Reduction of Nitrogen and Phosphorus from Livestock Waste : A Major Priority for Intensive Animal Production* - Review -

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ABSTRACT : In current animal production in Japan, a large surplus of nitrogen and phosphorus is given to animals as their feed which are mostly imported from outside of our own country. Today, an excess of nitrogen and phosphorus from animal manure has been spread out of the area of animal production and the surroundings. These components have become the major reason for eutrophication of ground, surface and inland water. Nutritional studies for the reduction of nitrogen and phosphorus from animal waste has been done by many researchers. The reduction of excess protein in animal feed and the supplementation of deficient essential amino acids to feed have a possibility to increase the biological value of feed and to reduce nitrogen excretion, especially, via urine. The use of phytase activity to degrade phytate and to release utilizable inorganic phosphorus make it possible to cut an excess supply of feed additive inorganic phosphorus and to reduce phosphorus excretion from animal waste. *(Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 4 : 651-656)*

Key Words : Animal Waste, Nitrogen, Phosphorus, Microbial Phytase, Amino Acids

INTRODUCTION

One of the big problem induced by an intensive animal production is an excess manure production. The number of livestock raised in rural area used to be balanced by the area available for feed and by the field acceptable for manure as fertilizer. In current animal production in Japan, a large surplus of nitrogen and phosphorus were given to animals as their feed which were mostly imported from outside of our own country. An excess of nitrogen and phosphorus from animal manure has been spread out the area of animal production and the surroundings. The amount of manure from livestock production in 1997 exceeded over 80 $x10^6$ t in Japan. The amounts of nitrogen (N) and phosphorus (P_2O_5) in the manure were accounted for 0.74×10^6 t and 0.32×10^6 t per year respectively. These nitrogen and phosphorus have become the major reason for eutrophication of ground, surface and inland water. Furthermore, ammonia and nitrous oxide emission from animal manure could contribute to produce acid rain and global warming.

Phosphorus, an essential nutrient for animals and plants, fortifies animal feed and artificial fertilizer. The source of phosphorus is phosphate rocks which is originated guano and restricted of the ground deposit in the earth. The absorbability of phosphorus to plants is relatively low, 5-15% from fertilizer, and over 85-95% of phosphorus in fertilizer should be fixed in ground materials and formed to insoluble complex. Saving rock phosphorus to feed is needed for sustainable agriculture.

In an animal body, most of nutrients ingested excessively were inevitably excreted into faeces and urine. An appropriate scheme for nutritional requirement provides a minimum but a sufficient nutrient for animals. The improvement of digestibility, absorbability and bioavailability of nutrients might be an effective method to reduce the indigested, unabsorbed and unutilized nutrients in excreta. Nutrition can contribute substantially to reduce N and P excretion in animals, by supplying modified feed. The addition to diets of synthetic amino acids and of the enzyme phytase decreased the intake of N and P and reduced the excretion of N and P from animals. The low protein diet supplemented with amino acids could become an ideal protein diet for animals and could reduce the nitrogen output in animal excreta. Using enzyme phytase which was needed for hydrolysis of phytate to inositole and inorganic phosphate must increase available phosphorus level in diet or digesta for monogastric animals which cannot produce enzyme phytase in themselves.

From the view point of animal nutrition, some kinds of dietary processing to increase available nutrients might reduce environmental pollution caused by intensive animal production.

REDUCING NITROGEN EXCRETION USING A LOW PROTEIN DIET

Nitrogen in manure may become a bigger problem since it results in leaching NO_3-nitrogen to surface, ground and drinking water. Furthermore, ammonia and

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nitrous oxide emitted from manure to the air as acid gas and global warming gas.

In dietary ingredients of common feed formulations, there is usually an excess of essential and non-essential amino acids. Animal can not convert the extra amount of amino acids into their body proteins. Therefore, the excess amount of amino acids must be excreted as waste in faeces and urine. The excess amount of amino acids does not only depress economical performance of feed efficiency, but also induce to environmental pollution by ammonia, nitrate and nitrous compound.

