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Original Article

Influence of Levels of Dietary Fiber Sources on the Performance, Carcass Traits, Gastrointestinal Tract Development, Fecal Ammonia Nitrogen, and Intestinal Morphology of Broilers

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Keywords

Rice hulls, soybean hulls, broilers, intestinal morphology, ammonia nitrogen.

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ABSTRACT

The present study was conducted to investigate the effects of dietary fiber source levels on the fecal ammonia nitrogen, growth performance, carcass traits, gastrointestinal tract development, and intestinal morphology of broilers. A total of 420 one-day-old unsexed broiler chicks were individually weighed and randomly divided into 5 groups, each with seven replicates of twelve chicks. Rice hulls (RH) and soybean hulls (SH) were ground through a hammer mill with a 2-mm screen. The RH and SH experimental diets were as follows: 0% (control); 2.5% RH; 2.5% SH; 5% RH; and 5% SH. No significant differences were found in growth performance and fecal ammonia nitrogen among the dietary treatment groups ($p>0.05$). Compared with the control, the experimental diets with 2.5% SH significantly decreased the wing weight of chickens ($p<0.05$), while no significant differences in the weight of the other visceral organs were observed. Compared with the control, broilers in the 5% SH group had a longer jejunum and ileum ($p<0.05$). Feeding the broilers SH and RH had no effect on the villus area and crypt depth of the intestine. Compared with the control, the experimental diet with 2.5% RH significantly increased the duodenal villus height of chickens ($p<0.05$). These findings suggest that the inclusion of 5% SH in the diets resulted in improved intestinal morphology without negatively affecting growth performance and carcass traits.

INTRODUCTION

The majority of ammonia (NH_3) emissions are from livestock production such as cattle, poultry, and swine farming (Battye *et al*., 1994). In Thailand, swine farms are the largest source of NH_3 emissions; however, poultry farms also cause air pollution through the release of NH_3. Nitrogen (N) in feces, containing undigested dietary N, endogenous N and microbial N (Jha & Berrocoso, 2016), is lost to the atmosphere as volatile ammonia (Ferket *et al*., 2002). NH_3 emission from manure has become a significant problem not only for human and chicken health but also in poultry production, with effects such as lower egg production and growth performance (Carlile, 1984; Miles *et al*., 2004). Consequently, many researchers have used diet composition improvement to decrease manure pollutants.

In Thailand, rice is the major locally produced crop, which amounted to 20.7 million metric tons in 2018/19 (USDA, 2018). Rice hulls (RH) account for 20% on average of the whole grain, and the most efficient use of this by-product is as a litter material for livestock production. Moreover, soybean hulls (SH) are also a by-product of the soybean oil processing. Some researchers have demonstrated that the inclusion of soluble fiber leads to a decrease in NH_3 emission from swine manure (Kreuzer *et al*., 1998; Beccacia *et al*., 2015). Bindelle *et al*. (2009) found that the inclusion of oat hulls did not reduce the urinary nitrogen excretion ratio. In contrast, the dietary10% corn dried distillers' grains with solubles, 7.3% wheat middlings, or 4.8% SH decreased NH_3 emission from laying hen manure (Roberts *et al*., 2007). As previously noted, dietary fiber extends the fermentation of microbes in the large intestine, and therefore the suitability of that fiber diets to lower NH_3 emission from the manure (Roberts *et al*., 2007). RH increase retention time in the upper part of the GIT, thus a well-developed gizzard will improve the nutrient absorption and digestibility (Mateos *et al*., 2002; Hetland *et al*., 2003; Hetland *et al*., 2005). Moreover, the insoluble fiber in broiler diets improved their intestinal morphology and growth performance (Sarikhan *et al*., 2010) and increased the secretion of hydrochloric acid (González-Alvarado *et al*., 2007). These improvements might be associated with increased nutrient digestibility (Amerah *et al*., 2009), as well as improved digestive tract health (Perez *et al*., 2011). Besides, the inclusion of feed ingredients with an adequate type and amount of fiber might improve the gastrointestinal tract (GIT), leading to reduced antibiotic use in the feed (Mateos *et al*., 2002; Montagne *et al*., 2003).

