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Effects of Rice Hulls and Acidifier Supplementation on Growth Performance and Gut Physiology in Broiler Chickens

Research Article

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ABSTRACT

The experiment was conducted to study how adding rice hulls and an acidifier to the diet affected performance, histomorphology, and microbial colonization in the gastrointestinal system of broiler chickens. A completely randomized design was used to test eight treatments and five replications of 1000 day-old-male Arbor Acres broiler chickens (45±1 g). Dietary treatments included control (no additives 1-42 days), acidifier (control+0.1% acidifier from 1-42 days), RH10 (3% rice hull from 1-10 days and control diet from 11-42 days), RH10 + acidifier (3% rice hulls from 1-10 days and control diet+0.1% acidifier from 11-42 days), RH24 (3% rice hulls from 1-24 days and control diet from 25-42 days), RH24 + acidifier (3% rice hulls from 1-24 days and control diet+0.1% acidifier from 25-42 days), RH42 (3% rice hulls from 1-42 days) and RH42 + acidifier (3% of the rice hulls+0.1% acidifier from 1-42 days). Feed conversion ratio significantly decreased ($P<0.05$) in the RH24 + acidifier group compared with RH10 and control groups after 24 and 42 days. The RH42 + acidifierbirds had lower mortality than RH24+acidifier birds ($P<0.05$). Also, at 42 days of age, feeding RH42 + acidifier diet increased villus height/crypt depth ($P<0.01$). Acidifiers increased the Lactobacillus population in the small intestine of broiler chickens. The lowest gizzard pH was observed in birds fed 3% rice hulls from 0 to 42 days of age ($P<0.05$). In general, the addition of 30 kg/ton of rice hulls to the broiler feed, from 0 to 42 days of age, caused the highest possible weight gain and the lowest mortality.

KEY WORDS rice hulls, broiler, gastrointestinal tract, gizzard, *lactobacillus*, liveability.

INTRODUCTION

Billions of chickens are raised every year to meet the need for animal protein. Millions of tons of food are required by these birds. To increase nutrient absorption and digestive efficiency in broiler chickens, experts all over the globe are investigating novel feed management methods. It has been demonstrated that nutrient absorption and production performance improve when broiler chickens have a healthy gut (Jha *et al.* 2019). Manipulating the dietary fiber content of broilers' diets has become more popular lately as a feeding strategy to improve gut health. Rice hull contains 2.4% crude protein (CP), 0.5% ether extracts (EE), 12.9% ash, 54.2% crude fibre (CF), 74% total fiber, 72% insoluble fiber, 2% soluble fiber, 54.7% acid detergent fiber (ADF) and 19.2% acid detergent lignin (ADL) (Jiménez-Moreno *et al.* 2016). Several studies showed that dietary inclusion of fiber sources at moderate levels improves broiler perform ance (Jiménez-Moreno *et al.* 2016), nutritional efficiency (Donadelli *et al.* 2019) and gut health of broiler chickens (Sozcu, 2019). Mateos *et al.* (2012) reported that dietary inclusion of insoluble fibers significantly reduced feed passing to the gizzard, resulting in higher gizzard activity, feed mixing, and digestive secretions, leading increase absorption of the ingested feed. Also, adding fiber to the diet improved the growth of health-promoting bacteria in the broiler's gut (De Maesschalck *et al.* 2019), decreased intestinal pH due to fermentation of fiber into short-chain fatty acids in the small and large intestines (Kheravii *et al.* 2018; Jha *et al.* 2019), and consequently increased mineral absorption (Bournazel *et al.* 2018), stimulated enterocyte proliferation (Jha *et al.* 2019), and improved immune system. Another strategy for improving gut health by lowering intestinal pH is to supplement organic acids in broilers' diets. Organic acids are used in poultry feeds to enhance feed stability and prevent microbial contamination (Van Immerseel *et al.* 2009). Acidifiers inhibit the growth of pathogenic microbes in the chicken gut, thereby improving growth and performance of broiler chickens (Palamidi *et al.* 2017). Also, acidifiers enhance nutrient absorption via changing in gut morphology (Adil *et al.* 2011). It has been well documented that organic acids provide numerous benefits for poultry gut health, nutrient digestibility, and growth performance (Abdollahi *et al.* 2020). Thus, adding organic acids and/or insoluble fibers may have beneficial togut development by affecting the height and depth of the duodenal villus, crypt depth, and the size and shape of the gizzard in broilers (Naderinejad *et al.* 2016; Sabour *et al.* 2019). Therefore, this study aimed to determine whether adding rice hullalone or along with an acidifier has beneficial effects on performanceof broiler chickens and their digestive system during their different rearing phases.

MATERIALS AND METHODS

Throughout this experiment, animal welfare standards were followed. All animal care and experimental methods were permitted by the Isfahan University of Technology's Animal Policy and Welfare Committee.

Birds and experimental diets

The experimental diets were developed using the Arbor Acres catalog as a guide (Tables 1, 2, and 3) (Aviagen, 2018). The Arbor Acres manuals were followed for rearing chicken (including light schedule and intensity, temperature, and humidity control) throughout the experiment (Aviagen, 2018). The experimental diets were iso-caloric and iso-nitrogenous, and the diets were all based on corn-soybeans. The pelleted form of feed was produced. The starter pellet was 2 mm in diameter, but the finishing pellet had a 4 mm diameter. Five replications of 25 broilers each were included in the eight treatments. The birds were housed in floor pens (150×130×65 cm) with feed and water supplied on an *ad-libitum* basis. Dietary treatments included a control diet (the basal pelleted feed from 1 to 42 days), acidifier (control diet+0.1% acidifier from 1 to 42 days), RH10 (dietary inclusion of 3% rice hull from 1 to 10 days and the control diet from 11 to 42 days of age), RH10+acidifier (3% rice hulls from 1 to 10 days and the control diet+0.1% acidifier from 11 to 42 days of age), RH24 (3% rice hulls from 1 to 24 days and the control diet from 25 to 42 days of age), RH24 + acidifier (3% rice hulls from 1 to 24 days and the control diet + 0.1% acidifier from 25 to 42 days of age), RH42 (3% rice hulls from 1 to 42 days of age) and RH42 + acidifier (3% of the rice hulls+the 0.1% acidifier from 0 to 42 days of age). The diets included 3.0% and 0.1% of rice hulls and acidifier, respectively. The acidifier was in powder form and consisted of propionic acid, formic acid, sodium butyrate, ammonium propionate, calcium formate, calcium lactate, and ammonium formate.

