

## Effect of Feeding Diets Combining Whole-Grain Paddy Rice and High Levels of Fat on Broiler Chicken Growth

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Previously, we showed that the growth of chicks fed a diet containing 43% whole-grain paddy rice and 10% soybean oil was retarded relative to a control group fed a corn-based diet containing 6% soybean oil. However, feeding chicks with 43% whole-grain paddy rice containing 6% soybean oil resulted in normal growth. It is possible that the observed growth retardation was caused by the high soybean oil content or resulted from the combination of whole-grain paddy rice and the high level of soybean oil which was added to the diet to maintain the overall energy content. The present study was therefore carried out to identify the reasons for the observed growth retardation. Thirty-six chicks (0-day-old) were divided into six equal-sized groups that were fed one of the following six experimental diets *ad libitum* for 28 d: two kinds of dehulled rice-based diets containing 5% or 10% soybean oil (DS5% or DS10%), another three whole-grain paddy rice-based diets containing 10% soybean oil, corn oil, or rendering oil (WS10%, WC10%, WR10%, respectively), and a WS10% diet supplemented with vitamin B<sub>12</sub>, methionine and ethoxyquin. The body weight gain of groups fed the WS10% and WC10% diets was significantly lower than the weight gain of birds fed the DS5% diet (control). In addition, the liver of birds fed the WS10% and WC10% diet exhibited significantly higher lipid peroxidation than that of the control group. In comparison, supplementation of the WS10% diet with vitamin B<sub>12</sub>, methionine and ethoxyquin dramatically improved growth and hepatic oxidation status. These results indicate that diets combining whole-grain paddy rice and high levels of soybean and corn oil adversely affect performance, presumably via lipid peroxidation in the liver.

**Key words:** broiler chicken, high fat diet, lipid peroxidation, oxidative stress, whole-grain paddy rice

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### 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, many types of grain have been proposed as substitutes for corn in broiler chicken diets, serving as alternative sources of dietary carbohydrates. Several studies have demonstrated that paddy rice, including brown rice, has potential as a substitute for corn in poultry feed (Honda *et al.*, 2011; Sittiya *et al.*, 2011).

Those findings are not entirely supported by our previous data (Nanto *et al.*, 2012), which showed that chicks fed a diet containing 43% whole-grain paddy rice and 10% soybean oil exhibited growth retardation relative to a control group fed a corn-based diet containing 6% soybean oil. However, feed-

ing chicks with diet containing 43% whole-grain paddy rice and 6% soybean oil resulted in normal growth. It is possible that the growth retardation was caused by the high soybean oil content or that it was due to the combination of the whole-grain paddy rice and the high level of soybean oil (which was added to the diets to maintain the overall energy content). Therefore, the present study was carried out to determine what caused the observed growth retardation. In particular, we investigated the effect of rice-based- diets containing or not containing rice hulls, and containing different types of oil (such as soybean oil, corn oil or rendering oil) on the growth performance of broiler chickens. We also investigated whether supplementation of the rice diet with vitamin B<sub>12</sub>, methionine and ethoxyquin could improve growth performance.

### Materials and Methods

#### Experimental Diets

The feed compositions are shown in Table 1. Six diets were formulated as follows: two kinds of dehulled rice-based diets containing 5% or 10% soybean oil (DS5%: con-

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Table 1. **Composition (%) of diets in the experiment**

Ingredient	DS5% control	DS10%	WS10%	WC10%	WR10%	WS10% B <sub>12</sub> +Met+EQ
Dehulled rice	54.6	48.8	—	—	—	—
Whole grain paddy rice	—	—	47.00	47.00	46.00	47.00
Soybean meal	35.30	36.00	37.40	34.70	37.50	37.40
Soybean oil	5.00	10.40	10.40	—	—	10.40
Corn oil	—	—	—	10.40	—	—
Rendering oil	—	—	—	—	11.55	—
Calcium carbonate	1.03	1.03	1.03	1.03	1.03	1.03
Dibasic calcium phosphate hydrate	1.75	1.75	1.75	1.75	1.75	1.75
Sodium chloride	0.33	0.33	0.33	0.33	0.33	0.33
Mineral mixture <sup>1</sup>	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mixture <sup>1</sup>	0.40	0.40	0.40	0.40	0.40	0.40
DL-methionine	0.12	0.14	0.22	0.22	0.22	0.57
Ethoxyquin	—	—	—	—	—	0.03
Vitamin B <sub>12</sub>	—	—	—	—	—	0.000009
Cellulose	1.07	0.75	1.07	1.07	0.82	0.71
Calculated						
Crude Protein (%)	20	20	20	20	20	20
Metabolisable Energy (kcal/kg)	3101	3416	3100	3100	3100	3100

