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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：　Janjira Sittiya
Name

学位論文題目：
Title of Dissertation

Study on the development of whole paddy feed rice as a poultry feed ingredient
(家禽の飼料原料としての飼料用籾米全粒の開発に関する研究)

学位論文要約：
Dissertation Summary

Poultry production has grown rapidly in the last decade throughout the world. As feed ingredient comprise the greatest proportion of the diets, the main problem that against this rapid expansion is insufficient supply of these feed ingredients, particularly corn. Corn is the major feed ingredient used in poultry diets, constituting about 50–70% of the total, and it is an important source of energy for broilers. It is also used for ethanol production and as a food source for people. This situation has led to the search for the new feed ingredients with the potential that could replace corn in poultry diets without detrimentally affecting performance.

Another obstacle which contributes to insufficient supply of some feed ingredients is high cost of poultry production. Several strategies have been proposed to reduce the incidence of these problems, and to advocate the development of the digestive tract including (1) the use of local feed ingredients, (2) whole grain feeding, and (3) diet dilution.

Interestingly, using rice as feed is currently becoming popular in Japan. This cereal grain is high in energy and rich in highly digestible starch. Starch is the main component in rice and it is an important provider of energy for animal. The Japanese government has advocated rice cultivation for animals (feed rice), estimates of the planted area of feed rice have increased rapidly (1,611 ha in 2008; Ministry of Agriculture, Forestry and Fisheries, 2008), which will then be used as a feasible ingredient in poultry diets. Among feed rice, Momiroman (feed-type rice) seems to be the most suitable cultivar for animal diets due to its high yield (203 kg/ha; Ministry of Agriculture, Forestry and Fisheries, 2008). Some poultry researchers have started to study the use of paddy rice as a feed ingredient to increase the use of local feedstuff. The use of home-grown cereals (whole-grain form) reduces feed costs due to reduced transportation and processing cost.

Objectives and Outline

The overall aim of this thesis was to investigate the possibility of using whole-grain paddy rice (WPR) as feed ingredient in poultry diets. This will encourage poultry farmers to use new locally grown grains as an alternative feed ingredient in chicken diets. The specific objectives are: **(I)** to examine the chemical composition, digestibility and metabolizable energy of WPR in order to confirm the nutritional value. **(II)** to investigate how feeding WPR would alter the performance and intestinal histology of chickens. **Experiment 1** is related to objective **I**, and **Experiment 2-5** are related to objective **II**.

This thesis provides a review and describes the results of five experiments in which different nutritional factors and feeding strategies were done in different type of chickens (Sanuki Cochin chickens, broiler chickens, and laying hens).

A short outline of all chapters was described as following:

In **Experiment 1,** the nutritional values of WPR are variable components according to different varieties, growing locations, and climate. There is a need to evaluate local feedstuffs for nutritional values and to provide information to be used for accurate formulation of poultry diets. Therefore, this experiment was carried out to examine the chemical composition, digestibility of crude fiber and energy, and metabolizable energy of WPR.

The objective of **Experiment 2** was to determine the performance and histological alterations of intestinal villi and epithelial cells in Sanuki Cochin chickens fed basal diets diluted with WPR. Diets were diluted with WPR, which may affect feed intake, body weight gain, feed efficiency and intestinal epithelial cells in chickens. Based on the results of **Experiment 2**, it was decided to continue in the next experiment. Therefore, **Experiment 3** describes an experiment in which the effects of WPR replacement with or without enzyme addition on broiler performance and intestinal morphology.

The objective of the work shown in **Experiment 4** was to investigate the effect of replacing corn with WPR in laying hen diets on egg production performance. In this chapter, the egg production and egg qualities are presented. The following experiment, **Experiment 5,** was done to evaluate the effects of replacing corn with WPR and brown rice (BR) in broiler diets on growth performance and intestinal morphology. The results from this thesis would be provided new information for poultry diet formulations, and also would be provided new alternative for poultry feed ingredients.

