Effects of Whole-Grain Paddy Rice on Growth Performance, Oxidative Stress and Morphological Alterations of the Intestine in Broiler Chickens Exposed to Acute and Chronic Heat Stress

Fumika Nanto, Chiaki Ito, Motoi Kikusato and Masaaki Toyomizu

Animal Nutrition, Applied Life Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan

We have previously shown that, under thermoneutral conditions, the growth performance of chicks fed a rice-based diet containing 6% soybean oil [metabolizable energy (ME): 2,800 kcal/kg] for 28 days was comparable to that obtained with a corn-based diet containing 6% soybean oil (ME: 3,100 kcal/kg). However, there is no information available concerning how such diets compare with respect to growth performance under heat stress conditions. The present study was therefore conducted to clarify differences in dietary effectiveness between corn-based and whole-grain paddy rice-based diets [formulated to be equivalent in terms of fat (6%), but not iso-caloric] under the two conditions of acute and chronic heat stress (Experiment 1 and Experiment 2, respectively). In Experiment 1, the body weight gain of a corn- and rice-fed group of chickens exposed to acute heat (33 deg C-12 h) was significantly decreased compared with that of a corn-fed control group (24 deg C), with similar results obtained for the two heat-treated groups. Malondialdehyde (MDA) levels in the M. pectoralis superficialis were significantly increased in corn-fed birds exposed to acute heat stress, but not in the rice-fed group (33 deg C-12 h). In Experiment 2, body weight gains in the corn- and rice-fed groups exposed to chronic heat (33 deg C-6 d) were significantly decreased, and to a similar extent, compared with the corn-fed control group (24 deg C). Muscle MDA levels in the corn- and rice-fed groups were similarly increased by the chronic heat exposure compared with the control group. These results suggest that, in response to acute heat stress (but not chronic heat stress) conditions, the feeding of whole-grain paddy rice diet to chickens may attenuate skeletal muscle oxidative damage compared with that in corn-fed chickens. The possible involvement of intestinal morphology (such as the villus height: crypt depth ratio observed here) on growth performance is also discussed.

Key words: broiler chicken, heat stress, intestinal morphology, oxidative stress, whole-grain paddy rice


Introduction

The global demand for corn to be used in the production of agricultural feed and fuel is increasing at a rapid rate (Edgerton, 2009). To offset this demand, several studies demonstrated that the paddy rice, including brown rice, shows potential for use as a substitute for corn in poultry feed (Honda et al., 2011; Sittiya et al., 2011). Under thermoneutral conditions, we previously reported that chicks fed a rice-based diet containing 6% soybean oil [metabolizable energy (ME): 2,800 kcal/kg] for 28 days exhibited normal growth, whereas chicks fed a whole-grain paddy rice-based diet containing 10% soybean oil (ME: 3,100 kcal/kg) for 28 days showed growth retardation compared with a control group fed a corn-based diet containing 6% soybean oil (ME: 3,100 kcal/kg) (Nanto et al., 2012). These findings suggested that whole-grain paddy rice could be a constituent compatible with corn feed for broiler chickens, as long as the dietary fat level in the whole-grain paddy rice-based diet is kept equal to that of the standard corn-based diet (ME: whole-grain paddy rice-based diet, 2,800 kcal/kg versus standard corn-based diet, 3,100 kcal/kg). However, to the authors' knowledge, virtually no data are available to confirm whether the paddy rice-based diet with a lower ME would still serve as a useful ingredient of poultry feeds in situations when birds are subjected to stressful conditions such as
environmental temperature extremes (Bottje and Harrison, 1985; Yahav et al., 1995). Therefore, the present studies were conducted with a view to clarifying the practical utility of whole-grain paddy rice-based diets (ME: 2,800 kcal/kg, fat content: 6%) on growth performance under the two conditions of acute and chronic heat stress. We also focused on oxidative stress and intestinal morphological alterations in broiler chickens, because alteration of mitochondrial reactive oxygen species (ROS) levels and oxidative damage to skeletal muscle were observed previously in acute and chronic heat-exposed broilers (Mujahid et al., 2005, 2007, 2009; Azad et al., 2010a, b), and changes to intestinal morphology also were observed in chronic heat-exposed chickens (Quinteiro-Filho et al., 2010).