To the best of our knowledge, little is known about the effect of the use of rice hulls and soybean hulls as a fiber source in broiler diets. The differences in the structure and properties of fiber sources might affect nutritional and physiological effects (Santos *et al*., 2019). Therefore, this study aimed to investigate the effects of dietary fiber source levels on the fecal ammonia nitrogen, growth performance, carcass traits, gastrointestinal tract development, and intestinal morphology of broilers.

MATERIALS AND METHODS

Birds, management, and diets

A total of 420 one-day-old unsexed broiler chicks (Ross 308) were individually weighed and randomly divided into five groups of chicks with similar mean body weights, each with seven replicates of twelve chicks. These chicks were placed in litter-floored pens under an average environmental temperature of 32°C (12 birds per pen). The RH and SH were ground in a hammer mill to pass through a 2-mm sieve. Subsequently, the chemical composition of the fiber samples was analyzed as described by AOAC (2000) and Van Soest *et al*. (1991) (Table 1). Experimental diets were in mash form and formulated according to NRC (1994). These diets including RH and SH were as follows: 0% (control); 2.5% RH; 2.5% SH; 5% RH; and 5% SH (Table 2, Table 3). Feed and water were given *ad libitum* for 42 d and light was provided 24 h a day.

Table 1 – Chemical composition of rice hull (RH) and soybean hull (SH).

Item	RH	SH
Dry matter(%)	94.98	93.74
Crude protein (%)	1.04	5.00
Ether extract (%)	5.67	8.46
Neutral detergent fiber (%)	71.07	61.57
Acid detergent fiber (%)	52.52	38.90
Acid detergent lignin (%)	18.60	3.58
Acid insoluble ash (%)	0.11	0.00
Gross energy, kcal/kg	3309.20	3866.90

The experiment was carried out following the guidelines and rules for animal experiments of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Thailand.

Growth performance and fecal ammonia nitrogen

During the experiment, feed intake and body weight were measured weekly, and the feed conversion ratio was calculated. At 22 d of age, birds with similar mean body weights were randomly allocated to the five dietary treatment groups (4 birds/group). They were moved to individual cages. Subsequently, feces were collected over three consecutive 24-h periods in each cage. The feces from each of the 24-h periods were pooled by a group and stored at -20°C until analysis. Fecal ammonia nitrogen was analyzed by the method of AOAC (2000).

Carcass traits and digestive organ development

At 42 d of age, seven birds from each group were weighed individually and slaughtered to determine digestive organ development and carcass traits. The head, digestive organs, and shanks were removed and then weighed. Wings, abdominal fat, thighs, and drumsticks were removed and weighted individually. In the digestive organs, the lengths of the duodenum, jejunum, and ileum were measured separately. The weights of the proventriculus, gizzard, duodenum, jejunum, and ileum were then recorded after the

Table 2 – Feed compositions and calculated nutrient value of experimental diets (0 – 21 days of age).

Item	Control	2.5% RH	2.5% SH	5.0% RH	5.0% SH
Ingredient (%)					
Corn	43.10	38.72	39.32	34.35	35.55
Soybean meal	40.10	40.68	40.38	41.26	40.67
Soybean oil	7.12	8.41	8.10	9.71	9.09
Rice bran	5.00	5.00	5.00	5.00	5.00
Rice hull	-	2.50	-	5.00	-
Soybean hull	-	-	2.50	-	5.00
Monocalcium phosphate	2.09	2.10	2.10	2.11	2.11
Limestone	1.22	1.21	1.22	1.21	1.21
Premix[a]	0.60	0.60	0.60	0.60	0.60
Salt	0.42	0.42	0.42	0.42	0.42
D,L-methionine	0.17	0.17	0.17	0.18	0.18
Choline Chloride	0.07	0.07	0.07	0.07	0.07
L-lysine	0.05	0.04	0.04	0.03	0.03
L-threonine	0.02	0.02	0.02	0.02	0.02
Calculated analysis					
Crude protein	23	23	23	23	23
Metabolizable energy (kcal/kg)	3,200	3,200	3,200	3,200	3,200
Crude fiber	3.20	4.06	3.96	4.92	4.71
Crude fat	9.70	11.06	10.83	12.32	11.87
Ash	5.90	5.93	6.02	5.91	6.09
Calcium	0.90	0.90	0.90	0.90	0.90
Available phosphorus	0.50	0.50	0.50	0.50	0.50
Available lysine	1.15	1.15	1.15	1.15	1.15
Available methionine	0.47	0.47	0.47	0.47	0.47

