Management of Excretion of Phosphorus, Nitrogen and Pharmacological Level Minerals to Reduce Environmental Pollution from Animal Production - Review -

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ABSTRACT : In order to prevent pollution from animal waste, P, N and pharmacological level minerals should be properly managed. Microbial phytase has been used successfully to control P excretion. Activity of natural phytase in certain plant feedstuffs is high enough to be considered in feed formulation. Nitrogen control can be achieved through amino acid supplementation and protein restriction in the diet. Supplementation with carbohydrases reduces output of excreta as well as N. Ammonia release from the manure could be reduced by using a low crude protein diet along with the supplementation with probiotics products. Excretion of minerals used at pharmacological level can be reduced by using chelated forms. Cu and Zn in the form of methionine chelate have been successfully used in the broiler and pig diets. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 3 : 384-394)*

Key Words : Phytase, Poultry, Environmental Pollution, Nitrogen, Minerals, Animal Mannure

INTRODUCTION

The animal industry must be environmentally sound to ensure its long-term sustainable growth. Livestock wastes, mostly manure, can be a valuable resource as a fertilizer or soil conditioner. But it can be a potential hazard to environment as well. Environmental concerns relate to water quality, soil degradation, air pollution and rural-urban interface issues. Land application of excessive quantities of nutrients is subject to surface run-off and leaching that may contaminate ground or surface waters. Phosphorus (P) entering surface waters can stimulate growth of algae and water plants. Decomposition of them results in an increased oxygen demand, which may interfere with the well-being of fish and wildlife. Nitrate leaching has been considered a major nitrogen (N) pollution concern with livestock farms. Ammonia toxicity to fish and altered effectiveness of chlorination are other concerns. Manure can be a major source of methane and nitrogen oxides which contribute to the accumulation of greenhouse gas. Volatilization of ammonia causes acid rain which results in forest dieback in western Europe (ApSimon et al., 1987). Emissions of nitrous oxide (N_2O) during nitrification and denitrification cause depletion of the stratospheric ozone layer (Christensen, 1983). Manure can be a source of odours which contribute to friction between urban and rural residents. Excessive contributions of some minerals from animal manure can create high salt concentration in the soil. High concentration of copper in the pig diet can cause accumulation of copper in the soil.

As described above, animal manure can be a valuable resource while it can be a major obstacle in the future development of animal industry if the impact on environment is not properly controlled. Major efforts are required to adopt all best available technologies capable of reducing excretion of pollutants from animal industry before further restrictive legislation is enacted to control the problem. There are a number of possible solutions to this problem.

The first option of manure management is developing an 'environmentally sound' nutritional management, that is, feeding program and feeds to result in less excreted nutrients that need to be managed. Once the manure is produced it can be best utilized as a fertilizer or a soil conditioner. In many countries the amount of manure that can be spread on land depends on the nutrient requirements of the crop being grown. The laws specify maximum application rates and not animal stocking rates. Farmers who reduce the N and P component of manure can release pressure on the environment without having to reduce the number of animals. There are alternative systems for housing and manure treatment which generate manures that are easier to handle and have less pollutants or more economic value. Treated animal wastes may also be used as a feedstuff or fuel source.

The present paper reports the results of experiments conducted at author's and some other laboratories regarding to the nutritional management to control environment pollution from animal production.

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PHOSPHORUS CONTROL

Microbial phytase

In broiler chickens, phytase supplementation at a level of 1,000 U/kg diet increased the bioavailability of P and Ca by 60% and 26%, respectively (Simons et al., 1990). The beneficial effects of phytase supplementation were illustrated by Zyla and Korelski (1993). The performance of birds fed available P deficient diets was improved by the addition of phytase to the diets. The *in vitro* activity (i.e. ability to dephosphorylate phytate) was also demonstrated, confirming the proposed mode of action of this enzyme. The direct benefits of dietary phytase supplementation on bone mineralization have been shown by Farrel and Martin (Annison and Choct, 1993) who reported that tibial ash deposition was enhanced in birds fed phytase supplemented diets. Simons and Versteegh (1993) summarized the results of several experiments conducted in Netherlands. A microbial phytase product from *Aspergillus niger* was added to broiler feed with a low inorganic P level. The availability of total P could be increased up to 70%. In comparison with feed with increased levels of inorganic feed phosphates, a significantly larger amount of the P consumed was absorbed. Improved utilization of P decreased its excretion by 40% or more. Growth and feed conversion ratios were comparable with feed to which inorganic feed phosphate was added. In layers the degradation of phytate and the absorption of P was slightly decreased by higher amounts of Ca in the diets (4.0% vs. 3.0% Ca in feed), nevertheless at both levels the efficacy of phytase addition was satisfactory. In broilers up to 500 units of phytase per kg feed, 250 units phytase was equivalent for P absorption with 0.5 g monocalcium phosphorus (MCP) P per kg feed. Addition of up to 300 units phytase per kg feed for laying hens resulted in a minimal equivalency of 0.3 g MCP P per 100 units phytase.

