

Growth performance, pH value of gizzard, hepatic enzyme activity, immunologic indicators, intestinal histomorphology, and cecal microflora of broilers fed diets supplemented with processed lignocellulose

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ABSTRACT This study was performed to investigate the hypothesis that supplementation of processed lignocellulose (PL) in the diets of broilers has a positive effect on growing performance, pH value of gizzard, hepatic enzyme activity, immunologic indicators, histomorphological character of small intestine, and cecal microflora populations. A total of 720 one-day-old Ross 308 broiler chicks were allotted to 4 treatment groups and fed maize–soybean meal based diets. The basal diet was supplemented with PL with an amount of 0 kg (control), 0.5 kg, 1 kg, and 2 kg per ton feed. Growing performance parameters, were determined weekly until 35 D of age. Blood samples for enzyme activities and immunoglobulins, jejunum and cecum samples for histomorphological characters for villus growth, and microbial population were collected from 12 broilers from each group. At 35 D of age, body weight of broilers supplemented with 1 kg of PL was found to be the highest with a value of 2305.0 g, when compared to the broilers supplemented with control, 0.5 and 2 kg of

PL groups (2154.0, 2201.0, and 2141.7 g, respectively, $P = 0.001$). An increased activity of aspartate amino transferase (AST) was observed in the control and 1 kg PL supplementation groups (633.6 and 597.4 IU/L, respectively), whereas alkaline phosphatase (ALP) activity was the highest in the control group (5404 IU/L, $P < 0.05$). Broilers in the control group had the lowest level of IgY and IgA (122.2 and 25.8 mg/dL, respectively, $P < 0.05$). Villus height increased by 22.0%, 40.7%, and 34.8% in 0.5, 1, and 2 kg PL supplementation groups, respectively, when compared to the control ($P < 0.001$). The processed lignocellulose supplemented as 1 kg of PL decreased the average count of *Staphylococcaceae*, *E. coli*, and *Enterobacteriaceae*, whereas it increased the population of *Lactobacillus* spp. in the cecum ($P < 0.05$). These data indicate that the supplementation of processed lignocellulose had positive effects for performance via changes in hepatic enzyme activities, immunoglobulin levels, villus growth in jejunum, and microflora in cecum.

Key words: processed lignocellulose, villus growth, feed efficiency, microflora, broiler

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INTRODUCTION

Currently, there is a growing interest for new feeding strategies for healthier digestive system development and growth, and more efficient utilization of feed in broiler nutrition. Recent studies have focused on the possible effects of fiber supplementation in broiler nutrition (Sklan et al., 2003; He et al., 2015; Hussein et al., 2017; Kheravii et al., 2017; Zhou et al., 2018; Makivic et al., 2019; Zeitz et al., 2019). Dietary fiber source can be classified into 2 subclasses: water soluble and insoluble fiber, each having different structural and physiological roles for digestive and absorptive processes of the gastrointestinal tract (Raninen et al., 2011). Water soluble fibers are fermentable fibers and have a viscous structure; whereas insoluble fibers are non-fermentable

and have a nonviscous structural component. Soluble fibers cause a reduction in intestinal passage rate, digestion of fat and protein, and an increase in viscosity of digesta (Mateos et al., 2012). In contrast, insoluble dietary fibers, such as cellulose and lignin, are beneficial to the gastrointestinal tract of chickens by promoting the growth of beneficial gut bacteria, and the activity of digestive enzymes (González-Alvarado et al., 2010; Jiménez-Moreno et al., 2010; Mateos et al., 2012).

There is a wide variation of these 2 fiber components in various plant materials. For example, cereals such as rice, sunflower, wheat bran, straw, hulls, and lignin have a rich content of insoluble fiber. Lignocellulose, made from wood, has recently begun to be used as a high quality fiber source, with an amount of 55% crude fiber as cellulose and hemicellulose, and aromatic polymers, such as lignin with a content of 25 to 30%, depending on the source of lignocellulose (Jørgensen et al., 2007; Harmsen et al., 2010; Bogusławska-Tryk et al., 2015). Preliminary studies have verified that lignocellulose supplementation in various amounts can

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be very beneficial for both performance and the digestive function and health of both broiler and layer chickens, as well as geese (Mateos et al., 2012; He et al., 2015; Jiménez-Moreno et al., 2016; Kheravii et al., 2017).

The critical balance between beneficial and pathogenic bacterial populations in the intestinal tract can be protected by stimulating beneficial bacterial growth, and reducing the abundance of pathogenic bacteria and thereby increase broiler performance by improving feed efficiency (Stanley et al., 2012). Therefore, broiler diets could be supplemented with feed additives that include insoluble fiber sources, e.g., lignocellulose, to improve intestinal health. Variation of the lignocellulose content could change the possible physiological effects depending on the source (Jørgensen et al., 2007; Kheravii et al., 2017). This study focused on the effects of a processed lignocellulose (PL) rich fiber source that contained 90% lignin, on growing performance during the first 35 D, hepatic enzyme activity, immunologic indicators, morphologic character of small intestine, and cecal microflora populations in broilers.

MATERIAL AND METHODS

Ethical Approval

The care and use of animals were in accordance with the laws and regulations of Turkey and approved by the ethics committee of Uludag University (License Number 2018-05/10).

