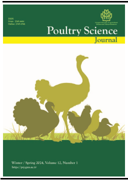

Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2023.21428.1944

GUASNR

Does the Type of Cereal Grain in Broiler Diets Affect the Susceptibility to Ascites Syndrome?

Ali Afifian Tehrani[1], Fariborz Khajali[1], Mohammad Amir Karimi Torshizi[2] & Mohammad Reza Akbari[1]

[1] Department of Animal Science, Faculty of Agriculture, Sharekhord University, Sharekhord, Iran
[2] Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

Poultry Science Journal 2024, 12(1): 35-41

Keywords
Diet
Broiler
Cereal grain
Ascites syndrome

Corresponding author
Ali Afifian Tehrani
aliafifiantehrani@gmail.com

Article history
Received: June 6, 2023
Revised: September 4, 2023
Accepted: October 16, 2023

Abstract
An experiment was conducted to address the relationship between the types of cereal grain and ascites syndrome in broiler chickens raised in a high-altitude region. A number of 252 one-day-old broiler chicks (Cobb 500 strain) were randomly assigned to three dietary treatments including a corn-soybean diet (corn diet), a wheat-soybean diet (wheat diet), and a barley-soybean diet (barley diet). Chicks were kept on experimental diets for 42 days, during which growth performance and carcass traits were assessed, and blood samples collected. Results indicated that corn substitution with wheat and barley significantly suppressed feed intake, growth rate, and lipogenesis as reflected in lower serum cholesterol, triacylglycerols, and abdominal fat deposition ($P = 0.0001$). Feeding barley resulted in down-regulation of TLR2 and GLUT2 genes but caused an up-regulation of FABP2 gene in jejunum as compared to the corn control. Feeding wheat and barley did not significantly affect the relative weights of the heart, and the right to total ventricular weight ratio (RV/TV). Mortality from ascites was not significant among treatment groups. In conclusion, substituting corn for wheat or barley could impair broiler performance, but under the conditions of this experiment had no significant impact on susceptibility to ascites.

Introduction

Modern poultry diet is mainly composed of corn and soybean meal. Recent attempts to use corn for the biofuel industry in major corn-producing countries (Alavijeh and Karimi., 2019) have led to increased demand for this feedstuff and subsequently impacted its availability and price for animal feed. Although prices of corn are volatile and unpredictable, more stability in the prices of wheat and barley, at least in some regions, making them alternative grains that can replace corn in broiler diets. Besides economic considerations, the production of wheat and barley is more sustainable. While corn production requires large amounts of water and fertilizer (N, P, K), wheat and barley can be grown in more diverse environments, making them more environmental-friendly and less resource-intensive (Grote *et al*., 2021).

Ascites syndrome is a serious health issue in broiler meat production worldwide, particularly at high altitudes (Hossain and Akter, 2022; Yousefi *et al*., 2013). The condition is characterized by the accumulation of massive transudate in the abdominal cavity of birds, leading to difficulty in breathing and even death (Saedi and Khajali, 2010). Nutrition has a crucial role in the incidence of broiler ascites. Numerous nutritional interventions have been developed to prevent ascites in broiler chicken (reviewed in Khajali and Wideman, 2016). Despite intense research, some questions have not been appropriately addressed. One of them, for example, is that whether the types of cereal grain in the diet could affect the susceptibility to ascites syndrome? This is vital information when corn is replaced by wheat, barley, or other cereal grains. Given little scientific evidence as to how the type of cereal grain may impact broiler ascites, it is very important to address the question through research.

Please cite this article as Ali Afifian Tehrani, Fariboruz Khajali, Mohammad Amir Karimi Torshizi & Mohammad Reza Akbari. 2024. Does the Type of Cereal Grain in Broiler Diets Affect the Susceptibility to Ascites Syndrome?. Poult. Sci. J. 12(1): 35-41.

With the increasing trend in the substitution of corn for other grains, the concern remains as to how this nutritional strategy affects ascites incidence.Therefore, the present study was conducted to shed light on the relationship between the types of cereal grain and ascites syndrome in broiler chickens raised in a high-altitude region.

