Final report

Project Title: Ammonia emission and intestinal morphological alteration of broilers affected by dietary fiber sources

By Janjira Sittiya

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บทคัดย่อ

รหัสโครงการ: MRG6080075

ชื่อโครงการ: การลดกลิ่นแอมโมเนียและการเปลี่ยนแปลงทางสัณฐานวิทยาของลำไส้ไก่กระทง หลังจากได้รับอาหารที่มีเยื่อใย

ชื่อนักวิจัย และสถาบัน: ดร.จันทร์จิรา สิทธิยะ คณะสัตวศาสตร์และเทคโนโลยีการเกษตร มหาวิทยาลัยศิลปากร

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บทคัดย่อ:

การเพิ่มขึ้นของอุตสาหกรรมการเลี้ยงสัตว์ในปัจจุบันส่งผลต่อปัญหาสิ่งแวดล้อมเพิ่มมากขึ้น เนื่องจากมูลสัตว์เป็นสาเหตุหนึ่งของการเกิดก๊าซแอมโมเนียซึ่งส่งผลกระทบโดยตรงต่อสุขภาพสัตว์และคน นอกจากนี้ ก๊าซแอมโมเนียยังเป็นอีกหนึ่งสาเหตุที่ทำให้เกิดฝนกรดโดยทำลายชั้นบรรยากาศ จากการรวบรวมข้อมูลงานวิจัยที่เกี่ยวข้องกับการใช้แกลบ (rice hulls; RH) และเปลือกหุ้มเมล็ดถั่วเหลือง (soybean hulls; SH) เพื่อเป็นแหล่งเยื่อใยในอาหารไก่ยังพบน้อย ดังนั้น งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของการใช้แหล่งของเยื่อใยในระดับต่างๆต่อระดับแอมโมเนียไนโตรเจนในมูล ประสิทธิภาพการเจริญเติบโต ลักษณะซาก การพัฒนาของระบบทางเดินอาหาร และลักษณะทางสัณฐานวิทยาของลำไส้ในไก่กระทง โดยไก่กระทงอายุ 1 วัน จำนวน 420 ตัว ถูกแบ่งออกเป็น 5 กลุ่ม กลุ่มละ 7 ซ้ำ ซ้ำละ 12 ตัว อาหารทดลองแบ่งเป็น 5 สูตรตามกลุ่มการทดลอง คือ Control, 2.5% RH, 2.5% SH, 5.0% RH และ 5.0% SH โดยแบ่งการทดลองออกเป็น 2 ช่วง คือ ไก่เล็ก (0 - 21 วัน) และไก่โต (22 - 42 วัน) จากผลการทดลองพบว่า ในช่วงไก่เล็ก น้ำหนักตัวที่เพิ่มขึ้นของไก่กระทงที่ได้รับอาหาร Control, 2.5% RH, 5.0% RH และ 5.0% SH มีค่าสูงกว่าไก่กระทงกลุ่มที่ได้รับอาหาร 2.5% SH ($p < 0.05$) ในขณะที่ไม่พบความแตกต่างในไก่โต ($p > 0.05$) ในช่วงไก่เล็กพบว่า ระดับแอมโมเนียไนโตรเจนในมูลของไก่กลุ่มที่ได้รับอาหาร 2.5% RH และ 5.0% SH ลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในขณะที่ไม่พบความแตกต่างในช่วงไก่โต เมื่อเปรียบเทียบกับกลุ่มควบคุม อาหารทดลองที่เสริมเยื่อใยทำให้ค่า pH ของกึ๋นลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในขณะที่ไม่พบความแตกต่างของ pH ในลำไส้เล็กส่วนต้น กลาง และปลาย ($p > 0.05$) ไก่กลุ่มที่ได้รับอาหาร 2.5% และ 5.0% SH มีวิลไลส่วนดูโอดินัมยาวกว่าไก่กลุ่มที่ได้รับอาหารควบคุม ($p < 0.05$) ในขณะที่อาหารทดลองที่เสริมเยื่อใยไม่มีผลต่อความลึกของคริปท์ ($p > 0.05$) จากผลการทดลองสรุปได้ว่า การเสริมเปลือกหุ้มเมล็ดถั่วเหลืองที่ระดับ 5.0% สามารถกระตุ้นการพัฒนาลักษณะสัณฐานวิทยาของลำไส้ไก่กระทง และลดระดับแอมโมเนียไนโตรเจนในมูล โดยไม่มีผลกระทบต่อการเจริญเติบโตและลักษณะซากของไก่กระทง

คำหลัก: แกลบ; เปลือกหุ้มเมล็ดถั่วเหลือง; ไก่กระทง; แอมโมเนียไนโตรเจน; สัณฐานวิทยาของลำไส้

Abstract

Project Code: MRG6080075

Project Title: Ammonia emission and intestinal morphological alteration of broilers affected by dietary fiber sources

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Project Period: 2 years (4 April, 2017 – 4 April, 2019)

Abstract:

At present, increasing levels of livestock production are causing many environmental problems. Animal manure from livestock production can be a cause of Ammonia (NH_3) emission impacting animal and human health. Moreover, NH_3 emissions contribute to acid rain and nitrogen deposition that damage natural ecosystems. To the best of our knowledge, no information is available on the use of rice hulls (RH) and soybean hulls (SH) as a fibre source in broiler diets. The nutritional and physiological effects must be considered; however, these effects are caused by differences in the structure and properties of fibre sources. Therefore, the aim of this study was to investigate the effects of dietary fibre source levels on the faecal ammonia nitrogen, growth performance, carcass traits, gastrointestinal tract development, and intestinal morphology of broilers. A total of 420 one-day-old broiler chicks were individually weighed and randomly divided into 5 groups of chicks with similar mean body weight, each with seven replicates of twelve chicks. The experimental diets included RH and SH were as follows: 0% (Control); 2.5% RH; 2.5% SH; 5% RH; 5% SH. In grower period, the body weight gain of broilers in the control, 2.5% RH, 5.0% RH, and 5.0% SH groups were significantly higher than those of the 2.5% SH group ($p < 0.05$). Moreover, the faecal ammonia nitrogen of broilers decreased in the 2.5% RH and 5.0% SH groups ($p < 0.05$), while, there was no significant difference in fecal ammonia nitrogen in finisher period. Fiber inclusion reduced gizzard pH ($p < 0.05$) but did not alter duodenum, jejunum, and ileum ($p > 0.05$). 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. 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, resulting in improved intestinal morphology and decreased faecal ammonia nitrogen, without negatively affecting growth performance and carcass traits in broilers.

Keywords: rice hull; soybean hull; broilers; ammonia nitrogen; intestinal morphology

Executive summary

In Thailand, increasing livestock production numbers can establish many environmental problems. Animal manure from livestock production can be a cause of Ammonia (NH_3) emission impacting animal and human health. Moreover, NH_3 emission is an environmental problem because it has been related with the nitrification and acidification of rain. Nitrogen (N) in the feces containing undigested dietary N, endogenous N and microbial N (Jha and Berrocoso, 2016) can lead to NH_3 emission to the atmosphere and be converted to nitrate during storage (Ferket *et al*., 2002). NH_3 emissions contribute to acid rain and nitrogen deposition that damage natural ecosystems.

More than 80% of total NH_3 emissions were from livestock production such as cattle, poultry and swine farming (Battye *et al*., 1994). In Thailand, swine farms being the largest source of NH_3 emission, however, poultry farms also cause air pollution through the release of NH_3. The NH_3 emission from manure has become a major problem not only for the human and chicken health, but also in the poultry production such as; lower egg production and growth performance (Carlile, 1984; Miles *et al*., 2004). Therefore, several methods have been evaluated for reducing NH_3 emission from poultry manure affecting animal and human health as well as environment. Many researchers used the diet composition improving method to decrease the manure pollutants. Interestingly, dietary fiber has been shown to lower NH_3 emission from pigs (Canh *et al*., 1997; Shriver *et al*., 2003) and laying hens (Roberts *et al*., 2007). Moreover, inclusion of feed ingredients with adequate type and amount of fiber might improve gastro-intestinal tract (GIT) leading to reduce antibiotic use in feed (Mateos *et al*., 2002; Montagne *et al*., 2003).

