

The Effects of Dietary Whole Rice Hull as Insoluble Fiber on the Flock Uniformity of Pullets and on the Egg Performance and Intestinal Mucosa of Laying Hens

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ABSTRACT

Our two experiments were intended to investigate the effects of dietary Whole Rice Hull (WRH) as insoluble fiber on the flock uniformity of pullets and the performance, egg quality and intestinal mucosa structure of laying hens. In experiment 1, a total of 1,500 chicks (4 weeks old) with the same uniform weight were randomly separated into three treatments of 500 birds each and fed diets containing 0 (control), 3 and 6% WRH. With increasing dietary WRH levels, body weight and feed intake were higher ($p < 0.05$); the 3 and 6% WRH groups were higher than the control group. The Feed Conversion Ratio (FCR) was lowest ($p < 0.05$) in the 3% WRH-fed birds. In addition, the percentage of flock uniformity tended to increase in both dietary WRH groups. In the experiment 2, a total of 48 of the highest-producing hens (32 weeks old) were divided into three groups of 16 birds each and fed diets containing 0, 3 and 6% WRH. Hen-day egg production was 2.56% higher in the 6% WRH group and 1.48% higher in the 3% WRH group than in the control, without any distinctly adverse effects on egg quality. Morphologically, no significant differences were observed in the light microscopic parameters, with the exception that the muscularis externa width showed a higher value in the duodenum of the 6% WRH group and in the ileum of both dietary WRH groups. Epithelial cellular phenomena of the jejunum and ileum were similar among treatments, except cell clusters with numerous protuberated epithelia were found in the 6% WRH group. In conclusion, the current data indicate that WRH can be used as a source of insoluble fiber in diets up to 6% to enhance growth and uniformity of pullet chicks and to improve egg production of laying hens without any harmful impact on egg quality or on the intestinal mucosa structure.

Keywords: Egg Production, Insoluble Fiber, Intestinal Mucosa Structure, Pullet Performance, Whole Rice Hull

1. INTRODUCTION

Fibrous ingredients have been restricted in poultry feed due to their evidenced depression of nutrient digestibility. However, recent scientific publications exhibited that insoluble fiber sources are profitable to gut structure (Gonzalez-Alvarado *et al.*, 2007; Hetland and Svihus, 2007; Jimenez-Moreno *et al.*, 2009;

Incharoen, 2013) and stimulate the production of hydrochloric acid, bile acids and digestive enzyme secretions (Hetland *et al.*, 2003; Svihus, 2011). These data demonstrated a strong positive correlation between insoluble fiber and nutrient digestibilities resulting in enhanced animal growth and health. For example, the inclusion of oat hulls and soy hulls in broiler diets improved Total Tract Apparent Retention

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(TTAR) of nutrients (Jimenez-Moreno *et al.*, 2009). Similarly, dietary oat hulls could improve wheat starch digestibility and stimulate gizzard activity (Hetland and Svihus, 2001) resulting in increased growth performance (Mateos *et al.*, 2012). Wang *et al.* (2009) noted that the addition of soybean hulls in diets decreased slurry pH and ammonia emissions without any adverse impact on the growth performance of finishing pigs.

In Thailand, the main co-product of rice processing in rice mills is the rice hull, one of the major components of which is insoluble fiber. Previous experiments justified that an inclusion of rice hull meal (100 g kg⁻¹) in broiler diets could improve production (Incharoen, 2013). Therefore, the author decided to investigate the effect of diets containing insoluble fiber from Whole Rice Hull (WRH) on the uniformity of pullet chicks in experiment 1. Performance, egg quality and morphological intestinal changes of laying hens were examined in experiment 2.

2. MATERIALS AND METHODS

2.1. Experiment 1

2.1.1. Birds and Experimental Design

The present feeding trial was conducted and funded in co-operation with a private company (Kaithep Farm Co., Ltd., Chachoengsao, Thailand). At 4 weeks of age, a total of 1,500 H and N Brown Nickpullet chicks with a similar uniform weight was randomly separated into 3 dietary groups (500 birds group⁻¹), each with 5 replicates of 100 birds. They were provided with ad libitum access to water and fed the basal grower diets (CP, 19%; ME, 2,950 kcal kg⁻¹) containing 0 (control), 3 and 6% WRH. All birds were housed in pens of the same size (3×5 m) in a deep litter material with a rice hull floor. The lighting program and temperature controls were automatically operated according to management recommendations for rearing pullets. Body weight and feed intake were recorded until 8 weeks of age; Feed Conversion Ratio (FCR) and flock uniformity were also calculated.