Birds, Diets, Housing, and Experimental Design

A total of 720 one-day-old Ross 308 broiler chicks were weighed using a balance (± 0.1 g precision), and then randomly placed in 24 floor pens (2.0×1.5 m² floor space per pen) to provide 6 replicate pens for each treatment group. Each replicate pen consisted of 30 chicks (15 males/15 females).

Broiler chicks were randomly assigned to the following 4 treatment groups: Control (no supplementation of PL); 0.5 kg, 1 kg, 2 kg supplementation of PL per ton feed. The PL product contained 90% lignin processed at low temperature (Lignochar, Global Nutritech Biotechnology LLC, Richmond, VA). The PL was applied into diets during growing period (between days 1 and 35). Corn–soybean meal-based diets for starter and for grower broilers were formulated according to the minimum nutrient requirements for Ross 308 broilers (Nutrition Specification for Ross 308, 2014). The chicks received a standard crumbled broiler starter diet between days 1 and 14, and then a grower diet between days 15 and 35 (Table 1). The approximate nutrient level of feeds was analyzed according to the AOAC procedures (2000). Water and feed were offered ad libitum throughout the experiment. Room temperature was 33°C at 1 D of age and was decreased by 3°C every

Table 1. The composition and nutrient level of broiler diets.

Ingredients (%)	Starter	Grower
Maize	54.20	58.80
Soybean meal (CP 48%)	37.80	32.05
Vegetable oil	3.50	5.00
Dicalcium phosphate	2.00	2.00
Limestone	1.50	1.25
Salt	0.35	0.25
L-Lysine-HCl (99%)	0.10	0.10
DL-Methionine (98%)	0.35	0.25
Premix ¹	0.20	0.30
Total	100	100
Nutrient level (%)		
ME (kcal/kg)	3014	3185
Dry matter	89.5	90.7
Crude ash	5.8	6.3
Crude protein	22.2	20.4
Crude fat	7.0	8.4
Crude fiber	3.4	3.8
Lysine	1.38	1.09
Methionine + Cysteine	0.94	0.87
Calcium	1.38	1.25
Available Phosphorus	0.48	0.44

¹Vitamin and mineral premix provided per kilogram of diet: vitamin A 4,000,000 IU; vitamin D₃ 800,000 IU; vitamin E 8,000 mg; vitamin K₃ 1,200 mg; vitamin B₁ 800 mg; vitamin B₂ 2,400 mg; vitamin B₆ 2,000 mg; vitamin B₁₂ 6 mg; vitamin C 20,000 mg; niacin 8,000 mg; biotin 40 mg; folic acid 400 mg; choline chloride 80,000 mg; manganese 32,000 mg; iron 24,000 mg; zinc 24,000 mg; copper 2,000 mg; iodine 400 mg; cobalt 80 mg; selenium 60 mg.

week until maintained at 20°C and 50 to 60% relative humidity till the end of the experiment. Wood shavings were used as litter material, which was laid at a thickness of 7 to 8 cm on the floors of the pens. Lighting schedule was applied according to the company's management guides and recommendations (Ross 308 Management Guide, 2014).

Growing Performance and pH Measurement of Gizzard

Body weight and feed intake were recorded weekly on a pen basis until the end of the 5th week. Body weight gain and feed consumption were determined weekly, and feed conversion ratio was calculated using the weekly weight gains and feed consumption values.

At 35 D of age, pH of the gizzards from 12 birds per treatment group were measured using a digital pH meter (Mettler Toledo, SevenCompact™ pH/Ion S220, Greifensee, Switzerland).

Hepatic Enzyme Activities and Immunologic Indicators

At 35 D of age, 4 mL blood samples were collected into non-heparinized tubes from the jugular veins of 12 birds (6 males/6 females) per treatment group. Serum was collected by the method of Calneck et al. (1992) and stored at -20°C for future analysis. The serum samples were analyzed for activity of aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) using an

automatic analyzer (Roche Cobas 6000 C501, Roche Diagnostics, Regensburg, Germany).

Plasma levels of immunoglobulins (IgY, IgA, and IgM) were measured by a commercial kit (Roche Cobas 6000 E601, Roche Diagnostics, Indianapolis, USA) according to the analysis techniques described by Carew et al. (1997).

Morphological Characters of Small Intestine

At 35 D of age, small intestine samples were collected from a total of 48 sacrificed birds ($n = 12$ birds from each experimental group, 6 male/6 female) for intestinal villus micrometry analysis. Small intestine samples were collected from the midpoints of the jejunum segments according to procedures of Mahmoud and Edens (2012). Samples were trimmed to 2 cm and washed with 0.9% NaCl to remove the intestinal contents. Samples were then fixed in a 10% buffered formaldehyde solution and dehydrated in graded alcohol series, gradients from 30%, 50%, 70%, 80%, 95% to 100% during a 24-hour period. Intestinal slices were embedded in paraffin, and then paraffin blocks were sectioned to 5 μm thickness by a microtome (Leica RM2155, Leica Biosystems, Nussloch, Germany) and put on a glass slide and stained with hematoxylin and eosin for microscopic evaluation (Gridley, 1960; Sakamoto et al., 2000).

For morphological evaluations, jejunal morphometric variables including villus height, crypt depth, *Tunica muscularis* thickness, and villus apparent surface area were measured by a light microscope (Leica, DM-500, Leica Microsystems, Heerbrugg, Switzerland) with a computerized image system (Leica Application Suite, LAS Version 3.7.0, Leica Microsystems) (Sakamoto et al., 2000). Crypt and *Tunica muscularis* were measured in 15 to 20 villus for each bird. Then, the ratio between villus height and crypt depth (**VH/CD**) were calculated for each treatment group.

