Effect of Fermented Product from *Bacillus subtilis* on Feed Conversion Efficiency, Lipid Accumulation and Ammonia Production in Broiler Chicks

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ABSTRACT : This study investigated the effects of fermented product from *Bacillus subtilis* (FPBS) on feed conversion efficiency, fat accumulation and ammonia production in broiler chicks. Sixty female broilers (strain Chunky, 7-day old) were divided into four groups and raised in individual cages. One group was fed a commercial diet without supplementation of FPBS as the control and the other three groups were fed commercial diets containing FPBS, either 0.5, 1.0 or 2.0%, for 21 days from 7 to 28 days of age. Water and feed were given *ad libitum*. Feed conversion efficiency was significantly improved in chicks supplemented with 0.5 or 1.0% of FPBS as compared with the control ($p<0.05$). The activities of hepatic acetyl-CoA carboxylase and fatty acid synthetase, and contents of triglyceride and cholesterol in the liver were significantly decreased in treatment groups ($p<0.05$) as compared with the control group. FPBS had no effect on the concentration of plasma triglyceride, phospholipids and cholesterol. Feeding FPBS at 1% or 2% levels reduced ammonia gas release ($p<0.05$). The inclusion of FPBS at 1% level may be recommended both to improve production efficiency and to reduce air pollution caused by ammonia gas release. For production efficiency to reach maximal profit, the inclusion of FPBS at 0.5% level can be recommended. Feeding FPBS reduced fat accumulation in the liver. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 3 : 333-337*)

Key Words : Fermented Product, *Bacillus subtilis*, Feed Conversion Efficiency, Ammonia Gas Release, Lipid Accumulation

INTRODUCTION

Recently, there is greater awareness amongst consumers concerning dietary fat and its adverse effects on human health. In order to consume meat with lower fat content, consumers tend to consume meat of broilers slaughtered at younger ages (28 days of age). However, economic returns are lower as compared to conventional slaughter at 42 days of age (Santoso et al., 1998). Therefore, an attempt should be made to improve production by improving feed conversion efficiency and body weight gain at younger ages (before 42 days). Although at an early age broiler chicks raised in a windowless house produced less ammonia gas than at later age (Nishiyama, 1994), the concentration is still higher than the safe level. Wijaya (2000) stated that ammonia at 20 ppm reduced body weight and feed conversion efficiency. Therefore, a lower ammonia gas release is needed.

Previous experiments showed that the inclusion of microorganisms in the diets improved feed conversion efficiency (Jirapochakul et al., 1990; Santoso et al., 1995) and reduced fat accumulation of broiler chicks at 42 days of age (Santoso et al., 1995; Tanaka et al., 1992).

Santoso et al. (1999) showed that feeding dried *Bacillus subtilis* culture at 0.5% inclusion level reduced the release of ammonia gas. The present study, therefore, investigated the effect of commercial fermented product from *Bacillus subtilis* (FPBS) on feed conversion efficiency, fat accumulation and ammonia gas release at 28 days of age.

MATERIALS AND METHODS

Female broiler chicks (strain Chunky) used in this study were obtained from a commercial hatchery. At 7 days of age, they were weighed individually, and sixty broiler chicks were selected, wing-banded, randomly distributed to four treatment groups and raised in individual cages. From 1 to 14 days of age, supplemental heat was provided, and thereafter the room temperature was kept at 22± 2℃ with the light on from 08:00 to 20:00. The chicks were raised to 28 days of age in individual cages.

One group was fed a commercial diet without supplementation of FPBS as the control, and the other three groups were fed commercial diets supplemented with either 0.5, 1.0 or 2.0% of FPBS for 21 days from 7 to 28 days of age. Commercial FPBS was produced by fermenting the media using *Bacillus subtilis*, dried, ground and pelleted. Water and feed were fed *ad libitum*. Body weight was individually measured on a weekly basis. The composition of diets is presented in table 1.