Material and Methods

Experimental Diets

The dietary feed compositions used in acute and chronic heat stress experimental conditions (Experiment 1 and 2, respectively) are shown in Table 1. Two diets were formulated to contain 48% corn or 43% whole-grain paddy rice with the same levels of animal fat (6%) and crude protein (CP: 20%), but with different ME levels (ME: 3,100 kcal/kg for the corn-diet versus 2,800 kcal/kg for the rice-diet). The diets included all nutrients necessary to fulfill the nutritional requirements of broiler chickens, except that the whole-grain paddy rice-diet had a slightly lower ME than recommended (Standard Tables of Feed Composition in Japan, 2009).

Animals and Experimental Design

Male broiler chickens (chunky strain) were obtained from a commercial hatchery (Economic Federation of Agricultural Cooperatives Hatchery, Miyagi, Japan) at 0 d of age for the two experiments. Twenty-four chicks for each experiment were randomly divided into 2 groups, each housed in electrically-heated batteries under continuous light for 11 days and provided with ad libitum access to water and one of the two experimental diets. Thereafter, chickens from each group were moved to individual cages. At 21 d of age, birds in the corn-fed group were randomly divided into two groups (n = 8), and then one of the two groups and the rice-fed group were exposed to heat stress while the remaining group was maintained at 24°C. The heat stress conditions for Experiment 1 and 2 were 33°C for 12 h and 33°C for 6 days, respectively. The body weight and feed intake of chickens were recorded over a 17 d period from 11 to 28 d of age, with all chickens sacrificed on this day by exsanguination. The pectoralis superficialis muscle, liver, abdominal fat, gizzard, proventriculus, heart and thigh were harvested and immediately weighed. Tissues were immediately frozen, powdered in liquid nitrogen and stored at −80°C until analyzed. All experiments were performed in accordance with institutional guidelines concerning animal use and every effort was made to minimize pain or discomfort to the animals.

Determination of Malondialdehyde (MDA) Content

Tissues were homogenized in buffer (1.15% KCl) and the supernatants collected. Lipid peroxidation was assayed colorimetrically as a function of the 2-thiobarbituric acid reactive substance (TBARS) content as described previously (Mujahid et al., 2007). In brief, 800 μL of tissue homogenate were mixed with 200 μL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid (pH 3.5), 50 μL of 0.8% butyl-hydroxytoluene, and 1.5 mL of 0.8% 2-thiobarbituric acid. After vortexing, samples were incubated on ice for 60 min and then heated at 95°C for 60 min in a water bath. After cooling, 1 mL of H2O and 5 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added and the samples were again mixed by vortexing. After centrifugation at 1000 × g for 10 min, the organic layer was extracted and read spectrophotometrically at a wavelength of 532 nm. The TBARS content was expressed as nmol of MDA per equivalent weight (g) of wet tissue. The samples were analyzed within
1 week of storage at −80°C.

