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

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 (NH₃) 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 NH₃ emissions; however, poultry farms also cause air pollution through the release of NH₃. 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). NH₃ 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 dietary 10% 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.

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

| 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  |

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

*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         | 1416.64             | 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).
**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\textsubscript{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\textsubscript{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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