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Received May 10, 2000; Accepted November 17, 2000

At the end of the experimental period (28 day of age), ten chicks of each group were killed, and livers were removed and weighed. Five gram of liver was placed in ice-cold saline to measure the activities of lipogenic enzymes, and the remainder of the liver was frozen and stored at -30℃ for the analysis of various lipid fractions. Blood was collected from the wing vein with heparinized syringe and then centrifuged at 2,500 rpm for 10 minutes. The plasma obtained was stored and frozen at -30℃ for the analysis of lipid fractions concentrations. The lipid fractions were separated by thin-layer chromatography on silica gel chromarod using hexane-diethylether-formic acid (60:10:1) and hexane-benzene (1:1) as developing solvent and quantified by IATROSCAN TH-10 TLC/FID Analyzer (Iatron Laboratories, Inc., Tokyo, Japan 101). Carcasses as previously described (Santoso et al., 1995) were cut, frozen, and stored in a sealed plastic bags at -30℃ for analysis of fat content.

Liver homogenates for enzyme activity were obtained by the method previously described (Santoso et al., 1993). Acetyl-CoA carboxylase (ACC) activity was assayed by the $H^{14}CO_3$ - fixation method (Qureshi et al., 1980). Fatty acid synthetase (FAS) activity was assayed by the 1-^{14}C-acetyl-CoA incorporation method (Hsu et al., 1965). The protein content of the solution used for enzyme assay was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Enzyme activities were expressed as nanomole of substrate converted to product per minute per milligram of protein at 37℃.

For the 21 days of the experimental period, a mixture of feces and urine was collected and weighed daily. Ten individual cages in each group were sprayed with 5% HCl to prevent fermentation of the urine and loss of ammonia, and the excreta were dried in an electric oven at 55℃ for uric acid, N-total and ammonia analysis. Five individual cages in each treatment group enclosed for the measurement of ammonia gas release using Kitagawa Gas Kenchikan AP-1. Uric acid content in poultry excreta was carried out by the modification of Dubbs (1956) and Pudelkiewiez et al. (1968) methods (Santoso et al., 1999). N-total and moisture of excreta and fat content of carcass were determined by the methods of AOAC (1980). Ammonia content in excreta was determined using a commercial kit (Wako Pure Chemical Industries, Ltd., Japan).

All data were statistically analyzed using the one-way analysis of variance (Shinjo, 1990). Significant differences between control and treatments were determined by three df orthogonal contrasts.

Table 1. Chemical composition of experimental diets (%)[1]

Chemical Constituents	Control	FPBS inclusion level		
		0.5%	1.0%	2.0%
Moisture	11.5	11.8	11.9	12.0
Crude protein	23.8	24.0	24.1	23.6
Crude fat	3.6	3.7	3.6	3.6
Fiber	5.0	5.0	5.0	5.0
Ash	8.0	8.0	8.0	8.0
NFE[2]	48.1	47.5	47.4	47.8
ME[3] (kcal/kg)	3,200	3,200	3,200	3,200

[1] All variables were analyzed four times.
[2] NFE (Nitrogen Free Extract) (calculated by difference).
[3] Metabolizable energy.

RESULTS

There was no effect of FPBS on body weight, body weight gain, and the weights of liver and abdominal fat. In comparison with the control, the inclusion of FPBS at 0.5 or 1.0% significantly reduced feed intake ($p<0.05$) and improved feed conversion efficiency ($p<0.05$) (table 2). Chicks fed the diet with 0.5% FPBS had lower caeca weights than chicks fed other diets ($p<0.05$). Caeca lengths were significantly shorter in chicks fed the diet with 1.0 or 2.0% FPBS than in others.

Table 3 shows the effect of FPBS on N excretion and ammonia gas release. Total-N, N-urate and N-ammonia excretion were not significantly changed ($p<0.05$), but ammonia gas release was significantly reduced in broiler chicks fed FPBS at level 1 or 2% inclusion level ($p<0.05$). Ammonia and moisture contents of caecum and its pH were not significantly affected.