Data and sample collection

For starters, growers, and finishers phases as well as the entire experimental period, the average feed intake, average weight gain, and average feed conversion ratio (average feed intake/average weight gain) were calculated. All the birds were weighed two hours after feed withdrawal. At 24 and 42 days of age, one bird from each pen was sacrificed to measure the weight of proventriculus, gizzard, duodenum, jejunum, ileum, and cecum, and expressed as the percentage of bird's weight. The pH of the gizzard and ileum contents were determined on day 42using the pH meter (HANNA HI2211) and by homogenizing one gram of the contents with 9 mL of distilled water (Pang and Applegate, 2007). On days 24 and 42, intestinal tissue samples (2 jejunal sub-samples of one bird of each pen) were collected, and gently flushed twice with physiological saline solution, and tissue sections were fixed in 10% formalin (Saadatmand *et al.* 2019). To analyze digesta microbial growth, the ileal contents of the birds and surrounding areas close to the intersection of the ileum and cecum were collected.

Statistical analyses

Data were analyzed using SAS software (SAS, 2003) in a completely randomized design (CRD). There were 15 and 10 replications for some treatments based on feeding the same diet during the starter and grower phases in this experiment. This led to an unbalanced analysis of data. Tukey's tests were used to determine the difference between treatments. The models used in this study were as:

For CRD design: $Y_{ij} = \mu + Ti + eij$

Where:
Y_{ij}: each observation.
μ: mean.
Ti: treatment effect.
eij: error effect.

For CRD design with the sample:
$Yijk = \mu + Ti + eij + \sigma ijk$

Where:
Yijk: each observation.
μ: mean.
Ti: treatment effect.
eij: error effect.
σijk: sampling error.

The significance levels were considered at $P < 0.05$.

RESULTS AND DISCUSSION

Daily feed intakes for starter, grower, and finisher phases were similar among the dietary treatments (Table 4). This indicates that neither rice hull nor acidifier inclusion in broilers' diet had an impact on feed consumption. Hetland *et al.* (2003) found that dietary inclusion of insoluble fiber from oat in a pelleted feed had no effect on feed intake. González-Alvarado *et al.* (2010) also reported that dietary inclusion of insoluble fiber, such as oat hulls, did not affect the daily feed intake of broiler chickens. In another study, Jiménez-Moreno *et al.* (2016) reported that broilers fed low-fiber diets tended to have better daily gain and feed conversion ratio when their diets were pelleted and insoluble fiber sources were added, but the effect of adding insoluble fiber sources on these traits were more pronounced when diets fed in the pelleted rather than mashed form.

There was no significant difference in daily feed intake between the birds fed diet supplemented with or without acidifier.

Table 1 Ingredient composition of the experimental diets during the starter period (1 to 10 days)

Items (g/kg)	Control	Acidifier	Rice hull (RH)	RH + Acidifier
Corn	504.15	503.15	449.55	448.55
SBM (44%)	392.80	392.80	401.10	401.10
Wheat gluten	40.00	40.00	40.00	40.00
Soy oil	23.40	23.40	40.70	40.70
Rice hull	0.00	0.00	30.00	30.00
Acidifier	0.00	1.00	0.00	1.00
Limestone	12.50	12.50	11.90	11.90
Mono Calcium	11.10	11.10	10.80	10.80
Salt	1.90	1.90	1.90	1.90
Lysine	2.70	2.70	2.60	2.60
Methionine	3.20	3.20	3.30	3.30
Threonine	1.00	1.00	1.00	1.00
Vitamin and mineral premix[1]	5.00	5.00	5.00	5.00
Sodium bicarbonate	2.20	2.20	2.10	2.10
Hostazim P 10000[2]	0.05	0.05	0.05	0.05
Nutrient analysis				
Metabolizable energy (kcal/kg)	3000	3000	3000	3000
Crude protein (g/kg)	239.80	239.80	240.10	240.10
Crude fiber (g/kg)	30.5	30.5	41.2	41.2
Digestible lysine (g/kg)	12.80	12.80	12.80	12.80
Digestible methionine (g/kg)	6.30	6.30	6.30	6.30
Methionine + digestible cysteine (g/kg)	9.50	9.50	9.50	9.50
Digestible threonine (g/kg)	8.60	8.60	8.60	8.60
Calcium (g/kg)	9.60	9.60	9.60	9.60
Available phosphorus (g/kg)	4.80	4.80	4.80	4.80
Sodium (g/kg)	1.60	1.60	1.60	1.60

[1] Premix provided per kg of feed: Vitamin A: 12000 IU; Cholecalciferol: 5000 IU; α-tocopherol: 45 IU; Menadione: 2.4 mg; Thiamin: 2.6 mg; Riboflavin: 6.6 mg; Niacin: 55 mg; Pyridoxine: 5.5 mg; D-pantothenate: 25 mg; Folic acid: 1.5 mg; Biotin: 0.1 mg; Choline: 1500 mg; Cyanocobalamin: 0.015 mg; BHT: 1 mg; Fe: 50 mg; Mn: 90 mg; Cu: 10 mg; I: 1 mg; Se: 0.25 mg and Zn: 85 mg.
[2] Huvepharma NV, Uitbreidingstraat, Belgium.

Table 2 Ingredient composition of the experimental diets during the grower period (11 to 24 days)

Items (g/kg)	Control	Acidifier	Rice hull (RH)	RH + Acidifier
Corn	550.35	548.35	494.65	492.55
SBM (44%)	361.80	362.10	370.90	371.30
Wheat gluten	20.00	20.00	20.00	20.00
Soy oil	32.70	33.40	50.20	50.90
Rice hull	0.00	0.00	30.00	30.00
Acidifier	0.00	1.00	0.00	1.00
Limestone	11.30	11.30	10.70	10.70
Mono Calcium	9.00	9.00	8.70	8.70
Salt	2.10	2.10	2.20	2.20
Lysine	2.10	2.10	2.00	2.00
Methionine	3.00	3.00	3.10	3.10
Threonine	0.70	0.70	0.70	0.70
Vitamin and mineral premix[1]	5.00	5.00	5.00	5.00
Sodium Bicarbonate	1.90	1.90	1.80	1.80
Hostazim P 10000[2]	0.05	0.05	0.05	0.05
Nutrient analysis				
Metabolizable energy (kcal/kg)	3100	3100	3100	3100
Crude protein (g/kg)	215.80	215.80	216.30	216.30
Crude fiber (g/kg)	29.4	29.4	40.2	40.1
Digestible lysine (g/kg)	11.50	11.50	11.50	11.50
Digestible methionine (g/kg)	5.80	5.80	5.80	5.80
Methionine + digestible cysteine (g/kg)	8.70	8.70	8.70	8.70
Digestible threonine (g/kg)	7.70	7.70	7.70	7.70
Calcium (g/kg)	8.70	8.70	8.70	8.70
Available phosphorus (g/kg)	4.30	4.30	4.30	4.30
Sodium (g/kg)	1.60	1.60	1.60	1.60