<sup>1</sup> Akiba and Matsumoto (1978)

Table 2. **Fatty acid composition (%) of the dietary fat sources**

Fatty acid (%)	Soybean oil	Corn oil	Rendering oil
C6:0	—	—	—
C8:0	—	—	0.1
C10:0	—	—	0.1
C12:0	—	—	0.4
C14:0	0.1	—	1.3
C14:1	—	—	0.2
C15:0	—	—	0.1
C16:0	9.6	10.5	20.6
C16:1	0.1	0.2	2.7
C17:0	0.1	0.1	0.3
C17:1	0.1	—	0.3
C18:0	3.1	1.9	8.4
C18:1	23.6	26.2	44.5
C18:2	53.8	56.9	14.5
C18:3	7.5	2.7	2.0
C20:0	0.3	0.3	0.3
C20:1	0.2	0.2	0.6
C20:2	—	—	0.3
C20:3	—	—	0.1
C20:4	—	—	0.3
C20:5	—	—	0.1
C22:0	0.1	0.1	0.2
Other	1.5	0.7	2.5
Total saturated fatty acids	13.3	12.9	32.6
Total unsaturated fatty acids	86.7	87.1	67.4

trol or DS10%); three kinds of whole-grain paddy rice-based diets containing 10% soybean oil, corn oil, or rendering oil (WS10%, WC10%, and WR10% respectively); and a WS 10% diet supplemented with vitamin B<sub>12</sub> (90 µg/kg), methionine (3.5 g/kg) and ethoxyquin (150 ppm) (WS10%+B<sub>12</sub>+

Met+EQ). The fatty acid profiles of the three fat sources (soybean oil, corn oil, and rendering oil) are given in Table 2. Fatty acids were analyzed as methyl esters by gas-liquid chromatography (Shimadzu GC-17A; Shimadzu Corp., Kyoto, Japan) on a capillary column (IntertCap Pure-WAX

0.25 mm, 30 m length, 0.25  $\mu$ m; GL Sciences, Tokyo, Japan). Column temperature was ramped from 160 to 210°C at a rate of 5°C/min, then kept at 210°C for 5 min, and then increased to 250°C at a rate of 2.5°C/min. The temperatures of the injector and detector were 260°C and 250°C, respectively. Rendering oil, which is widely employed in feedstock used in the production of broiler feed, is extracted from inedible parts of birds and domestic animals, such as the feathers, head and some internal organs, and is supplemented with 500 ppm ethoxyquin (EQ). EQ is widely used in animal feed in order to protect it against lipid peroxidation. The diets included all nutrients necessary to fulfill the nutritional requirements of broiler chickens (Standard Tables of Feed Composition in Japan, 2013).

#### **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 experiment. Thirty-six chicks were randomly divided into six 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 six experimental diets. Thereafter, chickens from each group were moved to individual cages. The body weight and feed intake of each chicken were recorded during the period from 11 d to 28 d. At the end of the experiment, all chickens were sacrificed by exsanguination and dissected. Within one minute, the skeletal muscle, *M. pectoralis superficialis* and liver samples were frozen and powdered in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until measurements were carried out. The experiment was performed in accordance with institutional guidelines for the Care and Use of Laboratory Animals (Tohoku University), 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  $\mu$ L of tissue homogenate were mixed with 200  $\mu$ L of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid (pH 3.5), 50  $\mu$ L of 0.8% butyl-hydroxyl toluene, 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 H<sub>2</sub>O 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  $\times$ 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 one week of storage at  $-80^{\circ}\text{C}$ .

#### **Determination of Fecal Total Bile Acid Content**

The fecal bile acid was extracted according to the method of Iwami *et al.* (2002). Feces from the chickens fed on each of the experimental diets were successively collected for

three days before the end of the experimental period. Feces were dried and milled. Each dried feces sample (10 mg) was suspended in 0.2 ml of 90% ethanol. After vortexing, samples were incubated at 65°C for 60 min. After centrifugation at 3,000 g for 5 min, the organic layer was extracted, evaporated and dried. Then, the dried residue was dissolved with 0.5 ml of 90% ethanol, and quantified by an enzymatic procedure carried out with commercially-available enzymatic assay kits (Wako, Osaka, Japan).