Table 4 shows the effect of FPBS on lipid fractions contents in liver and plasma. Hepatic triglyceride content was significantly reduced as compared with the control ($p<0.01$). Hepatic cholesterol content was also significantly lower in broiler chicks fed diets supplemented with FPBS ($p<0.05$). Hepatic phospholipid content was not significantly different and FPBS did not influence concentrations of triglyceride, cholesterol and phospholipid in plasma. There were no significant differences in the content of protein, moisture and fat of carcasses, whereas ash content was significantly reduced by 0.5 and 1.0% FPBS (table 5).

Activities of hepatic ACC and FAS were lower in chicks fed fermented product than the control ($p<0.05$) (table 6). Chicks fed diets with 1.0 or 2.0% FPBS had lower ACC but higher FAS than chicks fed the diet supplemented with 0.5% FPBS.

DISCUSSION

Continuous feeding of FPBS to animals may

Table 2. Effect of fermented product from *Bacillus subtilis* on broiler performance

Measurement	Control	FPBS inclusion level		P
		0.5%	1.0%	2.0%	
Body weight, g	980 ±45[1]	1,020 ±80	1,000 ±81	1,014 ±81	NS
BWG, g	866 ±14	906 ±33	886 ±81	900 ±81	NS
Feed intake, g/bird	1,404 ±36[b]	1,331 ±47[a]	1,321 ±38[a]	1,465 ±31[b]	p<0.05
Feed conversion efficiency[2], %	1.63 ± 0.04[a]	1.47 ± 0.03[b]	1.49 ± 0.02[b]	1.63 ± 0.04[a]	p<0.05
Liver wt., % BW	2.8 ± 0.2	2.7 ± 0.3	2.7 ± 0.3	2.7 ± 0.3	NS
Abdominal fat wt.[3], % BW	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.3	NS
Caecum weight[3], % BW	0.53 ± 0.11[b]	0.38 ± 0.06[a]	0.64 ± 0.11[b]	0.57 ± 0.1[b]	p<0.05
Caecum length[3], mm/100 g BW	2.7 ± 0.15[b]	2.6 ± 0.3[b]	2.4 ± 0.3[a]	2.3 ± 0.3[a]	p<0.05

[1] Values reported represent 15 chicks ± SD.
[2] Calculated with the following equation: Feed conversion efficiency=feed intake/BWG.
[3] Values reported represent 10 chicks ± SD.
NS: Not significant; C: as control.
P: Probability.

Table 3. Effect of fermented product from *Bacillus subtilis* on N excretion, N-urate and ammonia gas release

Measurement	Control	FPBS inclusion level		P
		0.5%	1.0%	2.0%	
Feces					
N-total, mg/100 g BW	321.6 ±11.3	326.5 ±26.9	329.8 ±10.2	323.2 ± 6.5	NS
N-urate, mg/100 g BW	111.4 ± 3.9	112.1 ± 5.7	115.5 ± 0.6	113.3 ± 7.7	NS
N-ammonia, mg/100 g BW	2.5 ± 0.1	2.4 ± 0.4	2.3 ± 0.3	2.5 ± 0.2	NS
Ammonia gas release, ppm[2]	20.0 ± 0.4[b]	18.0 ± 0.3[b]	9.8 ± 0.2[a]	8.4 ± 0.2[a]	p<0.05
Caecum					
pH of caecum	6.2 ± 0.1	6.5 ± 0.1	6.3 ± 0.2	6.5 ± 0.1	NS
N-ammonia, mg/g caecum wt.	4.2 ± 0.5	4.8 ± 0.6	4.6 ± 0.7	4.5 ± 0.5	NS
Moisture, %	78.7 ± 1.3	80.9 ± 2.3	81.6 ± 1.6	81.0 ± 1.7	NS

[1] Values reported represent 10 chicks ± SD.
[2] Values reported represent 5 chicks ± SD.
NS: Not significant; C: as control.
P: Probability.

