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EFFECT OF ASPERGILLUS NIGER ON BROILERS PERFORMANCE

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Abstract: *This study was conducted to evaluate fungus, Aspergillus niger, as probiotics in broiler chickens. The Chickens were divided into 4 treatments with average weight (375± 4 g). First treatment as control fed control diet and other chicks were fed diets supplemented with Aspergillus niger at the levels of 0.01, 0.05 and 0.1%. The birds were fed experimental diets until 27 d of age to evaluate the effect on growth, organs weights, abdominal fat content, muscle fat contents, muscle (TBARS, thiobarbituric acid reactive substance) and plasma biochemical parameters. Body weight gain was increased and feed intake and feed conversion ratio were decreased (P < 0.05) by the fungus. Plasma 3-methylhistidine as an index of skeletal muscle protein breakdown was decreased by the fungus. Due to the fungus, abdominal fat and plasma cholesterol were decreased (P<0.05), while fat content in the breast muscle was increased. Interestingly, muscle α-tocopherol content was increased (P < 0.01), and muscle TBARS as an index of lipid oxidation and (glutamic-oxalacetic transaminase activity, GOT) as an index of liver function were decreased (P < 0.001) by the fungus, indicating anti-oxidative activities of the fungus. In conclusion, feeding Aspergillus niger improved growth performance and meat quality. Thus, the fungus can be used as effective probiotics in broiler chickens.*

INTRODUCTION

Probiotics are defined as live microorganisms which beneficially affect the host animal by improving its intestinal microbial balance (Kahraman et al., 1996; Leeson and Summers, 1997). *Aspergillus niger* is fungus called "Koji" in Japan and have long been used for food processing. The products processed by *A. niger* are given (GRAS, Generally Recognized as Safe) status from FDA (Bigelis and Lasure, 1987). *A. niger* used for Japanese food processing such as Shochu a traditional Japanese liquor. As it has been reported that distillery by-product of Shochu contains unidentified growth factor for broiler chicken (Mahfudz et al. 1996a, b, 1997), it is very provable that *A. niger* produces a growth promoter during the fermentation. Furthermore, Koji is well known to produce enzymes enhancing digestions of carbohydrates and proteins (Gracia et al.2003). In the present study, we tested the possibility of using Koji as probiotics in broiler chickens.

MATERIALS AND METHODS

The animal experiment was conducted in accordance with the guideline of Kagoshima University, Japan.

Birds and Management

Twenty four 1d old male broiler chickens (Chunky strain) were supplied by a commercial hatchery (Kumiai Hina Center, Kagoshima, Japan). Chicks were housed in an electrically heated battery brooder, and provided with water and commercial starter diet [23% crude protein and 3081kcal/kg metabolizable energy] supplied by (Nichiwa Sangyou Company Kagoshima, Japan) until 12 d of age. Then chicks were fed the basal diet from 12 d of age to 15d of age. The composition of the basal diet (CP 22, 6%, ME 3081 kcal/kg) is shown in Table 1. Chicks were divided into 4 groups (n = 6): control and *A. niger* groups with 3 levels of fungi (0.01%, 0.05% and 0.1%). The fungi were mixed in the basal diet. The

numbers of spores were about 50×10^3, 25×10^4 and 50×10^4/g feed for diets treatments of 0.01, 0.05 and 0.1% fungi, respectively. The birds were given the experimental diets from 15 to 27 d of age. *A. niger* was given by Genkoji Research Institute (Kagoshima, Japan). The experiment was conducted in a temperature-controlled room with 14h light: 10 h dark cycle. Room temperature was kept at 25°C with relative humidity from 50 to 70 % throughout the experiment.

Sampling

Body weight was recorded every 6 days and feed intake was recorded daily during the experimental period. At the end of the experimental period, the birds were slaughtered and then dissected to measure the weights of breast muscle (*Musculus pectorails profundeus* and *Musculus pectoralis superficialis*), liver and abdominal fat. Blood samples were collected into heparinised test tubes, quickly centrifuged at 5,900 ×g for 10 minutes at 4°C to separate plasma, and stored at -30°C until analysis.

Biochemical Analysis

Blood and muscle total cholesterol level and GOT were measured by automated Fuji DRY-CHEM 3500 (Fuji Medical Systems, Tokyo, Japan) according to the manufacturer's instructions. Concentration of plasma thiobarbituric acid reactive substance (TBARS) was measured by the method of Ohkawa *et al.* (1979). The α-tocopherol concentration of the muscle was determined by Shimadzu HPLC model LC6A (Tokyo, Japan) with a Shim-Pack CLC-ODS column (6.0 × 150 mm) according to Faustman *et al.* (1989). The plasma 3- methylhistidine concentration was measured by HPLC method according to Hayashi *et al.* (1987).