[1] Premix provided per kg of feed: Vitamin A: 12000 IU; Cholecalciferol: 5000 IU; α-tocopherol: 45 IU; Menadione: 2.4 mg; Thiamin: 2.6 mg; Riboflavin: 6.6 mg; Niacin: 55 mg; Pyridoxine: 5.5 mg; D-pantothenate: 25 mg; Folic acid: 1.5 mg; Biotin: 0.1 mg; Choline: 1500 mg; Cyanocobalamin: 0.015 mg; BHT: 1 mg; Fe: 50 mg; Mn: 90 mg; Cu: 10 mg; I: 1 mg; Se: 0.25 mg and Zn: 85 mg.
[2] Huvepharma NV, Uitbreidingstraat, Belgium.

Table 3 Ingredient composition of the experimental diets during finisher period (25 to 42 days)

Items (g/kg)	Control	Acidifier	Rice hull (RH)	RH + Acidifier
Corn	595.35	593.75	540.05	537.95
SBM (44%)	319.50	319.50	328.30	328.70
Wheat gluten	20.00	20.00	20.00	20.00
Soy oil	32.00	32.60	49.40	50.10
Rice hull	0.00	0.00	30.00	30.00
Acidifier	0.00	1.00	0.00	1.00
Limestone	10.50	10.50	9.90	9.90
Mono Calcium	8.00	8.00	7.70	7.70
Salt	2.10	2.10	2.20	2.20
Lysine	2.10	2.10	2.00	2.00
Methionine	2.90	2.90	3.00	3.00
Threonine	0.60	0.60	0.60	0.60
Vitamin and mineral mix[1]	5.00	5.00	5.00	5.00
Sodium Bicarbonate	1.90	1.90	1.80	1.80
Hostazim P 10000[2]	0.05	0.05	0.05	0.05
Nutrient analysis				
Metabolizable energy (kcal/kg)	3150	3150	3150	3150
Crude protein (g/kg)	201.40	201.70	201.70	201.70
Crude fiber (g/kg)	27.9	27.8	38.6	38.6
Digestible lysine (g/kg)	10.60	10.60	10.60	10.60
Digestible methionine (g/kg)	5.60	5.60	5.60	5.60
Methionine + digestible cysteine (g/kg)	8.30	8.30	8.30	8.30
Digestible threonine (g/kg)	7.10	7.10	7.10	7.10
Calcium (g/kg)	8.10	8.10	8.10	8.10
Available phosphorus (g/kg)	4.05	4.05	4.05	4.05
Sodium (g/kg)	1.60	1.60	1.60	1.60

[1] Premix provided per kg of feed: Vitamin A: 12000 IU; Cholecalciferol: 5000 IU; α-tocopherol: 45 IU; Menadione: 2.4 mg; Thiamin: 2.6 mg; Riboflavin: 6.6 mg; Niacin: 55 mg; Pyridoxine: 5.5 mg; D-pantothenate: 25 mg; Folic acid: 1.5 mg; Biotin: 0.1 mg; Choline: 1500 mg; Cyanocobalamin: 0.015 mg; BHT: 1 mg; Fe: 50 mg; Mn: 90 mg; Cu: 10 mg; I: 1 mg; Se: 0.25 mg and Zn: 85 mg.
[2] Huvepharma NV, Uitbreidingstraat, Belgium.

The effectiveness of acidifiers mainly depends on their dose and formulation. The concentration of the acidifier used in this study was 0.1%. Some authors suggested that a higher level of acidifier is required to ensure their positive effects (Edmondset *al.* 2014). However, in a study (Biggs and Parsons, 2008), using high doses (1 to 6%) of acidifier (containing citric, fumaric, gluconic, and malic acids) did not improve the broiler performance. The acidifier in the present study contained propionic acid, formic acid, sodium butyrate, ammonium propionate, calcium formate, calcium lactate, ammonium formate, and calcium propionate. It's also possible that the lack of a significant effect was due to the chemical composition of our acidifier.

No significant difference in daily weight gain (DWG) among treatments during the starter, grower, and finisher phases was found (Table 4). The control diet provided minimal fiber to maximize growth performance, while the other dietary treatments which contained the higher fiber content did not restrict the growth performance of the birds. Similarly, DosSantos *et al.* (2019) showed that supplementing rice hulls and soybean hulls at 2.5% and 5% had no positive effect on broiler weight after 21 days. Further, Saadatmand *et al.* (2019) found that adding rice hulls to the diet of broiler chickens throughout the entire study period (1-42 days) did not reduce daily weight gain.

Adding the acidifier to the diet did not increase weight gain in the current study. Leeson *et al.* (2005) found that adding butyric acid, as an acidifier, to the broiler diets did not affect their performance or weight gain (Leeson *et al.* 2005). In another study, adding organic acids such as butyric acid and propionic acid to the diet had no effect on bird weight (Mountzouris *et al.* 2007). In addition, Abdollahi *et al.* (2020) found that feeding broilers with pelleted diets supplemented with acidifiers at 0.7 and 1% did not improve their performance. However, several studies were conducted under the standard management conditions, and the lack of response to acidifiers could be attributed to the excellent management in those flocks. Therefore, some researchers believe that improving the rearing conditions might reduce the effectiveness of acidifiers for broiler chickens (Abdollahi *et al.* 2020). Unlikethem, others indicated that organic acids could be supplemented in the diet to increase body weight at 21 and 28 days of age (Khalid *et al.* 2020). However, these investigations did not reveal any significant findings up to 14 days of age.