Table 4. Effect of fermented product from *Bacillus subtilis* on lipid fractions

Measurement	Control	FPBS inclusion level		P
		0.5%	1.0%	2.0%	
Liver, mg/g					
Triglyceride	6.6 ± 2.6[b]	3.6 ± 0.3[a]	3.5 ± 1.0[a]	3.4 ± 1.8[a]	p<0.01
Cholesterol	3.2 ± 0.3[b]	2.9 ± 0.3[a]	2.8 ± 0.1[a]	2.9 ± 0.1[a]	p<0.05
Phospholipid	46.8 ± 3.4	46.5 ± 3.6	42.7 ± 2.5	45.6 ± 2.5	NS
Plasma, mg/100 ml					
Triglyceride	38.5 ± 4.3	37.1 ± 6.9	34.7 ± 5.1	38.5 ± 4.3	NS
Cholesterol	166.6 ±24.0	167.3 ± 0.6	157.2 ±15.7	166.6 ±24.0	NS
Phospholipid	311.4 ± 37.0	325.9 ±34.1	313.5 ±56.9	298.4 ±75.6	NS

[1] Values reported represent 10 chicks ± SD.
NS: Not significant; C: as control.

provide a constant inoculum of *Bacillus subtilis* in the alimentary tract (Jiraphocakul et al., 1990). This organism may associate with gut wall and favor an increase in the number of natural *Lactobacillus*, which

will suppress undesirable enteric microorganisms such as *Escherichia coli* and thus improve feed conversion efficiency as noted in broiler chicks fed diets supplemented 0.5% or 1% FPBS.

An improved feed conversion efficiency in broiler chicks fed diets supplemented with 0.5% or 1% FPBS would benefit producers, because it will increase their profits if the price of FPBS is low. In addition, the tendency of higher body weight gain in broiler chicks fed commercial diets supplemented with FPBS would increase the yield of product per meter square. The inclusion of 0.5% FPBS showed the best improvement of feed conversion efficiency and body weight gain as compared with 1% or 2% FPBS group. 0.5% FPBS inclusion would reduce feed consumption by 9.8% and increase body weight gain by 4.6% as compared with control group. If the results of Santoso et al. (1998) were recalculated using that improvement, the profit obtained by producers at 28 days of age was greater than at 42 days of age (2% improvement of profit). Therefore, when both feed conversion efficiency and body weight gain are considered, for reaching maximal profit the inclusion of FPBS at 0.5% level should be recommended.

Intestinal microorganisms influenced the weight of gastrointestinal tract of chicks (Williams and Fuller, 1971), and a shorter caecum length might be caused by a change in the balance of microflora in gastrointestinal following *Bacillus subtilis* inclusion. It is interesting to note that caecum length could be used to estimate hepatic triglyceride: hepatic triglyceride (mg/g liver)=-11.98+6.5 caecum length (mm/100 g BW), r=0.77, p<0.05.

The present study showed that feeding FPBS at 1 to 2% inclusion levels reduced ammonia gas release. This observation confirmed the finding of Santoso et al. (1999) that dried *Bacillus subtilis* culture reduced ammonia gas release in a poultry house, and in feces of 42-day old broilers. The study also confirmed the previous results (Santoso et al., 1999) that *Bacillus subtilis* could not reduce N-excretion. No change in caecal N-ammonia in the current results indicated that microflora activity on producing ammonia gas from uric acid might not be reduced by FPBS supplementation. The current study may confirm the hypotheses of Santoso et al. (1999) that lower ammonia gas release caused by FPBS might be attributed to binding ammonia by substances produced by FPBS. Wijaya (2000) stated that ammonia at level 20 ppm reduced body weight and feed conversion efficiency. The current study, however, showed that higher feed conversion efficiency and slightly higher body weight gain in treatment groups might be not predominantly caused by lower ammonia gas release.