In turn to protein level in animal feed, a crude protein level of common commercial diet for chicken is accounted near by 20% which is 140% of the protein level recommended by Japanese Feeding Standard (1992). Ishibashi et al. (1996) reported that egg production was kept to be equal in laying hens fed the diet lowering crude protein from 20.2% to 15.1%. In this study, the amounts of nitrogen used for egg production and loss of feather and scurf by laying hens were not affected by dietary crude protein levels, which were 856 mg and 883mg, and 8mg and 15 mg in a day per bird, respectively. When dietary crude protein decreased from 20.2% to 15.1%, nitrogen excretion in excreta decreased from 1.92 to 1.41g in a day per bird (table 1).

Table 1. Effects of dietary CP levels on N excretion of laying hens

Dietary CP (%)	Feed Intake (g/day)	Nitrogen (g/hen/day)			
		Intake	Egg	Excreta	Debris
19.8[1]	98.4	2.77	0.868	1.942	0.010
20.2	97.8	2.86	0.856	1.961	0.011
17.5	100.6	2.59	0.883	1.701	0.008
15.1	100.5	2.25	0.880	1.071	0.016

[1] Commercial. (Ishibashi et al., 1996)

For swine production, the reduction of nitrogen excretion might be achieved by supplying appropriate nitrogen with requirement or introducing multiple-phase feeding.

An ideal balance of amino acids in feedstuffs will greatly contribute the animal response in terms of protein consumption and nitrogen excretion. Today, artificially synthesized amino acids become more available. The protein level of diet can be reduced by supplementation of these essential amino acids to the diet to meet amino acids requirement of the animals. The synthesized amino acids will become more usefull as feed additives for poultry and swine.

Equal production might be kept for broiler chicken fed the diet lowering of crude protein from 27.8% to 21.2% during 20 to 30 days of age and lowering of crude protein from 21.2% to 14.1% during 40 to 50 days of age (Ishibashi et al., 1996) (table 2). When the dietary crude protein was decreased from 27.8 to 21.2% during 20 to 30 days of age, feed intake and body weight gain were not affected by dietary crude protein levels, but nitrogen excretion in excreta decreased from 2.5g to1.7g/chick/day, which was about 32 % reduction of nitrogen excretion. When the dietary crude protein was decreased from 21.2% to 14.1% during 40 to 50 days of age, nitrogen excretion was decreased from 3.5g/day to 1.7g/day with no detrimental effect on body weight gain of broilers, which was 51% reduction of nitrogen excretion. These low protein diets were supplemented with Arg, Lys, Met and Trp for 14.1% CP diet and 21.2%CP diet , supplemented with Arg, Met and Trp for 27.8% CP diet to meet their amino acid requirements. From the results mentioned above, the dietary protein level for chickens might be reduced by up to 4% units without bad effect on growth and egg production when some kinds of essential amino acids were supplemented. In such feeding condition, nitrogen excretion might be depressed by 50% for laying hen and also be depressed by 50% for broiler chicken.

Table 2. Effects of dietary CP levels on N excretion of female broilers at 20 to 30 days of age and at 40 to 50 days of age

Dietary CP (%)	BW Gain (g/d)	Feed Intake (g/d)	Nitrogen (g/chick/day)		
			Intake	Excreta	Debris
20 to 30 days of age					
27.8	57.7	120.2	5.34	2.54	0.13
24.5	57.2	120.8	4.73	2.08	0.14
21.2	57.3	124.1	4.22	1.68	0.13
40 to 50 days of age					
21.2	72.9	161.8	5.48	3.46	0.29
17.6	72.9	162.8	4.43	2.43	0.27
14.1	73.3	160.7	3.61	1.68	0.32

(Ishibashi et al., 1996)

As same as a diet for chicken, the dietary protein level for swine can be lowered by amino acid supplementation. The balanced amino acids in feedstuffs are advantageous for pig production. In commercial diet for swine, a part of the dietary protein which is indigested was lost in faeces and a large proportion of nitrogen excretion appeared in urine (table 3).