Cecal Microbial Populations

Cecal contents were collected by squeezing cecum into sterile beakers for enumeration of *Staphylococcaceae*, *E. coli*, *Enterobacteriaceae*, and *Lactobacillus spp.* according to the method described by McDonald et al. (1983) and Horn et al. (1996). Samples were sent to the microbiology laboratory for further analysis. Each cecal digesta sample was diluted with Ringer's solution and homogenized for 3 min, then serially diluted from 10^{-1} to 10^{-7} . The dilutions were then plated on selective agar media for target bacterial groups, including Violet red bile agar for *Staphylococcaceae*, Eosin Methylene Blue agar media for *E. Coli*, MacConkey agar for *Enterobacteriaceae*, and De Man, Rogosa and Sharpe agar media for *Lactobacillus*. The plates for *Staphylococcaceae*, *E. coli*, and *Enterobacteriaceae* were incubated at 37°C for 24 to 48 h, whereas the plates for *Lactobacillus spp.* were incubated at 30°C in a microaerophilic environment for 48 h. The microbial pop-

ulations were expressed as a colony forming unit per gram of cecal digesta.

Statistical Analysis

In the study, data of intestinal morphology, hepatic enzymes, serum immunoglobulin levels, and broiler growth performance were analyzed using the GLM procedures of SAS statistical software (SAS, 2003). Significant differences among the treatment means were compared using Tukey test and the differences were considered at the statistical level of $P < 0.05$. Effects of the PL supplementation levels (0.5, 1, and 2 kg per ton feed) were determined by the contrast option of the GLM procedure. Orthogonal polynomial contrasts were also applied to determine the linear and quadratic responses to different levels of PL supplementation.

RESULTS

The effects of dietary supplementation of PL on growth performance are presented in Table 2. Body weight on day 1 varied from 45.1 to 46.2 g among the experimental groups. Birds supplemented with 1 kg of PL were found to be quadratically heavier compared to the other treatment diets throughout the growing period ($P < 0.01$). The highest BW was observed in birds supplemented with 1 kg of PL with a value of 2305.0 g at 35 D of age. Between 7 and 28 D, BW gain showed a quadratic response due to PL supplementation ($P < 0.05$). The PL supplementation with an amount of 1 kg recorded the highest BW gain at 28 D of age (723.3 g, $P < 0.0001$).

Table 3 presents the differences for feed consumption and feed efficiency among the treatment groups. As seen in Table 3, weekly feed consumption was found to be higher in birds fed the diet contained 1 kg PL compared to the other groups at 21, 28, and 35 D of age ($P < 0.05$). Cumulative feed consumption at 35 D of age was found to be the highest with a value of 3391.7 g, in the 1 kg PL supplementation group ($P = 0.004$). Contrast analysis revealed a quadratic effect for feed conversion rate at 14, 28, and 35 D ($P < 0.01$). It was found to be more efficient in the 0.5 kg and 1 kg supplementation of PL groups (1.51 and 1.50 respectively) at 35 D of age ($P = 0.002$).

PL supplementation caused significant differences in the mean pH values of gizzards in all treatment groups (Figure 1), ranging from 3.18 to 3.74. A lower pH was observed in birds fed with diets supplemented with 1 kg and 2 kg of PL, compared to the control and 0.5 kg supplementation of PL ($P = 0.024$).

Effects of dietary supplementation of PL on hepatic enzyme activities and immunologic indicators at 35 D of age are presented in Table 4. An increased activity of AST was linearly observed in birds fed the control and 1 kg supplementation of PL (633.6 and 597.4 IU/L respectively, $P < 0.01$), whereas the lowest level of ALP was observed in birds fed diet containing 2 kg PL

Table 2. The effect of processed lignocellulose supplementation on growth performance in broilers.

Supplementation amount (kg/tonne feed)	Growing period (D)					
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
	Body weight (g)					
Control	45.1	178.3 ^c	460.0 ^c	856.0 ^b	1504.1 ^c	2154.0 ^{b,c}
0.5	45.9	181.2 ^c	474.5 ^b	883.9 ^{a,b}	1570.1 ^b	2201.0 ^b
1	45.1	205.0 ^a	489.6 ^a	914.5 ^a	1637.7 ^a	2305.0 ^a
2	46.2	197.7 ^b	471.4 ^{b,c}	867.7 ^b	1515.9 ^{b,c}	2141.7 ^c
SE	1.08	3.6	4.9	15.6	22.4	20.6
<i>P</i> value						
Fixed	0.509	0.001	0.001	0.009	0.001	0.001
Linear	0.287	0.006	0.293	0.679	0.922	0.843
Quadratic	0.809	0.030	0.001	0.001	0.001	0.001
	Body weight gain (g)					
Control	–	133.2 ^b	281.7 ^{b,c}	396.0	648.1 ^b	649.9
0.5	–	135.3 ^b	293.4 ^a	409.4	686.1 ^{a,b}	630.9
1	–	159.8 ^a	284.7 ^{a,b}	424.9	723.3 ^a	667.2
2	–	151.5 ^a	273.7 ^c	396.3	648.2 ^b	625.8
SE	–	3.3	3.8	17.9	22.6	31.7
<i>P</i> value						
Fixed	–	0.001	0.002	0.235	0.010	0.414
Linear	–	0.015	0.052	0.954	0.891	0.538
Quadratic	–	0.040	0.015	0.048	0.001	0.466

^{a-c}Means bearing different superscripts within the same column are significantly different.

n: 6 replicate pens per treatment group (30 broilers/pen).