2.2. Experiment 2

2.2.1. Performance and Egg Quality

In experiment 2, a total of 48 of the highest-producing hens (percentage hen-day egg production of 93.7 ± 1.25) were chosen for this experiment. At 32

weeks of age, the selected hens were randomly divided into three experimental groups of 16 birds each. The control birds were fed a basal laying diet (CP, 18%; ME, 2,850 kcal kg⁻¹), while the other two groups were fed the basal laying diet containing 3 or 6% WRH. Water and feed were available ad libitum in each dietary treatment. All birds were placed in individual cages in an experimental controlled room under an automatic lighting program (17L: 7D). During the experimental period (12 weeks), all eggs and surplus feed were recorded to analyze data for percentage of hen egg production, egg mass, feed intake and feed efficiency. Collected eggs (n = 260) from each dietary treatment were assembled weekly to measure egg quality according to Incharoen and Yamauchi (2009). Analytical parameters were shell breaking strength, shell thickness, shell ratio, albumen ratio, yolk ratio and yolk color. The digital balance, egg shell strength meter, dial thickness gauge and yolk color fan were used to measure egg traits.

2.2.2. Morphological Procedure of the Small Intestine

At 44 weeks of age, 5 birds with uniform weights were selected from each group and decapitated under light ethyl ether. The entire intestine was cut and fixed into a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1M cacodylate buffer (pH 7.4). A specimen about 2-3 cm long was taken from the midpoint of the duodenum, jejunum and ileum and washed with 0.1M Phosphate Buffered Saline (PBS). The sampling sections were: (1) the duodenum, from the gizzard to the pancreatic and bile ducts; (2) the jejunum, from the duct to Meckel's diverticulum and (3) the ileum, from the end of the ileal point to the ileocaecal junction. Each segment was classified and prepared for light and scanning electron microscopy.

For light microscopic technique, the specimen was fixed in Bouin's solution and dehydrated with a graded series of ethanol. A sample was embedded in paraffin and sliced at 5 µm. Four transverse-sectional segments were mounted onto glass slides and stained with hematoxylin and eosin. Finally, all tissue slides were measured under light microscope. Intestinal morphology variables included villus height, crypt depth, villus height per crypt depth, mucosa thickness and muscularis externa width. These measurements were analyzed separately for each intestinal segment and performed according to Incharoen *et al.* (2010).

For scanning electron microscopy, an intestinal specimen was cut lengthwise and the digesta contents removed using a jet sprayer of 0.1M PBS (pH 7.4). These tissues were immediately pinned flat onto paraffin plates containing 4% paraformaldehyde + 3% glutaraldehyde buffered with 0.1M cacodylate (pH 7.4). The flattened specimens were incised into small square pieces with a razor blade and washed 3-4 times with 0.1M cacodylate buffer (pH 7.4) and soaked in several grades of ethanol. After dehydration, each intestinal sample was freeze-dried, coated with platinum and examined using a scanning electron microscope (Incharoen *et al.*, 2010).

2.3. Statistical Analysis

The results of the growth performance and uniformity of pullet chicks, as well as egg production, egg quality and light microscopic assessments of laying hens were statistically analyzed by one-way Analysis of Variance (ANOVA). The data from both experiments were reported as means \pm SE. Duncan's multiple range test was used to compare the means. The differences among the treatment groups were considered significant at $p < 0.05$.

3. RESULTS

3.1. Experiment 1

3.1.1. Performance of Pullet Chicks

In this trial, the data for the growth performance and flock uniformity of pullets fed the basal diets containing 0 (control), 3 and 6% WRH is shown in **Table 1**. In the current results, final body weight and weight gain were higher in both the dietary 3 and 6%WRH groups ($P < 0.05$) than in the control group. With increasing dietary WRH levels, feed intake significantly increased ($P < 0.05$) in the 3 and 6% WRH group, respectively. FCR was lowest ($P < 0.05$) in the 3% WRH fed-birds. In addition, the percentage of flock uniformity tended to increase in both dietary WRH groups.