Generally, the physico-chemical characteristics of fiber can be divide into two major subclasses; 1) soluble fiber (fermentable), 2) insoluble fiber (non-fermentable). Both of them have different roles in digestive and absorptive processes in the GIT (Asp, 1996; Sarikhan *et al*., 2010). Many researchers reported that the inclusion of soluble fiber led to a decrease of NH_3 emission from swine manure (Kreuzer *et al*., 1998; Beccacia *et al*., 2015). In insoluble fiber, Bindelle *et al*. (2009) found that the inclusion of oat hulls did not reduce the urinary nitrogen excretion ratio. In contrast, Roberts *et al*.

(2007) found that inclusion of 10% corn dried distillers grains with solubles (DDGS), 7% wheat middlings (WM), or 5% soy bean hulls (SH) as insoluble fiber in laying hen diets lowered total manure NH_3 emission and the NH_3 emission rate. Moreover, insoluble fiber in broiler diets improved the intestinal morphology and growth performance (Sarikhan *et al*., 2010).

As mentioned previously, NH_3 emission can be reduced from manure by inclusion of fiber (soluble and insoluble) in diets. In Thailand, rice is the major locally produced crop, which amounted to 20.7 million metric tons in 2018/19 (USAD 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 in Thailand that is generated in soybean oil processing. To the best of our knowledge, no information is available on the use of rice hulls and soybean hulls as a fibre source in broiler diets. The nutritional and physiological effects must be considered; however, these effects are caused by differences in the structure and properties of fibre sources. I hypothesized that inclusion of moderate amounts of fiber have been some of the alternatives proposed to reduce NH_3 emission affecting animal and human health as well as environment and improve the intestinal morphology.

Therefore the aim of this experiment was to investigate the effects of dietary fiber on performance, carcass traits, development of gastrointestinal tracts and digestibility, fecal ammonia nitrogen, and intestinal morphology of broilers. The experiment was conducted in accordance with the guidelines and rules for animal experiments of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Thailand. A total of 420 one-day-old broiler chicks were obtained from a commercial hatchery. Chicks were individually weighed and randomly divided into 5 groups of chicks with similar mean body weight, each with seven replicates of twelve chicks. The rice hull (RH) and soybean hull (SH) were ground through a hammer mill with a 2-mm screen. Experimental grower (0-21 d) and finisher (22-42 d) diets were in mash form and included RH and SH were as follows: 0% (Control); 2.5% RH; 2.5% SH; 5% RH; 5% SH. Feed and water were provided *ad libitum* for 42 d. During the experiment, feed intake and body weight were measured weekly, and feed efficiency

was calculated. The fecal ammonia nitrogen was measured during the last week of two periods. At 42 d of age, 7 birds from each group were weighed individually and slaughtered to determine digestive organ development and carcass traits. Another 7 birds per group were used for intestinal morphological observations.

In grower period, the body weight gain of broilers in the control, 2.5% RH, 5.0% RH, and 5.0% SH groups were significantly higher than those of the 2.5% SH group ($p < 0.05$). In grower period, the fecal ammonia nitrogen of broilers decreased in the 2.5% RH and 5.0% SH groups ($p < 0.05$), while, there was no significant difference in fecal ammonia nitrogen in finisher period. Fiber inclusion reduced gizzard pH ($p < 0.05$) but did not alter duodenum, jejunum, and ileum ($p > 0.05$). The chicks receiving 2.5 and 5% of SH were higher villus height in the duodenum than broiler fed the control diet ($p < 0.05$). However, none of dietary treatments led to remarkable changes in any crypt depth and villus area of these segments, included jejunum and ileum. These findings suggest that the inclusion of 5% SH in the diets, resulting in improved intestinal morphology, without negatively affecting growth performance and carcass traits.

Ammonia emission and intestinal morphological alteration of broilers affected by dietary fiber sources

1. Introduction to the research problem and its significance

In Thailand, increasing livestock production numbers can establish many environmental problems. Animal manure from livestock production can be a cause of Ammonia (NH_3) emission impacting animal and human health. Moreover, NH_3 emission is an environmental problem because it has been related with the nitrification and acidification of rain. Nitrogen (N) in the feces containing undigested dietary N, endogenous N and microbial N (Jha and Berrocoso, 2016) can lead to NH_3 emission to the atmosphere and be converted to nitrate during storage (Ferket *et al*., 2002). NH_3 emissions contribute to acid rain and nitrogen deposition that damage natural ecosystems.

More than 80% of total NH_3 emissions were from livestock production such as cattle, poultry and swine farming (Battye *et al*., 1994). In Thailand, swine farms being the largest source of NH_3 emission, however, poultry farms also cause air pollution through the release of NH_3. The NH_3 emission from manure has become a major problem not only for the human and chicken health, but also in the poultry production such as; lower egg production and growth performance (Carlile, 1984; Miles *et al*., 2004). Therefore, several methods have been evaluated for reducing NH_3 emission from poultry manure affecting animal and human health as well as environment. Many researchers used the diet composition improving method to decrease the manure pollutants. Interestingly, dietary fiber has been shown to lower NH_3 emission from pigs (Canh *et al*., 1997; Shriver *et al*., 2003) and laying hens (Roberts *et al*., 2007). Moreover, inclusion of feed ingredients with adequate type and amount of fiber might improve gastro-intestinal tract (GIT) leading to reduce antibiotic use in feed (Mateos *et al*., 2002; Montagne *et al*., 2003).

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As mentioned previously, NH_3 emission can be reduced from manure by inclusion of fiber in diets. In Thailand, rice is the major locally produced crop, which amounted to 20.7 million metric tons in 2018/19 (USAD 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 in Thailand that is generated in soybean oil processing. To the best of our knowledge, no information is available on the use of rice hulls and soybean hulls as a fibre source in broiler diets. The nutritional and physiological effects must be considered; however, these effects are caused by differences in the structure and properties of fibre sources.

Therefore the aim of this experiment was to investigate the effects of dietary fiber on performance, carcass traits, development of gastrointestinal tracts, fecal ammonia nitrogen, and intestinal morphology of broilers.

2. Literature review

NH_3 emission from animal farms is one of important pollutants impacting environment and public health (Aneja *et al*., 2006). The National Institute of Occupational Safety and Health (NIOSH) has established a threshold limit of 25 ppm averaged over 8 h. The United Egg Producers animal husbandry guidelines (United Egg Producers, 2006) reported that atmospheric NH_3 concentrations below 25 ppm in laying-hen houses. The NH_3 emission from manure has become a major problem not only for the human and chicken health, but also in the poultry production such as; lower egg production and growth performance (Carlile, 1984; Miles *et al*., 2004). Therefore, several approaches have been suggested for reducing NH_3 emissions from animal farms: 1) reduce animal production; 2) reduce nitrogen excretion through nutritional manipulation in diets; 3) reduce volatile NH_3 in the manure to stop NH_3 loss; 4) segregate urine from feces to reduce contact between urase and urine. Among these strategies, improving animal nutritional manipulation is the most interesting and feasible approach to reduce NH_3 emission from manure. Another report, Ferket *et al*. (2002) summarized the nutritional strategies to reduce nutrient emissions from poultry and swine (Table 1).

Table 1 Potential reduction in the excretion of nitrogen and phosphorus by various nutritional strategies in poultry and swine.