Materials and methods

Birds and experimental diets

This study was carried out at the Poultry Research Center of Shahrekord University, Shahrekord, Iran. The geological location had a 2100 m elevation. The experimental protocols used in the study were aligned with the Animal Care and Use Committee of Shahrekord University.

A total of 252 one-day-old broiler chicks (Cobb 500 strain) with uniform body weight (46 ± 0.60 g) were randomly assigned to 18-floor pens (2.8 × 2.8 m). Six such pens were randomly assigned to each of the three dietary treatments. Dietary treatments included: corn-soybean diet (corn diet), wheat-soybean diet (wheat diet), and barley-soybean diet (barley diet). Table 1 indicates the chemical composition of experimental diets in various feeding phases. The experimental period lasted for 42 days, composed of three feeding phases: starter (1-8 d), grower (9-18 d), and finisher (19-42 d).

Table 1. Composition of the experimental diets

Item (% unless noted)	Starter (1–8d)			Grower (9-18d)			Finisher (19–42d)		
	Corn	Wheat	Barley	Corn	Wheat	Barley	Corn	Wheat	Barley
Corn	55.5	-	-	62.66	-	-	65.5	-	-
Wheat	-	60.6	-	-	60.2	-	-	70	-
Barley	-	-	54.5	-	-	52	-	-	61
Soybean meal(44% CP)	36.5	31	32.5	30.5	31	34.7	27.5	21.6	25.12
Soy oil	3.4	4	8.5	2.5	4.8	9.4	3	4.5	10
Dicalcium phosphate	1.8	1.2	1.6	1.7	1.1	1.4	1.5	1	1.2
Oyster shell	1.29	1.6	1.4	1.3	1.5	1.3	1.1	1.4	1.3
Salt	0.38	0.37	0.37	0.34	0.35	0.35	0.44	0.35	0.35
DL-Methionine	0.33	0.38	0.33	0.3	0.33	0.25	0.28	0.35	0.33
L-Lysine	0.3	0.35	0.3	0.2	0.22	0.1	0.18	0.3	0.2
Mineral supplement*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin supplement **	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated composition									
AME (kcal/kg)	3000	3000	3000	3050	3050	3050	3100	3100	3100
CP	21.5	21.5	21.5	19.5	19.5	19.5	18.5	18.5	18.5
Met+Cys	1.05	1.05	1.05	0.85	0.85	0.85	0.81	0.81	0.81
Lys	1.45	1.45	1.45	1.28	1.28	1.28	1.20	1.20	1.20
Na+K–CL (mEq/kg)	180	180	180	181	179	181	185	184	183

* Provided the following per kg of diet: vitamin A (trans retinyl acetate), 3600IU; vitamin D3 (cholecalciferol), 800 IU; vitamin E (dl-α-tocopherol acetate), 7.2 mg; vitamin K_3, 1.6 mg; thiamine, 0.72 mg; riboflavin, 3.3 mg; niacin, 0.4 mg: pyridoxin, 1.2 mg; cobalamin, 0.6 mg; folic acid, 0.5 mg; choline chloride, 200 mg.

** Provided the following per kg of diet: Mn (from MnSO4-H2O), 40mg; Zn (from ZnO), 40mg; Fe (from FeSO4-7H2O), 20 mg; Cu (from CuSO4-5H_2O), 4 mg; I [from Ca (IO3)2-H2O], 0.64 mg; Se (from sodium selenite),0.08 mg.

Measurements

The chemical analysis of corn, wheat, barley, and soybean meal was obtained before feed formulation. This included crude protein and crude fiber. Body weight and feed consumption were recorded during the feeding phases and throughout the trial. Body weight gain and feed conversion ratio (FCR) were also calculated taking into account mortality weights. All mortality in the present trial was necropsied and the right to total ventricular weight ratio (RV/TV) was determined to confirm ascites. On day 42, blood samples (3 mL) were collected from two birds per pen (10 birds per treatment). The birds were then euthanized to determine carcass characteristics. The hearts were also removed and dissected to determine RV/TV.