Experiment 1: Chemical composition, digestibility of crude fiber and gross energy, and metabolizable energy of whole paddy rice of Momiroman

Aim: The nutritional value of an ingredient depends on some it's nutrients like energy content. The need for accurate information on the energy content of paddy rice is of importance to nutritionist. In feeding experiments using chickens, metabolizable energy (ME) is a necessity for the formulation of poultry diets and has been accepted as the preferred measure of the energy content of feedstuffs. The other basic precondition would be to know the nutrient digestibility content of individual feed ingredients. However, the information available on the ME and digestibility of paddy rice has not been established. Consequently, in the current study, the intention was to evaluate the chemical composition, the nutrient digestibility, and the ME of WPR for use in further formulations of poultry diets.

Matherial and Methods: The experiment was conducted in accordance with the guidelines and rules for animal experiments of Kagawa University, Japan (authorization number of the ethical approval: No. 28). WPR was obtained from a rice farm at Kagawa University. The chemical composition and gross energy (GE) of WPR were determined using an adiabatic bomb calorimeter and proximate analysis. The experiment was conducted as a two-period crossover design to determine the true digestibility (TD) and ME. In the first period, 30 g of WPR were fed through a plastic tube to the force-feeding group, with excreta collection per bird carried out for 24 hours (n=4). Another four birds served as the fasting group and were fasted for 24 hours; the excreta from each cage was collected for 24 hours then pooled together (n=1). In the second period, the birds were crossed over to the opposite experimental group. Consequently, eight sets of data from both the first period and the second were used to calculate TD and ME. The TD, true metabolizable energy (TME), and apparent metabolizable energy (AME) were calculated as follows:

$$\text{TD (\%)} = \frac{\left(F_i \times \%NU_i\right) - \left[\left(E_f \times \%NU_f\right) - \left(E_r \times \%NU_r\right)\right] \times 100}{F_i \times \%NU_i}$$

$$\text{AME (kcal/g)} = \frac{\left(GE_i \times F_i\right) - \left(GE_f \times E_f\right)}{F_i}$$

$$\text{TME (kcal/g)} = \frac{\left(GE_i \times F_i\right) - \left[\left(GE_f \times E_f\right) - \left(GEr \times E_r\right)\right]}{F_i}$$

Where

NU_i = Nutrient content in feed (%)

NU_f = Nutrient content in excreta from the fed group (%)

NU_r = Nutrient content in excreta from the fasted group (%)

GE_i = Gross energy of feed (kcal/g)

GE_f = Gross energy of excreta from the fed group (kcal/g)

GE_r = Gross energy of excreta from the fasted group (kcal/g)

F_i = Feed intake (g)

E_f = Excreta from the fed group (g)

E_r = Excreta from the fasted group (g)

Results: The chemical composition of WPR was 5.11% crude protein, 1.83% ether extract, 68.04% nitrogen-free extract and 10.54% crude fiber (CF) on air dry basis. The GE of WPR (3.77 kcal/g) is close to that of corn. This result suggests that WPR can probably be used as a feasible alternative grain source in poultry diets. The TD of CF and GE was 38.65% and 80.35%, respectively. AME and TME were 2.79 and 3.02 kcal/g on air dry basis, respectively. The AME value of WPR was slightly lower than that of corn in chickens.

Conclusion: The present results would provide new information for poultry diet formulations and suggest that WPR can probably be used as a good alternative cereal grain source in poultry diets, and can replace corn.

Experiment 2: Growth performance and histological intestinal alterations of Sanuki Cochin chickens fed diets diluted with untreated whole-grain paddy rice

Aim: The results obtained from the **Experiment 1** have revealed that WPR can probably be used as a good alternative cereal grain source in poultry diets. Consequently, it is in the interest of the using WPR as feed ingredient in the chicken diets. However, no reports had been done on the growth performance in chickens fed basal diets diluted with WPR. Moreover, diet dilution is likely to offer a convenient feed preparatory method for poultry farmers. Therefore, the objective of the present experiment was to determine the performance and histological alterations of intestinal villi and epithelial cells in chickens fed basal diets diluted with WPR.