Strategy	Reduction in nutrient excretion
Formulation closer to requirements	10 to 15% for N and P
Reducing feed spillage/waste	1.5% for all nutrients for every 1%
Pelleting	reduction
700 to 1,000 µm (fineness of grind)	5% for N, P, Zn, Cu
Use of highly digestible feed ingredients	5% for N, P, Zn, Cu
Reduce variability by quality control	5% for N and P
	10 to 25% for N and P
Reduced protein/amino supplementation	10 to 25% for N in poultry; 10 to 40% for
Low-phytate (HAP) corn	N in swine
Phytase/low dietary P	9% for N for every 1% reduction in dietary
Phytase/HAP corn	CP
Phytase/enzyme cocktails	25 to 50% for P
Phytase/1,25$(OH)_2D_3$[a]	2 to 5% for N, Zn, and 20 to 30% for P
Phytase/probiotics	2 to 5% for N, Zn, and 20 to 40% for P
Cellulases, xylanases, pentosanase, *ß*-	2 to 8% for N, Zn, and 20 to 40% for P
glucanase	2 to 5% for N, Zn, and 20 to 60% for P
Growth promotion feed additives	2 to 5% for N, Zn, and 20 to 40% for P
Phase feeding	5% for N and P for appropriate diet
Split-sex feeding	5% for all nutrients
Reducing microminerals/organic minerals	5 to 10% for N and P
	5 to 8% for N
	Up to 50% for Zn, Cu, Mn

[a]1,25$(OH)_2D_3$ = 1,25-dehydroxycholecalciferol

Source: Ferket *et al*. (2002)

Moreover, an inclusion of fiber in diets reduces the amount of NH_3 emission from manure of laying hens (Roberts *et al*., 2007) and pigs (Canh *et al*., 1997; Shriver *et al*., 2003). It has been reported that the inclusion of moderate amounts of different fiber sources in the diet improves digestive organ development (González-Alvarado *et al*., 2007; Hetland *et al*., 2007) resulting in growth performance (Sklan *et al*., 2003) and gastrointestinal tract health enhancement (Correa-Matos *et al*., 2003; Perez *et al*., 2011). However, fiber inclusion in diets for broilers has not been studied in NH_3 emission and intestinal morphological alteration.

2.1 NH_3 emission

Diet composition affects NH_3 emission from animal manure. N from animal diets transformed to animal products, such as meat and eggs. Moreover, approximately 50% of the N consumed by birds is excreted as uric acid, and about 15% of the N consumed by animal is lost in the feces (Figure 1). N in the feces containing undigested dietary N, endogenous N and microbial N (Jha and Julio, 2016) can lead to NH_3 emission to the atmosphere and be converted to nitrate during storage (Ferket *et al*., 2002).

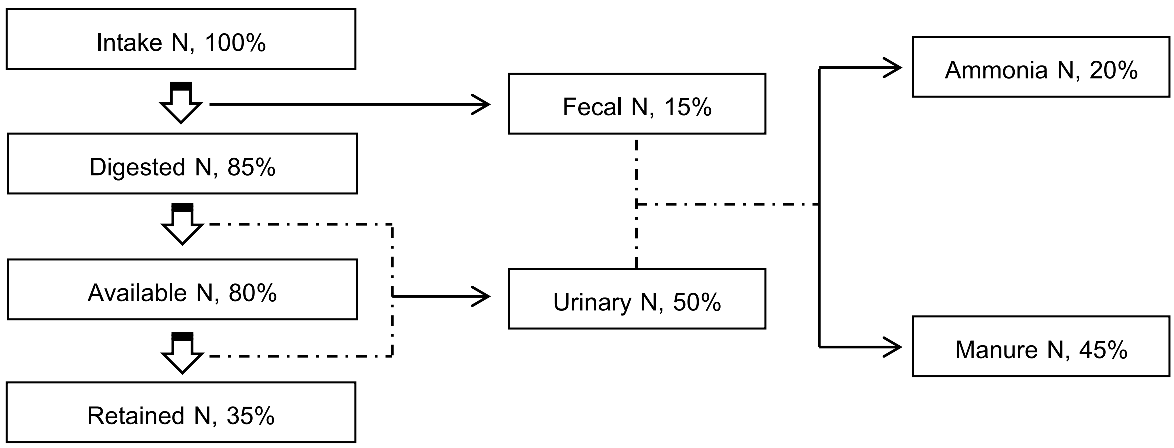

Figure 1 Nitrogen flow in poultry and swine.

Source: Ferket *et al*. (2002)

2.2 General effects of dietary fiber

Fiber can be defined as non-starch polysaccharides (NSP) and lignin in plant materials which are indigestible by animal endogenous enzymes (Urriola *et al*., 2013).

Chemically, dietary fiber is often defined as NSP (Englyst, 1989). The NSP are mainly cellulose, pectins, ß-glucans, pentosans and xylans. NSP are generally defined as water soluble and insoluble (Montagne *et al*., 2003). Solubility of NSP depends on their chemical structures and related with cell wall components. Both of soluble and insoluble NSP have different nutritional impacts in poultry diets resulting in digestive and absorptive processes of the GIT (Sarikhan *et al*., 2010; Mateos *et al*., 2012).

Soluble NSP is known to increase intestinal viscosity (Choct *et al*., 1996), and subsequently reduces the efficiency of nutrient digestion and absorption (Johnson and Gee, 1981; Lázaro *et al*., 2003). Moreover, the reduced digesta transit rate affects undigested nutrients remain in the gut for a longer period of time increasing the number of microbes in the intestine (Choct *et al*., 1996). Whereas, insoluble fiber sources, stimulate gizzard activity and reduce gizzard pH and the length of the GIT (González-Alvarado *et al*., 2007). The results of other studies indicate that the pH of the gastrointestinal tract correlates with dietary fiber (González-Alvarado *et al*., 2008; Jiménez-Moreno *et al*., 2009). These results are consistent with Duke (1986) who reported that dietary fiber stimulated the production of HCl in the proventriculus via mechanoreceptors, lowering the pH in the upper part of the GIT. Some reports have shown that inclusion of moderate concentration of fiber in diet affected GIT improvement (Montagne *et al*., 2003). Choct *et al*. (2001) found that insoluble fiber source can affect the gut transit time and gut motility of intestinal epithelium enhancing the ability of endogenous enzyme. Therefore, the poultry industry required a minimal amount of fiber in feed formulation.

2.3 Influence of dietary fiber on NH_3 emission and intestinal morphology

In poultry manure, the volatilization of ammonia has been attributed to microbial decomposition of nitrogenous compounds, mainly uric acid (Li *et al*., 2008). Roberts *et al*. (2007) reported that 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. Numerous studies found that the inclusion of soluble fiber led to a decrease of NH_3 emission from swine manure (Kreuzer *et al*., 1998; Beccacia *et al*., 2015). In insoluble fiber, Bindelle *et al*. (2009) found that the inclusion of oat hulls did not reduce the urinary nitrogen excretion ratio. Very little scientific literature exists that examines the effect of fiber source on NH_3 emission of poultry. There is some evidence that inclusion of fiber (10% corn dried distillers grains with solubles, 7% wheat middlings, or 5% soy bean hulls) in laying hen diets lowered total manure NH_3 emission and the NH_3 emission rate (Roberts *et al*., 2007).

A small number of studies have examined the changes in intestinal morphology caused by dietary fiber in diets. Some researchers have demonstrated that the physicochemical characteristics of dietary fiber are thought to induce physiological and histological changes in the intestine (Jankowski *et al*., 2009; Juskiewicz *et al*., 2009). Rezaei *et al*. (2011) reported that a diet supplemented with insoluble fiber increases the ileal villus height:crypt depth ratio and decreases ileal crypt depth. These observations correspond with Sittiya *et al*. (2016) that inclusion of whole-grain paddy rice (fiber rich) in broiler diets affected the intestinal morphology. In contrast, Jin *et al*. (1994) found that inclusion of high fiber in growing pig diets caused an enlargement of villi and deepening of the jejunal and ileal crypts.

3. Objectives

To investigate the effects of different level of fiber source on fecal ammonia nitrogen, growth performance, carcass traits, development of gastrointestinal tracts, and intestinal morphology of broilers.

4. Methodology

4.1 Animals and diets

A total of 420 one-day-old broiler chicks (ROSS 308) were obtained from a commercial hatchery. Chicks were individually weighed and randomly divided into 5 groups of chicks with similar mean body weights, each with seven replicates of twelve chicks. The RH and SH were ground through a hammer mill with a 2-mm screen. Subsequently, the chemical composition of the fibre samples was analysed as described by AOAC (2000) and Van Soest *et al*. (1991) (Table 2). Experimental grower (0-21 d) and finisher (22-42 d) 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 3, 4). Feed and water were provided *ad libitum* for 42 d.

The experiment was conducted in accordance with the guidelines and rules for animal experiments of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Thailand.

Table 2 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

4.2 Growth performance and fecal ammonia nitrogen

During the experiment, feed intake and body weight were measured weekly, and feed efficiency was calculated.