**Determination of Protein Carbonyl Content**

Skeletal muscle protein carbonyl content (used as a biomarker of oxidative stress) was determined by derivatization with 2,4-dinitrophenyldrazine (DPNH; Levine *et al.*, 1990; Dalle-Donne *et al.*, 2003). Briefly, frozen tissues were homogenized in ice cold-buffer comprising 50 mM potassium phosphate (pH 6.7) and 1 mM ethylenediaminetetraacetic acid, and the homogenates were centrifuged at 10000×g for 15 min at 4°C. To minimize the interference of nucleic acids in the estimation of the carbonyl content, homogenates were incubated at room temperature for 15 min with 1% streptomycin sulfate, which precipitates the nucleic acids. After further centrifugation of the samples at 6000×g for 10 min at 4°C, the supernatants were used for the determining the carbonyl content. The supernatants were incubated with either 10 mM DPNH in 2N-HCl (sample) or 2N-HCl only (blank) at room temperature for 1 h in the dark with vortexing every 15 min. Following the incubation, they were precipitated with 20% trichloroacetic acid (TCA) (w/v) and centrifuged at 10000×g for 10 min at 4°C. The supernatants were discarded, the protein pellets were washed once more with 20% TCA, and then washed three times with ethanol/ethyl acetate (1:1, v/v) to remove any free DPNH. Pellets were resuspended in 6 M guanidine hydrochloride (pH 2.3) at 37°C for 15 min with vortex mixing. The absorbances of samples and blanks were spectrophotometrically measured at a wavelength of 366 nm. The absorbance of the blank was then subtracted from the absorbance of the sample, which was taken as the corrected absorbance. Carbonyl content was determined with a molar extinction coefficient of 22,000 M⁻¹·cm⁻¹ (Levine *et al.*, 1994). Protein contents were also spectrophotometrically determined as the absorbance at a wavelength of 280 nm, with bovine serum albumin used as the standard. The results were expressed as nanomoles carbonyl per milligram protein.

**Intestinal Morphology**

A one cm length of intestinal tissue from each chicken was collected from the midpoint of the duodenum and stored in 10% formalin neutral buffer solution prior to morphological analysis, which was performed on 3-4 μm-thick sections cut on a microtome. Sections were stained using the hematoxylin-eosin method. Villus height and crypt depth were measured with the aid of a microscope from 10 randomly selected villi and associated crypts on two sections per chicken. The ratio of villus height to crypt depth as an index of the maturity and functional capacity of enterocytes (Hampson, 1986) was calculated from these measurements.

**Statistical Analysis**

Statistical analysis was performed with one-way analysis of variance followed by the Tukey-Kramer multiple comparison test (P<0.05). All data are expressed in the form of mean±standard error (SE, n=5–8 per measurement).

**Results**

**Experiment 1**

Body weight gains and ME intakes of both corn- and rice-fed groups exposed to acute heat stress (33°C−12 h) were significantly decreased (P<0.05) compared to the corn-fed thermoneutral group (24°C), with similar results obtained for the two heat-treated groups (33°C−12 h) (Fig. 1A and B). Average rectal temperatures for both groups subjected to acute heat exposure were significantly increased compared with the thermoneutral group, and to a similar extent (Fig. 1C).

No significant differences were observed among any of the groups (thermoneutral and heat-stressed corn-fed groups, and heat-stressed rice-fed group) with respect to the weight and yield (expressed in relative terms as tissue weight per 100 g body weight) of *M. pectoralis superficialis* thighs (including bone), heart and abdominal fat (Fig. 2). Compared with control, no significant alterations in the weights and yields of liver and gizzard were observed in the corn-fed chickens subjected to acute heat stress; significantly increased values were however recorded in the rice-fed group. The findings of increased size of gizzard by whole-grain paddy rice rich in dietary fiber is in accordance with the results of Hetland *et al.* (2003) who observed that gizzard weight increased with inclusion of hull-rich feed ingredients, such as oat hulls and whole wheat. In contrast, the weight and yield of the proventriculus were less in heat-stressed rice-fed chickens than in their corn-fed counterparts.

* M. pectoralis superficialis MDA levels were slightly increased in corn-fed birds exposed to acute heat, while MDA levels were significantly lower in rice-fed chickens compared with corn-fed counterparts (Fig. 3A). No changes in MDA levels in *M. iliotibialis lateralis* or plasma were observed among any of the groups (Fig. 3B and C). Liver MDA levels in the corn- and rice-fed groups were significantly increased compared with the rice-fed thermoneutral group (Fig. 3D). Protein carbonyl levels in *M. pectoralis superficialis* of corn-fed groups subjected to acute heat exposure were also slightly increased compared to the corn-fed thermoneutral group, with the increase for the rice-fed group being slightly more than that in the heat-stressed corn-fed group (Fig. 3E). No differences in protein carbonyl levels in the *M. iliotibialis lateralis* or plasma were observed among any of the groups (Fig. 3F and G).

