Effect of Feeding *Aspergillus Oryzae* Culture on Fecal Microflora, Egg Qualities, and Nutrient Metabolizabilities in Laying Hens[a]

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ABSTRACT : This experiment examined the effects of feeding *Aspergillus oryzae* (AO) culture to laying hens on fecal microbial populations, fecal pH and moisture content, egg quality, and metabolizabilities of several nutrients. Sixteen commercial 38-wk-old laying hens were randomly allotted to four diets: control; with 0.15% locally produced AO culture; with 0.3% locally produced AO culture, and; or with 0.3% imported AO. Each treatment consisted of four replicates (cages) containing one bird per cage according to a completely randomized design. After 4 wk, AO were recovered in the feces of birds fed the AO diets, indicating that AO might pass through the fore-gut alive and become active in the hind gut. The number of *Lactobacillus* spp. in feces was higher in all treated groups than that of the control, indicating that AO would provide a beneficial environment for the *Lactobacillus* spp. to proliferate in the intestine. The number of fecal *E. coli* was significantly reduced by the addition of AO. A similar trend was also found for aerobic bacteria. Although not significant, fecal moisture contents tended to be reduced by the addition of AO. Fecal pH was not significantly different among the treatments. The addition of AO did not affect the various economic traits of eggs. Metabolizabilities of gross energy and dry matter measured during the 5th wk were increased by the AO supplementation. It appears that AO culture alone could be used as a probiotic supplement for layers. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 3 : 417-421*)

Key Words : *Aspergillus Oryzae*, Laying Hen, Egg Quality, Fecal Microflora, Metabolizability

INTRODUCTION

In the modern intensive poultry production units, newly-hatched chicks have little chance to be in contact with their mothers; consequently a normal population of microflora is slow to develop in their intestines (Fuller, 1989). This situation makes chicks more likely to be affected by minor occurrences of pathogenic bacteria increasing the risks of diseases in the chickens and of food-borne diseases in human-beings (Pivnick and Nurmi, 1982).

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance, and have been used to help newly-hatched chicks develop normal microflora similarly to conventionally hatched chicks (Fuller, 1989). Probiotics also have been used as feed additives to improve the performance of poultry as described in several reviews (Jernigan et al., 1985; Barrow, 1992; Stavric and Kornegay, 1995; Jin et al., 1997).

Aspergillus oryzae (AO) and yeasts, particularly *Saccharomyces cerevisiae*, have been used as probiotics by many workers (Day et al., 1987; Wiedmeier et al., 1987; Harms and Miles, 1988; Gomez-Alarcon et al., 1990; Brake, 1991; Grimes et al., 1997; Rhee et al., 1997). Both *Aspergillus* spp. and *Saccharomyces* belong to the *Ascomycoyina* subdivision (Boyd, 1988), and have many industrial applications in the brewing, distilling and baking industries (Raper et al., 1965).

Line et al. (1998) reported that feeding live yeast to broiler breeders reduced colonization of *Salmonella* in their ceca, and Thayer and Jackson (1975) reported that live yeast culture improved phosphorus utilization in growing chickens. In contrast, yeast culture had no effect on the performance of laying hens and broilers (Day et al., 1987; Brake, 1991). Mohan et al. (1996) fed dried primary cultures of *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, Aspergillus oryzae and Torulopsis* to broilers and found these increased body weight and nitrogen retention. Grimes et al. (1997) used Fermacto 500, which is primarily an *Aspergillus* spp. fermentation extract, and reported consistent increases in protein and lipid digestibility. Until recently, however, studies investigating the effects of AO on fecal and/or intestinal microbial flora and performance of layers are very rare. The present study was conducted to determine the role of AO as a probiotic in layers.

MATERIALS AND METHODS

Experimental design and diets

There were four dietary treatments; each treatment consisted of four replicates containing one bird per replicate (cage). A corn-soybean meal based commercial layer diet (table 1) was used for the control treatment (T1). The T2 and T3 diets were prepared by including Natu-fermen (NF) at the levels of 0.15 and 0.3%, respectively. The T4 diet contained Amaferm (AF) at the level of 0.3% in the diet. NF is the brand name of an AO culture produced locally by fermenting the soybean oil meal with AO. AF is a commercial brand of dried AO fermentation extract (Biozyme Inc., St. Joseph, MO, USA). The chemical composition and colony forming units (cfu) of NF and AF are presented in table 2.

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Management of experimental birds

Sixteen commercial 38-wk-old laying hens (Lohmann Brown strain) were randomly allotted to individual wire-floored metabolism cages, one bird per cage. They were housed in a temperature-controlled room (18±1℃), and subjected to a 14L10D lighting programme. A 500 mL plastic bottle equipped with a nipple drinker was placed upside down at the top of each cage. Feed and water were provided ad libitum for a 2-wk adaptation period. After the adaptation period, all hens were given the experimental diets *ad libitum* for a 4-wk period.