The fecal ammonia nitrogen was measured during the last week of two periods (during wk 3 and wk 6 of the study). The 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, faeces was collected over 3 consecutive 24-h periods in each cage. The faeces from each of the 24-h periods was pooled by group and stored at -20°C until analysis. Faecal ammonia nitrogen was analysed by the method of AOAC (2000). The procedure was repeated for a second period.

Table 3 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.

Table 4 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.

4.3 Carcass traits and digestive organ development

At 42 d of age, 7 birds from each group were weighed individually and slaughtered to determine digestive organ development and carcass traits. Head, digestive organ, and shanks were removed and then weighed. Wings, abdominal fat, thighs and drumsticks were removed and weighed individually. In the digestive organs, the lengths of the duodenum, jejunum, ileum and ceca were measured individually. The weights of the proventriculus, gizzard, duodenum, jejunum, ileum and ceca were then recorded after the digesta content had been removed. The weight of empty organs was expressed relative to 100 g body weight. Samples of digesta of each bird were then diluted 1:3 (w/w) with deionized distilled water and stirrer-mixed. The pH was measured using a digital pH meter (AD12, Adwa Instruments, Hungary).

4.4 Intestinal morphological observation

At 42 days of age, another 7 birds per group were used for intestinal morphological observations. Immediately following decapitation, the midpoint of each intestinal segment (duodenum, jejunum and ileum) were removed and fixed in 10% neutral-buffered formalin. After dehydration through varying concentrations of alcohol, each intestinal segment were embedded in paraffin wax. A 4-μm-thick transverse section was cut and stained with hematoxylin-eosin, 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 regarded as the mean of each bird. The villus area was calculated from the villus height, basal width and apical width (Iji *et al*., 2001). The 8 calculations of villus area were expressed as the mean of each bird. Crypt depth was defined as the distance from the villus base to the muscularis layer, not including the intestinal muscularis (Rezaei *et al*., 2011).

4.5 Statistical analysis

The data from the experimental groups were statistically analyzed using one-way analysis of variance (ANOVA) in the SPSS statistical software package (version

19.0; IBM Corp. Armonk, NY, US). Significant differences among the treatments were determined with Tukey's tests. Statistical significance was accepted at $p < 0.05$.

5. Results

5.1 Growth performance and fecal ammonia nitrogen

Influence of different sources and levels of fiber on growth performance and fecal ammonia nitrogen in broiler chickens are presented in Table 5 and Table 6. In grower period (Table 5), the body weight gain of broilers in the control, 2.5% RH, 5.0% RH, and 5.0% SH groups were significantly higher than those of the 2.5% SH group ($p < 0.05$). In grower period (Table 5), the fecal ammonia nitrogen of broilers decreased in the 2.5% RH and 5.0% SH groups ($p < 0.05$), while, there was no significant difference in fecal ammonia nitrogen in finisher period (Table 6).

TABLE 5 The effects of different sources and levels of fiber on growth performance (mean ± SE; n = 7) and faecal ammonia nitrogen (mean ± SE; n = 4) in broiler chickens from 0 to 21 days of age

Item	Level of fiber (%)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio	Faecal ammonia nitrogen (mg/g)
Control	0	1190.00	852.62^{a}	1.39	0.93^{a}
RH	2.5	1161.42	827.37^{a}	1.40	0.13^{b}
	5.0	1170.85	831.27^{a}	1.40	0.72^{a}
SH	2.5	1100.83	747.00^{b}	1.48	0.58^{ab}
	5.0	1144.28	805.11^{a}	1.42	0.11^{b}
SEM		20.50	14.62	0.02	0.08
p-value		0.750	0.010	0.890	0.001

RH = rice hulls; SH = soybean hulls

^{a,b}Values 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 growth performance (mean ± SE; n = 7) and faecal ammonia nitrogen (mean ± SE; n = 4) in broiler chickens from 22 to 42 days of age

Item	Level of fiber (%)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio	Faecal 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 ($p > 0.05$)

5.2 Carcass traits and digestive organ development

Compared with the control, the experimental diets with 2.5% SH significantly decreased the wing weight of chickens ($p < 0.05$), while no significant differences in weight of the other visceral organs was seen (Table 7).

The gizzard pH was lower in broilers fed the 2.5% SH diet than in those fed the control diet ($p < 0.05$). The treatments, however, had no effect on the pH of duodenum and jejunum ($p > 0.05$). Moreover, a trend of improved ileum pH ($p = 0.072$) was found following inclusion of fiber (Table 8).

The intestinal weight and length of broilers are presented in Table 9. Compared with the control, broilers in 5.0% SH group had higher the length of jejunum and ileum ($p < 0.05$). In terms of intestinal weight and duodenal length, there were no significant difference among the dietary treatment groups ($p > 0.05$).

TABLE 7 The effects of different sources and levels of fiber on carcass and visceral organs weight (g/100 g BW) in 42 days 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,b]Values with different superscripts in the same column are significantly different ($p < 0.05$).

TABLE 8 The effects of different sources and levels of fiber on pH of gastrointestinal tract (GIT) in 42 days old broiler chickens (mean ± SE; n = 7)

Item	Level of fiber (%)	pH			
		Gizzard	Duodenum	Jejunum	Ileum
Control	0	3.90[a]	5.97	5.87	5.89
RH	2.5	3.45[ab]	6.10	5.95	6.41
	5.0	3.37[ab]	6.17	6.19	6.65
SH	2.5	2.96[b]	6.17	6.04	6.33
	5.0	3.44[ab]	6.13	5.83	6.09
SEM		0.09	0.05	0.05	0.09
p-value		0.018	0.742	0.240	0.072

RH = rice hulls; SH = soybean hulls

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

TABLE 9 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 ($p < 0.05$).

5.3 Intestinal morphological measurements

Feeding the broilers SH and RH had no effect on the villus area and crypt depth of the intestine (Table 10). Compared with the control, the experimental diets with 2.5% RH significantly increased the duodenal villus height of chickens ($p < 0.05$).

TABLE 10 The effects of different sources and levels of fiber on intestinal morphological measurements in 42 days old broiler chickens (mean ± SE; n = 5)

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

6. Discussion

It is commonly reported that dietary fibre decreases nutrient digestibility and chicken performance (Sklan *et al*., 2003). This result is similar to that from a previous study by Santos *et al*. (2019), who found that body weight gain from 1 to 21 d of age was lower for broilers fed a 2.5% SH diet than for those fed control, 2.5% RH, 5% RH, and 5% SH diets. This finding is in agreement with the results of Sklan *et al*. (2003), who found that turkeys (1 to 4 wk of age) fed 3% soybean hulls had a lower body weight than those fed 6 or 9% soybean hulls. However, the opposite effect was found on body weight gain at 14 wk of age: turkeys 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 fibre 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 fibre source (oat hulls or soybean hulls) reduced average daily feed intake without affecting average daily gain; consequently, the feed conversion ratio was improved by fibre inclusion. This finding may be explained by the differences in the level and type of dietary fibre, as well as the age of the bird.

The addition of fibre 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 normal cases, the nitrogen excreted in faeces consists of undigested dietary nitrogen and endogenous nitrogen, mainly as amino acids and bacterial protein. In poultry faeces, the volatilization of ammonia has been attributed to microbial decomposition of nitrogenous compounds, mainly uric acid (Li *et al*., 2008). Therefore, nitrogen content was determined in broiler faeces. It has been reported that the inclusion of moderate amounts of different fibre sources in pig diets affects the growth of bacterial populations in the large intestine, resulting in decreased NH_3 emission (Kirchgessner *et al*., 1994). This finding is in agreement with our previous study (Santos *et al*., 2019), in which the faecal 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 faecal ammonia nitrogen. However, in the present study, from 22 to 42 d of age, there was no significant difference in faecal ammonia nitrogen between the fibre groups and the control group. This might be due to the poor grinding activity of the gizzard in the present study.

Lu *et al*. (1996) reported that relative organ weight can 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. This finding is confirmed by the research of Shahin and Abdelazim (2005), in which high fibre inclusion in broiler diets decreased carcass weight. Mateos *et al*. (2012) reported that dietary fibre decreased the intestinal length and weight of the organs of birds. Consequently, these changes might reduce the carcass yield (Jørgensen *et al*., 1996).