Table 3. The effect of processed lignocellulose supplementation on feed consumption and feed efficiency in broilers.

Supplementation amount (kg/tonne feed)	Growing period (D)				
	Day 7	Day 14	Day 21	Day 28	Day 35
	Feed consumption (g)				
Control	147.7	323.0	585.7 ^b	976.3 ^{a,b}	1292.7 ^{a,b}
0.5	146.0	317.0	579.0 ^b	942.7 ^b	1266.0 ^{b,c}
1	146.7	323.3	623.0 ^a	985.0 ^a	1313.7 ^a
2	146.0	317.0	569.7 ^b	960.7 ^{a,b}	1265.7 ^c
SE	4.8	5.9	8.3	13.0	10.3
<i>P</i> value					
Fixed	0.968	0.403	0.008	0.018	0.001
Linear	0.717	0.407	0.630	0.832	0.386
Quadratic	0.850	0.853	0.044	0.937	0.252
	Cumulative feed consumption (g)				
Control	–	470.7	1056.3	2032.7	3325.3 ^{a,b}
0.5	–	463.0	1042.0	1984.7	3250.7 ^b
1	–	470.0	1093.0	2078.0	3391.7 ^a
2	–	463.0	1032.7	1993.3	3259.0 ^b
SE	–	8.4	13.3	20.0	34.8
<i>P</i> value					
Fixed	–	0.542	0.207	0.073	0.004
Linear	–	0.425	0.623	0.662	0.526
Quadratic	–	0.971	0.215	0.365	0.271
	FCR				
Control	1.11 ^a	1.13 ^a	1.30	1.39 ^a	1.58 ^a
0.5	1.08 ^{a,b}	1.08 ^{a,b}	1.24	1.30 ^b	1.51 ^b
1	0.92 ^c	1.06 ^b	1.26	1.30 ^b	1.50 ^b
2	0.96 ^{b,c}	1.09 ^{a,b}	1.26	1.36 ^{a,b}	1.55 ^{a,b}
SE	0.05	0.02	0.04	0.03	0.03
<i>P</i> value					
Fixed	0.002	0.025	0.333	0.031	0.020
Linear	0.015	0.199	0.338	0.694	0.823
Quadratic	0.064	0.005	0.218	0.006	0.002

^{a-c}Means bearing different superscripts within the same column are significantly different.

n: 6 replicate pens per treatment group (30 broilers/pen).

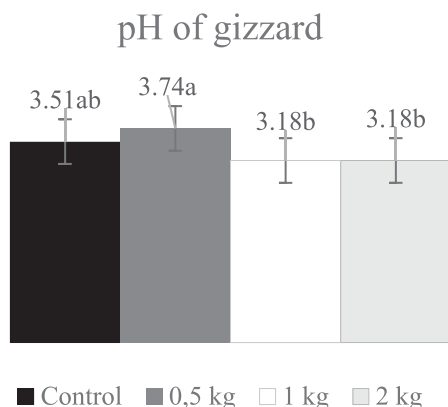


Figure 1. Mean pH values of gizzard in broilers fed by diets supplemented with PL (kg per ton feed). Bars represent mean \pm SE. Bars with different letters are different ($P < 0.05$; n: 12 samples per treatment group).

supplementation (4,775 IU/L, $P = 0.019$). The mean level of IgY and IgA were quadratically found to be the lowest in the control diet (122.2 and 25.8 mg/dL respectively) when compared to the other treatment diets ($P < 0.05$), while any significant differences observed among diets for the level of ALT and IgM at 35 D of age ($P > 0.05$).

The effects of dietary supplementation of PL on morphological characters of jejunum at 35 D of age are presented in Table 5. Results showed that the supplementation of PL had a stimulating effect on villus growth including villus height, villus apparent surface area, and crypt depth, compared to the control diet. PL supplementation showed a linear and quadratic increment in villus height ($P < 0.001$). A higher value of villus height was observed in birds fed with diets containing 1 kg and 2 kg supplementation of PL (1749.8 and 1675.9 μm , respectively, $P < 0.01$). Villus apparent surface area was found to be the lowest in the control group (309,716 μm^2). Contrast analysis showed a quadratic reduction in crypt depth in birds fed with diet supplemented 1 kg PL ($P < 0.05$). The ratio between VH/CD showed a linear decline in the control diet with a value of 9.6 ($P < 0.01$).

The microbial density of the cecum was affected by supplementation of PL at 35 D of age (Figure 2). A significant decline was observed for populations of *Staphylococcaceae*, *E. coli*, *Enterobacteriaceae* in the cecum of birds fed the diet contained 1 kg supplementation of PL, compared to the other diets. ($P < 0.05$). The total lactobacilli population in the cecum was the highest for the supplementation of 1 kg PL compared to other diets ($P < 0.05$).

