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The impact of different inclusion levels of whole barley in feed on growth performance, carcass, and gastrointestinal traits of broiler chickens

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Abstract: The aim of the present research was to evaluate the effect of feeding graded levels of whole barley (WB) on broiler chicken growth and carcass performance, gastrointestinal morphology, and function. A total of 800 male Ross 308 broiler chickens (1-day-old) were randomly assigned to four treatment groups with four replicate pens of 50 birds each. Diets differed in their WB dosage: without WB (NO-WB); low WB amount (LOW-WB) dosages: 4% (1–7 days of age), 8% (8–21 days of age), 15% (22–35 days of age); medium WB amount (MEDIUM-WB) dosages: 6% (1–7 days of age), 12% (8–21 days of age), 20% (22–35 days of age); high WB amount (HIGH-WB) dosages: 8% (1–7 days of age) 16% (8–21 days of age), 25% (22–35 days of age). Body weight, average daily gain, and mortality of chickens did not statistically differ between treatments ($P > 0.05$). The feed conversion ratio was higher in the treatments diluted with WB. There were no differences in ammonia nitrogen concentration except for increased butyrate concentration in the caecum and reduced digesta viscosity in the broiler ileum in groups fed LOW-WB, MEDIUM-WB, and HIGH-WB diets ($P < 0.05$). The high WB amount in the diets increased *Lactobacillus* spp. in the ileal contents and improved ileum morphology ($P < 0.05$). The inclusion of graded levels of WB in the diets had no effect on carcass performance but it seemed to enhance the gastrointestinal tract development.

Keywords: productivity; digestive processes; morphology; histomorphology; short-chain fatty acids

Recent years have witnessed the growing popularity of feeding whole grains to poultry. This development can be attributed to economic reasons, to the beneficial influence of whole grain on the development and health of birds' digestive tracts, and greater consumer acceptance towards more 'natural' feeding systems (Gabriel et al. 2008).

Until now, the scientific research has been mostly based on evaluating the effect of whole wheat in the diets on poultry production results, carcass

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traits, gastrointestinal tract development and function, its morphometric characteristics, as well as its microbiota profile (Amerah and Ravindran 2008; Gabriel et al. 2008; Zdunczyk et al. 2013; Truong et al. 2017). Studies have shown that feeding whole wheat improves birds' growth performance and feed efficiency (Ravindran et al. 2006). Most probably due to advanced development of the gastrointestinal tract (GIT) (Amerah and Ravindran 2008), including an increase in duodenal villus to crypt length and surface ratios (Gabriel et al. 2008). Additionally, large feed particles promote gizzard development and activity, and, consequently, feed stays longer in the gizzard and its particles are more uniform when moving to the small intestine, favouring their digestion (Amerah and Ravindran 2008; Svihus 2011).

Barley is one of the most extensively cultivated cereal grains in the world due to its compatible adaptability, controllable grain availability, and market price. Barley has a medium protein content, which falls between oats and wheat, and many varieties of barley are higher in protein and amino acid content than maize (Biel et al. 2020). The major dietary fibre components of barley are non-starch polysaccharides (NSP) such as pentosans and β-glucans which are predominantly found in the aleurone layers. Because NSPs are poorly soluble in water, barley β-glucans typically form gels in aqueous media, resulting in increased intestinal viscosity in birds (Raza et al. 2019). The NSPs are fermented by intestinal microbes during their passage through the intestinal tract and are converted into short-chain fatty acids (SCFA). Therefore, the SCFA content in the chyme is an indicator for the extension of NSP fermentation by microbiota (Gabriel et al. 2008). According to Sonnenburg and Backhed (2016), diet is a key mediator of the composition and metabolic function of the gastrointestinal microbiota. Because different bacterial species have different substrate preferences and growth requirements, the chemical composition of the digesta, to a large extent, determines the composition of the bacterial community in the GIT (Guyard-Nicodeme et al. 2015).