Table 3. Route of nitrogen excretion in growing pigs (24 kg BW)

	CP 19.5%[1]	CP 19.3%[2]
	(g/day)	
Feed Intake	992	1,205
Nitrogen Intake	24.8	32.2
Fecal Nitrogen	4.0	4.1
Urinary Nitrogen	5.1	9.0
Nitrogen Retention	15.7	19.1
	(%)	
Nitrogen Digestibility	83.9	87.1
Biological Value	75.4	68.0

[1] Yano and Matsuda 1996. [2] Taniguchi et al. 1998.

Results of balance test using pig (18-30 kg body weight) fed corn-soybean meal diet showed that 24%-32% of the digested (or absorbed) nitrogen were secreted in urine. In pigs fed 24.8 g N/day, 20.8 g N/day was absorbed, 4 g N was excreted in faeces and 5.1 g N was secreted in urine. When pig fed 32.2g N/day, 4.1 g N was excreted in faeces and 9.0 g N was lost in urine. The amount of nitrogen loss via urine was larger than that of faeces. This results suggested that 24% to 32% of absorbed crude protein (or abosrbed amino acids) could not be convert es to the body protein of these pigs. The nitrogen excretion in urine mainly come from the degradation of amino acids which can not be convert ed to body protein deposition. When the balance of amino acids in diets was met for amino acids requirements of animals, less protein is wasted and less nitrogen is secreted in urine.

Nitrogen retention was increased by amino acid supplementation to the low protein diet (table 4). The nitrogen digestibility was 77.8% in low protein diet and 82.1% in low protein+amino acids (Lys, Met. and Thr) diet. When pigs were fed amino acids supplemented low protein diet, nitrogen retention was increased about 5% unit and urinary nitrogen excretion was decreased about 2.3 g/day compared with the pig fed low protein diet. The apparent biological value was increased by 17% units as amino acid supplementation in low protein diet.

Table 4. Effect of amino acid supplementation on nitrogen excretion in pig fed a low protein diet

	CP 11.7%	CP 11.7%+AA
	(g/day)	
Nitrogen Intake	20.6	22.3
Fecal Nitrogen	4.6	4.0
Urinary Nitrogen	6.6	4.3*
Nitrogen Excretion	11.2	8.3*
Nitrogen Retention	9.4	14.0*
	(%)	
Nitrogen Digestibility	77.8	82.1*
Biological Value	59.3	76.0*

(Taniguchi et al., 1998)

* Significantly different from the value of CP 11.7%.

There still remains the problems of carcass fat deposition in concern of energy and protein balance of diet and of acid-base balance of animals caused by acid production from amino acid oxidation. A number of research have been conducted to clarify the optimal amino acids supplementation to get good performance of livestock production in low protein diets (Chung and Baker, 1992; Gatel and Grosjean, 1992; Hansen et al., 1993; Brudevold and Southern, 1994, Hahn and Baker, 1995; Kerr and Easter, 1995; Tuitoek et al., 1997)

REDUCING PHOSPHORUS EXCRETION USING MICROBIAL PHYTASE

The availability of phosphorus in plant origin feedstuffs, such as corn, soybean meal and wheat bran, is only 30 to 40% (Standard Table of Feed Composition in Japan 1995). The greater proportion of these phosphorus exists as a form of phytate-P, myo-inositole 1,2,3,4,5,6-hexakis. The compound is not hydrolyzed by the endogenous enzymes in mono-gastric animals and, therefore, is a low availability of phosphorus in pig and poultry. The diet for mono-gastric animals must be commonly supplemented with inorganic phosphorus to meet their phosphorus requirements, which increases the cost of the diet and phosphorus output from animals. Phosphorus requirement for growing-finishing pigs (30 to 70 kg) recommended by Japanese Feeding Standard for Swine (1993) is 0.45% of air-dried diet. However, phosphorus contents in commercial feed for pigs were commonly over 0.7%. Excess phosphorus is likely to excrete in pigs fed such a diet.