Table 1. Composition of the basal diet

Ingredient	Basal diet (%)
Yellow corn	49.40
Wheat, hard	15.00
Soybean meal (46%)	14.45
Calcium carbonate	8.38
Rice bran, polished	6.00
Rapeseed meal	5.00
Defluorinated phosphate	0.92
Salt	0.25
Coconut oil	0.17
Choline chloride	0.14
Mineral premix[1]	0.10
Vitamin premix[2]	0.10
DL-methionine	0.08
L-lysine-HCl	0.01
Total	100
Chemical analysis;[3]	
ME, kcal/kg	2835
Crude protein, %	15.00
Calcium, %	3.60
Phosphorus, %	0.60
Available phosphorus, %	0.28

[1] Mineral premix supplied followings per kg of diet: manganese, 88 mg; zinc, 65 mg; copper, 25 mg; iodine, 1.5 mg; and selenium, 0.1 mg.

[2] Vitamin premix provided followings per kg of diet : vitamin A (retinyl acetate), 3.03 mg; cholecalciferol, 0.04 mg; vitamin E (dl-α-tocopherol acetate), 5.0 mg; vitamin K, 1.0 mg; riboflavin, 6.0 mg; cyanocobalamin, 0.02 mg; niacin, 28 mg; pantothenic acid, 11.9 mg; biotin, 0.03 mg; and folic acid, 0.2 mg.

[3] Analytical values.

Table 2. Chemical composition and colony forming units (cfu) of Natu-fermen® (NF) and Amaferm® (AF)

	NF	AF
Chemical composition[1]		
Crude protein, %	46.32	17.10
Crude fat, %	0.18	0.73
Crude ash, %	16.78	7.19
Ca, %	0.59	0.10
P, %	0.66	1.17
cfu/g	8.4×10^3	4.2×10^4

[1] All data were expressed on dry matter basis.

Sampling and analyses

More than 10 g of fresh excreta samples from each bird were collected in sterilized tubes as soon as they were excreted from the cloaca on the last day of the 4-wk feeding period. The uric acid and cecal feces as visually identified were excluded from the collections. Three grams of fresh fecal samples were diluted with 10 mL of distilled water and completely vortexed to measure the pH (8417, Hanna, RI, USA). The remainder of the fecal samples collected were stored at 4℃ till further analysis.

One gram of wet sample from each bird was diluted with 10 mL of sterilized distilled water (SDW), of which 1 mL was further diluted with 9 mL of SDW. In this manner, samples were serially diluted from 10^{-1} to 10^{-7}. One-tenth mL quantities of each diluted sample were plated for measurements of microbial populations using appropriate media and culturing conditions as shown in table 3. The cfu were expressed as $\log_{10}$.

Table 3. The selective media and culturing conditions for enumeration of microbial populations in fresh feces

Microorganisms	Media[1]	Culturing condition
Aspergillus oryzae	PDA	Surface plate, 35℃ for 72 h
Lactobacillus spp.	MRS agar	Surface plate, 28℃ for 24 h
E. coli	MacConkay agar	Surface plate, 28℃ for 24 h
Aerobic bacteria	Nutrient agar	Surface plate, 28℃ for 24 h

[1] Difco, USA.

Egg quality measurements

All eggs laid during the last 3 days of the 4-wk feeding period were collected to determine egg quality traits including egg weight, eggshell breaking strength and shell thickness (strength meter and gauge of Fujihira Inc., Tokyo, Japan), yolk color, and Haugh unit. Two eggs from each replicate were selected and weighed individually. Subsequently, the eggs were broken on a glass plate to measure the albumen heights using a micrometer (Fujihira Inc., Tokyo, Japan), and Haugh units were calculated using the formula described by Roush (1981). Yolk color was determined with a Roche yolk color fan.

Metabolism trial

A metabolism trial was conducted after the fecal sample collections to determine the metabolizabilities of energy, DM, crude protein, and crude fat in the experimental diets. The amounts of diets were restricted to 80% of daily average intakes. After 4 days of adaptation, total excreta were collected into plastic containers with covers during two consecutive days at intervals of 30 min from 9 a.m. to 6 p.m. in order to measure moisture contents. The rest of the 24 h excreta

were collected at 8 a.m. on the next morning. All samples were dried in a forced-air oven at 60℃ until reaching constant weight, then weighed and pooled. The dried and pooled excreta samples were ground to pass through a 1 mm screen for chemical analysis. Analyses of proximate composition of the experimental diets and excreta were conducted according to the methods of AOAC (1990), and the gross energy content was measured using an adiabatic bomb calorimeter (1563, Parr, IL, USA).