According to Singh et al. (2014), the results published in the literature on the effects of whole grain feeding on performance and nutrient utilization of broilers are contradictory and are due to several confounding factors, including differences in feeding strategies, inclusion level of whole grain, its type and quality, length of feeding, and age of birds. Some reports show beneficial effects, while others fail to show any advantages, and some show poorer performance. However, whole wheat is still used more frequently, and research-based data on the effects of whole barley on broiler chickens remains limited. Therefore, the possibly beneficial effects of whole barley must be thoroughly investigated in light of the principles of the broiler chicken health status. So, this study was designed to demonstrate that feeding of whole barley (WB) is potentially beneficial for the development of whole gastrointestinal morphology and function in broiler chickens without negatively affecting carcass performance. Therefore, the objective of this study was to perform a feeding experiment by evaluating the effects of different WB levels as part of pelleted mixed feed (MF) on broiler chicken performance, carcass traits, development of the digestive tract, ileum histomorphology, viscosity, and *Lactobacillus* spp. counts in its digesta.

MATERIAL AND METHODS

Experimental design

The trial with broiler chickens was performed in accordance with EU Directive 2010/63/EEC and EC Recommendation 2007/526/EC for the accommodation and care of animals used for experimental and other scientific purposes. The trial was conducted following the regulations of the Republic of Lithuania for animal welfare and handling and a sub-statutory act by the State Food and Veterinary Service of the Republic of Lithuania regarding the confirmation of the order of the animals used for experiments, research, storage, maintenance, and operating requirements.

The trial with broiler chickens was carried out in an experimental poultry house. The feeding trial was performed on 800 1-day-old male Ross 308 broiler chickens, which were individually weighed and randomly assigned to four dietary treatments, with four replicate pens of 50 birds in each.

Treatments

Starting from day one, treatments were as follows: NO-WB – diet without whole barley supplementation; LOW-WB – diet with low WB supplementation

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[WB inclusion: 4% (day 1–7 of age, 1st period), 8% (days 8–21 of age, 2nd period) and 15% (day 22–35 of age, 3rd period)]; MEDIUM-WB – diet with medium WB supplementation [WB inclusion: 6% (day 1–7 of age, 1st period), 12% (days 8–21 of age, 2nd period) and 20% (day 22–35 of age, 3rd period)]; HIGH-WB – diet with high WB supplementation [WB inclusion: 8% (day 1–7 of age, 1st period), 16% (days 8–21 of age, 2nd period) and 25% (day 22–35 of age, 3rd period)].

Birds were fed a standard wheat-soybean meal compound diet *ad libitum* for five weeks, with the ingredient composition of the diets being identical for all treatments except for the proportion of WB. The diets were formulated to meet the nutrient and energy requirements for Ross 308 broiler chickens. The temperature and lighting program were consistent with the recommendations of Aviagen (2018). Birds in each pen were allowed free access to feed from a hanging feeder and fresh water from a drinker throughout the experiment. Calculated feed ingredients and analysed nutrients from one to 35 days in the diets are presented in Table 1.

Analysis of the feed chemical composition

The contents of crude protein (CP), crude fat (CF) and crude fibre (CFA) in the diets were determined according to AOAC (2011) using Methods No. 984.13, 978.10, and 991.42, respectively. Official methods were used to analyse the minerals Ca and P [Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA 1976)].

Growth and slaughter performance, sample collection

Body weight (BW), daily weight gain (DWG), feed conversion ratio (FCR) and mortality were recorded weekly and at the beginning of each period.

At the end of the feeding trial (35 days old), sixty birds per treatment, 15 birds per pen, were randomly selected, weighed, fasted overnight, and then slaughtered according to the recommendations for euthanasia of experimental animals (Close et al. 1997) in a commercial slaughterhouse. All samples were collected post-mortem.

Immediately after slaughtering, the eviscerated carcass, breast, thigh, abdominal fat, and removed separate parts of digestive tracts (e.g., intestines, gizzard, proventriculus, heart, liver, pancreas) were weighed and calculated as a proportion of live body weight. Before weighing the gizzard, proventriculus, and heart, the surrounding fat was removed and assigned to abdominal fat. Then the separated parts of the digestive tract were opened, the contents were removed, washed with physiological solution, blotted dry with filter paper, and weighed with a lab scale (KERN PBS/PBJ). The length of each intestinal segment (caecum, large and small intestine) was measured with flexible tape on a glass surface.