In the grower phase, the experimental treatments significantly affected feed conversion ratio. The RH24 + acidifier birds had better FCR than RH10 group in this phase ($P<0.05$). Also, RH24 + acidifier birds had better feed conversion ratio (FCR) than RH10 group in this phase (Table 4).

However, in the finishing phase, FCR was similar among the dietary treatments. Dietary inclusion of rice hull, as an insoluble fiber source, early in the growth period might explain such an improved FCR during the growth period, since insoluble fiber facilitates digestion and absorption of nutrients. According to some studies, oat hull increases starch digestion in the jejunum, enabling birds to absorb nutrients more effectively (Hetland *et al.* 2003). These authors believed that the release of digestive enzymes like amylase may be regulated more effectively by inclusion of insoluble fibers in broiler diets (Hetland *et al.* 2003). However, unlike our results, some authors showed that adding rice hulls to chicken diets at two levels (2.5% and 5%) did not affect feed conversion ratio after 21 days (DosSantos *et al.* 2019). It has been shown that acidifier improves nutrient absorption by adjusting the pH of the digestive tract. Adil *et al.* (2010) reported a significant improvement in FCR between the control and acidifier (butyric acid, formic acid, and lactic acid) supplemented birds. Our results indicate that both rice hulls and acidifier can positively affect the FCR of broiler chickens when applied during the first 24 days of rearing period. Therefore, rice hulls should be included in the diets until the end of the growing phase to ensure that the insoluble fiber benefits broiler chickens throughout the rearing period.

There was a significant difference in livability between treatments in the overall rearing period (Table 5). The lowest and the highest mortality was observed in RH42 + acidifiers and RH24 + acidifiers birds, respectively ($P<0.05$). Feeding birds with RH24 + acidifiers resulted in an increased mortality rate due to the sudden death syndrome. Although the causes of sudden death syndrome are complex, feeding birds with a high density of nutrients (like pellets) and rapid growth has been reported to exacerbate its incidence in broiler chickens (Kuleile *et al.* 2020). In this study, RH24 + acidifier significantly increased the incidence of the sudden death syndrome, possibly because of its low dietary fiber content ($P<0.014$). Changes in fiber content could significantly alter the digestive system of the bird. When rice hulls were used in this treatment, a better gastrointestinal tract (GIT) development was achieved at the beginning of rearing period, which might lead to a better absorption of dietary nutrients. Between 25 and 42 days, rice hulls were replaced with acidifiers so that the birds received a more dense and absorbable diet. Furthermore, the amount of feed consumed in this treatment increased from 25 to 42 days of age. Consequently, these birds obtained higher levels of nutrients through their high-density and low fiber diet, which makes the birds more susceptible to sudden death syndrome since they can absorb more nutrients (Scott, 2002).

Table 4 Effect of rice hull (RH) and acidifier on broiler growth performance (g)

Treatments[1, 2, 3]	Starter (1-10 d)[4]				Grower (11-24 d)[5]				Finisher (25-42 d)				Total (1-42 d)		
	BW (10 d)	AFI	ADWG	FCR	BW (24 d)	AFI	ADWG	FCR	BW (42 d)	AFI	ADWG	FCR	AFI	ADWG	FCR
Control	333.84	28.57	29.15	0.980	1414.50	97.16	73.149	1.328[a]	3165	161.85	102.94	1.574	104.34	71.98	1.449[a]
Acidifier	325.68	28.17	28.39	0.993	1404.15	95.55	73.021	1.309[ab]	3185	164.10	103.91	1.578	103.75	72.24	1.436[ab]
RH10	328.37	28.55	28.63	0.997	1390.28	95.88	71.808	1.336[a]	3133	161.49	103.67	1.558	103.11	72.00	1.432[ab]
RH10 + Acidifier	324.9	28.24	28.25	1.000	1408.03	96.58	73.402	1.315[ab]	3131	159.54	103.17	1.542	102.47	72.43	1.414[ab]
RH24	-	-	-	-	1411.31	96.08	73.356	1.311[ab]	3192	160.63	105.05	1.529	103.39	72.65	1.423[ab]
RH24 + Acidifier	-	-	-	-	1433.08	95.85	75.032	1.277[b]	3112	158.81	100.03	1.587	102.13	71.52	1.427[ab]
RH42	-	-	-	-	-	-	-	-	3181	161.18	104.85	1.538	103.01	73.21	1.407[ab]
RH42 + Acidifier	-	-	-	-	-	-	-	-	3217	162.34	105.47	1.538	103.52	73.93	1.400[b]
P-value	0.363	0.572	0.379	0.705	0.351	0.666	0.132	0.034	0.275	0.921	0.463	0.447	0.607	0.963	0.045
SEM	3.53	0.261	0.361	0.011	14.22	0.670	0.840	0.013	30.974	2.700	1.750	0.021	1.40	0.844	0.0132

[1] Control: basal pelleted feed from 1 to 42 days; Acidifier: basal pelleted feed + acidifier from 1 to 42 days; RH10: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed from 11 to 42 days; RH10 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed + acidifier from 11 to 42 days; RH24: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed from 25 to 42 days; RH24 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed + acidifier from 25 to 42 days; RH42: basal pelleted feed + 3% rice hull from 1 to 42 days and RH42 + Acidifier: basal pelleted feed + 3% rice hull + acidifier from 1 to 42 days.
[2] RH10, RH24, and RH42 fed similar diets during the starter phase (1-10 days), so, during this period their performance indexes were reported as one treatment with 15 repetitions.
[3] RH10 + Acidifier, RH24 + Acidifier, and RH42 + Acidifier fed similar diets during the starter phase (1-10 days), so, during this period their performance indexes were reported as one treatment with 15 repetitions.
4 RH24 and RH42 fed similar diets during the grower phase (11-24 days), so, during this period their performance indexes were reported as one treatment with 10 repetitions.
[4] BW: body weight: AFI: average feed intake; ADWG: average daily body weight gain and FCR: feed conversion ratio.
[5] RH24 + Acidifier and RH42 + Acidifier fed similar diets during the grower phase (11-24 days), so, during this period their performance indexes were reported as one treatment with 10 repetitions.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of rice hull (RH) and acidifier on broiler liveability