Materials and Methods: At 2 weeks of age, the 30 birds were randomly divided into 3 groups of 10 birds each. The control group was fed with basal diet and the other groups were fed with basal diet diluted with WPR at 20 and 40%. At the end of the feeding experiment, 5 birds from each group were weighed individually and killed by decapitation under light anaesthesia with diethyl ether. The weight of empty organs was expressed

relative to 100 g body weight. Another 4 birds per group were used for intestinal histological observations. The middle parts of the duodenum, jejunum and ileum were prepared for light microscopy and scanning electron microscopy. In light microscopic examination, samples of intestinal tissue were fixed in Bouin's fixative solution, dehydrated and paraffin-embedded. A 4-μm-thick transverse section was cut and stained with hematoxylin-eosin. The following measurements were performed: villus height, villus area, cell area and cell mitosis number. These measurements were performed using an image analyzer (Nikon Cosmozone 1S; Nikon Co., Tokyo, Japan). In scanning electron microscopic examination, each intestinal segment was slit longitudinally, cut into 5×5 mm squares, washed with 0.1 mol/L sodium cacodylate buffer (pH 7.4), and post-fixed with 1% osmium tetroxide. The specimens were then washed with deionized distilled water, dehydrated with varying concentrations of alcohol, and freeze-dried (Hitachi freeze dryer; Hitachi Ltd., Tokyo, Japan). These specimens were mounted on aluminium stubs and coated with platinum (E-1030 ion sputter; Hitachi Ltd., Tokyo, Japan), all villi were then observed with a scanning electron microscope (Hitachi S-4300SE/N; Hitachi Ltd., Tokyo, Japan) at eight kV.

Results: The growth performance, relative length of the intestines and relative weight of the visceral organs to 100 g body weight did not differ except that the weight of the gizzard increased significantly ($p<0.05$) in the WPR groups. Most parameters of villus height, villus area, cell area and cell mitosis numbers of the WPR groups did not show a significant decrease. In scanning electron microscopic results, the morphology of the villus apical surface in the WPR groups did not show damage due to WPR and had similar cells to the control (protuberated cells).

Conclusion: No significant differences were observed in growth performance or in most light microscopic parameters of all intestinal segments; as well, protuberated epithelial cells with no damage on the villus apical surface were observed, suggesting that chickens can be fed a basal diet diluted with WPR up to a level of 40% without negative effects on growth performance or intestinal histology.

Experiment 3: Effects of whole-grain paddy rice replacement with or without enzyme addition on broiler performance and intestinal morphology

Aim: The results of **Experiment 2** suggested that WPR can be diluted by up to 40% as a feed ingredient in chicken basal diets without negative effects on growth performance or intestinal histology. However, the use of WPR can cause severe problems because of the high crude fiber and non-starch polysaccharides (NSP). This problem may be overcome by dietary enzyme supplementation. Therefore, the objective of this study was to investigate the effect of replacing corn with different levels of WPR, with or without enzyme addition, on growth

performance and intestinal histological structures of broiler chickens from 14 to 49 days of age.

Materials and Methods: Marshall Chunky male broiler chicks at 14-d-old were individually weighed and randomly divided into 4 groups of chicks with similar mean body weight, each with four replicates of four chickens. These chicks were housed in floor pens covered with rice hulls under continuous lighting at an average environmental temperature of 16°C. The experimental diets containing different levels of WPR were as follows: (1) 0 g/kg (Control); (2) 141.5 g/kg, grower, and 125.0 g/kg, finisher (25WPR); (3) 283.0 g/kg, grower, and 250.0 g/kg, finisher (50WPR); (4) 283.0 g/kg, grower, and 250.0 g/kg, finisher (50WPR + enzyme). The 50WPR + enzyme diet was supplemented with 5 g/kg enzyme (Kemzyme^{TM} PS dry). During the experiment, feed intake and body weight were recorded on a group basis at weekly intervals, and feed efficiency was calculated. At the end of the feeding experiment, 7 birds from each group were weighed individually and killed by decapitation under light anaesthesia with diethyl ether. The weight of empty organs was expressed relative to 100 g body weight. Another 4 birds per group were used for intestinal histological observations. The protocol and measurements were similar with **Experiment 2**.