Digesta assay

The determination of SCFA concentrations in the caecum was conducted according to the method described by Kliseviciute et al. (2014) and ammonia nitrogen was measured by the Foss-Tecator method ASN 3302.

The viscosity of the ileal contents was measured at a shear rate of 30/s using a Brookfield Digital DV-II cone/plate viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA).

Lactobacillus spp. in the ileal contents were enumerated according to standard method ISO 15214:1998 (1998) (Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of mesophilic lactic acid bacteria – colony-count technique at 30 °C. International Standard ISO 15214, Geneva, Switzerland), using the plate count technique on de Man, Rogosa, and Sharpe (MRS) agar (Sigma, St. Louis, MO, USA), after incubation at 37 °C for 72 h under anaerobic conditions. After incubation, the total number of colonies was counted, and the results were expressed as the number of log of unit-forming colonies (log CFU/g).

Intestinal morphology analysis

Examinations of intestinal morphology were carried out according to the method of Iji et al. (2001). Samples were fixed with a 10% neutral formalin solution. Using standard procedures for histological evaluation, the tissues were embedded in paraffin and cut with a Leica RM 2235 rotary microtome (Leica Microsystems, Nussloch, Germany) into 4 µm thick tissue sections, which were stained with haematoxylin and eosin. Prepared histological

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Table 1. Calculated and analysed nutrients in the diets of chicken broilers fed different levels of whole barley (WB)

Indices	NO-WB[a]			LOW-WB[b]			MEDIUM-WB[c]			HIGH-WB[d]		
Period	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Days	1–7	8–21	22–35	1–7	8–21	22–35	1–7	8–21	22–35	1–7	8–21	22–35
WB dosage (%)	–	–	–	4	8	15	6	12	20	8	16	25
Ingredients (g/kg)												
Wheat	563	563	563	523	483	413	503	443	363	483	403	313
Soybean meal	248	248	248	248	248	248	248	248	248	248	248	248
Maize	100	100	100	100	100	100	100	100	100	100	100	100
Barley	–	–	–	40	80	150	60	120	200	80	160	250
Soybean oil	39	39	39	39	39	39	39	39	39	39	39	39
Fish meal	10	10	10	10	10	10	10	10	10	10	10	10
Salt	2	2	2	2	2	2	2	2	2	2	2	2
Monocalcium phosphate	12	12	12	12	12	12	12	12	12	12	12	12
Limestone	13	13	13	13	13	13	13	13	13	13	13	13
Sodium bicarbonate	3	3	3	3	3	3	3	3	3	3	3	3
L-Threonine	1	1	1	1	1	1	1	1	1	1	1	1
DL-Methionine	4	4	4	4	4	4	4	4	4	4	4	4
L-Lysine HCL	4	4	4	4	4	4	4	4	4	4	4	4
Coccidiostats Clinacox	1	1	1	1	1	1	1	1	1	1	1	1
Analysed and calculated nutritional value (g/kg)												
Crude protein (N × 6.25)	218	185	184	221	191	191	219	219	181	217	183	178
Crude fat	53.5	81.6	81.6	53.7	80.3	80.3	52.9	76.4	71.8	52.1	73.7	68.5
Crude fibre	24.4	29	29	24.5	28.9	28.9	24.5	28.7	28.5	24.5	28.6	28.4
Lysine[e]	12.7	11	11	12.5	11.2	11.2	12.3	10.7	10	12.2	10.4	10
Methionine/cysteine[e]	9.5	8.6	8.6	9.4	8.3	8.3	9.3	8	7.8	9.2	7.9	7.6
Threonine[e]	8.3	7.4	7.4	8.1	7.2	7.2	8.0	7	6.7	7.9	6.8	6.6
Thryptophan[e]	2.7	2.2	2.2	2.7	2.2	2.2	2.7	2.1	2.1	2.6	2.1	23
Calcium	9.0	8.5	8.5	8.8	8.3	8.3	8.7	7.8	7.2	8.5	7.5	6.8
Phosphorus	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.3	6.2	6.4	6.2	6
Sodium[e]	1.7	1.6	1.6	1.6	1.5	1.5	1.6	1.5	1.4	1.6	1.4	1.3
Chlorine[e]	2.1	2.5	2.5	2.2	1.8	1.8	2.2	1.7	1.6	2.1	1.6	1.5
Linoleic acid[e]	15.8	18.2	18.2	26.3	29.4	29.4	25.9	28.2	26.8	25.6	27.4	25.9
Metabolisable energy[e] (MJ/kg)	12.9	13.3	13.3	12.7	13.2	13.2	12.7	13.2	13.1	12.7	13.1	13.0