In a feeding trial with laying hens the effectiveness of microbial phytase in diets based on corn-soya and wheat-soya was tested (Peter and Jeroch, 1993). The supplement of phytase (500 U/kg diet) or inorganic P (0.1% of diet) had a positive effect on the performance of the corn-soya group but no effect on that of the wheat-soya group. The highest breaking strength of the egg shell was recorded with hens that received the phytase supplement in the corn-soya group. Mineralization of the tibia bone was also improved with phytase addition. Provided phytate P content and plant phytase activity are taken into account, it should be possible to mix layer diets which require minimum amount of supplementary inorganic P with 250 U phytase supplemented (Um et al., 1998) or do not require supplementary inorganic P sources with 500 U phytase supplemented (Um and Paik, 1998).

Although microbial phytases have shown an ability to enhance P availability in many varied situations, several technical problems still exist that prevent the universal application of such enzymes. These include instability to gastric pH and a severe loss in activity at elevated, present day processing temperatures. Such difficulties present a challenge to producers of microbial phytases, which the tools of biotechnology can address (Power, 1993).

Table 1. Effects of supplemental phytases on the productivity and P excretion of laying birds

Experiment	Level of NPP[1], %	Supplemental phytase, unit	Egg production	Feed/egg mass	P excretion
1	0.37	0	100	100	100
	0.37	500	102.2	99.6	88.5
	0.24	500	100.4	100.4	70.5
	0.12	500	100.4	100.4	59.0
2	0.27	0	100	100	100
	0.22	250	100.3	100.5	88.5
	0.16	250	101.4	98.6	67.3
	0.11	250	99.1	100.3	57.7
3	0.25 (Ca 4%)	0	100	100	100
	0.25 (Ca 4%)	300	103.1	99.8	94.4
	0.25 (Ca 3%)	0	102.1	96.1	102.8
	0.25 (Ca 3%)	300	104.5	91.6	86.1
	0.15 (Ca 4%)	0	97.6	130.4	86.1
	0.15 (Ca 4%)	300	97.2	101.6	72.2
	0.15 (Ca 3%)	0	100.6	89.9	83.3
	0.15 (Ca 3%)	300	101.5	95.0	80.6

[1] Nonphytate phosphorus.

Table 2. Effects of supplemental phytase on the productivity and P excretion of broiler

Experiment	Levelof NPP[1], %		Supplemental phytase, unit	Performance index		
	Stater	Finisher		Gain	Feed/gain	P excretion
1	0.45	0.40	0	100	100	100
	0.34	0.31	600	101.0	98.7	76.2
	0.23	0.21	600	99.3	101.3	54.8
	0.13	0.12	600	96.7	103.3	40.5
2	0.45	0.35	0	100	100	100
	0.35	0.25	0	89.4	100.6	84.8
	0.25	0.15	0	60.5	108.7	51.5
	0.25	0.15	600, Phyt-A[2]	82.2	109.3	39.4
	0.25	0.15	600, Phyt-B[3]	78.9	109.3	45.5
3	0.45	0.35	0	100	100	100
	0.45	0.35	500	99.9	100	107.4
	0.35	0.25	0	87.3	102.5	85.2
	0.35	0.25	500	97.0	100.6	70.4
	0.25	0.15	0	57.3	101.2	81.5
	0.25	0.15	500	65.1	108.1	55.6
4	0.45	0.35	0	100	100	100
	0.45	0.35	600	100.6	100	92.7
	0.35	0.25	0	92.5	103.0	78.6
	0.35	0.25	600	100.5	101.2	72.9

[1] Nonphytate phosphorus, [2] Crude phytase A (soup+cell), [3] Crude phytase B (soup).

Tables 1 and 2 shows summary of the feeding trials conducted with microbial phytases in author's laboratory.