Treatments[1]	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total (0-42 d)
Control	98.40	93.60	93.33	85.6[ab]
Acidifier	97.60	97.60	90.00	85.6[ab]
RH10	98.93	96.00	91.66	87.2[ab]
RH10 + Acidifier	98.93	97.60	84.16	80.0[bc]
RH24	-	96.80	87.50	87.2[ab]
RH24 + Acidifier	-	98.40	79.16	73.6[c]
RH42	-	-	89.16	86.4[ab]
RH42 + Acidifier	-	-	94.16	92.0[a]
P-value	0.622	0.262	0.628	0.014
SEM	0.75	1.01	4.13	2.615

[1] Control: basal pelleted feed from 1 to 42 days; Acidifier: basal pelleted feed + acidifier from 1 to 42 days; RH10: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed from 11 to 42 days; RH10 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed + acidifier from 11 to 42 days; RH24: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed from 25 to 42 days; RH24 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed + acidifier from 25 to 42 days; RH42: basal pelleted feed + 3% rice hull from 1 to 42 days and RH42 + Acidifier: basal pelleted feed + 3% rice hull + acidifier from 1 to 42 days.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

At 24 days of age birds fed RH24 and RH24 + acidifier had the heaviest gizzard relative weight (P<0.05; Table 6), which was attributed to inclusion of insoluble fiber in their diet because it has been shown that inclusion insoluble fiber enhances the function and capacity of the gizzard (Sacranie *et al*. 2012).

Moreover, dietary inclusion of insoluble fiber in laying hens has been reported to increase the weight of the gastrointestinal organs, which directly impacts the digestive system of the birds (Hetland *et al*. 2004).

Supplementing diets with rice hulls led to gizzard enlargement at 24 days of age. Previous studies have also shown that fiber either from native fiber (oat hulls) or synthetic fiber (isolated fiber sources) has a positive effect on the development of the gastrointestinal system (Sacranie *et al*. 2017). Comparing RH10 and RH10 + acidifier or RH24 and RH24 + acidifier at 24 days of age demonstrated a synergistic effect of rice hulls and the acidifier on gizzard development because the addition of acidifiers to a rice hulls-diet led to a larger gizzard at 24 days of age (Table 6).

Table 6 Effect of rice hull (RH) and acidifier on the relative weight of the digestive organs (% of live weight)

Treatments[1]	24 d							42 d						
	Gizzard	Proventriculus	Duodenum	Jejunum	Ileum	Caecum	Total	Gizzard	Proventriculus	Duodenum	Jejunum	Ileum	Caecum	Total
Control	1.169[c]	0.546	0.610	1.270	1.076	0.471	5.143	0.830	0.429	0.497	0.948	0.902	0.250	3.857
Acidifier	1.212[b]	0.572	0.630	1.312	0.995	0.489	5.213	0.944	0.377	0.515	0.947	0.755	0.246	3.786
RH10	1.186[b]	0.525	0.600	1.200	0.995	0.477	4.942	0.901	0.455	0.480	0.832	0.795	0.269	3.735
RH10 + Acidifier	1.340[ab]	0.616	0.611	1.294	1.021	0.509	5.392	0.831	0.350	0.452	0.931	0.797	0.225	3.538
RH24	1.424[a]	0.534	0.703	1.223	0.955	0.466	5.307	0.919	0.379	0.499	0.975	0.862	0.264	3.894
RH24 + Acidifier	1.517[a]	0.503	0.689	1.217	0.933	0.625	5.493	0.944	0.453	0.473	0.901	0.764	0.277	3.815
RH42	-	-	-	-	-	-	-	0.992	0.646	0.477	0.961	0.740	0.256	4.074
RH42 + Acidifier	-	-	-	-	-	-	-	0.881	0.399	0.443	0.913	0.700	0.207	3.545
P-value	0.030	0.774	0.159	0.860	0.414	0.126	0.290	0.739	0.096	0.840	0.687	0.126	0.123	0.332
SEM	0.091	0.056	0.372	0.072	0.050	0.053	0.174	0.070	0.066	0.035	0.054	0.049	0.017	0.162

[1] Control: basal pelleted feed from 1 to 42 days; Acidifier: basal pelleted feed + acidifier from 1 to 42 days; RH10: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed from 11 to 42 days; RH10 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed + acidifier from 11 to 42 days; RH24: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed from 25 to 42 days; RH24 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed + acidifier from 25 to 42 days; RH42: basal pelleted feed + 3% rice hull from 1 to 42 days and RH42 + Acidifier: basal pelleted feed + 3% rice hull + acidifier from 1 to 42 days. During the growth period (11-24 days of age), treatments (RH24, RH42) and (RH24+Acidifier, RH42+Acidifier) consumed a similar diet (n=10).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of rice hull (RH) and acidifier on the relative length of the digestive organs (cm/g)

Treatments[1]	24 d					42 d				
	Duodenum	Jejunum	Ileum	Caecum	Total	Duodenum	Jejunum	Ileum	Caecum	Total
Control	2.066	4.570	4.525	2.267	13.43	1.097	2.603	2.732	1.286	7.719
Acidifier	2.168	5.414	5.105	2.269	14.95	1.116	2.706	2.742	1.253	7.820
RH10	2.269	5.235	5.009	2.283	14.79	1.139	2.609	2.662	1.300	7.711
RH10 + Acidifier	2.197	5.732	5.267	2.511	15.70	1.133	2.791	2.684	1.296	7.905
RH24	2.241	5.069	4.859	2.348	14.51	1.211	3.064	2.989	1.345	8.610
RH24 + Acidifier	2.298	4.975	5.078	2.446	14.81	1.210	2.788	2.945	1.364	8.309
RH42	-	-	-	-	-	1.151	2.831	2.720	1.329	8.032
RH42 + Acidifier	-	-	-	-	-	1.124	2.876	2.816	1.269	8.087
P-value	0.688	0.157	0.646	0.565	0.372	0.861	0.354	0.418	0.834	0.500
SEM	0.105	0.280	0.291	0.118	0.639	0.062	0.139	0.118	0.054	0.322

[1] Control: basal pelleted feed from 1 to 42 days; Acidifier: basal pelleted feed + acidifier from 1 to 42 days; RH10: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed from 11 to 42 days; RH10 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed + acidifier from 11 to 42 days; RH24: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed from 25 to 42 days; RH24 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed + acidifier from 25 to 42 days; RH42: basal pelleted feed + 3% rice hull from 1 to 42 days and RH42 + Acidifier: basal pelleted feed + 3% rice hull + acidifier from 1 to 42 days. During the growth period (11-24 days of age), treatments (RH24, RH42) and (RH24+Acidifier, RH42+Acidifier) consumed a similar diet (n=10).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Organic acids may improve digestion by promoting healthier gastrointestinal tract (Adil *et al.* 2010).