HIGH-WB = diet with high WB; LOW-WB = diet with low WB; MEDIUM-WB = diet with medium WB; NO-WB = diet without WB

[a]Addition of 5.0% premix introduced to 1 kg of diet: vitamin A 1200 IU, vit. D_3 500 IU; Fe 54.00 mg/kg, Zn 65.00 mg/kg, Cu 9.0 mg/kg, Mn 58.5 mg/kg, I 0.90 mg/kg, Se 0.14 mg/kg, Co 0.18 mg/kg, vit. E 36 mg/kg, vit. K_3 3.50 mg/kg, vit. B_1 2.50 mg/kg, vit. B_2 7.20 mg/kg, vit. B_4 600 mg/kg, vit. B_6 5.0 mg/kg, vit. B_{12} 30 µg/kg, biotin 150 µg/kg, folic acid 1.8 mg/kg, nicotinic acid 40.5 mg/kg, calcium-D pantothenate 12.6 mg/kg

[b]Addition of 5.0% premix introduced to 1 kg of diet: vitamin A 1020 IU, vit. D_3 425 IU; Fe 51.00 mg/kg, Zn 59.50 mg/kg, Cu 8.5 mg/kg, Mn 55.25 mg/kg, I 0.85 mg/kg, Se 0.13 mg/kg, Co 0.17 mg/kg, vit. E 34 mg/kg, vit. K_3 2.98 mg/kg, vit. B_1 2.13 mg/kg, vit. B_2 6.80 mg/kg, vit. B_4 600 mg/kg, vit. B_6 4.3 mg/kg, vit. B_{12} 30 µg/kg, biotin 137 µg/kg, folic acid 1.3 mg/kg, nicotinic acid 38.25 mg/kg, calcium-D pantothenate 11.90 mg/kg

[c]Addition of 5.0% premix introduced to 1 kg of diet: vitamin A 960 IU, vit. D_3 400 IU; Fe 48.00 mg/kg, Zn 56.00 mg/kg, Cu 8.0 mg/kg, Mn 52.00 mg/kg, I 0.80 mg/kg, Se 0.12 mg/kg, Co 0.16 mg/kg, vit. E 32 mg/kg, vit. K_3 2.80 mg/kg, vit. B_1 2.00 mg/kg, vit. B_2 6.40 mg/kg, vit. B_4 600 mg/kg, vit. B_6 4.0 mg/kg, vit. B_{12} 29 µg/kg, biotin 128 µg/kg, folic acid 1.2 mg/kg, nicotinic acid 36.00 mg/kg, calcium-D pantothenate 11.20 mg/kg

[d]Addition of 5.0% premix introduced to 1 kg of diet: vitamin A 960 IU, vit. D_3 375 IU; Fe 45.00 mg/kg, Zn 52.50 mg/kg, Cu 7.5 mg/kg, Mn 48.75 mg/kg, I 0.75 mg/kg, Se 0.11 mg/kg, Co 0.15 mg/kg, vit. E 30 mg/kg, vit. K_3 2.63 mg/kg, vit. B_1 1.88 mg/kg, vit. B_2 6.00 mg/kg, vit. B_4 600 mg/kg, vit. B_6 3.8 mg/kg, vit. B_{12} 28 µg/kg, biotin 119 µg/kg, folic acid 1.1 mg/kg, nicotinic acid 33.75 mg/kg, calcium-D pantothenate 10.50 mg/kg

[e]Calculated according to the NRC (1994)

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samples were examined using an Olympus BX63 microscope (Olympus Corp., Tokyo, Japan), an Olympus DP72 video camera (Olympus Corp., Tokyo, Japan), and the computer Image-Pro® Plus program system for Windows, v7.0 (Media Cybernetics, Inc., Bethesda, MD, USA). Control and test treatments of the intestinal villus height and crypt depth in broilers were morphologically measured. Each sample was subjected to 10 intestinal villus measurements (best expressed) to measure the height, and in the same places 10 measurements were done to measure the thickness of the lining. The thickness of the lining of the villus height was obtained by subtracting the crypt depth ratio.