Statistical Analysis

The differences among treatments were analyzed by General Liner model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). The significant differences among

means of treatments were compared by Duncan's new multiple-range test. $P \leq 0.05$ was set as limit of significance.

RESULTS

Results concerning the effects of *A. niger* on body weight gain, feed intake, feed conversion ratio, muscle weight and plasma 3-methylhistidine concentration are summarized in Table 2. *A. niger* increased the body weight gain significantly ($P < 0.05$) when the level was 0 .01%. Feed intake was decreased ($P < 0.05$) in the treatment groups except 0.01% *A. niger* group. Feed conversion ratio was significantly decreased in the treatment groups compared to control. The muscle weight was significantly increased ($P < 0.05$) only in 0.01% *A. niger* group. Plasma 3-methylhistidine concentration was measured as an index of skeletal muscle protein degradation. Plasma 3-methylhistidine concentrations were lower in the treatment groups except 0.05% *A. niger* group.

The effects of plasma total cholesterol level and GOT are shown in Fig. 1. Plasma cholesterol levels (Fig. 1A) were significantly lower in the treatment groups. As an index of liver function, plasma GOT was measured. As shown in Fig. 1B, GOT was lower ($P < 0.05$) in all the treatment groups, indicating that *A. niger* had no side effect on liver function.

Results concerning the effects of abdominal fat and muscle fat are shown in Fig. 2. Abdominal fat contents were all lower in the treatment groups and the lowest value obtained from 0.1% *A. niger* groups (Fig. 2A). However, breast muscle fat content was increased in the treatment groups (Fig. 2B).

Effects of *A. niger* on muscle α-tocopherol content and muscle TBARS are shown in Fig.3A and B. Muscle α-tocopherol content was higher and TBARS was lower in groups containing higher levels of the fungi and statistically significant effects ($P < 0.05$) ware observed, indicating antioxidative property of the fungi.

DISCUSSION

The present study shows that feeding *A. niger* stimulated the growth in broiler chickens (P = 0.025). On the other hand, breast muscle weight was increased significantly when 0.01% *A. niger* was given. The growth promoting effects of the fungi are supported by the effects on plasma 3-methylhistidine concentration. The plasma 3-methylhisitidine concentration was decreased by the fungi (P = 0.020), indicating a decreased rate of skeletal muscle protein degradation. 3-Methylhisitidine is a component of skeletal muscle protein, actin and myosin. When skeletal muscle protein is degraded, 3-Methylhisitidine is excreted into the urine. 3-methylhisitidine is not reused for protein synthesis due to the lack of existing 3-Methylhisitidine tRNA. Thus 3-methylhisitidine release has been used as a marker of muscle protein degradation. Measurement of urinary 3-Methylhisitidine excretion is widely used as an index of muscle protein degradation, but it is difficult to measure 3-Methylhisitidine exertion in broilers. Thus, plasma 3-methylhisitidine concentration has been used to track changes in muscle protein degradation (Nagasawa et al. 1998). *Aspergillus* could improve nutritional quality of soybean due to their enzymes to degrade trypsin inhibitor.(Hong el al. 2004). The improvement in weight gain and feed efficiency due to *Aspergillus* may be due to increase in metabolisable energy (Mohan et al. 1996). Birds do not produce enzymes such as cellulase and xylanase which are required for the digestion of soluble non-starch polysaccharides (NSPs). However, these enzymes are produced by *Aspergillus* and improve digestibility. It is reported that an enzyme product contained activities of cellulase, hemicellulase, protease, α-amilase and α-galactosidase improves digestibility (Hajati 2010). These may be the reason for the efficient feed utilization due to *Aspergillus* feeding. The decrease in feed intake and improve in feed conversion by feeding *Aspergillus* were in agreement with the results of Willis and Reid (2008) and Roth and Kirchgessner (1986). The present results are also consistent with the results of Yamamoto *et al.* (2007) showing that when

broilers were fed on diets containing 0.05 and 1% of Koji-feed fermented by *A. awamori* improved growth in broiler chickens. Also our results are in agreement with the report of Chah *et al.* (1975) showing that diets containing full fat soybeans fermented by certain cultures of *A. oryzae* significantly improved broiler growth and feed utilization. On the other hand, Kamizono *et al.* (2010) have reported the growth promoting effect of Shochu distillery by-product. Shochu is made from many kinds of grains using *A. awamori* for sacharification. Yamamoto *et al.* (2007) noted that when broilers were fed on diets containing 0.05 and 1% of Koji-feed, carcass weight was significantly increased and the breast muscle weight tended to be increased and abdominal fat was decreased. It seems to be due to the growth promoter produced by *A. niger*.