Statistical analysis

All data were subjected to a one-way analysis of variance test (Steel and Torrie, 1980). Statistical significances among treatment means were determined by the method of the new multiple range test of Duncan (1955) when the F value was significant at 5% level.

RESULTS AND DISCUSSION

Fecal microbial count

The data on microbial populations in fresh fecal samples are shown in table 4. AO was not present in the control group (T1), but was found in all three groups given the probiotic (T2, T3 and T4). Although not significant, feeding NF and AF to hens appeared to increase the number of *Lactobacillus* spp. *E. coli* were significantly suppressed ($p<0.05$) by the feeding of NF and AF. Both NF and AF tended to decrease the number of aerobic bacteria in feces, and the decrement was significant especially at 0.3% NF feeding.

Table 4. Effect of feeding diets containing *Aspergillus oryzae* (AO) on populations of AO, *Lactobacillus* spp., aerobic bacteria, and *E. coli* in feces of laying hens

Treatment	AO	*Lactobacillus* spp.	Aerobic bacteria	*E. coli*
	$\log_{10}$ cfu/g feces			
T1	0^{b}	4.02 ± 1.05	$7.60^{a} \pm 0.77$	$6.29^{a} \pm 0.61$
T2	$0.83^{ab} \pm 0.99$	5.71 ± 1.15	$6.08^{ab} \pm 0.48$	$4.43^{b} \pm 1.18$
T3	$1.08^{a} \pm 0.57$	5.12 ± 0.86	$5.72^{b} \pm 1.15$	$3.95^{b} \pm 0.99$
T4	$1.58^{a} \pm 0.51$	5.47 ± 0.43	$6.52^{ab} \pm 0.16$	$3.41^{b} \pm 0.63$

[1] T1, control: T2, 0.15% Natu-fermen: T3, 0.3% Natu-fermen: T4, 0.3% Amaferm.

[a,b] Means ± SD having no common superscript in the same column differ significantly ($p<0.05$).

Rhee et al. (1997) reported a reduction of *E. coli* numbers when a diet containing 0.05% live yeast was fed to broiler chicks. Although yeasts do not belong to the natural microflora of animals, Line et al. (1998) reported that naturally occurring yeasts were found in chickens not given a yeast supplement; significant yeast populations were recovered from chickens fed yeast-supplemented diets. It has been proposed that yeasts actively eliminate *Salmonella* and *E. coli* prior to their adhesion to the intestinal epithelium due to its oxygen-scavenging role and binding effect on pathogenic bacteria (Rose, 1987; Jonvel, 1993; Line et al., 1998).

It is known that lactic acid bacteria have positive effects on the chicken intestinal microflora (Barrow, 1992) but there is a dearth of information on the probiotic effect of AO in poultry. There is evidence from our results that AO can survive along the gastrointestinal tract (GIT) of hens when added to their diets. It could be postulated that there were synergistic interactions between *Lactobacillus* spp. and AO in the GIT, thereby resulting in the increase in the population of *Lactobacillus* spp. in feces, rather than a direct effect of AO causing a reduction of *E. coli*.

Very often fecal microbial counts are not satisfactory to indicate the behavior of these organisms in the GIT (Pollmann et al., 1980; Ward and Nelson, 1982). Nevertheless, freshly excreted fecal samples were carefully collected in this experiment so that the values in table 4 probably give a reliable indication of the tendencies in the large intestine. It may be noted that fecal counts of *Lactobacilli* were normally higher than coliforms in healthy pigs and reversed in animals suffering from diarrhea (Mitchell and Kenworthy, 1976; Muralidhara et al., 1977).

Excreta pH and moisture contents

As shown in table 5, moisture contents were not significantly different among the treatments. However, droppings of hens fed 0.15% and 0.3% NF were less watery and more firmly-shaped than the control and AF-treated hens. The pH of the 0.3% NF group tended to be lower than the other treatents, but was not significantly different.

Table 5. Effect of diets containing *Aspergillus oryzae* on moisture and pH of excreta from laying hens

Treatments[1]	Moisture contents (%)	pH
T1	84 ± 5.3[2]	6.39 ± 0.87
T2	76 ± 3.7	6.51 ± 0.76
T3	79 ± 5.7	5.95 ± 0.42
T4	82 ± 1.8	6.44 ± 0.67

[1] T1, control: T2, 0.15% Natu-fermen: T3, 0.3% Natu-fermen: T4, 0.3% Amaferm.

[2] Mean ± SD.