Our present trial is in agreement with a few others (Preston *et al*., 2000; Taylor and Jones, 2001; Mourão *et al*., 2008) in which dietary fibre increased the relative length of the small intestine. The longer relative length of the small intestine in the fibre groups might be due to the increasing effort of this organ to adapt in order 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 studies. For example, Amerah *et al*. (2009) and Sklan *et al*. (2003) found that increasing the insoluble fibre in the diet reduced the length of the small intestine. These conflicting findings may be due to differences in the type and amount of fibre as well as its particle size (Mateos *et al*., 2012).

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 animal (Xu *et al.,* 2003). Some researchers have demonstrated that the physicochemical characteristics of dietary fibre induce physiological and histological changes in the intestine (Jankowski *et al*., 2009; Juskiewicz *et al*., 2009). In the present study, dietary fibre in broiler diets contributed to an increase in the duodenal villus height. Similarly, 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. Moreover, Sklan *et al*. (2003) found that the surface area of the small intestine of turkeys increased as the level of crude fibre 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 fibre groups. This combined change might be because of the increasing effort of the small intestine to adapt in order 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-fibre sunflower cake inclusion resulted in linear reductions in villus height.

7. Conclusion

According to the faecal ammonia nitrogen, growth performance, carcass traits, gastrointestinal tract development, and intestinal morphology, the appropriate level of fibre sources in broiler diets was 5% SH in the present study. Furthermore, 5% SH has some positive effects on intestinal morphology and faecal ammonia nitrogen without negatively affecting growth performance and carcass traits.

8. References

Aneja VP, Schlesinger WH, Niyogi D, Jennings G, Gilliam W, Knighton RE, Duke CS, Blunden J, Krishnan S. 2006. Emerging national research needs for agricultural air quality. Eos. 87(3):25-36.

AOAC. 2000. Official Method of Analysis. 17th ed. Gaithersburg (MD): Association of Official Analytical Chemists.

Amerah AM, Ravindran V, Lentle RG. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br Poult Sci. 50(3):366-375.

Asp NG. 1996. Dietary carbohydrates: classification by chemistry and physiology. Food Chem. 57(1):9-14.

Awad WA, Böhm J, Razzazi-Fazeli E, Ghareeb K, Zentek J. 2006. Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult Sci. 85(6):974-979.

Battye R, Battye W, Overcash C, Fudge S. 1994. Development and selection of ammonia emission factors, Durham (US): Research Triangle Park (NC). Report No.: PB-95-123915/XAB. Environmental Protection Agency, US.

Beccaccia A, Calvet S, Cerisuelo A, Ferrer P, Garcia-Rebollar P, De Blas C. 2015. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing-finishing pigs. I. Influence of the inclusion of two levels of orange pulp and carob meal in isofibrous diets. Anim Feed Sci Tech. 208:158-169.

Bindelle J, Buldgen A, Delacollette M, Wavreille J, Agneessens R, Destain JP, Leterme P. 2009. Influence of source and concentrations of dietary fiber on in vivo nitrogen excretion pathways in pigs as reflected by in vitro fermentation and nitrogen incorporation by fecal bacteria. J Anim Sci. 87(2):583-593.

Canh TT, Verstegen MWA, Aarnink AJA, Schrama JW. 1997. Influence of dietary factors on nitrogen partitioning and composition of urine and feces of fattening pigs. J Anim Sci. 75(3):700-706.

Carlile FS. 1984. Ammonia in poultry houses: A literature review. World's Poult Sci J. 40(2):99-113.

Caspary WF. 1992. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr. 55 Suppl:299S-308S.

Choct M. 2001. Enzyme supplementation of poultry diets based on viscous cereals in enzymes. Bedford MR, Partridge GG: Farm Animal Nutrition, CABI Publishing, UK.

Choct M, Hughes RJ, Wang J, Bedford MR, Morgan AJ, Annison G. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br Poult Sci. 37:609-621.

Correa-Matos NJ, Donovan SM, Isaacson RE, Gaskins HR, White BA, Tappenden KA. 2003. Fermentable fiber reduces recovery time and improves intestinal function in piglets following Salmonella typhimurium infection. J Nutr. 133:1845-1852.

Duke GE. 1986. Alimentary canal: secretion and digestion, special digestive functions, and absorption. Sturkie PD: Avian Physiology, Springer-Verlag, New York, NY. p. 289-302

Englyst H. 1989. Classification and measurement of plant polysaccharides. Anim Feed Sci Tech. 23:27-42.

Ferket PR, Van Heugten E, Van Kempen TA, Angel R. 2002. Nutritional strategies to reduce environmental emissions from nonruminants. J Anim Sci. 80 E-Suppl:E168-E182.

Geyra A, Uni Z, Sklan D. 2001. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br J Nutr. 86(1):53-61.

González-Alvarado JM, Jiménez-Moreno E, Lázaro R, Mateos GG. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult Sci. 86(8):1705-1715.

González-Alvarado JM, Jiménez-Moreno E, Valencia DG, Lázaro R, Mateos GG. 2008. Effects of fiber source and heat processing of the cereal on the development and pH of the gastrointestinal tract of broilers fed diets based on corn or rice. Poult Sci. 87:1779-1795.

Hetland H, Svihus B. 2007. Inclusion of dust bathing materials affects nutrient digestion and gut physiology of layers. Journal Appl Poult Res. 16:22-26.

Iji PA, Saki A, Tivey DR. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br Poult Sci. 42(4):505-513.

Jankowski J, Juskiewicz J, Gulewicz K, Lecewicz A, Slominski BA, Zdunczyk Z. 2009. The effect of diets containing soybean meal, soybean protein concentrate, and soybean protein isolate of different oligosaccharide content on growth performance and gut function of young turkeys. Poult Sci. 88(10):2132-2140.

Jha R, Berrocoso JF. 2016. Dietary fiber and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: A review. Anim Feed Sci Technol. 212:18-26.

Jiménez-Moreno E, Chamorro S, Frikha M, Safaa HM, Lázaro R, Mateos GG. 2011. Effects of increasing levels of pea hulls in the diet on productive performance, development of the gastrointestinal tract, and nutrient retention of broilers from one to eighteen days of age. Anim Feed Sci Technol. 168:100-112.

Jiménez-Moreno E, González-Alvarado JM, de Coca-Sinova A, Lázaro R, Mateos GG. 2009. Effects of source of fibre on the development and pH of the gastrointestinal tract of broilers. Anim Feed Sci Tech. 154:93-101.

Jin L, Reynolds LP, Redmer DA, Caton JS, Crenshaw JD. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J Anim Sci. 72:2270-2278.

Johnson IT, Gee JM. 1981. Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. Gut. 22:398-403.

Jørgensen H, Zhao XQ, Knudsen KEB, Eggum BO. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br J Nutr. 75(3):379-395.

Juskiewicz J, Jankowski J, Zdunczyk Z, Lecewicz A, Przybylska-Gornowicz B, Zieba M. 2009. Effect of diets with different contents of soybean [alpha]-galactosides and crude fibre on modification of duodenal microstructure and selected parameters of nutrient utilization in young turkeys. Pol J Vet Sci. 12(4):455.

Kalmendal R, Elwinger K, Holm L, Tauson R. 2011. High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens. Br Poult Sci. 52(1):86-96.

Kirchgessner M, Kreuzer M, Machmüller A, Roth-Maier DA. 1994. Evidence for a high efficiency of bacterial protein synthesis in the digestive tract of adult sows fed supplements of fibrous feedstuffs. Anim Feed Sci Technol. 46(3-4):293-306.

Kreuzer M, Machmüller A, Gerdemann MM, Hanneken H, Wittmann M. 1998. Reduction of gaseous nitrogen loss from pig manure using feeds rich in easily-fermentable non-starch polysaccharides. Anim Feed Sci Technol. 73(1-2):1-19.

Langhout DJ, Schutte JB, Van Leeuwen P, Wiebenga J, Tamminga S. 1999. Effect of dietary high-and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br Poult Sci. 40(3):340-347.

Lázaro R, Garcia M, Aranibar MJ, Mateos GG. 2003. Effect of enzyme addition to wheat-, barley-and rye-based diets on nutrient digestibility and performance of laying hens. Br Poult Sci. 44:256-265.

Li H, Xin H, Liang Y, Burns RT. 2008. Reduction of ammonia emissions from stored laying hen manure through topical application of zeolite, Al+ Clear, Ferix-3, or poultry litter treatment. J Appl Poult Res. 17(4):421-431.

