A NOTE ON THE REMOVAL OF PHYTATE IN SOYBEAN MEAL USING *Aspergillus usami*

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Summary

Soybean meal was fermented by *Aspergillus usami* in order to reduce phytate content. Aflatoxin B1 was not detected in the fermented soybean meal. The contents of crude protein, crude fiber, ether extract and crude ash were slightly increased following fermentation with a concomitant reduction in nitrogen free extract. Though the fermentation partly degraded proteins in the soybean meal, there was small difference in amino acid composition between the soybean meal and the fermented soybean meal. The results showed that the fermentation did not affect nutritional value of protein in soybean meal. Approximately 55% of phosphorus extracted by trichloroacetic acid was inositol hexaphosphate (phytate) in the soybean meal. The content of inositol tetra to hexaphosphates was not detected in the fermented soybean meal. These results indicated that the fermentation almost completely eliminated phytate in soybean meal. Phytase activity was not detected in the unfermented soybean meal. However, the enzyme activity in the fermented soybean meal was 167.7 U/g. When the fermented soybean meal is supplemented in formula feeds, phytase in the fermented soybean meal might partly degrade the phytate in other ingredients in the digestive tract. The fermented soybean meal is possibly used as a phytate-free protein source of feed, which contains high available phosphorus.

(Key Words : *Aspergillus usami*, Soybean Meal, Inositol Phosphate, Phytase)

Introduction

Soybean meal is the major protein supplement fed to monogastric animals. More than 50% of phosphorus in corn, soybean meal and other plants seeds is in the form of phytate, which is poorly available in the digestive tract of monogastric animals (Reddy et al., 1982). Only 25% of the phosphorus in soybean meal is available in pigs (NRC, 1988). Because of the low availability of phytate phosphorus, animals are often given more phosphorus than required and the excess phosphorus is excreted via feces. The increase in fecal phosphorus excretion causes environmental pollution in areas where the livestock population is high (Cromwell, 1991). Furthermore, phytic acid tightly binds essential dietary minerals and reduces their availability in the digestive tract (Morris, 1986; Shinoda and Yoshida, 1989).

Certain strains of *Aspergillus* sp. are high in phytase activity. It was indicated that dietary supplements of *Aspergillus niger* phytase improved utilization of phytate phosphorus in chicks (Nelson et al., 1971) and in pigs (Cromwell et al., 1993). *Aspergillus oryzae* can reduce phytate content in insoluble residues of homogenized soybean (Matsuo, 1989). The objective of this study was to determine if fermentation of soybean meal by *Aspergillus usami* could reduce phytate content.

It is known that several phytate derivatives, inositol mono- to pentaphosphates, are produced during fermentation (Nayini and Markakis, 1983). The conventional analytical method for phytate determination

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is based on precipitation with ferric ion (Oberleas, 1971). However, other phosphorus-containing compounds are precipitated along with the phytate. Furthermore, De Boland et al. (1975) showed that all of the inositol phosphates from the di- to hexaphosphates formed insoluble iron complexes, while appreciable amounts of mono- di- and triphosphates remained in soluble form. In addition, Lonnerdal et al. (1989) indicated that calcium and zinc absorption was inhibited by inositol penta- and hexaphosphate but tri- and tetraphosphate did not affect absorption. For these reasons, contents of phytate derivatives were measured in the fermented soybean meal. Phytase activity was also determined because the fermented soybean meal may be used as a supplementary source of phytase.

Materials and Methods

Commercial soybean meal (SBM) was used. Fermented soybean meal was prepared from the same batch of soybean meal three times according the following method, approximately 2×10^6 spores of *Aspergillus usami* (Hishiroku Co, Kyoto, Japan) were added to 100 g of steamed SBM and then fermented for 48 h. Following the first fermentation, water was added to bring the product to 50% content and fermented again for 12 h. After the second fermentation, fermented soybean meal (FSBM) was dried at 45℃ for 20 h. Soybean meal and FSBM were ground for analyses.