We found that supplementing rice hulls for 24 days compared to 10 days led to a better development of gizzard, but extending the rice hulls consumption to 42 days did not further improve gizzard weight (Table 6). Similarly, Bournazel *et al.* (2018) reported that feeding an insoluble fiber source (oat hulls) to birds until 21 days of age increased the weight of their gizzards, but prolonged supplementation with oat hulls (31 days) did not cause a heavier gizzard.

The dietary treatments had no effects on the length of different parts of the intestine (including duodenum, jejunum, ileum, and caecum) at 24 and 42 days of age (P>0.05). However, on day 24, RH10 + acidifier and control birds had the longest and shortest jejunum, respectively (P>0.05).

At 42 days, adding rice hulls and acidifiers had no significant effect on the length of the birds' gastrointestinal tracts (Table 7). The length of the GIT is crucial in poultry production. A more developed GIT increases nutrients digestion and absorption. Saki *et al.* (2011) found that feeding broiler chickens with a diet in which the ratio of soluble to insoluble fibers (pectin and cellulose) was 1:2 caused a longer jejunum at 21 and a larger cecum at 42 days of age, also, another study reported that feeding birds with a soluble fiber supplement (oligofructose) elongated digestive tracts and improved intestinal condition (Yusrizal and Chen, 2003). While, feeding wood chips (lignin) as an insoluble fiber source reduced and shorten the gastrointestinal length of birds (Amerah *et al.* 2009).

Table 8 Effect of rice hull (RH) and acidifier on intestinal histology (μm)

Treatments[1]	24 d						42 d					
	Villi length	Villi width	Crypt depth	Muscularis layer	Villi length/ crypt depth	Villi area	Villi length	Villi width	Crypt depth	Muscularis layer	Villi length/crypt depth	Villi area
Control	1253	151	136.3	485.8	10.45[a]	0.588[b]	1346[a]	165	133[ab]	330	10.12[ab]	0.740[a]
Acidifier	1314	196	132	395.2	10.47[a]	0.828[a]	1320[a]	180	140[a]	347	9.42[b]	0.746[a]
RH10	1315	151	139.4	435.5	9.39[ab]	0.600[b]	1060[c]	191	104[d]	376	10.19[ab]	0.626[ab]
RH10 + Acidifier	1254	167	155.5	397.5	8.24[ab]	0.657[ab]	1154[bc]	178	142[a]	347	8.12[c]	0.644[ab]
RH24	1144	170	138.2	409.4	8.44[ab]	0.596[b]	1181[bc]	176	134[ab]	383	8.81[b]	0.565[b]
RH24 + Acidifier	1058	151	148	415.9	7.05[b]	0.510[b]	1058[c]	158	145[a]	334	7.29[d]	0.522[b]
RH42	-	-	-	-	-	-	1231[ab]	161	130[ab]	345	9.46[b]	0.627[ab]
RH42 + Acidifier	-	-	-	-	-	-	1164[bc]	143	109[d]	355	10.67[a]	0.538[b]
P-value	0.273	0.126	0.373	0.142	0.050	0.036	0.012	0.273	0.0018	0.645	0.009	0.0306
SEM	99.03	12.31	7.86	23.28	0.87	0.174	59.62	13.15	7.66	21.99	0.56	0.528

[1] Control: basal pelleted feed from 1 to 42 days; Acidifier: basal pelleted feed + acidifier from 1 to 42 days; RH10: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed from 11 to 42 days; RH10 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed + acidifier from 11 to 42 days; RH24: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed from 25 to 42 days; RH24 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed + acidifier from 25 to 42 days; RH42: basal pelleted feed + 3% rice hull from 1 to 42 days and RH42 + Acidifier: basal pelleted feed + 3% rice hull + acidifier from 1 to 42 days. During the growth period (11-24 days of age), treatments (RH24, RH42) and (RH24+Acidifier, RH42+Acidifier) consumed a similar diet (n=10).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

During the first 24 days, neither fiber source nor acidifier supplementation affected the jejunal morphometric features (Table 8). Further extending of rice hulls and acidifiers supplementation to 42 days did not have an adverse effect on the appearance of intestinal villi. In contrast, feeding birds with RH10, RH10 + acidifier, RH24, and RH24 + acidifier diets caused severe tissue damage resulting in the keratinization of specific layers of villi. However, feeding birds with RH42 diet, in which rice hulls was included for the entire growth period, had no adverse effects on the villi length, mucosal layer and muscle layers. Chickens rapidly change the shape and size of their digestive system due to diet changes. Yason and Schat (1987) reported that digestive and nutritional stresses have a great impact on changing the morphology and structure of intestinal villi and their structure changes rapidly. Of course, the fiber of the rice hulls has the ability to shorten the length of the villi, which is actually considered a negative point, but in general, the rebuilding of the villi has been done at a lower rate in these treatments.Therefore, some changes observed in 24 days were restored by the body up to 42 days. Exclusion of the rice hulls from the diets at days 10 and 24onwards resulted in severe damages in the gastrointestinal villi (Figure 1). Also, regenerating the surface villi of the epithelium was seen in RH10 (Figure 1). The RH24 treatment improved intestinal tissue, with villus boundaries becoming marginally visible. However, the villi started to branch out, and 2-3 villi grew together from the mucosal surface (Figure 1).

In these villi, the absorption layer was thicker and keratinized as well. At 42 days of age, villi had consistent sizes, and they could be easily separated. When the rice hulls omitted, the intestinal villi of broilers underwent fundamental changes in appearance. Hence, it is highly recommended to supplement rice hulls throughout the rearing period to maintain the integrity of the digestive tract and to gain its beneficial effects on GIT. Jiménez-Moreno *et al.* (2013) also demonstrated that including oat hulls, as an insoluble fiber source, in broiler diet did not affect the morphometric features of the jejunum. In contrast, Kalmendal *et al.* (2011) fed four levels (0, 10, 20, and 30%) of high-fiber sunflower cake to broiler chickens from 15 to 31 days of age, and observed a significant decrease in villus height, thickness of muscular is mucosa, and the circular and longitudinal layers of muscular is in the jejunum compared to the control group. Abazari *et al.* (2016) showed that broiler chickens fed 7.5 to 15 g/kg rice hulls per day experienced a significant loss of villus height. Saadatmand *et al.* (2019) reported that adding 3% rice hulls to broiler diets significantly reduced villus height and villus height/crypt depth in the jejunum. This reduction was explained by the abrasive action of insoluble fiber on intestinal mucus (Montagne *et al.* 2003).