It has been well known that many fungi, bacteria and yeast produce enzyme phytase, which is hydrolyze phytate to release inorganic phosphate. It was shown that microbial phytase from *Aspergillus ficuum* was evaluated by Nelson et al. (1971) as a feed additive. The microbial phytase could improve phosphorus availability in chicken fed a corn-soybean meal diet. The cost production and purification of this enzyme was considerably too expensive to compete inorganic phosphorus additives at that time. However, the cost of the enzyme has been tried to be decreased by a new processing method on using biotechnology of recombinant. And the practical application of microbial phytase to animal diet has been focussed on the reduction of phosphorus in excreta.

When phytase was supplemented in feeds, phosphorus availabilities were increased in chicks, broilers, hen and ducklings (Schoner et al., 1992; Broz et al., 1994). Today, some phytase substances derived from *Aspergillus niger* or transgenetic microbes has been sold on the market as additives for feed of swine (Cromwell et al., 1993; Yi et al., 1996; Kemme et al., 1997). Also, in Japan, several experiments for evaluation of phytase activity from *Aspergillus niger* have been carried out using chicken and pig. In these experiments, supplementation of 500 to 1500 unit per kg diet improved phosphorus retention from 14% to 48%, reduced total phosphorus contents in those diet by 21-40%, and also reduced fecal phosphorus excretion by 16-35% (table 5, 6, 7, 8).

Table 5. Effects of phytase on phosphorus availability in chicks

Phytase (unit/kg diet)	Improvement of P availability (%)	Reduction of P in diet (g/kg)	References
1,000	45→63	7.5→4.5	Simons et al.
1,500	41→62	7.5→4.5	(1990)
800	54→67	5.7→4.5	Schoner et al.
1,500	49→70	5.3→3.5	(1992)
500	44→53		Broz et al.
500	38→44		(1994)

Table 6. Effects of phytase on phosphorus digestibility in pigs

Phytase (unit/kg diet)	Apparent P digestibility (%)	Reduction of P in feces (g/kg)	Reference
1,000	20→46	21.0→13.6	Simons et al.
1,000	34→56	16.3→10.9	(1990)
1,500	26.4→44.9	21.0→13.6	Jongbloed et al.
1,500	16.4→45.8	15.8→10.4	(1992)
500	38.3→47.9	5.7→4.8*	Saitoh et al.
1,000	38.3→52.7	5.7→4.4*	(1995)

* g/day/head.

Table 7. Effects of phytase on P excretion in chicks

	Phytase (unit/kg)		
	0	0	1,000
P in diet (g/kg)	4.5	7.5	4.5
P availability (%)	49.8[a]	44.6[b]	62.5[c]
P in manure (g/kg feed intake)	2.7[a]	4.9[b]	2.0[c]
Growth 0-24 d (g)	338[a]	683[b]	690[c]

a,b $p<0.05$. (Simons et al., 1990)

Table 8. Effects of phytase on P excretion in swine (41 kg)

	Phytase (unit/kg)		
	0	500	1000
P in diet (g/kg)	5.7	5.7	5.73
P availability (%)	38.3	47.9	52.7
P in feces (g/day)	5.7	4.8	4.4

(Saitoh et al., 1995)

Since long years ago in Japan, many kinds of fermentation techniques have been developed for traditional food prosessing, that is, MISO (fermentation soybean past), Syoyu (soy-source) and SAKE (Japanese wine made from rice). *Aspergillus usami*, isolated in the process of MISO making, has a high ability to produce a large amount of enzyme phytase. Soybean meal fermented with *Aspergillus usami* was almost completely degraded phytate-phosphorus (Ilyas at al., 1994). The fermentation may increase inorganic phosphorus in the soybean meal and also enhance phosphorus absorption. Digestion trial were carried out with rats, chicks and pigs using the fermented soybean meal as a feed ingredient.