Lu CD, Schoknecht PA, Ellis KJ, Shypailo R, Su DR, Pond WG. (1996). Differential compensatory organ growth in young pigs after short-term rehabilitation from protein deficiency. Nutr Res. 16(4):627-637.

Mateos GG, Jiménez-Moreno E, Serrano MP, Lázaro RP. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J Appl Poult Res. 21(1):156-174.

Mateos GG, Lázaro R, Gracia MI. 2002. The feasibility of using nutritional modifications to replace drugs in poultry feeds. J Appl Poult Res. 11(4):437-452.

Miles DM, Branton SL, Lott BD. 2004. Atmospheric ammonia is detrimental to the performance of modern commercial broilers. Poult Sci. 83(10):1650-1654.

Montagne L, Pluske JR, Hampson DJ. 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 Technol. 108(1-4):95-117.

Mourão JL, Pinheiro VM, Prates JAM, Bessa RJB, Ferreira LMA, Fontes CMGA, Ponte PIP. 2008. Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. Poult Sci. 87(4):733-743.

National Research Council. 1994. Nutrient Requirements of Poultry, 9th edn. National Academy Press, Washington, DC, USA.

Perez VG, Jacobs CM, Barnes J, Jenkins MC, Kuhlenschmidt MS, Fahey Jr GC, Parsons CM, Pettigrew JE. 2011. Effect of corn distillers dried grains with solubles and Eimeria acervulina infection on growth performance and the intestinal microbiota of young chicks. Poult Sci. 90(5):958-64.

Preston CM, McCracken KJ, McAllister A. 2000. Effect of diet form and enzyme supplementation on growth, efficiency and energy utilisation of wheat-based diets for broilers. Br Poult Sci. 41(3):324-331.

Rezaei M, Karimi Torshizi MA, Rouzbehan Y. (2011). The influence of different levels of micronized insoluble fiber on broiler performance and litter moisture. Poult Sci. 90(9):2008-2012.

Roberts SA, Xin H, Kerr BJ, Russell JR, Bregendahl K. 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. Poult Sci. 86(8): 1716-1725.

Santos dos S, Laosutthipong C, Yamauchi K, Thongwittaya N, Sittiya J. 2019. Effects of dietary fiber on growth performance, fecal ammonia nitrogen, and gastrointestinal tract pH in broilers from 1 to 21 days of age. Proceedings of the 4th Industrial Revolution and Its Impacts; March 27-30; Thailand: Walailak Procedia. p. 4-73.

Sarikhan M, Shahryar HA, Gholizadeh B, Hosseinzadeh MH, Beheshti B, Mahmoodnejad A. 2010. Effects of insoluble fiber on growth performance, carcass traits and ileum morphological parameters on broiler chick males. Int J Agric Biol. 12(4):531-536.

Shahin KA, Abd Elazeem F. 2005. Effects of breed, sex and diet and their interactions on carcass composition and tissue weight distribution of broiler chickens. Arch Anim Breed. 48(6):612-626.

Shriver JA, Carter SD, Sutton AL, Richert BT, Senne BW, Pettey LA. 2003. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. J Anim Sci. 81(2):492-502.

Sittiya J, Yamauchi K, Takata K. 2016. Effect of replacing corn with whole-grain paddy rice and brown rice in broiler diets on growth performance and intestinal morphology. J Anim Physiol Anim Nutr. 100(2):381-390.

Sklan D, Smirnov A, Plavnik I. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br Poult Sci. 44(5):735-740.

Taylor RD, Jones GPD. 2001. The effect of whole wheat, ground wheat and dietary enzymes on performance and gastro-intestinal morphology of broilers. Proceedings of Australian Poultry Science Symposium; Sydney, Australia: Proc. Aust. Poult. Sci. Sym. p. 187-190.

United Egg Producers. 2006. United Egg Producers Animal Husbandry Guidelines for U.S. Egg Laying Flocks. United Egg Producers, Alpharetta, GA.

Urriola PE, Cervantes-Pahm SK, Stein HH. 2013. Fiber in swine nutrition. Sustainable swine nutrition. p. 255-276.

USDA. 2018. Grain: World Markets and Trade. p. 25.

Van Soest PV, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74(10):3583-3597.

Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci. 82(6):1030-1036.

Yason CV, Summers BA, Schat KA. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. Am J Vet Res. 48(6):927-938.

Output

1. International journal publication

One international journal manuscript was submitted as follow

Title: Influence of levels of dietary fibre sources on the performance, carcass traits, gastrointestinal tract development, faecal ammonia nitrogen, and intestinal morphology of broilers from 22 to 42 days of age

Authors: Janjira Sittiya, Koh-en Yamauchi, Wasunan Nimanong and Narin Thongwittaya

Journal: Italian Journal of Animal Science (Agricola, CABI, Scopus, Science Citation Index; Impact factor 2017 = 0.990)

2. Research utilization

2.1 Farm owners, academician of feed and animal production can get the academic information that the inclusion of 5% SH in the diets, resulting in improved intestinal morphology, without negatively affecting growth performance and carcass traits.

2.2 Inclusion of fiber is one of the nutritional strategies recommended to improve gut health leading to reduce antibiotic use in feed. These strategies may be used for organic animal production.

3. Others

Santos dos S, Laosutthipong C, Yamauchi K, Thongwittaya N, **Sittiya J.** 2019. Effects of dietary fiber on growth performance, fecal ammonia nitrogen, and gastrointestinal tract pH in broilers from 1 to 21 days of age. Proceedings of the 4th Industrial Revolution and Its Impacts; March 27-30; Thailand: Walailak Procedia. p. 4-73.

Appendix

(Submitted manuscript)

Influence of levels of dietary fibre sources on the performance, carcass traits, gastrointestinal tract development, faecal ammonia nitrogen, and intestinal morphology of broilers from 22 to 42 days of age

Journal:	*Italian Journal of Animal Science*
Manuscript ID	Draft
Manuscript Type:	Paper
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Complete List of Authors:	Sittiya, Janjira; Silpakorn University - Phetchaburi Information Technology Campus, Animal Science and Agricultural Technology Yamauchi, Koh-en; Kagawa University, Laboratory of Animal Science, Faculty of Agriculture Nimanong, Wasunan; Silpakorn University - Phetchaburi Information Technology Campus, Faculty of Animal Sciences and Agricultural Technology Thongwittaya, Narin; Maejo University, Faculty of Animal Sciences and Technology
Keywords:	rice hulls, soybean hulls, broilers, intestinal morphology
Abstract:	The present study was conducted to investigate the effects of dietary fibre source levels on the faecal ammonia nitrogen, growth performance, carcass traits, gastrointestinal tract development, and intestinal morphology of broilers from 22 to 42 d of age. A total of 420 one-day-

	old broiler chicks were obtained from a commercial hatchery. 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 faecal ammonia nitrogen among the dietary treatment groups ($p > .05$). Compared with the control, the experimental diets with 2.5% SH significantly decreased the wing weight of chickens ($p < .05$), 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 ($p < .05$). 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 ($p < .05$). 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.

Influence of levels of dietary fibre sources on the performance, carcass traits, gastrointestinal tract development, faecal ammonia nitrogen, and intestinal morphology of broilers from 22 to 42 days of age

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Keywords: rice hulls; soybean hulls; broilers; intestinal morphology

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 faeces, containing undigested dietary N, endogenous N and microbial N (Jha and Berrocoso 2016), is lost to the atmosphere as volatized ammonia (Ferket et al. 2002). NH_3 emission from manure has become a major 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.

It has been reported that the inclusion of soluble fibre 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, Roberts et al. (2007) reported that the inclusion of 10% corn dried distillers' grains with solubles (DDGS), 7% wheat middlings (WM), or 5% soybean hulls (SH) as insoluble fibre in laying hen diets decreased total manure NH_3 emission. Moreover, insoluble fibre 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). In addition, the inclusion of feed ingredients with an adequate type and amount of fibre might improve the gastrointestinal tract (GIT), leading to reduced antibiotic use in feed (Mateos et al. 2002; Montagne et al. 2003).