The villus height in duodenal tissue from corn-fed chickens exposed to acute heat was no different to that of the corn-fed thermoneutral group, while that of the rice-fed chickens exposed to acute heat was significantly increased compared with that of the heat-stressed corn-fed chickens (Fig. 4A). The crypt depth in corn-fed chickens exposed to acute heat stress was slightly deeper compared to the corn-fed thermoneutral group, with this difference being even more pronounced in the rice-fed group (Fig. 4B). The villus height to crypt depth ratio was significantly decreased in the heat-stressed corn-fed group, but not in the rice-fed group treated in the same manner (Fig. 4C).

**Experiment 2**

Three birds in the rice-fed group died on the first and second day of the chronic heat stress period (33°C−6 d), but
Fig. 1. Effects of corn- and whole-grain paddy rice-based diets on body weight gain (A), ME intake (B) and rectal temperature (C) of broiler chickens exposed to acute heat stress (33℃, 12 h). Values are means±SE, n=8 per group. a,b P<0.05 for each treatment; values with different letters are statistically different.

Fig. 2. Effects of corn- and whole-grain paddy rice-based diets on the absolute and relative weights (yields) of M. pectoralis superficialis, thigh (including bone), heart, liver, abdominal fat, proventriculus and gizzard of broiler chickens exposed to acute heat stress (33℃, 12 h). Values are means±SE, n=8 per group. a,b P<0.05 for each treatment; values with different letters are statistically different.
no deaths were recorded for corn-fed birds under the same conditions. Body weight gains and ME intakes of the corn- and rice-fed groups exposed to chronic heat were significantly decreased compared to the corn-fed thermoneutral group (24°C), with similar results obtained for the two heat-treated groups (Fig. 5A and B). The rectal temperatures of both groups exposed to chronic heat stress (33°C–6 d) were significantly increased compared to the corn-fed thermoneutral group (Fig. 5C).

The weights of the *M. pectoralis superficialis* and heart of the corn- and rice-fed groups exposed to chronic heat stress were significantly decreased, and to a similar extent, compared with the corn-fed the thermoneutral group (24°C) (Fig. 6). Yields for the *M. pectoralis superficialis* and heart of the corn- and rice-fed chickens exposed to chronic heat stress showed similar tendencies to those seen for weights. No significant differences in the weights and yields of thighs (including bone) and proventriculus were observed between the thermoneutral and heat-stressed corn groups, but that of the rice-fed group exposed to chronic heat was slightly decreased compared with heat-stressed corn-fed groups (Fig. 6). No significant differences in the weights and yields of liver were observed among all three groups. Moreover, no significant differences in the weights and yields of abdominal fat were seen for the corn-fed chickens exposed to chronic heat compared with the thermoneutral group, but that of the rice-fed group exposed to chronic heat was slightly increased compared with that of the corn-fed counterparts. In relation to gizzard weights, corn-fed chickens exposed to chronic heat exhibited a decreased weight compared to the corn-fed thermoneutral group (24°C), while the rice-fed group exhibited a significantly higher value than those seen for the heat-stressed corn-fed groups. The yields of gizzard in corn-fed chickens were not changed by the chronic heat treatment, while the yield of the rice-fed group was significantly higher than that of the heat-stressed corn-fed group.

No significant differences in MDA levels for the *M. pectoralis superficialis, M. iliotibialis lateralis*, plasma or liver were observed among the three groups (Fig. 7A-D). Similarly, no significant differences in protein carbonyl levels in *M. pectoralis superficialis* or plasma were observed among the three groups (Fig. 7A-D). However, protein carbonyl levels in the *M. iliotibialis lateralis* were significantly increased in the corn-fed heat-stressed group compared to the thermoneutral group (24°C), and even more so in the rice-fed group (Fig. 7F).