RH = rice hulls; SH = soybean hulls.

[a]Premix included the following (per kg of diet): retinol, 8,250 IU; cholecalciferol, 2,750 IU; tocopherol, 17.9 IU; menadione, 1.1 mg; thiamine, 1.4 mg; riboflavin, 5.5 mg; pyridoxine, 1.1 mg; cyanocobalamin, 12 µg; niacin, 41.3 mg; pantothenic acid, 11 mg; biotin, 41 µg; folic acid, 1.4 mg; manganese, 125 mg; iron, 282 mg; copper, 27.5 mg; zinc, 275 mg; iodine, 844 µg; selenium, 250 µg.

digesta content had been removed. The weight of empty organs was expressed relative to 100 g of body weight.

Intestinal morphological observation

At 42 d of age, another seven birds per group were used for intestinal morphological observations. Immediately following the decapitation, the midpoint of each intestinal segment (duodenum, jejunum, and ileum) was removed and fixed in 10% neutral-buffered formalin. After dehydration through varying concentrations of alcohol, each intestinal part was embedded in paraffin wax. A 4-µm-thick transverse section was cut and stained with hematoxylin-eosin, and the following values were measured using Toup View 3.7 software (Irwin, U.S.A).

The villus height was measured as the length from the tip to the base, excluding the intestinal crypt. A total of 8 villus heights from eight sections were measured and averaged for each bird. The villus area was calculated from the villus height, basal width, and apical width (Iji *et al.*, 2001). The eight calculations of villus area were averaged for each bird. Crypt depth was measured as the distance from the villus base to the muscularis layer, not including the intestinal muscularis (Rezaei *et al.*, 2011).

Statistical analysis

The data from the experimental groups were statistically analyzed using one-way analysis of variance (ANOVA) in SPSS statistical software (version 19.0; IBM Corp. Armonk, NY, US). The Tukey's test was used to compare mean. Statistical significance was accepted at $p<0.05$.

RESULTS

Growth performance and fecal ammonia nitrogen

The influence of different sources and levels of fiber on growth performance and fecal ammonia nitrogen in broiler chickens is presented in Table 4. No significant differences were observed in feed intake, body weight gain, or feed efficiency among the dietary treatment groups ($p>0.05$). Feeding the chickens fiber did not affect the fecal ammonia nitrogen ($p>0.05$).

Table 3 – Feed compositions and calculated nutrient value of experimental diets (22 – 42 days of age).

Item	Control	2.5% RH	2.5% SH	5.0% RH	5.0% SH
Ingredient (%)					
Corn	52.22	47.85	48.45	43.47	44.69
Soybean meal	32.40	32.98	32.69	33.56	32.95
Soybean oil	5.69	6.99	6.68	8.28	7.67
Rice bran	5.00	5.00	5.00	5.00	5.00
Rice hull	-	2.50	-	5.00	-
Soybean hull	-	-	2.50	-	5.00
Monocalcium phosphate	1.87	1.88	1.88	1.89	1.89
Limestone	1.36	1.35	1.35	1.34	1.35
Premix[a]	0.60	0.60	0.60	0.60	0.60
Salt	0.42	0.42	0.42	0.42	0.42
D,L-methionine	0.16	0.17	0.16	0.17	0.17
Choline Chloride	0.70	0.07	0.07	0.07	0.07
L-lysine	0.11	0.11	0.11	0.10	0.10
L-threonine	0.04	0.04	0.04	0.04	0.05
Calculated analysis					
Crude protein	20	20	20	20	20
Metabolizable energy (kcal/kg)	3,200	3,200	3,200	3,200	3,200
Crude fiber	3.10	3.96	3.86	4.81	4.61
Crude fat	8.65	9.91	9.69	11.18	10.73
Ash	5.53	5.51	5.60	5.49	5.67
Calcium	0.90	0.90	0.90	0.90	0.90
Available phosphorus	0.45	0.45	0.45	0.45	0.45
Available lysine	1.03	1.03	1.03	1.03	1.03
Available methionine	0.43	0.43	0.43	0.43	0.43