Villus width plays an important role in determining the absorption area of the villus; therefore, the wider the villus, the greater the absorption area. The width of the villus was similar among the treatment groups at 24 days.

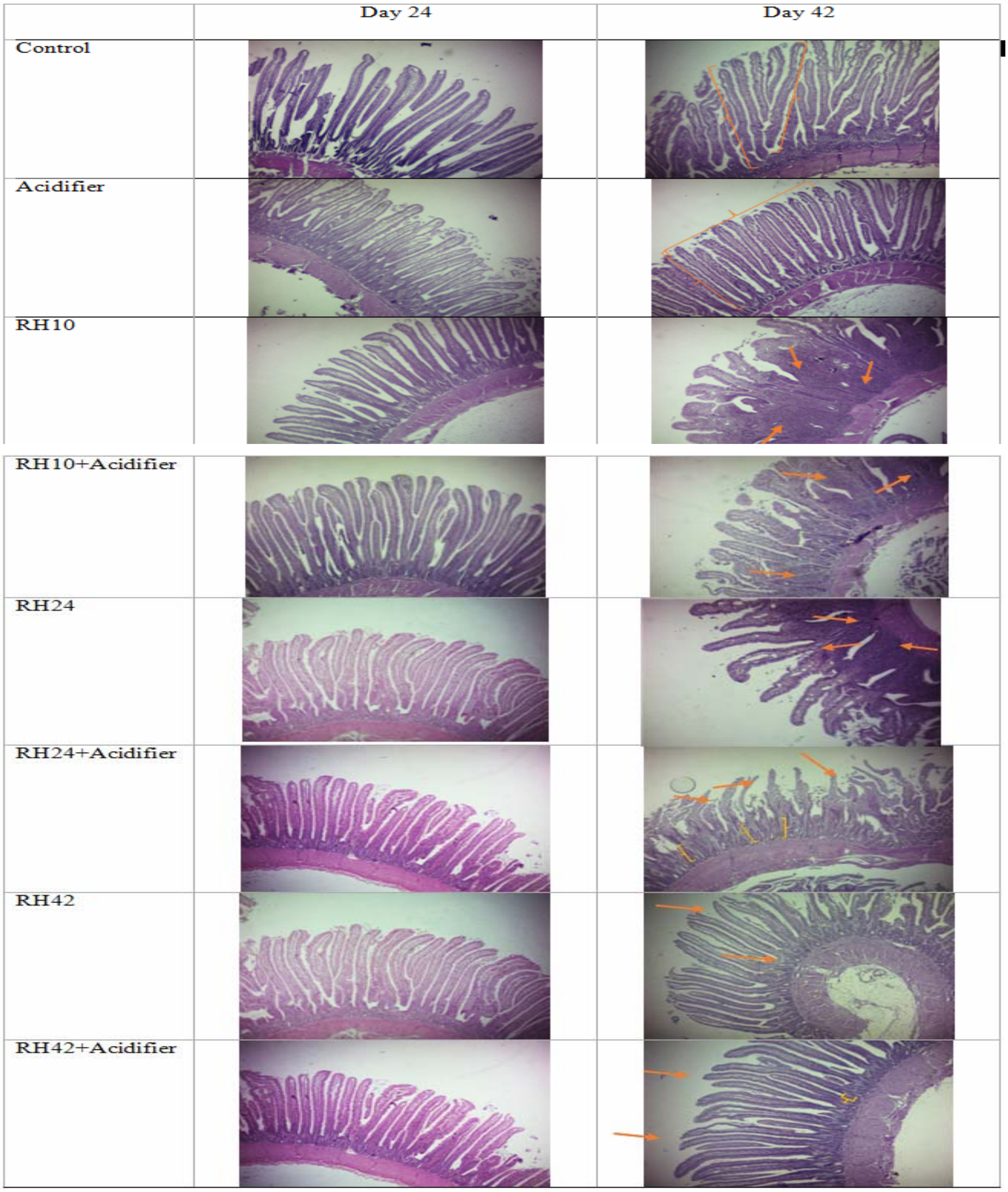

Figure 1 Effect of dietary treatments on jejunal villus histomorphometry in broilers at 24 and 42 days of age
Control: basal pelleted feed from 1 to 42 days; Acidifier: basal pelleted feed + acidifier from 1 to 42 days; RH10: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed from 11 to 42 days; RH10 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed + acidifier from 11 to 42 days; RH24: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed from 25 to 42 days; RH24 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed + acidifier from 25 to 42 days; RH42: basal pelleted feed + 3% rice hull from 1 to 42 days and RH42 + Acidifier: basal pelleted feed + 3% rice hull + acidifier from 1 to 42 days

Table 9 Effect of rice hull (RH) and acidifier on microbial population, pH of gizzard and ileum

Treatments[1]	*Lactobacillus* bacteria × 10^6 CFU/g	*Escherichia coli* × 10^5 CFU/g	Gizzard pH	Ileum pH
Control	4.172[c]	3.771	3.81[a]	6.37
Acidifier	5.421[a]	3.527	3.62[ab]	6.00
RH10	4.246[c]	3.542	3.26[bc]	5.67
RH10 + Acidifier	5.318[a]	4.024	3.58[ab]	5.67
RH24	4.679[ab]	4.058	3.18[bc]	6.17
RH24 + Acidifier	5.361[a]	3.898	3.47[bc]	6.11
RH42	4.920[ab]	3.706	2.98[c]	6.28
RH42 + Acidifier	4.962[ab]	3.877	3.22[bc]	6.29
P-value	0.029	0.850	0.046	0.111
SEM	0.305	0.288	0.177	0.241

[1] Control: basal pelleted feed from 1 to 42 days; Acidifier: basal pelleted feed + acidifier from 1 to 42 days; RH10: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed from 11 to 42 days; RH10 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 10 days and basal pelleted feed + acidifier from 11 to 42 days; RH24: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed from 25 to 42 days; RH24 + Acidifier: basal pelleted feed + 3% rice hull from 1 to 24 days and basal pelleted feed + acidifier from 25 to 42 days; RH42: basal pelleted feed + 3% rice hull from 1 to 42 days and RH42 + Acidifier: basal pelleted feed + 3% rice hull + acidifier from 1 to 42 days.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Neither the muscular is layer nor the crypt depth or the villus area were affected by the experimental treatments at 24 days. However, feeding birds with diets containing the acidifiers tended to decrease the muscular is layer. It was predicted that chickens fed treatments containing acidifiers (Acidifier and RH10+acidifier) would have the thinnest muscular is layer diameter at 24 days of age. Adil *et al.* (2010) showed that feeding birds with organic acid resulted in a non-significant decrease in muscular is thickness in all segments of the intestine.