Blood samples were centrifuged at 2500 × g for 10 min to separate sera for the determination of cholesterol, triglycerides, low-density lipoprotein-cholestrol (LDL-c), and high-density lipoprotein-cholestrol (HDL-c) using commercial kits according to the manufacturer's guidelines (Pars Azmun Kits; Pars Azmun, Tehran, Iran). Serum malondialdehyde (MDA) and nitric oxide (NO) were also measured according to Behrooj *et al*., (2012) and Nair and Turner (1984), respectively. An aliquot of blood was used to enumerate heterophils and lymphocytes using the May-Grunwald and Giemsa staining. All chemicals were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

RNA extraction, cDNA synthesis, and Quantitative Real-time PCR Analysis

Samples of jejunum from two birds per pen (10 birds per treatment) were cut at 2×2 cm^2 and kept in liquid

nitrogen until further analysis. The samples were homogenized and treated with a digestion buffer (RNX-Plus reagent; Sina Clon Bioscience, Karaj, Iran). An aliquot of 200 µL chloroform was added to each homogenate, mixed, and centrifuged at 16,000 g for 15 min at 4°C. Supernatants were collected and used for RNA extraction by mixing with equal volumes of isopropanol and rinsing with 75% ethanol, followed by centrifugation at 10000 g. The resulting pellets were dissolved in diethyl dicarbonate (DEPC)-treated water and treated with a DNase (Sina Clon Bioscience) to enhance RNA purity. RNA was subsequently quantified by the absorbance of A260/280 using a bio photometer (Eppendorf, USA). RNA fraction having the A260/280 ratio between 1.8 and 2.2 was used for cDNA synthesis using the Prime Script™ RT Reagent Kit (Takara Bio Inc., Japan). The levels of toll-like receptor2 (TLR2), mucin2 (MUC2), glucose transporter2 (GLUT2), fatty acid binding protein2 (FABP2), and tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein zeta (YWHAZ) transcripts were determined by real-time RT-PCR using SYBR® Premix Ex Taq™ II kit (TliRnase H Plus; Takara Bio Inc.). YWHAZ was considered an endogenous reference gene to normalize the input load of cDNA across samples (Hassanpour *et al*., 2018). Table 2 depicts the primers (forward and reverse), designed with the online software of Primer-Blast (www.ncbi.nlm.nih. gov/tools/primer-blast/index.cgi?LINK_LOC=Blast Home).
Transcripts in three replications per tissue sample were amplified in a real-time PCR cycler (Rotor-Gene Q 6000; Qiagen, USA). An amount of 1 µL cDNA, 10 µL of SYBR Premix Ex Taq II Mix, and 0.5 µmol/L of specific primers were mixed and brought to a total volume of 20 µL. Thermal cycles are set at 95°C for 30 s, 94°C for 40 s, 64°C for 35 s, and 72°C for 30 s. Data were subjected to LinReg PCR software version 2012.0 (Amsterdam, the Netherlands) (Ruijter *et al*., 2009). The relative transcript levels (gene / YWHAZ) were estimated using the Paffl method (Dorak, 2006; Hassanpour *et al*., 2018).

Table 2. Details of the primers used for quantitative real-time PCR

Target	Primers	PCR product (bp)	Annealing Temperature (C)	Accession no.
TLR2	F:5´-GGTGTTCCTGTTCATCCTCATCCT-3´ R:5´-TTGGGCTTCCGCTTGGCTT-3´	238	62	XM_01530138001
Muc2	F:5´-CTGGACTTCACGGACACCTG-3´ R:5´-CTCAACACAGCCCCCTCTAC-3´	442	62	XM_421035
GLUT2	F:5´-CGCAGAAGGTGATAGAAGCA-3´ R:5´-ATGGTGAGGATGTGAGGGG-3´	577	63	Z22932
FABP2	F: 5´-TGGAAGCAATGGGCGTGA-3´ R: 5´-GTCGATGGTACGGAAGTTGC-3´	143	60	Z_55050
YWHAZ	F:5´-AGGAGCGAGCTGTCCAATG-3´ R:5´-TCCAAGATGACCTACGGGCTC-3´	84	60	NM_001031343.1

Abbreviations: TLR2: toll-like receptor2; MUC2: mucin2; GLUT2: glucose transporter 2; FABP2: fatty acid binding protein 2; YWHAZ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; bp, base pair.

Statistical Analysis
Data were analyzed in a completely randomized design (CRD) using SAS (2007) software and treatment means were compared by Duncan's multiple range test.