RH = rice hulls; SH = soybean hulls.

[a]Premix included the following (per kg of diet): retinol, 8,250 IU; cholecalciferol, 2,750 IU; tocopherol, 17.9 IU; menadione, 1.1 mg; thiamine, 1.4 mg; riboflavin, 5.5 mg; pyridoxine, 1.1 mg; cyanocobalamin, 12 µg; niacin, 41.3 mg; pantothenic acid, 11 mg; biotin, 41 µg; folic acid, 1.4 mg; manganese, 125 mg; iron, 282 mg; copper, 27.5 mg; zinc, 275 mg; iodine, 844 µg; selenium, 250 µg.

Table 4 – The effects of different sources and levels of fiber on growth performance (mean ± SE; n = 7) and fecal ammonia nitrogen (mean ± SE; n = 4) in 42 days old broiler chickens.

Item	Level of fiber (%)	Feed intake (g)	Bodyweight gain (g)	Feed conversion ratio	Fecal ammonia nitrogen (mg/g)
Control	0	3134.71	1405.04	2.23	0.06
RH	2.5	3159.85	1469.27	2.14	0.10
	5.0	3283.00	1494.20	2.20	0.10
SH	2.5	3311.83	1414.64	2.34	0.08
	5.0	3175.71	1401.77	2.26	0.09
SEM		47.87	14.99	0.03	0.007
p-value		0.732	0.167	0.503	0.354

RH = rice hulls; SH = soybean hulls.

There are no significant differences between each groups ($p>0.05$).

Carcass traits and digestive organ development

Compared with the control, the experimental diets with 2.5% SH significantly decreased the wing weight of chickens ($p<0.05$) (Table 5). In addition, no significant differences in the weight of the other visceral organs were observed (Table 5). The intestinal weight and length of broilers are presented in Table 6. Compared with the control, broilers in the 5% SH group had a longer jejunum and ileum ($p<0.05$). In terms of intestinal weight and duodenal length, there were no significant differences among the dietary treatment groups ($p>0.05$).

Intestinal morphological measurements

Feeding the broilers SH and RH did not affect the villus area and crypt depth of the intestine (Table 7). Compared with the control, the experimental diets with 2.5% RH significantly increased the duodenal villus height of chickens ($p<0.05$).

Table 5 – The effects of different sources and levels of fiber on carcass and visceral organs weight (g/100 g BW) in 42 day old broiler chickens (mean ± SE; n = 7).

Item	Level of fiber (%)	Carcass	Thigh+ drumstick	Wings	Abdominal fat	Liver	Proven-triculus	Gizzard
Control	0	87.01	23.05	8.31^{a}	1.56	2.13	0.44	1.22
RH	2.5	86.25	22.89	8.09^{ab}	1.52	2.09	0.44	1.29
	5.0	84.35	22.81	7.84^{ab}	1.45	2.13	0.42	1.37
SH	2.5	84.59	22.14	7.26^{b}	1.53	2.38	0.43	1.30
	5.0	82.91	21.62	7.95^{ab}	1.49	2.36	0.44	1.38
SEM		0.62	0.25	0.11	0.10	0.06	0.01	0.03
p-value		0.248	0.344	0.026	0.997	0.352	0.982	0.558

RH = rice hulls; SH = soybean hulls.

a,bValues with different superscripts in the same column are significantly different ($p<0.05$).

Table 6 – The effects of different sources and levels of fiber on intestinal weight (g/100 g BW) and length (cm/100 g BW) in 42 days old broiler chickens (mean ± SE; n = 7).