#### **Determination of Vitamin B<sub>12</sub> Content**

Plasma vitamin B<sub>12</sub> concentrations were determined using the Bayer Diagnostics ADVIA Centaur folate assay, which is a competitive immunoassay using direct chemiluminescence technology (LSI Medience Corporation, Tokyo, Japan).

#### **Statistical Analysis**

Statistical analysis was performed with one-way analysis of variance followed by Duncan's least significance multiple-range test ( $P < 0.05$ ). All data are expressed in the form of mean  $\pm$  standard error (SE,  $n = 6$  per measurement).

## **Results**

Most of the chickens fed the WS10% and WC10% diets exhibited ataxia; that is they exhibited clumsiness and an unsteady gait with a tendency to fall. As shown in Fig. 1A, the body weight gain of birds fed the WS10%, WC10% and DS10% diets was significantly lower than that of birds fed the DS5% diet (control) ( $P < 0.05$ ), while the weight gain of birds fed the WR10% and WS10%+B<sub>12</sub>+Met+EQ was comparable to that of the control group. ME intake by chickens in each group (Fig. 1B) varied in a similar manner to the body weight gain. The ratio of body weight gain to ME intake of birds fed the WS10% and WC10% diets was significantly less than that of the control group, while that of birds fed the DS10%, WR10% and WS10%+B<sub>12</sub>+Met+EQ was comparable to that of the control group (Fig. 1C).

Liver MDA levels in birds fed the WS10% and WC10% diets were significantly higher than in the control group, but this was not the case for groups fed DS10%, WR10% and WS10%+B<sub>12</sub>+Met+EQ diets (Fig. 2A). Plasma MDA levels in birds fed the WC10% diet were significantly higher than in controls, while no significant differences were observed between the control and other groups (Fig. 2B). Compared with the control, the MDA levels in the skeletal muscle, *M. pectoralis superficialis* in birds fed the DS10% and WS10% diets were significantly higher, while no significant differences in those levels were observed in the WC10%, WR10% and WS10%+B<sub>12</sub>+Met+EQ groups (Fig. 2C).

No significant differences either in the plasma vitamin B<sub>12</sub> content or fecal total bile acid content were observed between any of groups (Fig. 3A, B).

## **Discussion**

Previously we showed that the growth of chicks fed a diet containing 43% whole-grain paddy rice and 10% soybean oil was retarded relative to the growth of a control group fed a corn-based diet containing 6% soybean oil, while feeding of