Eighteen male rats of 7 week old were fed the fermented soybean meal (FSBM) diet for 2 weeks in digestion trial. Apparent phosphorus absorption was increased from 73.3% to 88.6% and phosphorus excretion in feces was reduced by 60%, i.e. from 42.7 to 17.0 mg/day (table 9).

Thirty WL chicks of 1 week old were fed the fermented soybean meal diet (FSBM), regular soybean meal diet (SBM) and regular soybean meal supplemented with inorganic phosphorus (SBM+P) diet to meet P requirement for 4 weeks. The body weight gain and bone characteristics were not affected, however, the total phosphorus content in diets could be reduced from 7.7 to 5.5 g/kg. The amount of phosphorus in excreta was also reduced from 337 to 234 mg/bird/day, which was counted 30% reduction of phosphorus excretion (table 10).

Table 9. Effect of fermented soybean meal on P metabolism in rats

Diets	SBM	FSBM
Intake (mg/d)	162.8	148.0*
Feces (mg/d)	42.7	17.0*
Apparent absorption (% of intake)	73.7	88.6*

(Hirabayashi et al., 1994)

* Significantly different from SBM ($p<0.05$).

Table 10. Effect of fermented soybean meal on P metabolism in chicks

Diet	SBM	FSBM	SBM+P
Total P in diet (%)	0.55	0.56	0.77
P in excreta (mg/d)	241[a]	234[a]	337[b]
BW gain (g/d)	12.4[a]	13.6[b]	14.2[b]

a,b $p<0.05$. (Nakajima et al., 1996)

Digestion trials was carried out using fifteen growing LW pigs of 2 months old weighing 23.6 kg. The animals were fed a diet consisted 20% fermented soybean meal (FSBM), a diet consisted 20% regular soybean meal (SBM) and a diet consisted 10% fermented soybean meal and 10% soybean meal (Mixed) for 10 days. The apparent phosphorus digestibility was increased from 26.1 to 41.9%, when pig was fed FSBM diet. The amount of fecal phosphorus excretion was reduced by 1 g/head/day (table 11).

Table 11. Effect of fermented soybean meal on P metabolism in pigs

Diet	SBM	FSBM	SBM+P
P intake (g/d)	7.46	7.62	7.55
Fecal P (g/d)	5.43	4.44	5.08
Apparent P digestibility (%)	26.1[a]	41.9[b]	32.9[ab]

a,b $p<0.05$. (Yano and Matsuda, 1997)

The phytase activity in the fermented soybean meal was 167.7 U/g. When feed consists of 20% FSBM, dietary phytase activity is counted 33.5 U/g. The phytase activity in FSBM is seemed relatively low to degrade phytate perfectly in a formula feed when the feed contained 20% of FSBM. However, the existence of phytase activity in FSBM may partly dephosphorylate phytate in other ingredients of feed.

Twelve growing pigs of 2 months old weighing 20.1 kg were fed three types of corn-soybean meal diet for 10 days in balance trial. Total feces and total urine were taken in this experiment. Phosphorus digestibility was increased from 33.3% to 40.5% when pig were fed fermented soybean meal diet. The total phosphorus excretion in pig fed fermented soybean meal diet was reduced by 0.7 g/day compared with pig fed soybean meal+phosphorus diet (table 12).