Item	Level of fiber (%)	Intestinal weight (g/100 g BW)			Intestinal length (cm/100 g BW)		
		Duodenum	jejunum	Ileum	Duodenum	jejunum	Ileum
Control	0	0.63	1.14	0.90	1.24	3.04^{b}	2.96^{c}
RH	2.5	0.65	1.11	0.84	1.26	3.06^{b}	3.03^{bc}
	5.0	0.60	1.10	0.88	1.32	3.55^{ab}	3.62^{ab}
SH	2.5	0.66	1.11	0.89	1.35	3.14^{b}	3.18^{bc}
	5.0	0.67	1.16	0.96	1.42	3.72^{a}	3.90^{a}
SEM		0.01	0.02	0.02	0.02	0.07	0.08
p-value		0.636	0.963	0.584	0.103	0.002	<0.001

RH = rice hulls; SH = soybean hulls.

a,b,c Values with different superscripts in the same column are significantly different ($p<0.05$).

Table 7 – The effects of different sources and levels of fiber on intestinal morphological measurements in 42 days old broiler chickens (mean ± SE; n = 7).

Item	Level of fiber (%)	Villus height (mm)			Villus area (mm^2)			Crypt depth (mm)		
		Duodenum	jejunum	Ileum	Duodenum	jejunum	Ileum	Duodenum	jejunum	Ileum
Control	0	1.04^{b}	1.04	0.61	0.15	0.14	0.07	0.27	0.25	0.17
RH	2.5	1.38^{a}	0.87	0.71	0.19	0.11	0.08	0.26	0.20	0.15
	5.0	1.14^{ab}	0.95	0.68	0.16	0.10	0.07	0.26	0.20	0.16
SH	2.5	1.35^{ab}	0.86	0.74	0.21	0.10	0.09	0.31	0.26	0.21
	5.0	1.34^{ab}	0.91	0.67	0.21	0.12	0.07	0.32	0.23	0.16
SEM		0.043	0.039	0.033	0.010	0.007	0.005	0.009	0.011	0.010
p-value		0.020	0.673	0.842	0.338	0.401	0.719	0.163	0.393	0.444

RH = rice hulls; SH = soybean hulls.

a,bValues with different superscripts in the same column are significantly different ($p<0.05$).

DISCUSSION

Effect of different sources and levels of fiber on growth performance and fecal ammonia nitrogen

It is commonly reported that dietary fiber decreases nutrient digestibility and chicken performance (Sklan *et al*., 2003). This result is similar to that from Santos *et al*. (2019), who found decreased body weight gain when diets with 2.5% SH were fed to broilers from 1 to 21 d of age. Similarly, Sklan *et al*. (2003) found that the inclusion of up to 3% soybean hulls in diets decreased body weight gain in turkeys (1 to 4 wk of age). However, turkeys at 14 wk of age fed 6 or 9% soybean hulls had more body weight gain than those fed 3% soybean hulls. Similar to the present findings, there was no significant difference between the fiber groups and the control group in broilers from 22 to 42 d of age. Contrary to these findings, González-Alvarado *et al*. (2007) reported that the inclusion of a fiber source (oat hulls or soybean hulls) reduced average daily feed intake without affecting average daily gain and is consequence of the improved feed conversion ratio. This finding might be due to the differences in the level and type of dietary fiber, as well as the age of the chicken.

The addition of fiber to diets reduces the amount of NH_3 emitted from the manure of laying hens (Roberts *et al*., 2007) and pigs (Canh *et al*., 1997; Shriver *et al*., 2003). In typical cases, the nitrogen excreted in feces consists of undigested dietary nitrogen and endogenous nitrogen, mainly as amino acids and bacterial protein. In poultry feces, the ammonia volatilization has been attributed to the microbial decomposition of nitrogenous compounds, primarily uric acid (Li *et al*., 2008). Therefore, nitrogen content was determined in broiler feces. Kirchgessner *et al*. (1994) reported that the inclusion of moderate amounts of different fiber sources in pig diets affects the growth of bacterial populations in the large intestine, resulting in decreased NH_3 emission. Consistent with this finding, our previous study (Santos *et al*., 2019) found that the fecal ammonia nitrogen of broilers from 1 to 21 d of age decreased in the 2.5% RH and 5.0% SH groups. González-Alvarado *et al*. (2007) found that a large and well-developed gizzard improves nutrient utilization, resulting in decreased fecal ammonia nitrogen. However, in the present study, from 22 to 42 d of age, there was no significant difference in fecal ammonia nitrogen between the fiber groups and the control group. This effect might be due to the reduced grinding activity of the gizzard in the present study.