The samples were analyzed for dry matter, crude protein (micro-Kjeldahl; total NX 6.25), ether extract, crude fiber and crude ash using AOAC procedures (1980). Aflatoxin B1 content in SBM and FSBM was also measured by an enzyme-linked immunosorbent assay (Hongyo et al., 1992) using a commercial kit (Ube Industries, Ube, Japan). For the determination of total phosphorus and calcium content, the samples were digested by nitric acid and perchloric acid. Phosphorus concentration in digests were measured by the method of Gomori (1942). Calcium concentration was determined by atomic absorption spectrophotometry. Amino acid composition of SBM and FSBM was determined by high performance liquid chromatography using O-phtalaldehyde (Ishida et al., 1981) after hydrolysis with HCl for 22 h at 110℃. Proteins extracted from SBM or FSBM by water were separated using gel electrophoresis with 12% of polyacrylamide gel and 0.1% sodium dodecyl sulfate according to the method of Weber and Osborn (1969). After stained with Coomassie R-250, molecular weights of proteins were determined.

Phytic acid and its derivatives in SBM or FSBM were measured using ion exchange chromatography (Nayini and Markakis, 1983). Inositol phosphates were extracted by 3% trichloroacetic acid, followed by centrifugation at 12,000 × g for 15 min. The supernatant was chromatographed on a 1.1 × 10.5 cm of Dowex-1 × 8 (200-400 mesh, Cl^- from) column. Linear gradient elusion was performed with 600 ml of 0 to 1 mol/l HCl. Ten ml fractions were collected, dried and digested with perchloric acid. The phosphorus content in the digest was measured by Allen's method (1940).

Phytase activity in SBM and FSBM was determined by the following method. Homogenates of SBM and FSBM were centrifuged for 15 min at 12,000 × g. The supernatant was dialyzed in water to remove inorganic phosphate. The solution used in the assay was 0.1 mol/l sodium acetate buffer (pH 5) with 2.1 mmol/l sodium phytate (Han et al., 1987). Into this solution, 6 mmol/l $MgCl_2$ was added. After a 15 minute incubation, true inorganic phosphorus concentration in the solution was measured by the method of Takahashi (1955). One unit of enzyme activity was defined as nmol phosphorus production in 1 min.

Results and Discussion

Aflatoxin B1 was not detected (less than 5 ug/kg) in SBM and FSBM. Aflatoxin B1 is the well known toxic metabolite of *Aspergillus* sp. such as *Aspergillus flavus* and *Aspergillus parasiticus* (Chu., 1971). The present results suggest that FSBM produced by *Aspergillus usami* does not contain the toxic level of aflatoxin B1.

Table 1 shows the chemical composition of SBM and FSBM. Content of crude protein, crude fiber, ether extract and crude ash were slightly increased by the fermentation. On the other hand, the content of nitrogen free extract was decreased. These results indicated that carbohydrates other than fibers were used for microbial growth and the reduction of nitrogen free extract resulted in increased concentration of the other components. Dry matter loss during fermentation was not determined in this experiment. However, the dry matter loss estimated from the changes in crude ash content was approximately 7%.

Table 2 shows the amino acids composition of SBM and FSBM. The amino acid composition of SBM and FSBM were not different. In SBM, there are many proteins of which molecular weights were more than 50,000 but these high-molecular weight proteins were not found. In FSBN, it is clear that the fermentation partly breaks down proteins in soybean meal but does not adversely affect nutritional value of protein.

As shown in table 3, approximately 72% of extracted

phosphorus in SBM are inositol phosphates and 55% of phosphorus is inositol hexaphosphate (phytate). Using the precipitation method with ferric ion, Nelson et al. (1968), indicated that 61 to 70% of phosphorus in soybean meal was phytate, which is higher than the content of inositol hexaphosphate and lower than the total content of the inositol phosphates in this experiment. The difference is reasonable because iron salts of the mono-, di- and triphosphates have been shown to be too soluble to afford reliable quantification (De Boland et al., 1975).

Inositol tetra-, penta- and hexaphosphates were not detected in FSBM. These results suggest that fermentation of soybean meal by *Aspergillus usami* reduced phytate phosphorus levels. This reduction suggests that fermentation could improve phosphorus availability in soybean meal.