Abdominal fat and plasma cholesterol were decreased while breast muscle fat content was increased in our experiment. Kim *et al.* (2003) also found that *A. oryzae* at 0.1% in diet significantly lowered serum cholesterol in broiler chickens. Mechanism underlying the cholesterol lowering effect of *Aspergillus* could be related to an inhibitor of 3-hydroxyl-3-methylglutaryl-coenzyme reductase (Hajjaj et al. 2005). It is well known that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, called Statin, was extracted from a fungus and inhibits the rate-limiting step in cholesterol synthesis. Statin is now widely used to treat patients with hypercholesterolemia and is recognized as safe (Serruys et al. 2004). HMG-CoA reductase inhibitor may be also responsible for decreasing carcass fat deposition. On the other hand, *Aspergillus* may affect fat deposition in broilers by influencing the activities of hormone sensitive lipase and malate dehydrogenase enzyme in adipose tissues (Mersmann, 1998 and Shen et al. 1991). However, we have no data to support this assumption.

Feeding *A. niger* increased muscle α-tocopherol content and decreased TBARS and GOT, indicating that lipid peroxidation was decreased. This indicates that *A. niger* produce antoxidative substances.

In conclusion, this study shows that feeding *A. niger* as probiotic improved growth performance due to their effects on skeletal muscle protein breakdown in broiler. In addition, muscle vitamin E content was increased and lipid per-oxidation in the muscle was decreased by *A. Niger*.

Table .1. Composition and nutrient analysis of basal diet

Ingredients,%	Diet
Corn	50.19
Alfalfa	2.64
Soybean	39.01
Corn oil	4.4
Lysin	0.01
Methionine	0.18
Mineral[1] mix	3.31
Vitamin[1] mix	0.26
Calculated Analysis	
CP, %	22.6
ME, kcal/kg	3081

1-Supplies per kg of diet: 6.62 g CaCO3; 20.86 g CaHPO4; 5.06 g NaCl; 0.22 g MnSO4.7H2O; 0.13 g ZnSO4.5H2O; 0.20 g FeSO4; 7.71 mg CuSO4.5H2O; 0.56 mg NaIO3; 0.27 mg Na2SeO3.

2-Supplies per kg of diet: 1.37 mg retinol; 0.13 mg cholecalciferol; 6.50 mg riboflavin; 2.60 mg thiamine hydrochloride; 1.30 mg pyridoxamine hydrochloride; 0.03 mg cyanocobalamin; 10.40 mg D-pantothenic acid; 26.00 mg nicotinic acid; 1.05 mg vitamin K3; 0.52 mg pteroylglutamic acid; 0.78 mg choline chloride; 0.07 mg biotin; 6.50 mg DL-α-tocopherol acetate; 2.54 g sucrose.

Table.2. Effect of dietary *Aspergillus niger* on broilers performance (15-27 d old), breast muscle weight (BMW), f and plasma 3-methylhistidin (3-MH).

	Control	*Aspergillus niger*		
		0.01	0.05	0.1
BWG, g (15-27d)	635 ± 63^{c}	820 ± 46^{a}	715 ± 7^{b}	730 ± 18^{b}
FI, g (15-27d)	1235 ± 32^{a}	1215 ± 69^{a}	1070 ± 41^{c}	1160 ± 32^{b}
FCR	1.9 ± 0.01^{a}	1.5 ± 0.02^{c}	$1.5 \pm .01^{c}$	1.6 ± 0.05^{b}
BMW, g/100g BW	27.5 ± 0.9^{b}	29.3 ± 1.5^{a}	27.1 ± 0.3^{b}	27.2 ± 0.8^{b}
Plasma 3-MH, µmol/ml.	148 ± 42^{ab}	126 ± 12^{b}	149 ± 11^{a}	106 ± 13^{c}

Values are expressed as means ±standard error. ; $^{a-c}$ Means with different superscripts differ from each other significantly.

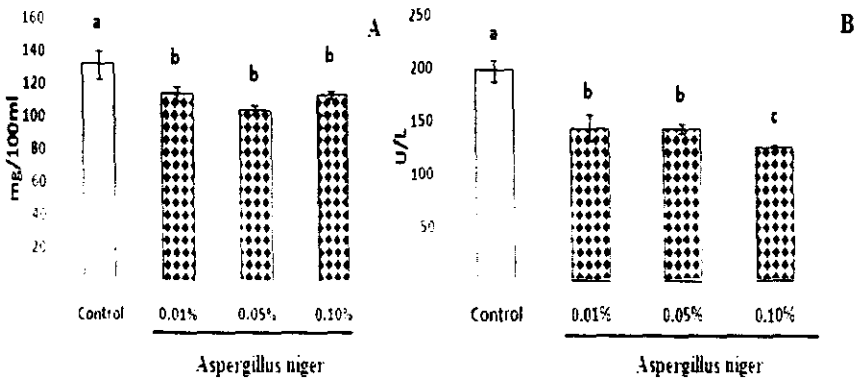

Fig.1. Effect of dietary *Aspergillus niger* on plasma cholesterol (A) plasma GOT (B). Values are expressed as means ±standard error. ; $^{a-c}$ Means with different superscripts differ from each other ($P < 0{\cdot}05$).