Results: There were no differences among the diets on the growth performance and digestive organ size. The villus height and cell mitosis number of all intestinal segments did not change in all groups. The ileal villus area, duodenal cell area, duodenal and jejunal goblet cell number in the 50WPR group increased significantly relative to the control but not when enzyme was included. In the scanning electron microscope results, all experimental groups showed clear protuberant cells and cell clusters on the villus apical surface of the duodenum. In the jejunum, cell clusters and areas having cells with no micro-villi were frequently found in both the 50WPR and 50WPR + enzyme groups.

Conclusion: Replacing corn with WPR in broiler diets showed hypertrophied villi of duodenum and ileum and epithelial cells in duodenum and jejunum, especially in the 50WPR group, without negatively affecting growth performance. These results may encourage poultry farmers to use locally grown grains as an alternative feed ingredient in chicken diets. These findings suggest that WPR can replace corn up to a level of 50% (283.0 g/kg, starter, and 250.0 g/kg, finisher) in broiler diets without enzyme supplementation.

Experiment 4: Effects of replacing corn with whole-grain paddy rice in laying hen diets on egg production performance

Aim: The results of **Experiment 3** confirmed that WPR can replace corn up to a level of 50% (283.0 g/kg, starter, and 250.0 g/kg, finisher) in broiler diets without enzyme supplementation. From these results,

enzyme addition is not beneficial to the WPR-based diets. To my knowledge, I have not found any report comparing the effects of corn and WPR when included in diets without enzyme addition for laying hens. Therefore, as the next step, the present experiment was carried out to study the effect of replacing corn with WPR in laying hen diets on egg production performance and quality.

Materials and Methods: This experiment comprises 2 trials. Commercial layers (Sonia) were used in both Trial 1 and 2. In Trial 1, 80 layers were placed into 4 groups of 20 birds each: the corn in the basal diet was replaced with 0, 10, 30 and 50%WPR. Each group of 20 birds had 10 replicates of 2 birds. In Trial 2, 45 layers were placed into 3 groups of 15 birds each: the corn in the basal diet was replaced with 0, 70 and 100%WPR. Feed and water were provided *ad libitum* during the experimental period. All birds were housed in individual cages in an environmentally controlled room with a 16-h photoperiod. Egg production was recorded daily throughout the experiment. Feed consumption was determined at the end of each week during the feeding period. Eggs from each group were collected biweekly to measure egg weight, shell-breaking strength, shell thickness, shell ratio, albumen ratio, yolk ratio, yolk color and Haugh units. Values for shell ratio, albumen ratio, yolk ratio and Haugh units were calculated for each individual egg as follows:

$$\text{Shell ratio} = \frac{\text{Shell weight} \times 100}{\text{Egg weight}}$$

$$\text{Albumen ratio} = \frac{\text{Albumen weight} \times 100}{\text{Egg weight}}$$

$$\text{Yolk ratio} = \frac{\text{Yolk weight} \times 100}{\text{Egg weight}}$$

$$\text{Haugh units} = 100 \log (H - 1.7\, W^{0.37} + 7.6)$$

where

H = Observed height of albumen (mm)

W = Weight of egg (g)

Results: Egg production performance and quality were not different among the groups ($P>0.05$), except for a decreased ($P<0.05$) shell ratio in the 100%WPR group. Moreover, yolk color score decreased ($P<0.05$) with increasing levels of WPR (50%WPR or more).

Conclusion: the present results reveal that WPR can totally replace corn in laying hen diets without harming egg production performance and quality. This suggests that WPR could be used as a feed ingredient in

laying hen diets.