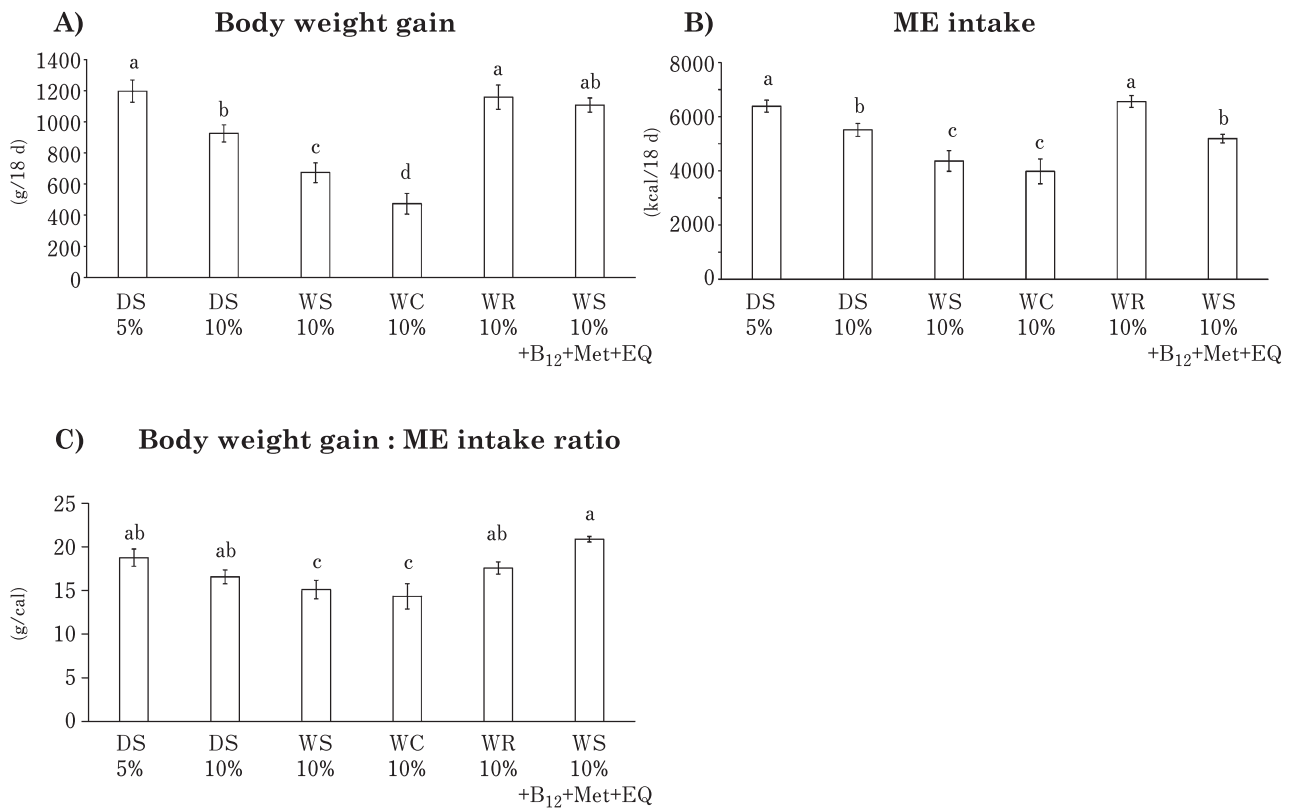


Fig. 1. Effects of whole-grain paddy rice-based diets containing high levels of different fat sources and supplementation of active ingredients on body weight gain (A), ME intake (B), and body weight gain to ME intake ratio (C) of broiler chickens. Values are means  $\pm$  SE,  $n=6$  per group. <sup>a, b, c, d</sup>  $P < 0.05$  for each treatment; values with different letters are statistically different.

chicks with 43% whole-grain paddy rice with 6% soybean oil resulted in growth comparable to that of the control group (Nanto *et al.*, 2012). This result implies that feeding a whole-grain paddy rice diet containing high levels of soybean oil to broiler chickens might negatively affect performance. However, the reason for the observed growth retardation was not known. We postulated that the use of rice hull in combination with the high levels of soybean oil in the diet could be responsible for the growth retardation. Rice hulls are an agricultural residue which contains organic materials, such as lignin, cellulose, and carbohydrates. Lignin, a major component of rice hull, has been reported to have a high binding affinity for bile acid (Story and Kritchevsky, 1976). It was demonstrated that inclusion of rice hull in diet inhibited digestion and absorption of fat and fat-soluble vitamins by decreasing the levels of free bile acid. Soybean oil is rich in unsaturated fatty acids compared to rendering oil (Table 2) and it has been shown (Harris *et al.*, 1963), both in humans and experimental animals, that increases in dietary unsaturated fat result in increased requirements for vitamin E, which is a primary antioxidant in the cell antioxidant defense system. Furthermore, Fox *et al.*

(1956) showed that the incorporation of 20% fat into a corn-soybean meal diet increased ten- to twentyfold the vitamin B<sub>12</sub> required for optimal growth at four weeks of age. This suggests that vitamin B<sub>12</sub> deficiency in chickens also might be induced by high dietary fat content. Since vitamin B<sub>12</sub> is the co-factor of methionine synthase, the first limiting amino acid in the poultry diet, vitamin B<sub>12</sub> deficiency could cause methionine deficiency. Therefore, we carried out this study to determine the effect of rice hulls (WS10% vs DS10%), or different types of oil (WS10% or WC10% vs WR10%), on the growth performance of broiler chickens, and whether supplementation of the WS10% diet with vitamin B<sub>12</sub>, methionine and ethoxyquin, which is one of the best known feed antioxidants, could improve growth performance.