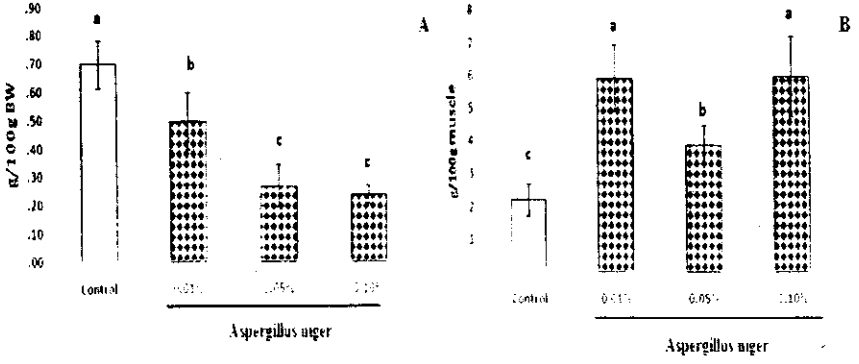

Fig.2. Effect of dietary *Aspergillus niger* on abdominal fat weight (A) and breast muscle fat content (B). Values are expressed as means ±standard error. : $^{a-c}$ Means with different superscripts differ from each other ($P < 0{\cdot}05$).

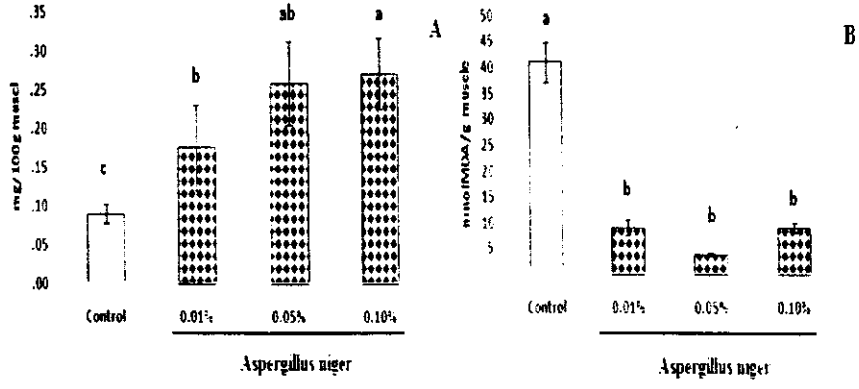

Fig.3. Effect of dietary *Aspergillus niger* on muscle α-tocopherol (A), muscle TBARS (B). Values are expressed as means ±standard error. ; $^{a-c}$ Means with different superscripts differ from each other ($P < 0{\cdot}05$).

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الملخص العربى

تاثير الاسبراجللس نيجر علي الاداء الانتاجي لكتاكيت التسمين

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اجريت هذة الدراسة لمعرفة تاثير فطر الاسبراجللس نيجر كابروبيوتيك في علائق دجاج التسمين.تم استخدام الكتاكيت عند عمر ١٥ يوم وقسمت الكتاكيت الي اربعة مجاميع. المجموعة الاولي هي مجموعة الكنترول وتم تغذيتها علي عليقة الكنترول اما المجاميع الاخري غذيت علي عليقة الكنترول مضاف اليها فطر الاسبراجللس نيجر بنسب(٠.٠١, ٠.٠٥, ٠.١ %) تمت تغذية الكتاكيت علي هذة العلائق حتي عمر ٢٧ يوم لتقييم تاثير اضافة الفطر علي النمو ومحتوي الدهن البطني ومحتوي العضلات من الدهن وفيتامين E وTBARSو بعض القياسات البيوكيميانية في بلازما الدم. وكتنت اهم النتائج المتحصل عليها زيادة معنوية في وزن الجسم وانخفاض العلف المستهلك وتحسن معدل التحويل الغذائي للعلف كما لوحظ انخفاض في محتوي بلازما الدم من الكوليستيرل و الميثيل هستدين وانخفاض في نسبة الدهن البطني بينما حدثت زيادة في نسبة الدهون في العضلات ومن المثير للاهتمام زيادة محتوي اللحم من فيتامين E وانخفاض نسبةTBARS. ويمكن ايجاز النتائج في ان تغذية كتاكيت اللحم علي علائق تحتوي علي فطر الاسبراجللس نيجر حسن معدل الاداء الانتاجي وكذلك حسن من جودة اللحم لذا يمكن استخدامه كبروبيوتيك في علائق كتاكيت التسمين.