TABLE 1. CHEMICAL COMPOSITION (g / 100 g DRY MATTER) OF SOYBEAN MEAL AND FERMENTED SOYBEAN MEAL

	SBM[a]	FSBM[b]
Crude protein	47.00	53.20 ± 2.30[c]
Crude fiber	4.00	4.70 ± 0.20
Ether extract	2.00	2.60 ± 0.50
Crude ash	6.90	7.40 ± 0.10
Nitrogen free extract	40.10	32.10 ± 2.50
Calcium	0.27	0.30 ± 0.01
Phosphorus	0.72	0.79 ± 0.01

[a]SBM; Soybean meal.
[b]FSBM; Fermented soybean meal.
[c]Mean ± SD. for 3 replicated fermentation of the same batch of soybean meal.

TABLE 2. AMINO ACID COMPOSITION OF SOYBEAN MEAL AND FERMENTED SOYBEAN MEAL g / 100 g OF TOTAL AMINO ACIDS

	SBM[a]	FSBM[b]
Threonine	4.13	4.10 ± 0.02[c]
Valine	4.84	5.16 ± 0.06
Methionine	1.45	1.54 ± 0.04
Isoleucine	4.47	4.66 ± 0.07
Leucine	7.80	8.07 ± 0.10
Phenylalanine	5.21	5.31 ± 0.09
Histidine	2.80	2.89 ± 0.05
Lysine	6.29	5.84 ± 0.09
Arginine	7.14	6.93 ± 0.11
Aspartic acid	11.63	11.76 ± 0.05
Serine	5.44	5.33 ± 0.03
Glutamic acid	18.16	18.19 ± 0.05
Proline	5.34	5.39 ± 0.05
Glycine	4.35	4.55 ± 0.08
Alanine	4.37	4.28 ± 0.06
Tyrosine	3.49	3.66 ± 0.09

[a]SBM; Soybean meal.
[b]FSBM; Fermented soybean meal.
[c]Mean ± SD. for 3 replicated fermentation of the same batch of soybean meal.

TABLE 3. COMPOSITION OF PHYTIC ACID DERIVATIVES AND PHYTASE ACTIVITY OF SOYBEAN MEAL AND FERMENTED SOYBEAN MEAL

	SBM[a]	FSBM[b]
None inositol phosphate	28.2[c]	91.4 ± 8.0[f]
IP1[e]	0.4	6.8 ± 1.5
IP2	1.0	1.3 ± 0.1
IP3	1.5	0.5 ± 0.4
IP4	5.9	tr[g]
IP5	8.2	tr
IP6	54.8	tr
Phytase (U/g)[d]	tr[h]	167.7 ± 4.4

[a]Soybean meal.
[b]Fermented soybean meal.
[c]Percentages of total extracted phosphorus.
[d]One unit (U) was defined as nmol phosphorus production in 1 min.
[e]IP1 to IP6; Inositol containing 1 to 6 phosphate.
[f]Mean ± SD. for 3 replicated fermentation of the same batch of soybean meal.
[g]tr; less than 0.05%.
[h]tr; less than 10 U/g.

Phytase activity was not detected in SBM. However, the enzyme activity was 167.7 U/g in FSBM. Cromwell et al. (1993) indicated that the addition of graded levles of phytase, 250 U/g to 1,000 U/g, linearly increased growth rate and bone strength in pigs. If a formula feed consists of 20% FSBM, dietary phytase activity would be 33.5 U/g. This level of phytase activity from FSBM may be too low to degrade phytate completely in a formula feed. Meanwhile, the existence of phytase activity in FSBM may partly degrade phytate from other ingredients of the formula feed though it has not been reported whether the addition of this low level of phytase effectively stimulates phosphorus availability. On the other hand, it is possible that the changes in conditions of fermentation increase phytase activity in FSBM and a strain of *Aspergillus usami* containing high phytase activity is selected. Therefore, it is possible that FSBM which contains higher phytase activity may be more useful as a supplementary

source of phytase.

It seems that the fermentation can convert phytate phosphorus in soybean meal to available phosphorus. If FSBM substitutes for SBM, phosphorus supplied as feed additive can be reduced. Furthermore, fecal excretion of phosphorus could be decreased by the substitution, which alleviates phosphorus pollution by reducing phosphorus swine manure applied to the land. In addition, FSBM contains phytase activity. If the enzyme effectively degrades phytase in the digestive tract of pigs, phytase in FSBM can be degraded phytate from other ingredients of a ration, therefore in a ration consisting of SBM and FSBM, the additional amount of FSBM may be cut down because phytase in FSBM may degrade phytate in SBM.

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