In the present study, compared with birds fed the control (DS5%) diet, birds fed the WS10% and WC10% diets exhibited growth retardation and ataxia. In contrast, birds fed the DS10% diet showed normal growth, similar to that of the control group. Therefore, it may be concluded that the rice hulls in the high fat diets may be involved in the observed growth retardation and ataxia. However, reduced reabsorption of bile acid due to binding of the bile acid to the rice

### MDA content

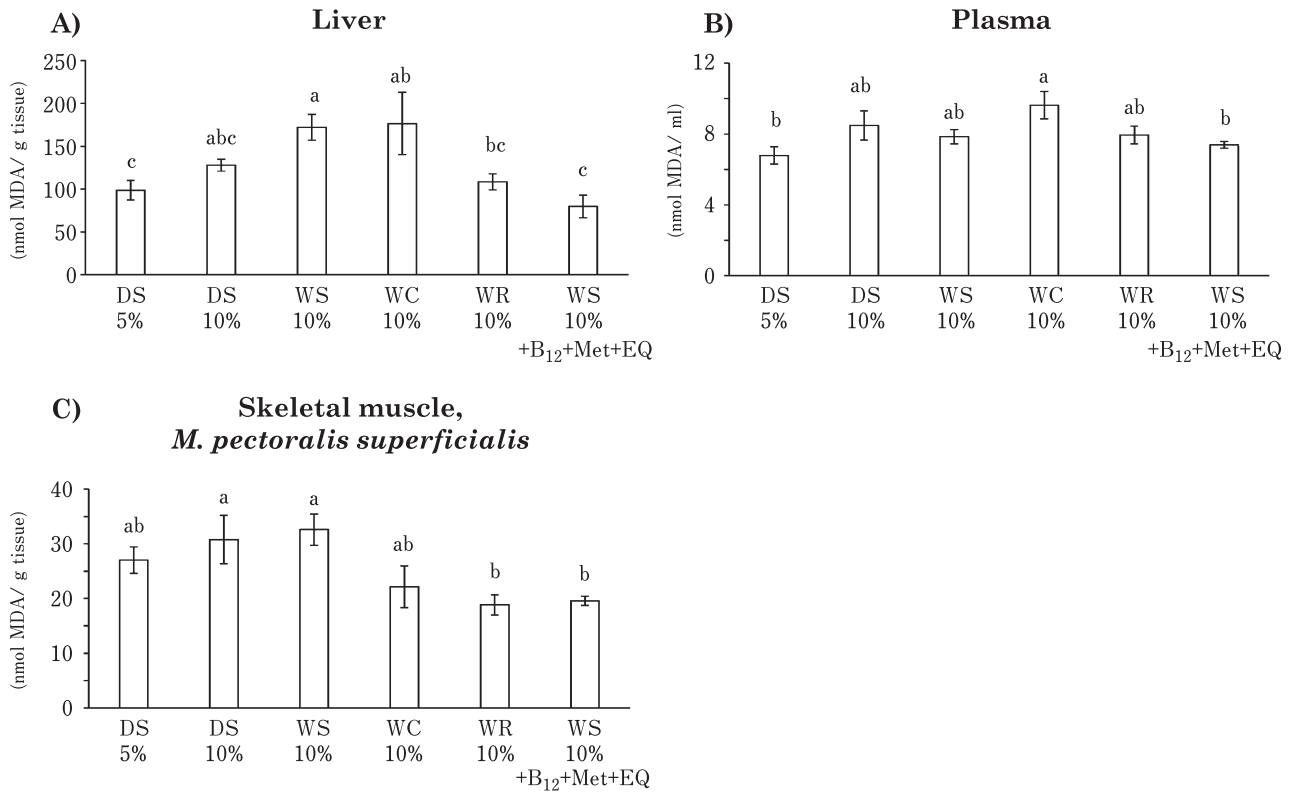


Fig. 2. Effects of whole-grain paddy rice-based diets containing high levels of different fat sources and supplementation of active ingredients on MDA concentrations in liver (A), plasma (B) and the skeletal muscle, *M. pectoralis superficialis* (C) of broiler chickens. Values are means  $\pm$  SE,  $n=6$  per group. <sup>a,b,c</sup>  $P < 0.05$  for each treatment; values with different letters are statistically different.