Table 3. Total P, phytate P content and phytase activity of plant origin feedstuffs

Ingredients	Phytate-P mg/100 g	Total-P mg/100 g	Phytate-P % of total P	Pytase activity, U/kg
Corn	60	182	32.7	0.2
Lupin	55	307	17.8	3.2
Tapioca	7	59	11.9	18.8
Wheat	199	295	67.5	1120
Sesame meal	542	816	66.4	3.0
Soybean meal	286	577	49.6	7.5
Cottonseed meal	303	678	44.7	2.4
Cocunut meal	204	539	37.8	350
Corn germ meal	32	130	24.4	12.6
Corn gluten meal	287	536	53.5	170
Corn gluten feed	896	1099	81.5	14.8
Rapseed meal	535	1016	52.7	103
Wheat bran	742	893	83.1	2935
Rice bran	1201	1886	63.7	-
Rice bran (fat-free)	1077	1899	56.7	114

Plant phytase

It is generally accepted that approximately one third of phosphorus in the plant origin feedstuffs are available to monogastric animals. However, proportion of phytate P of total P varies from 12% in tapioca to 83% in wheat bran. Natural phytase content also varies widely from almost none in corn to 2395U in wheat bran (table 3). Such differences should be considered in calculating available P content of diets.

Characteristics of wheat phytase and microbial

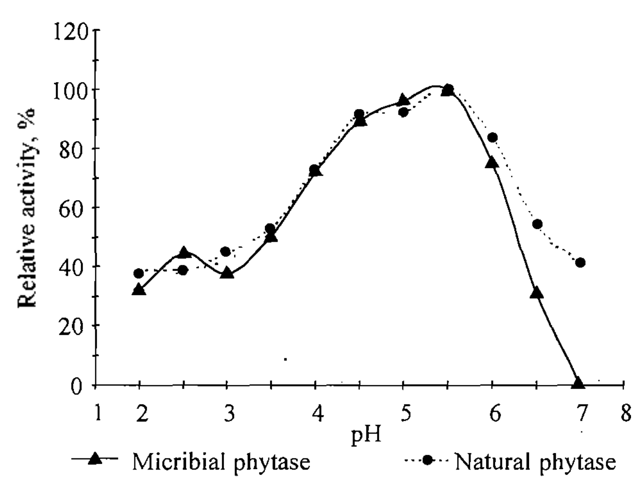

Figure 1. Activity of microbial and plant origin natural phytase at different pH

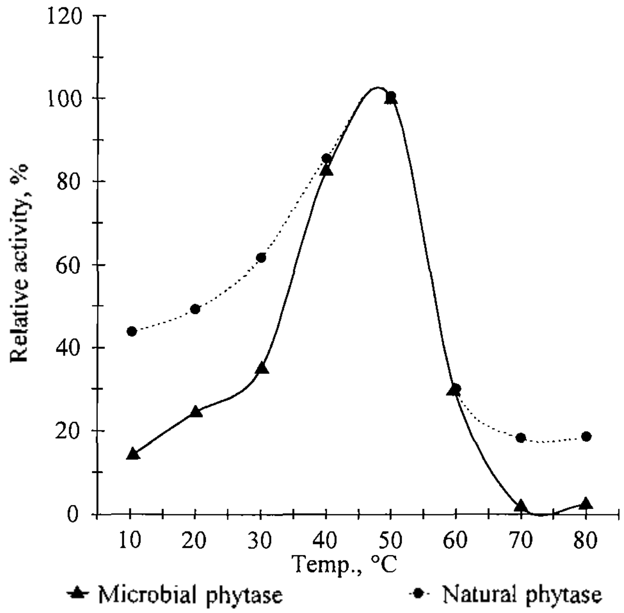

Figure 2. Activity of microbial and plant origin natural phytase at different temperature

phytase were compared. Both of them showed similar characteristics at varying pH and temperature. Maximum activities were achieved around pH 5.5 and 50℃ (figures 1 and 2). Considering these characteristics, plant phytase may be as effective as microbial phytase to the animals.

NITROGEN CONTROL

Amino acids supplementation and protein restriction

Table 4 shows the effect of dietary protein levels and enzyme supplementation on daily N output in broiler. Broilers fed reduced protein diets supplemented with amino acids performed as well as, if not better than, the broilers on the control (normal) protein diets and showed a significant reduction in daily N ouput (24% at 2 wk and 17% at 5 wk).

A similar experiment with laying hens indicated that reducing crude protein levels from 17% to 13.5% with supplementation of synthetic amino acids significantly reduced the daily N output (24.8% and 35.6% in collection 1 and 2, respectively) with no significant effect on egg production except one treatment with phytase (table 5).