In Thailand, rice is the major locally produced crop, which amounted to 20.7 million metric tons in 2018/19 (USAD 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 in Thailand that is generated in soybean oil processing. To the best of our knowledge, no information is available on the use of rice hulls and soybean hulls as a fibre source in broiler diets. The nutritional and physiological effects must be considered; however, these effects are caused by differences in the structure and properties of fibre sources. Therefore, the aim of this study was to investigate the effects of dietary fibre source levels on the faecal ammonia nitrogen, growth performance, carcass traits, gastrointestinal tract development, and intestinal morphology of broilers from 22 to 42 d of age.

Materials and methods

Birds, management and diets

A total of 420 one-day-old broiler chicks (ROSS 308) were obtained from a commercial hatchery. Chicks were individually weighed and randomly divided into 5 groups of chicks with similar mean body weights, each with seven replicates of twelve chicks. The RH and SH were ground through a hammer mill with a 2-mm screen. Subsequently, the chemical composition of the fibre samples was analysed 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). Feed and water were provided *ad libitum* for 42 d.

The experiment was conducted in accordance with the guidelines and rules for animal experiments of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Thailand.

Growth performance and faecal ammonia nitrogen

During the experiment, feed intake and body weight were measured weekly, and feed efficiency 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, faeces was collected over 3 consecutive 24-h periods in each cage. The faeces from each of the 24-h periods was pooled by group and stored at -20°C until analysis. Faecal ammonia nitrogen was analysed by the method of AOAC (2000).

Carcass traits and digestive organ development

At 42 d of age, 7 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 weighed individually. In the digestive organs, the lengths of the duodenum, jejunum and ileum were measured individually. The weights of the proventriculus, gizzard, duodenum, jejunum and ileum were then recorded after the 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 7 birds per group were used for intestinal morphological observations. Immediately following 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 segment was embedded in paraffin wax. A 4-µm-thick transverse section was cut and stained with haematoxylin-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 8 calculations of villus area were averaged for each bird. Crypt depth was defined 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 analysed using one-way analysis of variance (ANOVA) in SPSS statistical software (version 19.0; IBM Corp. Armonk, NY, US). Significant differences among the treatments were determined with Tukey's test. Statistical significance was accepted at $p < .05$.

Results

Growth performance and faecal ammonia nitrogen

The influence of different sources and levels of fibre on growth performance and faecal ammonia nitrogen in broiler chickens is presented in Table 3. No significant differences were found in feed intake, body weight gain or feed efficiency among the dietary treatment groups ($p > .05$). Feeding the chickens fibre had no effect on the faecal ammonia nitrogen ($p > .05$).

Carcass traits and digestive organ development

Compared with the control, the experimental diets with 2.5% SH significantly decreased the wing weight of chickens ($p < .05$), while no significant differences in the weight of the other visceral organs were observed (Table 4). The intestinal weight and length of broilers are presented in Table 5. Compared with the control, broilers in the 5% SH group had a longer jejunum and ileum ($p < .05$). In terms of intestinal weight and duodenal length, there were no significant differences among the dietary treatment groups ($p > .05$).

Intestinal morphological measurements

Feeding the broilers SH and RH had no effect on the villus area and crypt depth of the intestine (Table 6). Compared with the control, the experimental diets with 2.5% RH significantly increased the duodenal villus height of chickens ($p < .05$).

Discussion

Effect of different sources and levels of fibre on growth performance and faecal ammonia nitrogen

It is commonly reported that dietary fibre decreases nutrient digestibility and chicken performance (Sklan et al. 2003). This result is similar to that from a previous study by Santos et al. (2019), who found that body weight gain from 1 to 21 d of age was lower for broilers fed a 2.5% SH diet than for those fed control, 2.5% RH, 5% RH, and 5% SH diets. This finding is in agreement with the results of Sklan et al. (2003), who found that turkeys (1 to 4 wk of age) fed 3% soybean hulls had a lower body weight than those fed 6 or 9% soybean hulls. However, the opposite effect was found on body weight gain at 14 wk of age: turkeys 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 fibre 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 fibre source (oat hulls or soybean hulls) reduced average daily feed intake without affecting average daily gain; consequently, the feed conversion ratio was improved by fibre inclusion. This finding may be explained by the differences in the level and type of dietary fibre, as well as the age of the bird.

The addition of fibre 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 normal cases, the nitrogen excreted in faeces consists of undigested dietary nitrogen and endogenous nitrogen, mainly as amino acids and bacterial protein. In poultry faeces, the volatilization of ammonia has been attributed to microbial decomposition of nitrogenous compounds, mainly uric acid (Li et al. 2006). Therefore, nitrogen content was determined in broiler faeces. It has been reported that the inclusion of moderate amounts of different fibre sources in pig diets affects the growth of bacterial populations in the large intestine, resulting in decreased NH_3 emission (Kirchgessner et al. 1994). This finding is in agreement with our previous study (Santos et al. 2019), in which the faecal 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 faecal ammonia nitrogen. However, in the present study, from 22 to 42 d of age, there was no significant difference in faecal ammonia nitrogen between the fibre groups and the control group. This might be due to the poor grinding activity of the gizzard in the present study.

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

Lu et al. (1996) reported that relative organ weight can 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. This finding is confirmed by the research of Shahin and Abdelazim (2005), in which high fibre inclusion in broiler diets decreased carcass weight. Mateos et al. (2012) reported that dietary fibre decreased the intestinal length and weight of the organs of birds. Consequently, these changes might reduce the carcass yield (Jørgensen et al. 1996).

Our present trial is in agreement with a few others (Preston et al. 2000; Taylor and Jones 2001; Mourão et al. 2008) in which dietary fibre increased the relative length of the small intestine. The longer relative length of the small intestine in the fibre groups might be due to the increasing effort of this organ to adapt in order 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 studies. For example, Amerah et al. (2009) and Sklan et al. (2003) found that increasing the insoluble fibre in the diet reduced the length of the small intestine. These conflicting findings may be due to differences in the type and amount of fibre as well as its particle size (Mateos et al. 2012).

Effect of different sources and levels of fibre 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 animal (Xu et al. 2003). Some researchers have demonstrated that the physicochemical characteristics of dietary fibre induce physiological and histological changes in the intestine (Jankowski et al. 2009; Juskiewicz et al. 2009). In the present study, dietary fibre in broiler diets contributed to an increase in the duodenal villus height. Similarly, 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. Moreover, Sklan et al. (2003) found that the surface area of the small intestine of turkeys increased as the level of crude fibre 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 fibre groups. This combined change might be because of the increasing effort of the small intestine to adapt in order 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-fibre sunflower cake inclusion resulted in linear reductions in villus height.

Conclusion

According to the faecal ammonia nitrogen, growth performance, carcass traits, gastrointestinal tract development, and intestinal morphology, the appropriate level of fibre sources in broiler diets was 5% SH in the present study. Furthermore, 5% SH has some positive effects on intestinal morphology without negatively affecting growth performance and carcass traits.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Highlights

- 5% SH has some positive effects on intestinal morphology without negatively affecting growth performance and carcass traits in broilers.
- This study determined that fibre can be beneficial for improved intestinal morphology in broilers.
- The nutritional and physiological effects must be considered; these effects are caused by differences in the structure and properties of fibre sources.

References

AOAC. 2000. Official Method of Analysis. 17th ed. Gaithersburg (MD): Association of Official Analytical Chemists.

Amerah AM, Ravindran V, Lentle RG. 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Br Poult Sci. 50(3):366-375.

Awad WA, Böhm J, Razzazi-Fazeli E, Ghareeb K, Zentek J. 2006. Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult Sci. 85(6):974-979.

Battye R, Battye W, Overcash C, Fudge S. 1994. Development and selection of ammonia emission factors, Durham (US): Research Triangle Park (NC). Report No.: PB-95-123915/XAB. Environmental Protection Agency, US.

Beccaccia A, Calvet S, Cerisuelo A, Ferrer P, Garcia-Rebollar P, De Blas C. 2015. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing-finishing pigs. I. Influence of the inclusion of two levels of orange pulp and carob meal in isofibrous diets. Anim Feed Sci Tech. 208:158-169.

Bindelle J, Buldgen A, Delacollette M, Wavreille J, Agneessens R, Destain JP, Leterme P. 2009. Influence of source and concentrations of dietary fiber on in vivo nitrogen excretion pathways in pigs as reflected by in vitro fermentation and nitrogen incorporation by fecal bacteria. J Anim Sci. 87(2):583-593.