Statistical analysis

SPSS for Windows, v25.0 was used for data analysis (IBM SPSS Inc., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used, and a post-hoc test (Fisher's least significant difference test) was applied to detect differences between groups; P-values less than 0.05 ($P < 0.05$) were considered statistically significant.

RESULTS

Growth performance

The growth performance results are presented in Table 2. Body weight, average daily gain, and mortality (during the entire trial period) of broiler chickens did not differ between treatments on days 1–7, 8–21, 22–35, or in the total period (1–35) ($P > 0.05$). However, FCR in MEDIUM-WB and HIGH-WB groups between days 8 and 21 was higher as compared to the NO-WB group and in the HIGH-WB group as compared with the MEDIUM-WB group ($P < 0.05$) between days 21 and 35. Also, FCR results in the HIGH-WB group were higher ($P < 0.05$) as compared with the NO-WB and MEDIUM-WB groups during all trial periods (Table 2).

Slaughter performance

Feeding MEDIUM-WB and HIGH-WB diets compared to diets without WB supplementation resulted in the increased ($P < 0.05$) relative weight

Table 2. Performance of Ross 308 male broiler chickens fed different levels of whole barley (WB)

Period (days)	NO-WB	LOW-WB	MEDIUM-WB	HIGH-WB	SEM	P-value
Body weight (g)						
1	46.85	46.86	46.86	46.88	0.02	0.070
7	174.05	176.29	178.54	175.60	6.98	0.533
21	955.64	949.15	921.38	933.88	38.86	0.395
35	2 335.89	2 319.24	2 276.73	2 283.05	67.19	0.396
Average daily gain (g)						
1–7	18.17	18.49	18.49	18.81	1.00	0.534
8–21	55.83	55.20	55.20	53.06	2.51	0.291
22–35	98.59	97.86	97.86	96.81	3.00	0.473
1–35	65.40	64.92	64.92	63.71	1.92	0.396
Feed conversion ratio (%)						
1–7	0.92	0.94	0.89	0.89	0.03	0.204
8–21	1.54^{a}	1.69^{ab}	1.73^{b}	1.76^{b}	0.09	0.024
22–35	1.99^{ab}	1.99^{ab}	1.90^{a}	2.11^{b}	0.09	0.050
1–35	1.74^{a}	1.80^{ab}	1.76^{a}	1.88^{b}	0.05	0.014
Mortality (%)						
1–35	1.25	1.63	0.75	1.38	0.57	0.153

HIGH-WB = diet with high WB; LOW-WB = diet with low WB; MEDIUM-WB = diet with medium WB; NO-WB = diet without WB

^{a,b}Means in a row with different superscripts differ ($P < 0.05$)

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of the gizzard (Table 3). Moreover, in broiler chickens from MEDIUM-WB group an increase of the relative heart weight in comparison with those from LOW-WB and HIGH-WB groups was found (Table 4). In contrast, the relative weight of the proventriculus and relative weight of the intestine were lower ($P < 0.05$) in broiler chickens of LOW-WB and HIGH-WB groups. Carcass yield, relative weight of thigh and breast, relative weight of abdominal fat, relative weight of liver, pancreas, intestinal length without caeca or caecal length of birds in our study were not statistically significant between animals from all treatment groups (Table 4).

Digesta properties

There was no treatment effect on ammonia nitrogen concentration in the caecal contents of birds at 35 days of age between treatments ($P > 0.05$; Table 5). The concentration of butyric acid increased in the caecal contents of the chickens from LOW-WB and MEDIUM-WB groups in comparison with the NO-WB group ($P < 0.05$; Table 5). More specifically, the inclusion of a medium WB dose increased the concentration of butyric acid by 68% compared to a diet without WB supplementation ($P < 0.05$).

