johnston SL, Williams SB, Southern LL *et al.,* 2004. Effect of phytase addition and dietary calcium and phosphorus level on plasma metabolites and total-tract nutrient digestibility in pigs. J Anim Sci 82: 705-714.

Khadem A, Lourenço M, Delezie E *et al.,* 2016. Does release of encapsulated nutrients have an important role in the efficacy of xylanase in broilers? Poult Sci 95: 1066-1076.

Knudsen KEB, 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult Sci 93: 2380-93.

Parsaie S, Shariatmadari F, Zamiri M *et al.,* 2007. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers compared with a maize-based diet. Brit Poult Sci 48:594-600.

Pirgozliev V, Rose S and Kettlewell P, 2006. Effect of ambient storage of wheat samples on their nutritive value for chickens. Brit Poult Sci 47: 342-9.

Ravindran V, Johnston S, Camden B, *et al.,* 2001. Influence of storage of New Zealand wheats on energy availability for poultry. Rec Adv Anim Nut Aust 13: 30-18.

Rehman A, Hesheng H, Rahman MAU *et al.,* 2019. Evaluation of efficacy of compound chinese medicinal herbs against mycoplasma synoviae using different lab tests in mouse and chicken. Int J Agric Biol 22: 647-54.

Rostagno H, Albino L, Donzele J *et al.,* 2011. Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Animal Science Department UFV, Viçosa, MG, Brazil.

Sakamoto K, Hirose H, Onizuka A *et al.,* 2000. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. Journal of Surgical Research 94:99-106.

Saki A, Matin HH, Zamani P, *et al.,* 2011. Various ratios of pectin to cellulose affect intestinal morphology, DNA quantitation, and performance of broiler chickens. Livestock Science 139: 237-244.

Shahid I, Sharif M, Yousaf M *et al.,* 2020. Emulsifier supplementation response in ross 308 broilers at 1-10 days. Braz J Poult Sci 22: 1-6. http://dx.doi.org/10.1590/1806-9061-2020-1301

Shahid I, Sharif M, Yousaf M *et al.,* 2021. Effect of exogenous emulsifier (lyso-phospholipid) supplementation in the broiler diet, on the feed intake and growth performance during grower phase. Braz J Poult Sci 23: 1-8. http://dx.doi.org/10.1590/1806-9061-2020-1354

Swennen Q, Janssens GPJ, Millet S *et al.,* 2005. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: Endocrine functioning and intermediary metabolism. Poul Sci 84:1051-7.

Van der Klis, J, Van Voorst A and Van Cruyningen C, 1993. Effect of a soluble polysaccharide (carboxy methyl cellulose) on the physico-chemical conditions in the gastrointestinal tract of broilers. Brit Poult Sci 34: 971-983.

Yaghobfar A and Kalantar M, 2017. Effect of non-starch polysaccharide (NSP) of wheat and barley supplemented with exogenous enzyme blend on growth performance, gut microbial, pancreatic enzyme activities, expression of glucose transporter (SGLT1) and mucin producer (MUC2) genes of broiler chickens. Braz J Poult Sci 19: 629-38.