Canh TT, Verstegen MWA, Aarnink AJA, Schrama JW. 1997. Influence of dietary factors on nitrogen partitioning and composition of urine and feces of fattening pigs. J Anim Sci. 75(3):700-706.

Carlile FS. 1984. Ammonia in poultry houses: A literature review. World's Poult Sci J. 40(2):99-113.

Caspary WF. 1992. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr. 55 Suppl:299S-308S.

Ferket PR, Van Heugten E, Van Kempen TA, Angel R. 2002. Nutritional strategies to reduce environmental emissions from nonruminants. J Anim Sci. 80 E-Suppl:E168-E182.

Geyra A, Uni Z, Sklan D. 2001. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br J Nutr. 86(1):53-61.

González-Alvarado JM, Jiménez-Moreno E, Lázaro R, Mateos GG. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult Sci. 86(8):1705-1715.

Iji PA, Saki A, Tivey DR. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br Poult Sci. 42(4):505-513.

Jankowski J, Juskiewicz J, Gulewicz K, Lecewicz A, Slominski BA, Zdunczyk Z. 2009. The effect of diets containing soybean meal, soybean protein concentrate, and soybean protein isolate of different oligosaccharide content on growth performance and gut function of young turkeys. Poult Sci. 88(10):2132-2140.

Jha R, Berrocoso JF. 2016. Dietary fiber and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: A review. Anim Feed Sci Technol. 212:18-26.

Jiménez-Moreno E, Chamorro S, Frikha M, Safaa HM, Lázaro R, Mateos GG. 2011. Effects of increasing levels of pea hulls in the diet on productive performance, development of the gastrointestinal tract, and nutrient retention of broilers from one to eighteen days of age. Anim Feed Sci Technol. 168:100-112.

Jørgensen H, Zhao XQ, Knudsen KEB, Eggum BO. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br J Nutr. 75(3):379-395.

Juskiewicz J, Jankowski J, Zdunczyk Z, Lecewicz A, Przybylska-Gornowicz B, Zieba M. 2009. Effect of diets with different contents of soybean [alpha]-galactosides and crude fibre on modification of duodenal microstructure and selected parameters of nutrient utilization in young turkeys. Pol J Vet Sci. 12(4):455.

Kalmendal R, Elwinger K, Holm L, Tauson R. 2011. High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens. Br Poult Sci. 52(1):86-96.

Kirchgessner M, Kreuzer M, Machmüller A, Roth-Maier DA. 1994. Evidence for a high efficiency of bacterial protein synthesis in the digestive tract of adult sows fed supplements of fibrous feedstuffs. Anim Feed Sci Technol. 46(3-4):293-306.

Kreuzer M, Machmüller A, Gerdemann MM, Hanneken H, Wittmann M. 1998. Reduction of gaseous nitrogen loss from pig manure using feeds rich in easily-fermentable non-starch polysaccharides. Anim Feed Sci Technol. 73(1-2):1-19.

Langhout DJ, Schutte JB, Van Leeuwen P, Wiebenga J, Tamminga S. 1999. Effect of dietary high-and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br Poult Sci. 40(3):340-347.

Li H, Xin H, Liang Y, Burns RT. 2008. Reduction of ammonia emissions from stored laying hen manure through topical application of zeolite, Al+ Clear, Ferix-3, or poultry litter treatment. J Appl Poult Res. 17(4):421-431.

Lu CD, Schoknecht PA, Ellis KJ, Shypailo R, Su DR, Pond WG. (1996). Differential compensatory organ growth in young pigs after short-term rehabilitation from protein deficiency. Nutr Res. 16(4):627-637.

Mateos GG, Jiménez-Moreno E, Serrano MP, Lázaro RP. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J Appl Poult Res. 21(1):156-174.

Mateos GG, Lázaro R, Gracia MI. 2002. The feasibility of using nutritional modifications to replace drugs in poultry feeds. J Appl Poult Res. 11(4):437-452.

Miles DM, Branton SL, Lott BD. 2004. Atmospheric ammonia is detrimental to the performance of modern commercial broilers. Poult Sci. 83(10):1650-1654.

Montagne L, Pluske JR, Hampson DJ. 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 Technol. 108(1-4):95-117.

Mourão JL, Pinheiro VM, Prates JAM, Bessa RJB, Ferreira LMA, Fontes CMGA, Ponte PIP. 2008. Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. Poult Sci. 87(4):733-743.

Perez VG, Jacobs CM, Barnes J, Jenkins MC, Kuhlenschmidt MS, Fahey Jr GC, Parsons CM, Pettigrew JE. 2011. Effect of corn distillers dried grains with solubles and Eimeria acervulina infection on growth performance and the intestinal microbiota of young chicks. Poult Sci. 90(5):958-64.

Preston CM, McCracken KJ, McAllister A. 2000. Effect of diet form and enzyme supplementation on growth, efficiency and energy utilisation of wheat-based diets for broilers. Br Poult Sci. 41(3):324-331.

Rezaei M, Karimi Torshizi MA, Rouzbehan Y. (2011). The influence of different levels of micronized insoluble fiber on broiler performance and litter moisture. Poult Sci. 90(9):2008-2012.

Roberts SA, Xin H, Kerr BJ, Russell JR, Bregendahl K. 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. Poult Sci. 86(8): 1716-1725.

Santos dos S, Laosutthipong C, Yamauchi K, Thongwittaya N, Sittiya J. 2019. Effects of dietary fiber on growth performance, fecal ammonia nitrogen. Proceedings of the 4th Industrial Revolution and Its Impacts; March 27-30; Thailand: Walailak Procedia. p. 4-73.

Sarikhan M, Shahryar HA, Gholizadeh B, Hosseinzadeh MH, Beheshti B, Mahmoodnejad A. 2010. Effects of insoluble fiber on growth performance, carcass traits and ileum morphological parameters on broiler chick males. Int J Agric Biol. 12(4):531-536.

Shahin KA, Abd Elazeem F. 2005. Effects of breed, sex and diet and their interactions on carcass composition and tissue weight distribution of broiler chickens. Arch Anim Breed. 48(6):612-626.

Shriver JA, Carter SD, Sutton AL, Richert BT, Senne BW, Pettey LA. 2003. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. J Anim Sci. 81(2):492-502.

Sittiya J, Yamauchi K, Takata K. 2016. Effect of replacing corn with whole-grain paddy rice and brown rice in broiler diets on growth performance and intestinal morphology. J Anim Physiol Anim Nutr. 100(2):381-390.

Sklan D, Smirnov A, Plavnik I. 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. Br Poult Sci. 44(5):735-740.

Taylor RD, Jones GPD. 2001. The effect of whole wheat, ground wheat and dietary enzymes on performance and gastro-intestinal morphology of broilers. Proceedings of Australian Poultry Science Symposium; Sydney, Australia: Proc. Aust. Poult. Sci. Sym. p. 187-190.

USDA. 2018. Grain: World Markets and Trade. p. 25.

Van Soest PV, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74(10):3583-3597.

Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci. 82(6):1030-1036.

Yason CV, Summers BA, Schat KA. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. Am J Vet Res. 48(6):927-938.

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

Table 2. Feed compositions and calculated nutrient value of experimental diets.

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 3. 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 broiler chickens from 22 to 42 days of age.

Item	Level of fiber (%)	Feed intake (g)	Body weight 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 ($p > .05$).

437 **Table 4.** The effects of different sources and levels of fiber on carcass and visceral
438 organs weight (g/100 g BW) in 42 days 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

439 RH = rice hulls; SH = soybean hulls.
440 [a,b]Values with different superscripts in the same column are significantly different ($p <$
441 .05).
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458 **Table 5.** The effects of different sources and levels of fiber on intestinal weight (g/100
459 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

460 RH = rice hulls; SH = soybean hulls.
461 [a,b,c]Values with different superscripts in the same column are significantly different ($p <$
462 .05).

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Table 6. The effects of different sources and levels of fiber on intestinal morphological measurements in 42 days old broiler chickens (mean ± SE; n = 5).

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,b]Values with different superscripts in the same column are significantly different ($p < .05$).