Table 1. Growth performance and flock uniformity of pullets fed the basal diets containing 0 (control), 3 and 6% WRH (mean \pm SE)

Parameters	Control	3% WRH	6% WRH
Initial body weight, g	526 \pm 1.38	521 \pm 2.51	527 \pm 1.41
Final body weight, g	1107 \pm 4.17 ^b	1129 \pm 2.81 ^a	1130 \pm 2.51 ^a
Body weight gain, g	580 \pm 4.93 ^b	608 \pm 5.29 ^a	602 \pm 2.65 ^a
Feed intake, g	2063 \pm 2.46 ^c	2132 \pm 10.50 ^b	2205 \pm 16.53 ^a
Feed conversion ratio (FCR)	3.56 \pm 0.03 ^a	3.50 \pm 0.04 ^a	3.66 \pm 0.02 ^b
Flock uniformity within \pm 10% of each pen mean (%)	85.0 \pm 1.70	87.4 \pm 1.84	86.8 \pm 1.93

^{a,b} Means within each dietary treatment with different letter designations differ ($p < 0.05$)

3.2. Experiment 2

3.2.1. Production Performance and Egg Evaluations

In the current experiment, the production performance and egg quality of laying hens fed basal diets containing 0 (control), 3 and 6% WRH is displayed in **Table 2**. Egg mass, feed intake and feed efficiency of the laying hens was similar ($P > 0.05$) among groups. In addition, hen-day egg production was 2.56% higher in the 6% WRH group and 1.48% higher in the 3% WRH group than the control group. There were no significant ($p > 0.05$) differences in shell thickness or shell ratio among the dietary treatments. With increasing dietary WRH levels, yolk color and yolk ratio tended to decrease, with a significant decrease in the 6% WRH group ($p < 0.05$). Conversely, shell-breaking strength and albumen ratio tended to increase, significantly increasing in the 6% WRH group ($p < 0.05$).

3.2.2. Morphological Analysis of the Villus Parameters

No significant differences ($p > 0.05$) in villus mucosa were observed in the villus height, crypt depth, villus height per crypt depth or mucosal thickness among the dietary treatments (**Fig. 1a-d**). However, muscularis externa width significantly increased in the duodenal part of the 6% WRH group and in the ileum of both dietary WRH groups ($p < 0.05$), when compared to the control group (**Fig. 1e**). The scanning electron microscopy views recorded the cellular alteration on the villus apical surface. Duodenal cellular morphology of birds fed the control diet (**Fig. 2a**) and 3% WRH (**Fig. 2b**) showed a similar appearance characterized by villus surface composed with many single epithelial cells. Nevertheless, abundant cell clusters aggregated by numerous protuberated epithelia were found around the central sulcus of birds fed diets containing 6% WRH (**Fig. 2c**). However, the cellular phenomenon on the villus tip surface of the jejunum and ileum were similar among the dietary groups (**Fig. 2d-i**).