Effect of different sources and levels of fiber on carcass traits and digestive organ development

Lu *et al*. (1996) reported that relative organ weight could be used as an indicator of organ function. In the present study, the experimental diets with 2.5% SH significantly decreased the wing weight of chickens compared with the control. Similarly, Shahin & Abdelazim (2005) found that high fiber inclusion in broiler diets decreased carcass weight. Mateos *et al*. (2012) reported that dietary fiber decreased the intestinal length and weight of the organs of birds. Consequently, these changes might reduce carcass yield (Jørgensen *et al*., 1996).

Our present trial is in agreement with a few others (Preston *et al*., 2000; Taylor & Jones, 2001; Mourão *et al*., 2008) in which dietary fiber increased the relative length of the small intestine. The longer relative length of the small intestine in the fiber groups might be due to the increased effort of this organ to adapt to improve feed consumption and nutrient uptake (Mourão *et al*., 2008). However, the results of our study did not agree with those of a few other reviews. For example, Amerah *et al*. (2009) and Sklan *et al*. (2003) found that increasing the insoluble fiber in the diet reduced the length of the small intestine. These conflicting findings may be due to the differences in the physicochemical characteristics and amount of the fiber sources as well as its particle size (Mateos *et al*., 2012).

Effect of different sources and levels of fiber on intestinal morphology

Histologically, increased villus height and cell mitosis number in the intestine are indicators of activated villus function (Langhout *et al*., 1999). Intestinal crypt development affects the maintenance of crypt-cell turnover rates and intestinal maturation. Therefore, deeper crypts result in an increased intestinal absorption surface area (Geyra *et al*., 2001). Caspary (1992) reported that greater villus height contributes to an increased surface area for greater absorption of available nutrients. A deeper crypt indicates faster tissue turnover and higher demand for new tissue (Yason *et al*., 1987). This higher demand for faster turnover lowers the efficiency of the animals (Xu *et al*., 2003). Some researchers have demonstrated that the physicochemical characteristics of dietary fiber induce physiological and histological changes in the intestine (Jankowski *et al*., 2009; Juskiewicz *et al*., 2009). In the present study, dietary fiber in broiler diets contributed to an increase in the duodenal villus height. Sittiya *et al*. (2016) and Jiménez-Moreno *et al*. (2011) reported an improvement in the villus height:crypt depth ratio in broilers with the inclusion of whole rice grain and pea hulls, respectively. In addition, Sklan *et al*. (2003) found that the surface area of the small intestine of turkeys increased as the level of crude fiber in the diet increased from 2.7 to 7.9%. Awad *et al*. (2006) found that a greater villus height contributed to an increased surface area for greater absorption of available nutrients. The higher duodenal villus of broilers fed 2.5% RH might be related to the longer relative length of the small intestine in the fiber groups. This combined change might be because of the increasing effort of the small intestine to adapt to improve feed consumption and nutrient uptake (Mourão *et al*., 2008). However, the results of our study did not agree with those of Kalmendal *et al*. (2011), who observed that high-fiber sunflower cake inclusion resulted in linear reductions in villus height.

CONCLUSION

The inclusion of different fiber sources in the broiler diets led to the development of intestinal morphology, whereas there were few changes in the digestive organ. These results suggest that the inclusion of fiber in the diet enhances the development of the gastrointestinal tract without negatively affecting growth performance and carcass traits.

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CONFLICT OF INTEREST DECLARATION

No potential conflict of interest was reported by the authors.

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