DISCUSSION

The findings of this study clearly indicate that the supplementation of PL stimulates growth performance in broilers during 35-day feeding period. The amount of PL supplemented with an amount of 1 kg PL per ton feed resulted in more effective and better BW and FCR compared to the other treatment groups. Similarly, Sarikhan et al. (2010) reported that broilers fed with diets supplemented with insoluble fiber in an amount of 0.25 to 0.75% between weeks 3 and 6 had heavier BW and more efficient FCR. Such increments in growing performance could be related to an advance in protein and fat digestibility that was also reported by Bogulawska-Tryk et al. (2016) and Makivic et al. (2019). The supplementation of lignocellulose could improve the gizzard function and the activities of proteolytic enzymes in digestive organs, such as in the proventriculus and pancreas (Jiménez-Moreno et al., 2016; Farran et al., 2017). Another hypothesis may be that the lower pH observed in the gizzards of the birds fed the diet supplemented with 1 kg of PL could have resulted in the positive effects observed in this study. According to Guinotte et al. (1995) and Jiménez-Moreno et al. (2009), a lower gizzard pH could increase the solubility and absorption of mineral salts and pepsin activity.

However, few studies found no significant improvement in growing performance with the supplementation of lignocellulose in broilers (Bogulawska-Tryk et al., 2015; Yokhana et al., 2015). Furthermore, supplementation of lignocellulose in excess of 1 kg/ton feed

Table 4. The effect of processed lignocellulose supplementation on hepatic enzyme activities and immunologic indicators in broilers (35 D of age).

Supplementation amount (kg/ton feed)	Hepatic enzyme activities (IU/L)			Immunologic indicators (mg/dL)		
	AST	ALT	ALP	IgY	IgA	IgM
Control	633.6 ^a	3.9	5404 ^a	122.2 ^b	25.8 ^b	7.2
0.5	514.4 ^b	2.3	5068 ^{a,b}	129.6 ^a	28.4 ^{a,b}	7.9
1	597.4 ^a	2.4	5022 ^{a,b}	134.6 ^a	29.0 ^a	8.2
2	467.7 ^b	2.3	4775 ^b	131.0 ^a	28.4 ^{a,b}	7.9
SE	30.1	1.1	285.9	3.4	1.4	0.6
<i>P</i> value						
Fixed	<0.01	0.085	0.019	<0.01	0.015	0.100
Linear	0.001	0.077	0.064	0.015	0.054	0.149
Quadratic	0.838	0.102	0.075	0.001	0.009	0.060

^{a,b}Means bearing different superscripts within the same column are significantly different. n: 12 blood samples per treatment group.

Table 5. The effect of processed lignocellulose supplementation on morphological characters of jejunum in broilers (35 D of age).

Supplementation amount (kg/ton feed)	Villus height (μm)	Villus width (μm)	VASA (μm^2)	Crypt depth (μm)	VH/CD
Control	1243.7 ^c	106.8	309716 ^b	129.3 ^a	9.6 ^c
0.5	1517.7 ^b	121.9	345953 ^{a,b}	122.9 ^{a,b}	12.3 ^b
1	1749.8 ^a	136.6	404105 ^a	112.3 ^b	15.6 ^a
2	1675.9 ^a	118.2	363063 ^{a,b}	121.3 ^{a,b}	13.8 ^{a,b}
SE	62.8	18.6	32,491	7.3	1.0
<i>P</i> value					
Fixed	<0.001	0.129	0.003	0.004	<0.001
Linear	<0.001	0.428	0.052	0.784	<0.001
Quadratic	<0.001	0.129	0.003	0.011	0.118

^{a-c}Means bearing different superscripts within the same column are significantly different.

n: 12 samples per treatment group.

VASA: Villus apparent surface area, VH/CD: Villus height/crypt depth. Magnification 100 x, scale bar 100 μm .

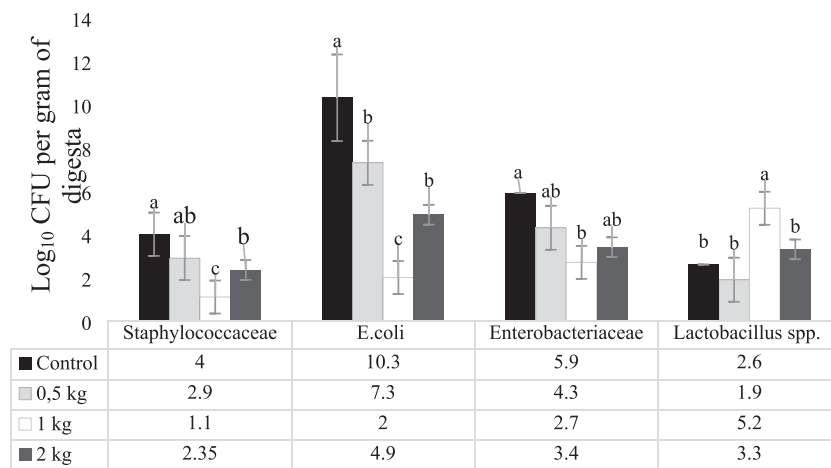


Figure 2. Total *Staphylococcaceae*., *E. coli*, *Enterobacteriaceae* and *Lactobacillus* spp. populations in the cecum of broilers at 35 D of age (kg supplementation per ton feed). Bars represent mean \pm SE. ($P < 0.05$, n: 12 samples per treatment group).

decreased BW and feed efficiency. Therefore, 1 kg of PL should be the optimum recommended amount per ton of feed for broilers. According to other studies, the amount of lignocellulose recommended should be between 2.5 to 3.5% (Jiménez-Moreno et al., 2011) and 3.98% (Makivic et al., 2019) of the dietary fiber content of the diet.