Several methods have been used to reduce moisture contents in droppings, i. e.,feeding diets containing fillers like kaolin, bentonite, or clay. Latif and Quisenberry (1968) reported a significant reduction in excreta moisture in pullets fed diets containing western bentonite and montmorillonite clay. Similarly, kaolin in corn-soybean diets seemed to reduce the moisture content of freshly voided droppings (Charles and Wildey, 1975). Sellers et al. (1980) observed a statistically significant decrease in excreta moisture for broilers but no effect on laying hens when 5.0% attapulgite was included in diets. They reported that excreta moisture was 73.7% and 74.5% for hens receiving control and 5.0% attapulgite diets, respectively. Their figures were slightly

Table 6. Effects of diets containing *Aspergillus oryzae* on egg quality

Treatments[1]	Egg weight(g)	Yolk color[2]	Egg breaking strength (kg/㎠)	Eggshell thickness ($mm10^{-2}$)	Haugh unit
T1	60.2[3]±2.03	6.8±0.79	4.2±0.13	33.3±5.58	82.1±0.93
T2	60.1±3.09	6.7±0.48	4.7±0.97	37.8±5.15	78.4±3.28
T3	62.6±4.53	6.6±0.26	4.3±0.93	40.3±1.80	78.6±7.62
T4	62.9±4.70	6.7±0.17	4.8±0.58	40.4±4.03	75.6±3.15

[1] T1, control: T2, 0.15% Natu-fermen : T3, 0.3% Natu-fermen : T4, 0.3% Amaferm.
[2] Roche yolk color fan score.
[3] Mean±SD (n=8).

lower than our values shown in table 5. This difference was probably due to the methods used for collecting excreta. They collected fecal droppings once a day, whereas we collected fresh excreta every 30 minutes.

Egg qualities

No significant differences were found in various egg quality traits among the four treatments (table 6). Several authors reported that egg production was significantly increased by including *Lactobacillus* spp. in the diets of laying hens (Krueger et al., 1977; Miles et al., 1981; Nahashon et al., 1994) although Cerniglia et al.(1983) observed no difference in egg production from probiotic supplement. An increase in the percentage of larger eggs by feeding *Lactobacillus* spp. has been reported (Hargis and Creger, 1978; Nahashon et al., 1994; Nahashon et al., 1996). Thayer et al. (1978) reported an improvement in egg production, egg weight, and egg specific gravity for turkey breeder hens fed diets containing a low phosphorus level and a live yeast culture. In the present experiment, however, the egg production rate was not determined because too small a number of birds was employed.

Brake (1991) reported that the addition of live yeast culture had no effect on egg production, egg weight, and eggshell weight in broiler breeder hens. Similar results were observed in laying hens (Day et al., 1987). Feeding Fermacto 500® to laying hens resulted in significant effects on egg quality (Harms and Miles, 1988) but Grimes et al. (1997) reported to the contrary.

Metabolism trial

The metabolizability of gross energy was improved significantly by AO (table 7). Dry matter metabolizabilities in T2 and T4 were significantly greater than in the control group (T1), but metabolizabilities of crude protein and crude fat were not affected.

Both AO and yeasts, particularly *Saccharomyces cerevisiae*, have many industrial applications including the brewing, distilling and baking industries. AO plays an important role in the alcoholic and soy food industries in the Far East due to its active amylolytic and proteolytic enzymes (Raper et al., 1965). Therefore, it is possible that the enhanced metabolizability of dry matter shown in this study could result from the action of these enzymes on the consumed nutrients. An increased digestibility of dry matter was also reported when AO was fed to ruminants (Wiedmeier et al., 1987; Gomez-Alarcon et al., 1990). Similarly, feeding yeasts to animals could enhance the digestibility of dry matter due to enzymes released by yeasts (Jonvel, 1993).

Table 7. Effect of feeding diets containing *Aspergillus oryzae* on nutrient metabolizabilities in laying hens

Treatment[1]	Metabolizability (%)			
	Gross energy	Dry matter	C. Protein	C. Fat
T1	66^{b}±3.11	62^{b} ±3.78	33±7.55	68±3.32
T2	73^{a}±2.99	69^{a} ±4.11	34±11.59	75±5.00
T3	71^{a}±1.83	67^{ab}±2.58	31±8.23	65±4.35
T4	73^{a}±1.73	70^{a} ±1.50	41±6.85	73±4.92

[1] T1, control: T2, 0.15% Natu-fermen: T3, 0.3% Natu-fermen: T4, 0.3% Amaferm.
[a,b] Means±SD having no common superscripts in the same column differ significantly ($p<0.05$).

Grimes et al. (1997) reported that metabolizabilities of protein and energy improved, although not significantly, in layers fed 0.2% Fermacto® (commercial AO fermentation extracts), mainly due to a prolonged GIT transit time. Chan et al. (1975) reported growth promoting effects of fermented soybean for broilers. They used soybeans fermented by AO to provide 50% of dietary protein levels in experimental diets and observed a better use of dietary nitrogen and dry matter.

In conclusion, it appears that AO culture could be used as a probiotic supplement to diets of laying hens. It reduced *E. coli* and increased *Lactobacillus* spp. in the intestine. By improving the intestinal microbial ecosystem, it enhanced the utilization of dietary nutrients.

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