Experiment 5: Effect of replacing corn with whole-grain paddy rice and brown rice in broiler diets on growth performance and intestinal morphology

Aim: The results of **Experiment 3** confirmed that WPR can replace corn up to a level of 50% (283.0 g/kg, starter, and 250.0 g/kg, finisher) in broiler diets without enzyme supplementation. Moreover, it has been suggest that feeding WPR to laying hens as a replacement for corn in diets led to a similar production performance to that of those fed basal diets, which demonstrated that WPR can safely replace up to 100% of corn in the laying hen diets (**Experiment4**).

From the above experiment, the rations were made isocaloric by increasing the level of animal fat to elevate the WPR level in the diets, resulting in oily diet. Therefore, whole-grain brown rice (BR) was supplemented to reduce animal fat in the diet. Subsequently, BR was not considered as a principal factor. The aim of this study was to investigate replacing corn with different levels of WPR and its effect on growth performance and intestinal histological structures of broiler chickens from 14 to 49 days of age.

Materials and Methods: 14-day-old chicks were individually weighed and randomly divided into 5 groups of chicks with similar mean body weight, each with four replicates of four chickens. These chicks were housed in floor pens covered with rice hulls under continuous lighting at an average environmental temperature of 28°C. In the dietary treatments, corn in the basal diet was replaced with WPR and BR. The chickens received five experimental diets consisting of corn, WPR and BR in ratios of 100:0:0 (Control), 50:0:50 (50Corn + 50BR), 50:25:25 (50Corn + 25WPR + 25BR), 25:50:25 (25Corn + 50WPR + 25BR), and 0:50:50 (50WPR + 50BR), respectively. Feed and water were provided *ad libitum* for 35 d. During the experiment, feed intake and body weight were measured weekly, and feed efficiency was calculated. At the end of the feeding period, 7 birds with body weights close to the mean were selected from each group and killed by decapitation under light anesthesia with diethyl ether. The gizzard content from each bird was diluted 1:3 (w/w) with deionized distilled water and mixed by stirring. The pH was measured using a digital pH meter (M-12; Horiba, Ltd., Kyoto, Japan). Subsequently, the weights of the proventriculus, gizzard, duodenum, jejunum, ileum and ceca were recorded after its digesta content had been removed. The weight of empty organs was expressed relative to 100 g body weight. Another 4 birds per group were used for intestinal histological observations. The protocol and measurements were similar with **Experiment 2**.

Results: No significant differences were found in feed intake, body weight gain and feed efficiency

among the treatment groups. The relative weights of the gizzard in the 50Corn + 25WPR + 25BR, 25Corn + 50WPR + 25BR and 50WPR + 50BR groups were significantly higher than that of the Control and 50Corn + 50BR groups ($P<0.05$). The gizzard pH of the experimental groups was lower than those of Control ($P<0.05$). The ileal crypt of birds on the Control diets was deeper ($P<0.05$) than those observed in the experimental birds. Moreover, the ileal villus height:crypt depth ratio increased ($P<0.05$) in the 50WPR + 50BR group ($P<0.05$) compared with the Control group. No specific changes were observed in the epithelial cells on the duodenal apical surface among the groups except that the villus of the 25Corn + 50WPR + 25BR group had cell clusters. The jejunal and ileal villus apical surface of the experimental groups showed similar morphology to the Control group.

Conclusion: Replacing corn with WPR and BR in broiler diets led to the development of gizzard function, whereas there were few changes in intestinal morphology. Histological results indicate that there were greater villi of the ileum and epithelial cells in the duodenum, especially in the WPR- and BR-fed birds. These results may encourage poultry farmers to include local grains in broiler diets. These findings suggest that WPR and BR can totally replace corn in broiler diets without negatively affecting growth performance.

Finally, the results from this thesis demonstrate that WPR can use as a feed ingredient in poultry diets. This development of WPR as a new alternative for poultry feed ingredients would be highly evaluated for poultry industry.