Results
Table 3 represents the growth performance of broiler chickens that received different cereal grains throughout the experiment. Corn substitution with wheat and barley significantly plunged feed intake during the starter (*P*=0.0001), grower (*P*= 0.0001), finisher (*P*=0.0004), and entire trial (*P*=0.0001). The substitution also led to a reduction in body weight gain in all feeding phases (*P*=0.0001). FCR deteriorated when corn was replaced by wheat or barley in the starter (*P*=0.0004), grower (*P*= 0.0050), finisher (*P*=0.0398), and entire trial (*P*=0.0483).
Effects of different types of cereal grains on carcass composition and ascites mortality are presented in Table 4. Dietary treatments did not affect carcass yield, breast yield, and thigh yield. However, the relative weight of intestines was significantly (*P*=0.0117) increased in broilers fed with barley. Feeding wheat and barley resulted in less accumulation of fat in the abdomen (*P*=0.0001) when compared to the corn-fed control. The relative weights of the heart, liver, bursa, spleen and the RV/TV ratio were not significantly affected by dietary treatments. Mortality from ascites was not significantly changed when dietary treatments were applied.

Table 3. Effects of type of cereal grain in broiler diets on growth criteria

	Dietary treatments			*SEM*	*P*-value
	Corn	Wheat	Barley		
Weight gain (g/bird)					
1-8 d	108.8[a]	77.6[b]	91.4[b]	2.99	0.0001
9-18 d	306.8[a]	247.1[b]	237.9[b]	4.34	0.0001
19-42 d	1595.1[a]	1444.7[b]	1252.6[c]	41.32	0.0004
1-42 d	2011.1[a]	1770.1[b]	1582.1[c]	47.13	0.0001
Feed intake (g/bird)					
1-8 d	151.7[a]	130.6[b]	144.4[b]	2.51	0.0001
9-18 d	430.3[a]	360.5[b]	382.2[b]	8.21	0.0001
19-42 d	3107.8[a]	2958.6[b]	2737.5[c]	49.58	0.0155
1-42 d	3690.1[a]	3450.2[b]	3266.4[c]	56.64	0.0004
FCR					
1-8 d	1.40[b]	1.69[a]	1.59[a]	0.036	0.0004
9-18 d	1.42[b]	1.46[b]	1.61[a]	0.038	0.0050
19-42 d	1.94[b]	2.05[b]	2.18[a]	0.045	0.0398
1-42 d	1.84[b]	1.96[ab]	2.08[a]	0.0601	0.0483

[a,b,c]Means in the same raw with different letters are significantly different.

Table 4. Effects of type of cereal grain on carcass characteristics and ascites mortality (42 days of age)

(as % BW)	Dietary treatments			*SEM*	*P*-value
	Corn	Wheat	Barley		
Carcass yield	66.4	66.5	64.1	1.04	0.1995
Breast yield	25.1	25.1	24.2	0.42	0.1885
Thigh yield	18.2	18.8	19.1	0.46	0.1655
Intestines	5.06[b]	5.46[b]	6.45[a]	0.298	0.0117
Abdominal fat	1.61[a]	1.22[b]	1.23[b]	0.048	0.0001
Liver	2.61	2,51	2.46	0.012	0.5016
Bursa	0.08	0.07	0.08	0.005	0.3989
Spleen	0.11	0.12	0.12	0.012	0.5639
Heart	0.43	0.47	0.47	0.016	0.3668
RV:TV†	0.22	0.23	0.24	0.011	0.7971
Ascites mortality (%)	15.5	18.9	21.1	4.62	0.6321

[a,b,] Means in the same raw with different letter superscripts are significantly different.
† RV:TV – right ventricle to total ventricle weight ratio.

Table 5 shows serum and blood variables in broilers fed corn, wheat, and barley. Feeding wheat and barley increased serum levels of malondialdehyde but decreased serum levels of nitric oxide. Substitution of corn for wheat and barley was associated with reduced circulatory levels of triacylglycerols, (66.7 *vs* 58.5 and 49.9 mg/dL) total cholesterol (133.5 *vs* 116.1 and 115.5 mg/dL), LDL-cholesterol (34.3 *vs* 30.4 and 26.5 mg/dL), and glucose (228 *vs* 215 and 191 mg/dL). Feeding wheat and barley caused a significant increase in the heterophils to lymphocytes ratio (P=0.0016) and aspartate transaminase (AST; P=0.0003).