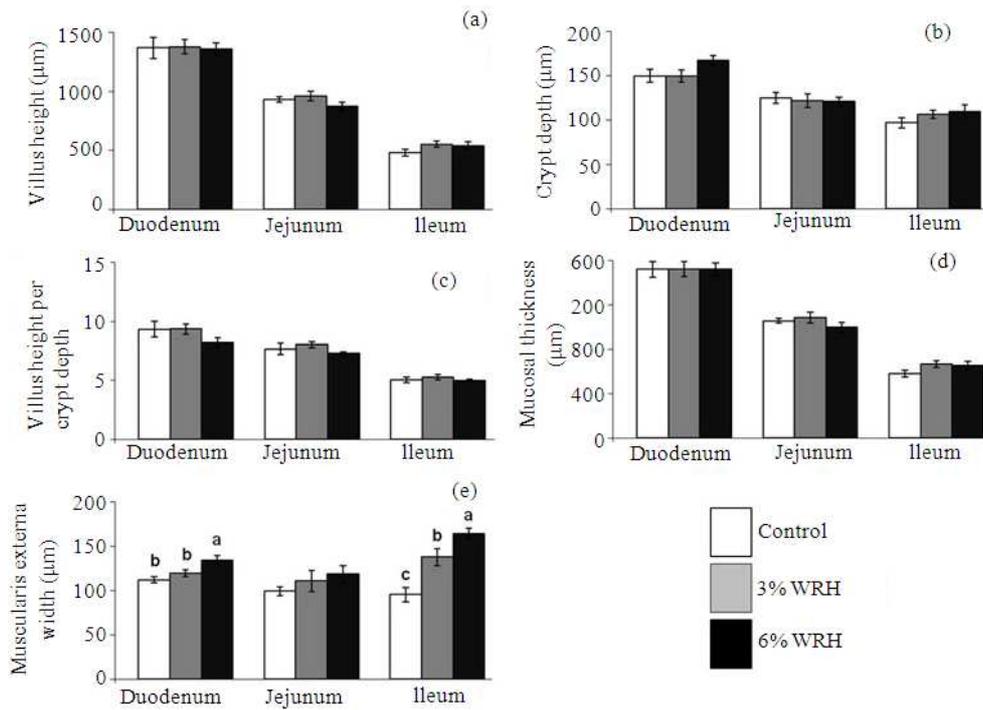


Fig. 1. Light microscopic parameters (villus height, crypt depth, villus height per crypt depth, mucosa thickness and muscularis externa width) of laying hens fed the basal diets containing 0 (control), 3 and 6% WRH (mean \pm SE)

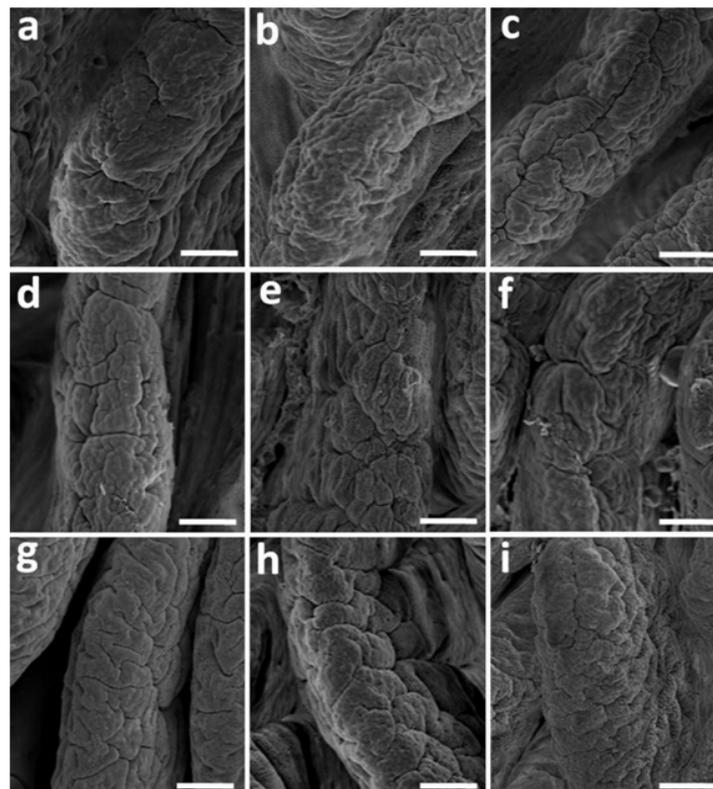


Fig. 2. Scanning electron microscopic observations of intestinal villi apical surface in duodenum (a-c), jejunum (d-f) and ileum (g-i) of laying hens fed the basal diets containing 0 (control), 3 and 6% WRH, respectively. Scale bar = 100 μ m, magnification: \times 300

Table 2. The production performance and egg quality of laying hens fed the basal diets containing 0 (control), 3 and 6% WRH (mean±SE)

Parameters	Control	3% WRH	6% WRH
Egg production			
Hen-day egg production, %	89.81±0.74	91.14±2.05	92.11±1.15
Egg mass, g/(hen*day)	55.69±0.59	55.86±2.64	58.12±1.58
Feed intake, g/(hen*day)	125.13±3.10	124.41±2.69	128.37±3.99
Feed efficiency	0.45±0.02	0.45±0.01	0.46±0.01
Egg quality (n=260)			
Shell-breaking strength, kg/cm ²	2.99±0.055 ^b	3.10±0.047 ^b	3.35±0.051 ^a
Shell thickness, mm	0.39±0.002	0.38±0.001	0.39±0.001
Shell ratio, %	11.19±0.063	11.07±0.056	11.24±0.062
Albumen ratio, %	56.49±0.225 ^b	56.59±0.189 ^b	57.34±0.27 ^a
Yolk ratio, %	28.08±0.124 ^a	28.18 ±0.169 ^a	27.58±0.130 ^b
Yolk color	13.05±0.053 ^a	13.07±0.047 ^a	12.91±0.045 ^b

^{a,b} Means within each dietary treatment with different letter designations differ (p<0.05)