No differences in villus height or villus height: crypt depth ratio were observed between the thermoneutral and heat-stressed corn-fed groups (Fig. 8A and C), with the heat-stressed rice-fed chickens showing slightly decreased values...
Fig. 4. Effects of corn- and whole-grain paddy rice-based diets on the villus height (A), crypt depth (B) and the villus height: crypt depth ratio (C) of duodenal tissue from broiler chickens exposed to acute heat stress (33°C, 12 h). Values are means±SE, n=8 per group. a,b p<0.05 for each treatment; values with different letters are statistically different.

Fig. 5. Effects of corn- and whole-grain paddy rice-based diets on body weight gain (A), ME intake (B) and rectal temperature (C) of broiler chickens exposed to chronic heat stress (33°C, 6 d). Values are means±SE, n=8/group fed corn and n=5/group fed rice. a,b P<0.05 for each treatment; values with different letters are statistically different.
for both parameters compared to their corn-fed counterparts. No significant differences in the crypt depth were observed among the groups (Fig. 8B).

**Discussion**

Under thermoneutral conditions, we demonstrated that the growth performance of chicks fed a rice-based diet containing 6% soybean oil (ME: 2,800 kcal/kg) for 28 days was comparable to that of chicks fed a corn-based diet containing 6% soybean oil (ME: 3,100 kcal/kg) (Nanto et al., 2012). In order to know how the two type of diets bear comparison with each other under heat stress conditions, the present study was conducted to clarify differences in dietary effectiveness between corn-based and whole-grain paddy rice-based diets [formulated to be equivalent in terms of fat (6%), but not iso-caloric] under the two conditions of acute and chronic heat stress. Exposure to acute heat stress (33°C, 12h; Experiment 1) caused significant reductions in body weight gain and ME intake of corn-fed birds, and the negative effects of heat stress on growth of rice-fed birds were similar to the case of the corn-fed birds. Additionally, no significant differences in the weights and yields of M. pectoralis superficialis and thighs (including bone) were observed between corn- and rice-fed birds under acute heat stressed conditions. These results suggest that, under acute heat stress conditions, the feeding of whole-grain paddy rice diet to chickens may show growth performance comparable to that achieved with corn-based diets.

Results of the present study show that, in rice-fed birds, the negative effects of acute heat stress on oxidative damage and intestinal morphology were reduced compared with corn-fed birds. MDA levels in M. pectoralis superficialis of the rice-fed group exposed to acute heat stress were significantly lower than that of corn-fed counterparts. The lower MDA levels in rice-fed chickens might result from a reduction in ROS production and/or an improved antioxidant defense system. The latter point is feasible, given that rice bran, which is obtained from whole-grain paddy rice, contains tocopherols and tocotrienols, which are powerful free radical scavengers (Cabrini et al., 2001). Therefore, the anti-oxidative effects of paddy rice are evident not only under thermoneutral conditions as shown in our previous studies (Nanto et al., 2012), but also under acute heat stress conditions. For evaluation of intestinal morphology, villus height and crypt depth as well as the villus height to crypt depth ratio are useful parameters indicative of gut health in animals (Pluske et al., 1996; Xu et al., 2003). Increased values for these parameters are directly correlated with an increased epithelial turnover (Fan et al., 1997). In the present study, the villus height to crypt depth ratio in the rice-fed group exposed to acute heat stress was significantly higher than that of the heat-stressed corn-fed group. Singh et
al. (2012) showed that the lipid peroxidation-inhibiting antioxidant α-tocopherol succinate protected the intestinal tissue of irradiated mice as evidenced by enhancements of crypt and villus number, villus length and mitotic cell division. Furthermore, Jiménez-Moreno et al. (2011) reported that at 15d of age, broilers fed diet containing 2.5% peahulls showed a significantly higher villus height to crypt depth ratio than broilers receiving diet with 0% peahulls, though a further increase to 7.5% of peahulls had an opposite effect on the ratio, meaning that moderate amount of dietary fiber can improve intestinal morphology. Therefore, intestinal function in the rice-fed chickens in our study might have been protected due to the enhanced antioxidant activity of tocotrienols as well as tocopherols in paddy rice and/or stimulated by moderate content of fiber in the rice-based diet which was 2 times higher than that of the corn-based diet. In contrast to the results of Experiment 1, exposure of rice-fed chickens to chronic heat stress (33°C–6 d; Experiment 2) resulted in a slightly lower muscle mass compared with that of corn-fed chickens maintained under the same conditions. Oxidative damage to the M. iliotibialis lateralis and intestinal morphological damage were more severe in heat-stressed rice-fed chickens than in corn-fed chickens maintained under the same conditions, which suggests that rice-fed chickens might have a lower resistance to chronic heat exposure than corn-fed chickens. These findings raise an important question: Why do rice-fed chickens respond differently when exposed to acute and chronic heat stress? One possible answer could be that ME level of the rice-based diet (2,800 kcal/kg) was too low for chickens subjected to chronic heat exposure compared with that of corn-based diet (3,100 kcal/kg). High energy feed has commonly been used for chickens in warm regions, probably to overcome the negative effects of low feed intake and to dissipate excess body heat (Daghir, 1995). Another possible answer could be that the oxidative stability of whole-grain paddy rice diets is lower than that of corn-based diets. We found that the rice-based diet containing 50% whole-grain rice and 6% animal fat exhibited a 2.3 times higher chemiluminescence intensity [which was proposed by Miyazawa et al. (1991) to be an indicator of free radical production and lipid peroxidation] than that of the corn-based diet containing 51% corn and 6% animal fat (data not shown). In fact, the administration of diets containing highly oxidized oils was shown to result not only in increased lipid oxidation in the plasma of broiler chickens (Engberg et al., 1996) and distortion of villus morphology in mice (Obembe et al., 2011), but also in reduced appetite, poorer feed conversion,