Table 5. Effects of type of cereal grain on serum and blood variables

Metabolites	Dietary treatments			*SEM*	*P*-value
	Corn	Wheat	Barley		
Malondialdehyde (μmol/L)	7.35[c]	9.37[a]	8.12[b]	0.12	0.0001
Nitric Oxide (μmol/L)	20.8[a]	18.1[b]	11.0[c]	0.62	0.0001
Triacylglycerol (mg/dL)	66.7[a]	58.5[b]	49.9[b]	2.16	0.0001
Cholesterol (mg/dL)	133.5[a]	116.1[b]	115.5[b]	1.13	0.0001
HDL (mg/dL)	83.1[a]	80.0[a]	76.1[b]	1.25	0.0032
LDL (mg/dL)	34.3[a]	30.4[b]	26.5[c]	0.92	0.0001
Hematocrit (%)	43	42	42	0.47	0.2564
H/L ratio†	0.38[a]	0.46[b]	0.46[b]	0.014	0.0016
Blood glucose (mg/dL)	228[a]	215[b]	191[c]	1.01	0.0001
AST (U/L) ††	169.1[b]	174.5[a]	173.0[a]	0.72	0.0003

[a,b,c] Means in the same raw with different letter superscripts are significantly different.
†Heterophils/Lymphocytes, †† Aspartate aminotransferase

Table 6 represents the effects of different types of cereals on jejunal gene expression in broiler chickens. TLR2 and GLUT2 genes were significantly down-regulated (P=0.0309 and P=0.0007, respectively) when barley was replaced with corn. On the other hand, FABP2 showed a significant up-regulation (*P*=0.0009) when corn was replaced by barley or wheat. MUC2 was not significantly influenced by dietary grain substitution.

Table 6. Effect of type of cereal grain on gut gene expression

	Dietary treatments			*SEM*	*P*-value
	Corn	Wheat	Barley		
TLR2	1.482[a]	1.128[ab]	0.982[b]	0.124	0.0309
MUC2	1.357	2.018	2.031	0.304	0.2289
GLUT2	0.871[a]	0.616[a]	0.298[b]	0.085	0.0007
FABP2	0.710[c]	2.191[b]	4.404[a]	0.572	0.0009

[a,b,c] Means in the same raw with different letter superscripts are significantly different.

Abbreviations: TLR2, toll-like receptor2; MUC2, mucin2; GLUT2, glucose transporter2; FABP2, fatty acid binding protein2; bp, base pair

Discussion

Birds fed on wheat and barley diets significantly reduced their feed consumption compared to the corn control. The reduced feed intake partly accounts for reduced weight gain and poor FCR. In line with our findings, Kalantar *et al*. (2016) reported that broilers received corn, wheat, and barley consumed on average 46, 41.5, and 43.2 g/d during 1 to 21 days and consequently gained 37.1, 32.8, and 33.7 g/d. The poor performance of birds receiving wheat and barley is also linked to the high contents of non-starch polysaccharides (NSPs) in these cereal grains, which have anti-nutritional effects (Choct and Annison, 1992). Non-starch polysaccharides contents of wheat are mainly arabinoxylan polymers and those in barley are mainly composed of β-glucans. Both types of NSP impact the digestibility of energy and protein in broiler chickens through an increase in digesta viscosity (Choct and Annison, 1992) or trapping nutrients (cage effect; Khadem *et al*., 2016), which account for poor weight gain and FCR in the present experiment.

A significantly higher proportion of intestines in broilers fed with barley is associated with a higher crude fiber (CF) content in barley. The crude fiber content of barley (5.4% by analysis) was twice as high as corn and wheat (2.4 and 2.7%, respectively). The (1,3–1,4)-β-glucans in the endosperm cell walls of barley are responsible for the high viscosity of digesta, and thickening the gut wall is, in fact, a response to high viscosity (Von Wettstein *et al*., 2000). The liver weight tended to be lower in wheat and barley diets but the difference was insignificant with the corn control. Similarly, Zaefarian *et al*. (2019) reported that broilers fed with different cereal sources (corn, barley, and wheat) had lower weight gain and feed intake, but liver weights were unaffected by dietary treatments.