In birds fed MEDIUM-WB and HIGH-WB diets, we determined lower ileal digesta viscosity compared

Table 3. Viscosity and counts of *Lactobacillus* spp. in ileal digesta and the histomorphology of the ileum in 35-day-old broilers fed different levels of whole barley (WB)

Items	NO-WB	LOW-WB	MEDIUM-WB	HIGH-WB	SEM	*P*-value
Viscosity (mPa.s)	4.02^{a}	3.54^{a}	2.62^{b}	3.12^{b}	0.32	0.001
Lactobacillus spp. (log CFU/g)	6.24^{a}	6.88^{ab}	7.64^{b}	7.34^{b}	0.38	0.002
Villus height (μm)	597.38^{a}	804.98^{b}	865.66^{c}	952.5^{d}	22.03	0.000
Crypt depth (μm)	183.57^{a}	240.64^{b}	196.34^{a}	204.82^{a}	7.27	0.000
Villus height/crypt depth	3.26^{a}	3.35^{a}	4.42^{b}	4.66^{b}	0.18	0.000

HIGH-WB = diet with high WB; LOW-WB = diet with low WB; MEDIUM-WB = diet with medium WB; NO-WB = diet without WB

[a–d]Means in a row with different superscripts differ ($P < 0.05$)

Table 4. Eviscerated carcass yields, relative organ weights, and intestine length in 35-day-old broilers fed different levels of whole barley (WB)

Items	NO-WB	LOW-WB	MEDIUM-WB	HIGH-WB	SEM	*P*-value
Eviscerated carcass yield (%)	73.12	74.61	72.47	73.35	2.29	0.364
Live weight (%)						
Breast	27.50	29.50	26.30	29.00	1.68	0.075
Thigh	18.50	19.80	18.90	17.70	1.27	0.118
Gizzard	1.40^{a}	1.51^{a}	2.09^{b}	1.82^{b}	0.18	0.001
Proventriculus	0.66^{a}	0.45^{b}	0.53^{ab}	0.45^{b}	0.08	0.016
Heart	0.76^{ab}	0.69^{a}	0.86^{b}	0.70^{a}	0.06	0.013
Liver	3.03	2.84	3.35	2.88	0.26	0.064
Pancreas	0.34	0.27	0.33	0.34	0.04	0.073
Abdominal fat	1.05	0.95	1.10	1.03	0.26	0.575
Intestine	9.17^{a}	6.58^{b}	8.70^{a}	7.07^{b}	0.68	0.001
Intestinal length without caeca (cm)	232.60	222.40	217.80	213.60	10.94	0.102
Caecal length (cm)	41.40	39.00	41.60	38.40	1.61	0.084

HIGH-WB = diet with high WB; LOW-WB = diet with low WB; MEDIUM-WB = diet with medium WB; NO-WB = diet without WB

[a,b]Means in a row with different superscripts differ ($P < 0.05$)

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Table 5. Ammonia nitrogen and short-chain fatty acid (SCFA) concentration in the caecum of 35-day-old broilers fed different levels of whole barley (WB)

Items	NO-WB	LOW-WB	MEDIUM-WB	HIGH-WB	SEM	*P*-value
Ammonia nitrogen (mg%)	267.48	311.36	240.29	244.33	39.70	0.092
Short-chain fatty acids (µmol/g)						
Acetic acid	20.52	20.72	20.43	26.61	5.47	0.276
Propionic acid	6.43	8.25	7.79	5.88	1.59	0.155
Butyric acid	5.22[a]	8.93[b]	8.75[b]	6.47[ab]	1.68	0.042
Total SCFA	32.16	37.90	36.98	38.96	7.36	0.370

HIGH-WB = diet with high WB; LOW-WB = diet with low WB; MEDIUM-WB = diet with medium WB; NO-WB = diet without WB

[a,b]Means in a row with different superscripts differ ($P < 0.05$)

with diets without WB ($P < 0.05$) (Table 3). *Lactobacilli* spp. concentrations in the ileum tended to be higher with the increasing amount of WB inclusion (Table 5). The highest *Lactobacilli* spp. counts were found in the ileal digesta of chickens fed MEDIUM-WB and HIGH-WB diets compared to the chickens on NO-WB diets ($P < 0.05$).