In this study, supplementation with PL changed the hepatic enzyme profiles, including AST and ALT, at 35 D of age. There is little data, however, focused on the effect of insoluble fiber on changes in enzyme levels in the livers of broilers. The mean levels of AST and ALP showed fluctuations, but these fluctuations did not directly correlate to any specific dose of PL. Hepatic enzyme activity is an indicative parameter for liver health and functionality (Cheesborough, 1991). When degenerative changes occur in the liver, liver enzymes are released (Johnston, 1999). While ALT is a better indicator than AST or ALP for estimation of cell damage in liver, the ALT level remained stable among the treatment groups at 35 D of age. This could be attributed to the hepato-protective effect of the PL

product. This was also supported by the lower levels of AST and ALP in birds fed PL diets compared to the control diet. Interestingly, birds fed the diet contained 1 kg supplementation of PL had a higher level of AST than the 0.5 kg and 2 kg of PL supplementation groups. This could be explained by regenerative changes in the liver, which resulted in a higher BW in birds fed the diet contained 1 kg supplementation of PL.

Immunoglobulins, including IgY, IgA, and IgM, provide a protection against pathogenic bacteria (Piquer, 1990). Results from this study found that diets supplemented with PL resulted in an immune-modulating reaction compared to the control diet due to observed increases in IgY and IgA concentrations, but not IgM concentration, of birds at 35 D of age. The main role of IgY and IgA is to prevent pathogenic bacteria from adhering onto the intestinal mucosal layer (Burke et al., 1988; Muir et al., 2000; Ohashi et al., 2014). Therefore, the higher levels of IgY and IgA in the birds fed with the diet supplemented with 1 kg of PL is consistent with lower populations of pathogenic bacteria, such as

Staphylococcaceae, *E. coli* and *Enterobacteriaceae* in cecal digesta.

The intestinal health is directly related to nutrient metabolism via digestion and absorption, and subsequently affects the growth and general health status of birds (Leung, 2014). Morphological characteristics of the small intestine including villus height, villus width, villus apparent surface area, and crypt depth, are accepted as an indicator for functionality of the small intestine (Montagne et al., 2003; Pelicano et al., 2005). Villus height is especially significant for absorptive capacity of the intestine (Iji et al., 2001). Crypts that are localized on the surface of the small intestine have a role in the formation of new villus (Geyra et al., 2001). Because changes in crypt depth is related with the formation of villus, the ratio between VH/CD is accepted as an indicator for estimating the absorptive capacity of the small intestine (Mateos et al., 2012). A higher mean of this ratio indicates the improvement in function and maturity of intestine (Baurhoo et al., 2007).

In the present study, villus and crypt development in jejunum of broilers were clearly stimulated by PL supplementation. The PL supplementation enhanced the villus height by about 22.0%, 40.7%, and 34.8%, and villus apparent surface area by about 11.7%, 30.5%, and 1.2% in 0.5 kg, 1 kg, and 2 kg supplementation of PL, respectively, when compared to the control diet. The highest increment for villus growth was observed in birds fed the diet contained 1 kg supplementation of PL. These findings are supported by the better growth performance in this treatment group. Furthermore, a higher ratio between VH/CD in the 1 kg supplementation of PL group was also accepted as an indicator for improvement in the digestion and absorption of nutrients. These results are in accordance with the findings of Iji et al. (2001) and Makivic et al. (2019), who reported that diets supplemented with insoluble fiber sources resulted in improvement in digestive tract development, and subsequently, growth performance.

Results from this study found that supplementation of PL affected the population of intestinal bacterial by increasing the beneficial bacterial population, such as *Lactobacillus*, and decreasing the number of pathogenic bacteria, such as *Staphylococcaceae*, *E. coli*, and *Enterobacteriaceae*, in cecal digesta at 35 D of age. These results are supported by Bogusławska-Tryk et al. (2015) and Abazari et al. (2016) and Makivic et al. (2019). The decline in population of pathogenic bacteria could be related to the antibacterial effect of lignin containing many phenolic monomers, and also an abrasive effect of lignocellulose that restricts the adhering of pathogenic bacteria on the surface of the intestinal mucosa (Jimenez-Moreno et al., 2011; Bogusławska-Tryk et al., 2015). Furthermore, the increase of the *Lactobacillus* population could result in the decrease of the pathogenic bacteria population by releasing bacteriocins that prevent the proliferation of these bacteria in intestinal mucosa (O'Shea et al., 2012).

In conclusion, this study found that the supplementation of PL as an insoluble fiber source affected notable changes in broilers. PL supplementation in the amount of 1 kg/ton of feed stimulated BW and FCR, and produced a lower pH value in the gizzard in birds at 35 D of age. Also noted in this study was a hepato-protective effect and an immune-modulating reaction, as well as better villus growth and development, and increased beneficial bacteria population in the cecum at 35 D of age. Furthermore, the optimum recommendation of PL supplementation should be 1 kg PL per ton of feed in broilers during growing period.