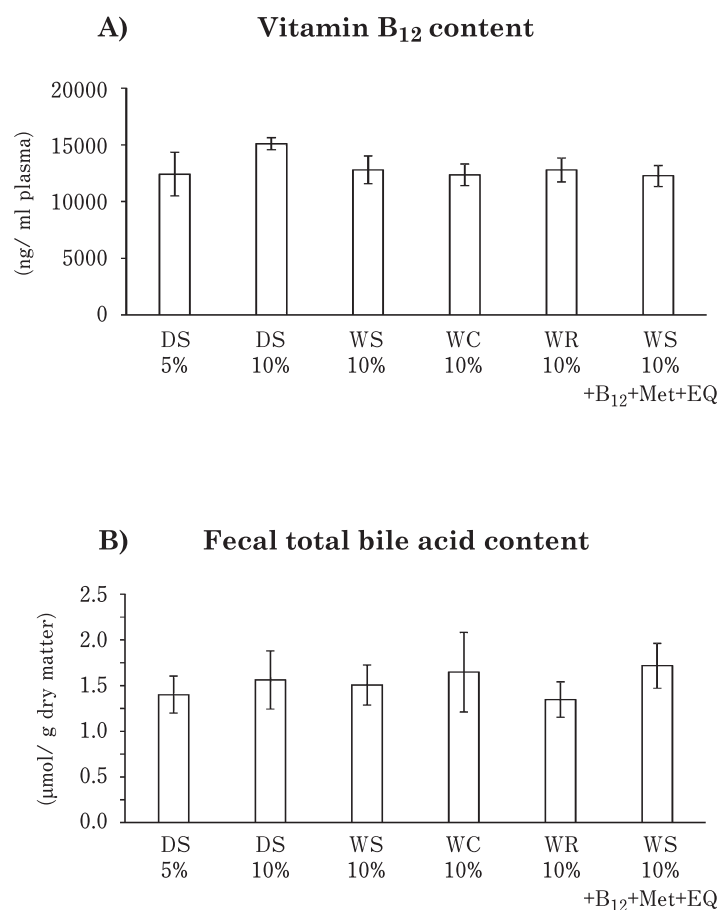
hulls can be ruled out as a cause because no significant differences in the fecal total bile acid content were observed between the groups.

It should be noted that birds fed the WR10% diets showed growth comparable to that of the control group, while birds fed the WS10% and WC10% diets exhibited growth retardation and ataxia, suggesting that high levels of soybean oil or corn oil, in whole-grain paddy rice diets may also contribute to growth retardation and ataxia. Soybean oil and corn oil showed a similar fatty acid composition, and their unsaturated fatty acid concentrations were 3.5 times higher than that of rendering oil, implying that requirements for vitamin E might be higher in birds fed the WS10% and WC10% diets compared with birds fed the WR10%. We observed that the liver tissue in birds from the WS10% and WC10% diet groups exhibited significantly higher concentrations of MDA compared with birds fed the WR10% group. Given that the MDA content of a tissue is indicative of the degree of lipid peroxidation due to overproduction of reactive oxygen species (ROS) and/or lowered scavenging activity, the growth retardation in the WS10% and WC10%

diet fed group might be a reflection of the higher levels of liver MDA which were induced by a weakened antioxidant defense system related to increased requirements for vitamin E.

Supplementation of the WS10% diet with vitamin B<sub>12</sub>, methionine and ethoxyquin dramatically improved growth performance and ataxia, suggesting that one or more of the supplements could inhibit the growth retardation. In this study, no significant differences in the plasma vitamin B<sub>12</sub> content were observed between the different treatments. Therefore, it may be ruled out that vitamin B<sub>12</sub> deficiency and/or methionine deficiency related to vitamin B<sub>12</sub> deficiency occurred in the WS10% and WC10% diet-fed groups. Consequently, ethoxyquin supplementation of the WS10% diet is most likely responsible for the observed attenuation of the growth retardation.

In conclusion, these results clearly indicate that diets combining untreated whole-grain paddy rice and 10% soybean or corn oil have negative effects on performance, probably via enhanced lipid peroxidation.



**Fig. 3. Effects of whole-grain paddy rice-based diets containing high levels of different fat sources and supplementation of active ingredients on the plasma vitamin B<sub>12</sub> content (A) and fecal total bile acid content (B) of broiler chickens.** Values are means  $\pm$  SE,  $n=6$  per group.

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