Histological data is presented in Table 3. In animals fed LOW-WB, MEDIUM-WB, and HIGH-WB diets, the higher mean villus height of the ileum was observed in comparison with those fed a NO-WB diet ($P < 0.05$). However, the highest mean crypt depth in the ileum was recorded in broiler chickens fed LOW-WB diets compared to the chickens in the other groups ($P < 0.05$). Animals from the MEDIUM-WB and HIGH-WB groups were characterized by the higher villus height/crypt depth ratio in the ileum in comparison with those from the NO-WB and LOW-WB groups ($P < 0.05$).

DISCUSSION

The different WB levels (LOW-WB, MEDIUM-WB, and HIGH-WB groups) as part of pelleted MF have no effect on the broiler chicken production performance (BW, ADG, and mortality) except for the FCR in comparison with the group without WB (NO-WB group). Our findings disagree with a study by Dahloum et al. (2017), who reported that the addition of barley up to 50% of the broiler diet resulted in equal or even better performance when compared to the diet without WB supplementation. Most other studies that have evaluated the post-pelleting inclusion of whole grain have reported no adverse effects on weight gain but a decrease or no effect on feed intake and FCR (Amerah and Ravindran 2008; Singh et al. 2014).

It is well established that higher structural components in diets increase gizzard grinding activity and stimulate its development (Svihus 2011). When structural components were added to the diet of broiler chickens, the gizzard volume increased significantly, as observed by Svihus (2011) and Truong et al. (2017). In their experiments, it was suggested that birds are able to grind WB efficiently in the gizzard, and they concluded that it is possible that WB can replace rolled or ground barley without a negative effect on performance. In the present study, feeding a pelleted diet supplemented with higher amounts of WB increased the empty weight of the gizzard, the relative weight of the heart, and reduced the weight of the proventriculus. There is a scarcity of data on the effects of WB feeding on the proventriculus and heart size. Taylor and Jones (2004) reported the reduced proventriculus proportional mass by 22% and 16%, respectively, in broilers fed diets containing either whole wheat or WB compared to those receiving ground grains. In contrast, several other authors did not observe any change in the relative weight of proventriculus or heart with the inclusion of whole grain (Nahas and Lefrancois 2001; Ravindran et al. 2006; Amerah and Ravindran 2008). Taylor and Jones (2004) speculated that the absence of the proventriculus dilation with whole grain feeding would have positive effects on bird health by lowering the incidence of ascites.

Since gizzard weights are increased with whole grain feeding, some reduction in carcass yield could be expected if the gizzards are not recovered or saleable (Singh et al. 2014). In our study, the feeding

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of different amounts of WB had no effect on carcass yield and the relative weight of breast, thigh, and abdominal fat. Unchanged abdominal fat content was reported earlier by Nahas and Lefrancois (2001) in an experiment with broiler chickens fed grower diets with 15% of WB. Our findings could only partially confirm the results reported by Asadi and Eydivandi (2011), who observed that chickens fed diets containing barley at 20% and 40% did not show a significant difference in carcass percentage but chickens that consumed diets with 40% barley had increased abdominal fat.

In this trial, there were no significant differences between treatments in ammonia nitrogen concentration in the caecal contents of birds at 35 days of age. Ammonia is one of the microbial fermentation products with toxic effects on animals (Visek 1978). The production of ammonia is closely related to bacterial activity and is associated with certain toxic events in the GIT (Salminen et al. 1998). We expected that by increasing the amount of WB in chicken diets, the development of the bird's GIT would be improved, inducing a better resorption of amino acids in the intestine by leaving fewer amino acids for bacterial fermentation. So, less ammonia nitrogen will be absorbed into the bloodstream, thereby improving the overall health status (Ghadban 2002). However, this was not the case in this study.