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REFERENCES

- Abazari, A., B. Navidshad, F. Mirzaei Aghjehgheshlagh, and S. Nikbin. 2016. The effect of rice husk as an insoluble dietary fiber source on intestinal morphology and *Lactobacilli* and *Escherichia coli* populations in broilers. *Iran J. Vet. Med.* 10:217–224.
- AOAC—Association of Official Analytical Chemists. 2000. *Official Methods Of Analysis*, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- Aviagen Brand. Ross 308 Broiler Management Handbook. 2014. Accessed Feb. 2018. http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Handbook-2014i-EN.pdf.
- Aviagen Brand. Ross 308 Broiler Nutrition Specification Guide. 2014. Accessed Feb. 2018. http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross308BroilerNutritionSpecs2014-EN.pdf.
- Baurhoo, B., C. A. Ruiz-Feria, and X. Zhao. 2007. Purified lignin: nutritional and health impacts on farm animals — a review. *Anim. Feed. Sci. Technol.* 144:175–184.
- Bogusławska-Tryk, M., R. Szymeczko, A. Piotrowska, K. Burlikowska, and K. Śliżewska. 2015. Ileal and cecal microbial population and short-chain fatty acid profile in broiler chickens fed diet supplemented with lignocellulose. *Pakistan Vet. J.* 35:212–216.
- Bogusławska-Tryk, M., A. Piotrowska, R. Szymeczko, K. Burlikowska, and B. Głowińska. 2016. Lipid metabolism indices and fatty acids profile in the blood serum of broiler chickens fed a diet with lignocellulose. *Braz. J. Poult. Sci.* 18:451–456.
- Burke, D. S., A. Nisalak, D. E. Johnson, and R. M. Scott. 1988. A prospective study of dengue infections in Bangkok. *Am. J. Trop. Med. Hyg.* 38:172–180.
- Calneck, B. W., H. J. Barnes, C. W. Beard, M. W. Reid, and H. W. Yoder, Jr. 1992. *Diseases of Poultry*. 9th ed. Wolfe Publishing Ltd., Gloucestershire, England.
- Carew, L. B., K. G. Evarts, and F. A. Alster. 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. *Poult. Sci.* 76:1398–1404.
- Cheesborough, M. 1991. *Medical Laboratory Manual for Tropical Countries*. 2nd ed. Tropical Health Technology and Butterworth Scientific Limited Cambridge and Edinburgh, England.
- Farran, M. T., H. A. Akilian, A. M. Hamoud, G. W. Barbour, and I. P. Saoud. 2017. Lignocellulose improves protein and amino acid digestibility in roosters and egg hatchability in broiler breeders. *J. Poult. Sci.* 54:197–204.
- Geyra, A., Z. Uni, and D. Sklan. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. *Poult. Sci.* 80:776–782.
- González-Alvarado, J. M., E. Jiménez-Moreno, D. González-Sánchez, R. Lázaro, and G. G. Mateos. 2010. Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive performance and digestive traits of broilers from 1 to 42 days of age. *Anim. Feed Sci. Technol.* 162:37–46.