Feeding wheat and barley caused a significant decline in abdominal fat deposition, along with reductions in circulatory levels of cholesterol, triacylglycerols, and LDL-c. The lipid-lowering mechanism of wheat and barley is presumably linked to the presence of high NSPs contents, which reduced the digestibility of lipids on one hand, and increase biliary clearance on the other hand (Knarrenborg *et al*., 2002; Taheri *et al*., 2016). In the present experiment, fatty acid-binding protein 2 (FABP2) which facilitates the diffusion of fatty acids into enterocytes showed an over-expression in wheat and barley groups. This finding also confirms the suppression of lipid utilization in these treatments, and the FABP2 up-regulation seems to be a compensatory response to overcome the situation.

Substituting corn for wheat and barley resulted in a decline in blood glucose levels. Seemingly, two reasons are responsible for this finding. First, glucose-transporter 2 (GLUT2) in the gut was down-regulated in the wheat and barley groups. Second, NSPs contents of wheat and barley are resistant to enzymatic hydrolysis, and less glucose had eventually absorbed into the bloodstream.

The main focus of this work was the occurrence of mortality from ascites in broiler chickens fed with different cereal grains. While mortality from ascites was numerically higher in the wheat and barley groups, the differences were not statistically significant compared to the corn group. Meanwhile, relative heart weights and RV/TV were comparable among treatment groups. These findings collectively indicate that the type of cereal grain in broiler diets may not affect susceptibility to ascites. There seem to be two inconsistent opinions on the relationship between the type of cereal grain and susceptibility to ascites in broiler chickens. On one hand, NSPs content in wheat and barley could augment ammonia production in the gut, predisposing birds to ascites (Balog *et al*., 1994; Anthony *et al*., 1994). On the other hand, wheat and barley NSPs suppress lipogenesis, as observed herein, and suppressed lipogenesis help prevent ascites (reviewed in Khajali, 2022). The findings of the present study are better matched with the latter opinion. The reduced growth rate of birds in wheat and barley groups could also explain insignificant ascites mortality among the treatment groups. It is noteworthy that ascites is a syndrome linked to a fast growth rate. The reduced growth rate in the wheat and barley diets could explain the lack of a significant difference between these treatment groups with the corn control concerning the occurrence of ascites syndrome. However, more research needs to be conducted to fully understand underlying mechanisms.

Conclusion

Replacement of corn for wheat or barley could impair broiler performance without any significant impact on susceptibility to ascites. An insignificant ascites mortality could be linked to reduced lipogenesis and reduced growth rate when corn was replaced by wheat or barley.

References

Alavijeh MK & Karimi K. 2019. Biobutanol production from corn stover in the US. Industrial Crops and Products Journal, 129: 641-653. DOI: 10.1016/J.Indcrop.2018.12.054

Anthony NB, Balog JM, Staudinger FB, Wall CW, Walker RD & Huff WE. 1994. Effect of a urease inhibitor and ceiling fans on ascites in broilers: 1. Environmental variability and incidence of ascites. Poultry Science Journal, 73(6): 801-809. DOI: 10.382/ps.0730801

Balog JM, Anthony NB, Wall CW, Walker RD, Rath NC & Huff WE. 1994. Effect of a urease inhibitor and ceiling fans on ascites in broilers: 2. Blood variables, ascites scores, and body and organ weights. Poultry Science Journal, 73(6): 810-816. DOI: 103382/ PS. 0730810

Behrooj N, Khajali F & Hassanpour H. 2012. Feeding reduced-protein diets to broilers subjected to hypobaric hypoxia is associated with the development of pulmonary hypertension syndrome. British Poultry Science, 53: 658–664. DOI:10.1080/00071668.2012.727082

Choct M & Annison G. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: Roles of viscosity and gut microflora. British Poultry Science Journal, 33: 821–834. DOI: 10.1080/00071669208417524

Dorak M. 2006. Real time PCR. Taylor & Francis, Oxford University Publication: UK. DOI: 10.4324/9780203967317.