According to Kheravii et al. (2018), adding different sources and levels of dietary fibre on top of the diet is a potential tool to manipulate SCFA production and concentration. In the work of Biggs and Parsons (2009), SCFA concentrations in the caecal digesta were not correlated with dietary whole wheat content. But our study demonstrates that the higher the amount of WB, the higher the concentration of butyric acid in the chicken caecal contents. Similar results were reported by Zdunczyk et al. (2013) in an experiment performed with turkeys fed a higher amount of whole wheat grain, which increased SCFA concentrations in the caecal digesta. Besides, our study confirmed the results of Boguslawska-Tryk et al. (2015) and Den Besten et al. (2013), who suggested that diets with larger particle size can increase a retention time in the crop, resulting in enhanced fermentation activity and SCFA production.

In our experiment, the viscosity of broiler chicken ileal digesta was lower than 10 mPa s in all treatments. Thus, according to Bedford and Apajalahti (2001), the passage rate in the birds was not reduced, and grain endogenous enzymes can act in a normal way. The inclusion of MEDIUM-WB and HIGH-WB in the diet resulted in a marked decrease in ileal digesta viscosity. The results are different from those of Lazaro et al. (2003), who found that WB increases the viscosity of the intestinal chyme and reduces the digestibility of nutrients. According to Yasar (2003), feeding wheat-based diets with a fine particle size was found to increase digesta viscosity compared to birds fed a whole wheat diet. The degree of digesta viscosity is dependent upon the amount of NSP, which varies amongst cereal grains (Amerah and Ravindran 2008). It is possible that the relatively low amount of NSP of WB in this study contributed to the lack of an effect of WB inclusion on the viscosity of the digesta.

Besides the lower viscosity, the broiler chickens fed MEDIUM-WB and HIGH-WB diets showed a favourable effect on beneficial bacterial counts in the ileum, i.e., a higher abundance of *Lactobacillus* spp. These results confirm the findings of other researchers who reported that diets containing whole grain showed higher counts of *Lactobacillus* spp. and lower counts of Coliform bacteria (Santos et al. 2008). Engberg et al. (2002) stated that an increase in *Lactobacillus* spp. is usually considered beneficial to the host because it can prevent the colonization of pathogenic bacteria such as *Escherichia coli*.

The composition and activity of the microbiota are known to affect the intestinal morphology, such as improving the intestinal villus structure (Bedford and Apajalahti 2001). However, there is a lack of experiments evaluating the effects of WB inclusion on intestinal morphology in broiler chickens. In the few experiments evaluating the effects of diet composition on intestinal morphology (Husveth et al. 2015), whole wheat grain had no influence on villus height or crypt depth of the broiler ileum. In another study, lower crypt depth was observed in the duodenum, but no differences were found in the histological parameters of the ileum of chickens fed diets with 20% wheat grain (Gabriel et al. 2008). The present study showed that the dietary dilution with a higher amount of WB is followed by an increase in the villus height, in the depth of ileum crypts, and the villus height to crypt depth ratio, and this effect was accompanied by beneficial changes in the caecal microbiota composition.

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CONCLUSION

Different WB levels (from 4% in starter diets up to 25% in finisher diets) as part of pelleted broiler chicken diets had no effect on productivity parameters, except a negative effect on feed conversion ratio. However, the inclusion of higher amounts of non-degradable carbohydrates from WB was fermented by intestinal microbes during their accelerated passage through the intestinal tract, resulting in better digestive processes, such as higher amounts of butyric acids, lowered viscosity of digesta, enhanced *Lactobacillus* counts, increased villus height, crypt depth in the ileum and their ratio. These events are helpful in suppressing the development of the main pathogenic microorganisms such as *Salmonella*, *Clostridium*, and *Escherichia coli*, and may positively affect the health of the birds. Feeding a pelleted diet supplemented with a higher amount of WB (up to 25%) resulted in a significant increase in the relative weight of the gizzard and heart and a decreased relative weight of the proventriculus. None of the levels of WB inclusion had an effect on the eviscerated carcass yield, relative weights of breast, thigh, and abdominal fat, or other analysed weights of digestive tract.

Conflict of interest

The authors declare no conflict of interest.

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