**Fig. 7.** Effects of corn- and whole-grain paddy rice-based diets on MDA concentrations in M. pectoralis superficialis (A), M. iliotibialis lateralis (B), plasma (C) and liver (D), and protein carbonyl content in M. pectoralis superficialis (E), M. iliotibialis lateralis (F) and plasma (G) of broiler chickens exposed to chronic heat stress (33°C, 6 d). Values are means±SE, n=8/group fed corn and n=5/group fed rice. a,b P<0.05 for each treatment; values with different letters are statistically different.
and growth depression in poultry (Shermer and Calabotta, 1985; Cabel et al., 1988; Engberg et al., 1996). Therefore, it can be speculated here that the negative effects of dietary oxidized oil on biological parameters and growth performance as shown above might be much more profound in rice-fed chickens exposed to chronic heat stress than acute heat stress. Moreover, the chronic heat stress may have been too severe for paddy rice-based diet-fed chickens to recover from oxidative damage due to peroxidation of the rice-based diet, even though whole-grain paddy rice in diets for experiment 1 and 2 contains similarly anti-oxidant nutrients, such as tocopherols and tocotrienols.

In summary, the feeding of a rice-based diet to chickens under acute heat stress conditions attenuated the level of oxidative damage to skeletal muscle and improved intestinal morphology, but this was not the case under chronic heat stress conditions. Further study is needed to determine how the negative effects associated with whole-grain paddy rice use under chronic heat stress conditions can be alleviated.

Acknowledgments

This study was supported by a Grant-in-Aid for “Research for Production of Valuable Livestock by Feeding Self-Sufficient Forage Crops” from the Ministry of Agriculture, Forestry and Fisheries, and a Research Fellowship from the Japan Society for the Promotion of Science (JSPS) for Young Scientists (No. 13J06451; F.N.). Acknowledgment is also made to the Japan Poultry Science Association for providing a travel grant to F.N. to allow presentation of some of the findings at the 24th WPC in Salvador, Brazil.

References


Jiménez-Moreno E, Chamorro S, Frikha M, Safaâ HM, Lázaro R and Mateos GG. 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. Animal Feed Science and Technology, 168: 100–112. 2011.