Table 12. Effect of fermented soy bean meal on P metabolism in pigs

Diet	SBM	FSBM	SBM+P
P intake (g/d)	3.40[a]	3.47[a]	4.20[b]
Fecal P (g/d)	2.23[a]	2.09[ab]	2.60[b]
Urinary P (g/d)	0.10[ab]	0.02[a]	0.20[b]
P retention	1.91	2.19	2.40
Total P excretion (g/d)	2.33[ab]	2.11[a]	2.80[b]
Apparent digestibility (%)	33.3[a]	40.5[b]	38.2[b]

[a,b] $p<0.05$. (Matsuda et al., 1997)

The fermentation with *Aspergillus* might increase phosphorus availability of soybean meal in monogastric animals, caused by microbial phytase activity to degrade phytate phosphorus. When phytase activity in FSBM is used, the amount of inorganic phosphorus supplementation to an animal diet might be reduced. Reducing phosphorus intake can substantially contrbute to reducing phosphorus output from animal production systems. In addition, there is a possibility that the change in conditions of fermentation or the use of some other *Aspergillus sp.* producing more active phytase increases phytase activity.

According to NRC, phosphorus requirement of growing finishing pigs (30-70 kg) is 0.45% of air dried diet. Most of commercial diet containes over 0.7-0.8% of phosphorus to eliminate phosphorus defficiency in animals. Such a kind of overloading of phosphorus in diets may induce a large amount of phosphorus loss into manure and may result in water pollution.

SIMULTANEOUS REDUCING NITROGEN AND PHOSPHORUS EXCRETIONS

When pigs were fed a fermented soybean meal diet, nitrogen digestibility was improved 6% unit compared with pigs fed a regular soybean meal diet and the amount of fecal nitrogen excretion reduced. Protease activity and other hydrolysys activityies were found in the microbes. Some structural change may occur in soybean meal protein and other organic conpounds when soybean meal is fermented with *Aspergillus usami* (table 13). However, in the 2nd experiment using fermented soybean meal, nitrogen digestibility was not affected with the fermented soy bean meal diet. The increase of non-phytate phosphorus with fermentation in FSBM of 2nd experiment was lower than in FSBM of 1st experiment. The microbes activity during fermentation processing may be different between experiment 1 and experiment 2. If the procedure of fermentation processing was improved, more effective soybean meal on nutrients digestibilities could be provided for animal feeds.

The enzyme phytase has been used to degrade phytate-phosphorus as a feed additive. Additionally, the improvement of crude protein digestibility was reported in pig fed phytase supplementation compared to pigs fed no phytase (Saitoh et al., 1995). However, the biological value of dietary protein and the amount of urinary nitrogen excretion were not changed by phytase supplementation (Taniguchi and Kado, 1998). The co-relative effect of amino acid supplementation and phytase supplementation on nitrogen metabolism was expected from the results above mentioned. When an appropriate amount of essential amino acids and phytase are added to a low protein and low phosphorus diet, both of nitrogen and phosphorus intake could be reduced. As a result, nitrogen and phosphorus excretion from animals could be simultaneously reduced.

Table 13. Effect of fermented soybean meal on nitrogen digestibility and excretion in feces of growing pigs (exp.1)

	SBM	FSBM	Mixed
N intake (g/d)	34.65	35.63	35.43
Fecal N (g/d)	11.62	9.50	9.08
N Digestibility (%)	66.4[a]	73.4[b]	74.4[b]
DM Digestibility (%)	70.5[a]	77.3[b]	76.4[b]

[a,b] $p<0.05$. (Yano and Matsuda, 1997)

CONCLUSION

Sustainable animal production will not be able to be succeeded without control of animal waste. A large amount of waste from animal production might result in area pollution and further global pollution. Recycling and treatment of animal waste have been studied by a number of microbiologists. We, animal nutritionist, may contribute to reduce eutrophical components, nitrogen, phosphorus and heavy metals from animal waste by nutritional aproaches. Nutritional improvement of feedstuffs means to increase bioavailability of nutrients and to prevent endogenous loss of nutrients. A low protein diet supplemented with appropriate essential amino acids and a low phosphorus diet supplemented with phytase activity, and the combination of both techniques might be useful to reduce nitrogen and phosphorus excretion from animal waste with no detrimental effect on animal production.

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