- Gridley, M. F. 1960. *Manual of Histologic and Special Staining Technique*. MacGraw-Hill Book Company, New York, NY.
- Guinotte, F., J. Gautron, Y. Nys, and A. Soumarmon. 1995. Calcium solubilization and retention in the gastrointestinal tract in chicks (*Gallus domesticus*) as a function of gastric acid secretion inhibition and of calcium carbonate particle size. *Br. J. Nutr.* 73:125–139.
- Harmsen, P. F. H., W. J. J. Huijgen, L. M. Bermúdez López, and R. R. C. Bakker. 2010. *Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass*. Wageningen: Wageningen University & Research Centre. Accessed Feb. 2019. <ftp://ftp.ecn.nl/pub/www/library/report/2010/e10013.pdf>.
- He, L. W., Q. X. Meng, D. Y. Li, Y. W. Zhang, and L. P. Ren. 2015. Influence of feeding alternative fiber sources on the gastrointestinal fermentation, digestive enzyme activities and mucosa morphology of growing Greylag geese. *Poult. Sci.* 94:2464–2471.
- Horn, K. G., C. A. Gedris, and K. M. Rodney. 1996. Selective isolation of vancomycin-resistant enterococci. *J. Clin. Microbiol.* 34:924–927.
- Hussein, S. M., S. Yokhana, and T. L. Frankel. 2017. Supplementing the feeds of layer pullets, at different ages with two different fiber sources improves immune function. *Poult. Sci.* 96:2718–2727.
- Iji, P. A., A. A. Saki, and D. R. Tivey. 2001. Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. *Anim. Feed Sci. Technol.* 89:175–188.
- Jiménez-Moreno, E., J. M. González-Alvarado, A. de Coca-Sinova, R. Lázaro, and G. G. Mateos. 2009. Effects of source of fibre on the development and pH of the gastrointestinal tract of broilers. *Anim. Feed Sci. Technol.* 154:93–101.
- Jiménez-Moreno, E., J. M. González-Alvarado, D. González-Sánchez, R. Lázaro, and G. G. Mateos. 2010. Effects of type and particle size of dietary fiber on growth performance and digestive traits of broilers from 1 to 21 days of age. *Poult. Sci.* 89:2197–2212.
- Jiménez-Moreno, E., C. Romero, J. D. Berrocoso, M. Frikha, and G. G. Mateos. 2011. Effects of the inclusion of oat hulls or sugar beet pulp in the diet on gizzard characteristics, apparent ileal digestibility of nutrients, and microbial count in the ceca in 36-day-old broilers reared on floor. *Poult. Sci.* 90:153. (Abstr.).
- Jiménez-Moreno, E., A. de Coca-Sinova, J. M. González-Alvarado, and G. G. Mateos. 2016. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth performance and water intake. *Poult. Sci.* 95:41–52.
- Johnston, D. E. 1999. Special considerations in interpreting liver function tests. *Am. Fam. Physician* 59:2223–2230.
- Jørgensen, H., J. B. Kristensen, and C. Felby. 2007. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels Bioprod. Bioref.* 1:119–134.
- Kheravii, S. K., R. A. Swick, M. Choct, and S. B. Wu. 2017. Article navigation coarse particle inclusion and lignocellulose-rich fiber addition in feed benefit performance and health of broiler chickens. *Poult. Sci.* 96:3272–3281.
- Leung, P. S. 2014. *The Gastrointestinal System: Gastrointestinal, Nutritional and Hepatobiliary Physiology*, Springer, New York, NY.
- Mahmoud, K. Z., and F. W. Edens. 2012. Breeder age affects small intestinal development of broiler chicks with immediate or delayed access to feed. *Br. Poult. Sci.* 53:32–41.
- Makivic, L., M. Glisic, M. Boskovic, J. Djordjevic, R. Markovic, M. Baltic, and D. Sefer. 2019. Performances, ileal and cecal microbial populations and histological characteristics in broilers fed diets supplemented with lignocellulose. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 25:83–91.
- Mateos, G. G., E. Jiménez-Moreno, M. P. Serrano, and R. P. Lázaro. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *J. Appl. Poult. Res.* 21:156–174.
- McDonald, L. C., C. R. Hackney, and B. Ray. 1983. Enhanced recovery of injured *Escherichia coli* by compounds that degrade hydrogen peroxide or block its formation. *Appl. Environ. Microbiol.* 45:360–365.
- Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Tech.* 108:95–117.
- Muir, W. I., W. L. Bryden, and A. J. Husband. 2000. Investigation of the site of precursors for IgA producing cells in the chicken intestine. *Immun. Cell Biol.* 78:294–296.
- O'Shea, E. F., P. D. Cotter, C. Stanton, R. P. Ross, and C. Hill. 2012. Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: bacteriocins and conjugated linoleic acid. *Int. J. Food Microbiol.* 152:189–205.
- Ohashi, Y., M. Hiraguchi, and K. Ushida. 2014. The composition of intestinal bacteria affects the level of luminal IgA. *Biosci. Biotechnol. Biochem.* 70:3031–3035.
- Pelicano, E. R. L., P. A. Souza, H. B. A. Souza, D. F. Figueiredo, M. M. Boiago, S. R. Carvalho, and V. F. Bordon. 2005. Intestinal mucosa development in broiler chickens fed natural growth promoters. *Rev. Br. Cien. Avi.* 7:221–229.
- Piquer, F. J. 1990. Post-hatching changes in the immunoglobulin A concentration in the small intestine of turkeys. M.Sc. Thesis, Department of Animal Science, Iowa State University, Ames, IA.
- Raninen, K., J. Lappi, H. Mykkanen, and K. Poutanen. 2011. Dietary fiber type reflects physiological functionality: comparison of grain fiber, inulin, and polydextrose. *Nutr. Rev.* 69:9–21.
- Sakamoto, K., H. Hirose, A. Onizuka, M. Hayashi, N. Futamura, Y. Kawamura, and T. Ezaki. 2000. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. *J. Surg. Res.* 94:99–106.
- Sarikhani, M., H. A. Shahryar, B. Gholizadeh, M. H. Hosseinzadeh, B. Beheshti, and A. Mahmoodnejad. 2010. Effects of insoluble fiber on growth performance, carcass traits and ileum morphological parameters on broiler chick males. *Intern. J. Agric. Biol.* 12:531–536.
- SAS Institute Inc. 2003. *SAS/STAT User's Guide*, version 9.1. SAS Institute Inc., Cary, NC.
- Sklan, D., A. Smirnov, and I. Plavnik. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. *Br. Poult. Sci.* 44:735–740.
- Stanley, D., S. E. Denman, R. J. Hughes, M. S. Geier, T. M. Crowley, H. Chen, V. R. Haring, and R. J. Moore. 2012. Intestinal microbiota associated with differential feed conversion efficiency in chickens. *Appl. Microbiol. Biotechnol.* 96:1361–1369.
- Yokhana, J. S., G. Parkinson, and T. L. Frankel. 2015. Effect of insoluble fiber supplementation applied at different ages on digestive organ weight and digestive enzymes of layer-strain poultry. *Poult. Sci.* 95:550–559.
- Zeitz, J. O., K. Neufeld, C. Potthast, A. Kroismayr, E. Most, and K. Eder. 2019. Effects of dietary supplementation of the lignocelluloses FibreCell and OptiCell on performance, expression of inflammation-related genes and the gut microbiome of broilers. *Poult. Sci.* 98:287–297.
- Zhou, H., W. Guo, T. Zhang, B. Xu, D. Zhang, Z. Teng, D. Tao, Y. Lou, and Y. Gao. 2018. Response of goose intestinal microflora to the source and level of dietary fiber. *Poult. Sci.* 97:2086–2094.