Peristalsic movement in the small intestine becomes stronger as the muscular is layer thickness increases. Denbow (2015) states that a thicker muscular is layer increases the weight of the gastrointestinal tract, which is a negative characteristic as a significant portion of energy goes to its formation, which increases the maintenance energy expenditure.

The villus area largely determines small intestine morphology. Increasing the intestinal surface area and surface area of a villous will increase the rate of nutrient absorption. Two important factors in determining villus area are villus length and villus width. The acidifier treatment had a significant influence on these parameters. The longest villus was found in control and acidified groups (Table 8). However, a recent study found that rice hulls-fed birds had a shorter villus length than control birds (Adibmoradi *et al.* 2016).

Even though the villi in the control birds were the longest, the size of the GIT was significantly smaller than the other treatment groups at 24 days (Table 8). Accordingly, although dietary inclusion of rice hulls had a negative impact on villi size, but it had a positive impact on GIT length. Birds fed RH10 had a wider villus on day 42 than those fed RH42+acidifier. As opposed to this, Saki *et al.* (2011) showed that villi was widest in broilers fed a control diet with no fiber supplementation.

The depth of the crypt was influenced by the treatments at 42 days of age so that acidifier, RH10 + acidifier, and RH24 + acidifier birds had the greatest crypt depth, while RH10 and RH42 + acidifier birds presented the lowest depth of crypt (P<0.001). In a study, inclusion of 1.5 and 0.75% of rice hulls into broilers' diet up to 42 days of age had no effect on crypt depth, but increased villus length/crypt depth compared to control birds (Abazari *et al.* 2016). The villi length/crypt depth was significantly lower in the RH24+acidifier compared to RH42 + acidifier at 42 days (P<0.001). However, the RH24 + acidifier group had a better performance until 24 days of age. After the growing period and removal of rice hulls from the diet, the gastrointestinal morphology of the birds showed severe impairment (25 to 42 days of age). The digestive tract of chickens was altered as a result of changes in their diet. Intestinal villi can undergo rapid changes in morphology and structure when subjected to digestive and nutritional stress (Yason and Schat,1987).

The lactobacillus population in the control group was significantly lower than the acidifier group (P < 0.05) (Table 9). Additionally, we found no significant differences in *Escherichia coli* populations among the treatments. One of the goals of acidifying poultry feeds is to promote the growth of beneficial bacteria over the harmful ones. By suppressing the harmful bacteria, the intestinal bacteria become less competitive, and nutrients utilize by the host more efficiently (Thompson and Hinton, 1997). It has been shown that feeding broiler chickens with acidified rations reduced the presence of harmful bacteria such as Salmonella in their gastrointestinal tract (Iba and Berchieri, 1995). This shows that acidity affects the ability of beneficial bacteria (*Lactobacilli*) to adapt to low pH levels in the gastrointestinal tract. In a study, birds received rice hulls showed significantly higher *Lactobacillus* and lower *Escherichia coli* bacteria than the control birds (Abazari *et al.* 2016).

According to some reports, adding 0.25, 0.5, or 1% of *Lignocellulose* to the broiler diet either increased beneficial bacteria like *Bifidobacteria* and *Lactobacillus* or reduced harmful bacteria like *E. Coli* (Boguslawska-Tryk *et al.* 2015). These two bacteria families, *Lactobacilli* and *Bifidobacteria*, can prevent the growth of harmful bacteria by reducing the pH of the intestine (Wang and Gibson, 1993). It is also essential to note that short-chain fatty acids, which are resulting from the digestion of poultry feed in the gut, could eliminate *Salmonella* and *Escherichia coli*, thereby preventing villi damage. Edmonds *et al.* (2014) found that commercial acidifiers decreased *Escherichia coli* bacteria, but not *Salmonella*. Fascina *et al.* (2012) reported that adding a mixture of organic acids (30% lactic acid, 25.5% benzoic acid, 7% formic acid, 8% citric acid, and 6.5% acetic acid) into the broiler diet improved the final weight of the bird, feed conversion ratio, carcass weight andnutrients digestion (Fascina *et al.* 2012).

In comparison with the RH42 and RH24 groups, the pH of the gizzard was significantly higher in the control group ($P < 0.05$) (Table 9). The birds fed 3% rice hulls from 0 to 42 days of age (RH42) had the lowest gizzard pH. Some researchers have reported that supplementing 3% oat hulls and sugar beet pulp in broiler feed reduced gizzard pH, but feeding with 3% cellulose had no effect on gizzard Ph (Jiménez-Moreno *et al.* 2009). Furthermore, in another study, gizzard pH was lower in broilers fed diet containing 3% oat hulls than the control birds (Jiménez-Moreno *et al.* 2009).

CONCLUSION

The data of the present project showed that fiber supplements added to the diet are only effective in improving the food conversion ratio during the growth period. However, in other parameters such as daily weight gain and feed consumption, they were not effective in any of the periods. Also, no significant difference was observed between the control and other treatments in terms of feed consumption, daily weight gain and feed conversion ratio in the whole period. Generally, the rice hulls, occupying a small part of the diet and its very low price, not only did not cause a decrease in performance and efficiency, but also obtained the highest average final weight of the bird in the entire period. Therefore, according to the information obtained in this project, it can be reported that adding rice hulls with acidifier for 42 days improves the absorption conditions in birds. This treatment had the best results compared to other treatments in two important factors of small intestine morphology including crypt depth and crypt length-to-depth ratio, too.

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