4. DISCUSSION

4.1. Experiment 1

4.1.1. Pullets Performance

Recent scientific publications stated that an appropriate level of insoluble fiber added to poultry diets can stimulate production performance in broilers (Gonzalez-Alvarado *et al.*, 2007; Jimenez-Moreno *et al.*, 2011), breeder hens (Mohiti-Asli *et al.*, 2012) and turkeys (Sklan *et al.*, 2003). Likewise, the use of oat hulls for their insoluble fiber properties could also improve the Total Tract Apparent Retention (TTAR) of nutrients (Jimenez-Moreno *et al.*, 2009) and enhance wheat starch digestibility and stimulate gizzard activity (Hetland and Svihus, 2001), resulting in increased growth performance (Mateos *et al.*, 2012). The current observations agreed with a previous report regarding broilers fed dietary 5 and 10% rice hull meal (Incharoen, 2013). According to Adeniji (2010), FCR was significantly better in pullet chicks that received dietary 7.5% rice hull. Similarly, the inclusion of rice hulls in duck diets could enhance body weight gain and FCR compared with the control duck group (Wu *et al.*, 2012). In addition, the better percentage of flock uniformity in dietary WRH groups might be correlated to a prolonged peak of egg production. Our obtained data indicate that WRH can be used as a source of insoluble fiber in diets at levels up to 6% to enhance the growth performance and uniformity of pullet chicks.

4.2. Experiment 2

4.2.1. Egg Production and Egg Quality

This result suggests that both 3 and 6% WRH in the laying diet are appropriate levels for egg performance without any harmful impact on egg quality. However, Deatona *et al.* (1977) reported that

the dietary fiber from pine shavings had no adverse effect on body weight and feed efficiency, nor on hen-day production. Likewise, it was found that 4.8% soybean hull added to contribute equal amounts of neutral detergent fiber in a normal diet did not alter egg production or nitrogen excretion (Roberts *et al.*, 2007). As the chemical component of the diets was almost identical, the current tendency of better egg production seems to be supported by WRH. It is well known that corn contains high concentrations of xanthophylls, which are responsible for dark yolk colors. In this trial, a decrease in yellowness of the egg yolk was expected because 6% WRH was substituted in the laying corn-based diet. According to our data, it is not clear at present why the values of shell strength and albumen ratio were found to be higher in the 6% WRH groups than in the control group, but this is possibly associated with the functional properties of the WRH.

4.2.2. Villus Morphological Analysis

In the small intestine, many villi and microvilli generate an extended surface area, increasing the absorption efficiency of nutritional composition along the intestinal lumen. Intestinal villi develop rapidly and respond sensitively to the chemical components of digested feed. The current finding could demonstrate that there are no harmful impacts of WRH on the intestinal villi structure. However, muscularis externa width of duodenum and ileum increased in both dietary WRH groups. These results are in harmony with our earlier study (Incharoen, 2013), which noted increased muscularis externa width in the duodenum, jejunum and ileum of birds fed rice hull meal. In the fact, the prime function of the muscularis externa is that of peristalsis, which helps with the continued movement of digested feed along the

intestinal lumen. This increase in width appears to correlate with the more functional peristaltic movement of the muscularis externa due to the presence of dietary insoluble fiber in the intestinal lumen.

As the intestinal crypt produces the epithelial cells, they climb upward along the villus surface to perform nutrient absorptive activities within the intestinal lumen. Subsequently, these cells are extruded into the intestinal lumen within 48-96 hours (Imondi and Bird, 1966; Potten, 1998). Yamauchi *et al.* (2010) reported that the turnover of these cells appears to evince specific types of cell morphologies on the villus tip surface depending upon the intestinal function. In the present research, many cell clusters could demonstrate the stimulative effects of insoluble fiber properties on the renewal and turnover of cells. A similar quantity of protuberated cells and cell clusters was reported in chickens with higher production performance (Yamauchi *et al.*, 2006; Incharoen *et al.*, 2010). Therefore, this trial could suggest that many protuberated epithelial cells and in consequence, cell cluster formations in the duodenum correlate to a tendency to enhanced egg production.

5. CONCLUSION

A greater enhancement of growth rate and percentage of flock uniformity were observed in pullet chicks fed both dietary WRH levels, compared to birds in the control group. In addition, dietary 3 and 6% WRH could improve hen-days egg production, as well as the shell-breaking strength and albumen ratio of laying hens without any harmful impact on the parameters of other egg qualities or on the intestinal mucosa structure. Therefore, the present results indicate that WRH can be used as a source of insoluble fiber in diets for pullet chicks and laying hens at levels of up to 6%.

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