Grote U, Fasse A, Nguyen TT & Erenstein O. 2021. Food security and the dynamics of wheat and maize value chains in Africa and Asia. Frontiers in Sustainable Food Systems.4: p.617009. DOI: 10.3389/fsufs.2020.617009

Hassanpour H, Bahadoran S, Farhadfar F, Fallahi Chamali Z, Nazari H & Kaewduangta W. 2018. Identification of reliable reference genes for quantitative real-time PCR in lung and heart of pulmonary hypertensive chickens. Poultry Science Journal, 97: 4048-4056. DOI: 10.3382/ps/pey258

Hossain ME & Akter N. 2022. Further insights into the prevention of pulmonary hypertension syndrome (ascites) in broiler: a 65-year review. World's Poultry Science Journal, 78(3): 641-688. DOI:10.1080/00439339. 2022.2090305

Kalantar M, Khajali F & Yaghobfar A. 2016. Effect of cereal type and enzyme addition on performance, pancreatic enzyme activity, intestinal microflora and gut morphology of broilers. Poultry Science Journal, 4(1): 63-71. DOI: 10.22069/psj.2016.2973

Khadem A, Lourenço M, Delezie E, Maerten L, Goderis A, Mombaerts R, Höfte M, Eeckhaut V, VanImmerseel F & Janssens GPJ. 2016. Does release of encapsulated nutrients have an important role in the efficacy of xylanase in broiler? Poultry Science Journal, 95(5): 1066-1076. DOI: 10.3382/ps/pew002

Khajali F & Wideman RF. 2016. Nutritional approaches to ameliorate pulmonary hypertension in broiler chickens. Journal of Animal Physiology and Animal Nutrition, 100(1): 3-14. DOI:10.1111/jpn.12315

Khajali F. 2022. Managing broiler production challenges at high altitude. Veterinary Medicine and Science, 8(4): 1519-1527. DOI: 10.1002/vms3.784

Knarrenborg A, Engberg RM, Jensen SK & Jensen BB. 2002. Quantitative determination of bile salt hydrolase activity in bacteria isolated from the small intestine of chickens. Applied and Environmental Microbiology, 68: 6425-6428. DOI: 10.1128/ AEM.68.126425-6428.2002

Ruijter J, Ramakers C, Hoogar SW, Karlen Y, Baker O, Vandenhoff M & Moorman A. 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Research. 37: 1-12. DOI: 10.1039/nar/gkp.45

Nair V & Turner G. 1984. The thio barbituric acid test for lipid peroxidation: Structure of the adduct with malondialdehyde. Lipids, 19(10): 804–805. DOI: 10.1007/BF02534475

Saedi M & Khajali F. 2010. Blood gas values and pulmonary hypertension as affected by dietary sodium source in broiler chickens reared at cool temperature in a high-altitude area. Acta Veterinaria Hungarica 58(3): 379-388. DOI: 10.1556/avet.58.2010.3.10

SAS Institute Inc. 2007. SAS/STAT user`s Guide Release 9. SAS Institute Inc., Cary, NC.

Taheri HR, Tanha N & Shahir MH. 2016. Effect of wheat bran inclusion in barley-based diet on villus morphology of jejunum, serum cholesterol, abdominal fat and growth performance of broiler chickens. Journal of Livestock Science and Technologies, 4(1): 9-16. DOI: 10.221.3/JLST.2016.1374

Von Wettstein D, Mikhaylenko G, Froseth JA & Kannangara CG. 2000. Improved barley broiler feed with transgenic malt containing heat-stable (1, 3–1, 4)-β-glucanase. Proceedings of the National Academy of Sciences Journal, 97(25): 13512-13517. DOI: 10.1073/pnas.97.25.13512

Yousefi A, Khajali F, Hassanpour H, & Khajali Z. Dietary L-carnitine improves pulmonary hypertensive response in broiler chickens subjected to hypobaric hypoxia. Journal of Poultry Science 50(2): 143-149. DOI:10.2141/jpsa.0120145

Zaefarian F, Abdollahi MR, Cowieson A & Ravindran V. 2019. Avian liver: the forgotten organ. Animals Journal, 9(2): 63-71. DOI: 10.1080/00071669208417524