Williams and Fuller (1971) stated that there is ample evidence that certain bacteria can split conjugated bile acids into taurine or choline and the constituent acid which can be further degraded. When this occurs in the upper part of the gut, fat will impair absorption. Furthermore, the present study showed that the inclusion of FPBS reduced hepatic acetyl-CoA carboxylase activity which may indicate lower hepatic fatty acid synthesis leading to lower hepatic triglyceride synthesis (Scorve et al., 1993). Therefore, lower fat absorption and lower hepatic fatty acid synthesis might be the factors lowering hepatic triglyceride content. Arbeeny et al. (1992) reported that in hamsters, inhibition of fatty acid synthesis decreases VLDL triglyceride secretion into the blood and therefore reduces plasma triglyceride. In the present study, however, triglyceride concentration in plasma was not changed, nor were carcass fat and abdominal fat contents. Plasma triglyceride levels are determined by a delicate balance between hepatic triglyceride synthesis on one hand and plasma triglyceride clearance on the other. Thus, no change in plasma triglyceride might due to lower plasma triglyceride clearance. Since an increase in plasma triglyceride concentration was correlated with an increased in body/carcass fat content (Griffin et al., 1982; Whitehead and Griffin, 1982), no change in plasma

Table 5. Effect of fermented product from *Bacillus subtilis* on carcass composition

Measurement	Control	FPBS inclusion level			P
		0.5%	1.0%	2.0%	
Moisture	70.9 ±0.7	71.3 ±0.8	70.0 ±0.5	69.3 ±1.8	NS
Crude protein	16.3 ±0.4	16.6 ±0.9	16.6 ±0.3	16.3 ±0.4	NS
Crude fat	9.1 ±0.4	9.0 ±0.3	10.1 ±0.4	10.4 ±1.5	NS
Ash	3.7 ±0.2^{b}	3.1 ±0.2^{a}	3.3 ±0.2^{a}	4.0 ±0.2^{b}	p<0.05

[1] Values reported represent 10 chicks±SD.
NS: Not significant; C: as control.
P: Probability.

Table 6. Effect of fermented product from *Bacillus subtilis* on hepatic-lipogenic enzyme activities (nmol/min/mg protein)

Measurement	Control	FPBS inclusion level			P
		0.5%	1.0%	2.0%	
Acetyl-CoA carboxylase	3.58 ±0.7^{c}	2.87 ±0.45^{b}	2.12 ±0.66^{a}	2.23 ±0.26^{a}	p<0.05
Fatty acid synthetase	3.64 ±0.38^{c}	2.70 ±0.34^{b}	3.11 ±0.26^{a}	3.11 ±0.66^{a}	p<0.05

[1] Values reported represent 10 chicks±SD.
NS: Not significant; C: as control.
P: Probability.

triglyceride would cause no change in carcass fat content.

The present results did not agree with the observation of Santoso et al. (1995) who reported that dried *Bacillus subtilis* culture significantly reduced abdominal fat content in 42-day-old broilers with a tendency to lower carcass fat content. It is likely that the fat accumulation in carcass and abdomen could be easily modified at a later than at younger age.

In some developing countries, internal organs such as liver, gizzard, heart etc. are consumed by humans. Therefore, lower lipid accumulation in the liver may benefit consumer health. Santoso et al. (1995) found that the fat content of broiler carcass (strain Chunky) was 14.5% at 42 days of age. The present result showed that the fat content of (broiler chicks raised in the same season in the following year) was 9.1% at 28 days of age. Therefore, it can be understood if consumers tend to consume broiler chicks at a younger age to avoid excessive fat consumption.

Williams and Fuller (1971) stated that certain intestinal microflora impaired the absorption of cholesterol and bile acid. In addition, Tanaka et al. (1992) found that fermented product reduced hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity, a rate-limiting enzyme in cholesterol synthesis. Therefore, the factors mentioned above might explain the occurrence of lower hepatic cholesterol. It is unknown however, why plasma cholesterol was not affected. It is possible that FPBS may cause lower cholesterol clearance from the blood.

In conclusion, the inclusion of FPBS at 1% level may be recommended both to improve production efficiency and to reduce air pollution caused by ammonia gas release. When production efficiency to reach maximal profit is considered, the inclusion of FPBS at 0.5% level can be recommended. Feeding FPBS reduced fat accumulation in the liver.

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