Carbohydrase supplementation

Non-starch polysaccharides (NSP) in some feed grains (e.g., pentosans or arabinoxylans in wheat and rye, and β-glucans in barley and oat) are soluble fibers. Their presence can either block digestion of other nutrients (e.g., protein and starch), or can seriously inhibit absorptive capacity. Therefore, the digestibility of NSP is low in monogastric animals (table 6).

It was found that the results of using enzymes (xylanase or b-glucanase) did not stem from complete hydrolysis of the non-starch polysaccharides but that relatively minor hydrolysis altered the ability of the medium to form a viscous solution and act as a barrier to endogenous enzyme activity.

In the past few years a number of different feed enzymes have been developed. The use of multi-enzyme preparations in traditional wheat-based poultry diets was examined (Graham, 1992). The results demonstrated that with a diet based on 60% wheat, a mixed enzyme preparation was capable of increasing the rate of live-weight gain (+17%) and at the same time reducing feed conversion ratio (1.46 to 1.29). There was also an increase in the N utilization

Table 4. The effect of dietary protein and enzymes on the growth performance and daily N output of broilers

Diet description			Weight gain		Feed intake		Feed conversion		Daily N output (g/bird)	
Protein	Phytase	Pentosanase	2 wk	5 wk	2 wk	5 wk	2 wk	5 wk	2 wk	5 wk
Control[1]	no	No	643	2272	758[ab]	2942	1.27[b]	1.81	0.67[abc]	2.12[a]
Control	yes	No	626	2277	794[a]	2907	1.37[a]	1.76	0.73[ab]	1.66[bc]
Control	yes	Yes	641	2326	793[a]	2959	1.33[ab]	1.76	0.80[a]	1.80[bc]
Control	no	Yes	642	2325	761[ab]	2961	1.28[b]	1.77	0.63[bcd]	1.92[ab]
	Average		638	2300	777	2942	1.31	1.78	0.71	1.88
Reduced[2]	no	No	635	2230	747[ab]	2882	1.27[b]	1.82	0.58[bcd]	1.91[ab]
Reduced	yes	No	608	2138	735[b]	2765	1.31[ab]	1.81	0.53[cd]	1.74[bc]
Reduced	yes	Yes	598	2106	754[b]	2747	1.36[a]	1.82	0.48[d]	1.39[cd]
Reduced	no	Yes	646	2250	758[ab]	2894	1.26[b]	1.80	0.56[cd]	1.56[bcd]
	Average		622	2181	749	2822	1.30	1.81	0.54	1.65

[a,b,c,d] $p<0.05$. (Jacob et al., 2000a)

[1] The protein levels of control protein diet were 23% CP in starter and 21% CP in grower.

[2] The Protein levels of reduced protein diet were 21% CP in starter and 17.5% CP in grower. Reduced protein diets were supplemented with synthetic amino acids to meet their requirements.

Table 5. The effect of dietary protein and enzymes on the egg production and daily outputs of dry matter and nitrogen of laying hens

Diet description			Egg production, %	Daily output (g/layer)			
				DM		N	
Protein	Phytase	β-glucanase		Collection 1	Collection 2	Collection 1	Collection 2
17	no	no	85.8^{a}	28.99^{a}	31.40^{a}	1.21^{a}	1.38^{ab}
17	yes	no	86.9^{a}	26.47^{ab}	26.17^{ab}	1.13^{abc}	1.21^{b}
17	yes	yes	89.1^{a}	30.24^{a}	30.92^{a}	1.36^{a}	1.45^{a}
17	no	yes	86.6^{a}	28.74^{a}	29.25^{a}	1.30^{ab}	1.38^{ab}
	Average		87.1	28.61	29.44	1.25	1.36
13.5[1]	no	no	85.0^{ab}	26.79^{ab}	26.17^{ab}	1.06^{bc}	0.98^{c}
13.5	yes	no	78.4^{b}	21.34^{c}	18.67^{c}	0.91^{c}	0.78^{c}
13.5	yes	yes	82.6^{ab}	21.92^{c}	22.53^{bc}	0.86^{c}	0.94^{c}
13.5	no	yes	84.9^{ab}	23.78^{bc}	22.51^{bc}	0.92^{c}	0.79^{c}
	Average		82.73	23.46	22.47	0.94	0.87

^{a,b,c} $p<0.05$. (Jacob et al., 2000b)

[1] 13.5% CP diets were supplemented with synthetic amino acids to meet their requirements.

percentage (37.4 to 45.3%). Such improvements were attainable even after pelleting which in itself was capable of solubilizing starch (Pettersson et al., 1991). A commercial multi-enzyme preparation from *Trichoderma viride* contained 11,150 U/g cellulase, 27,600 U/g glucanase and 37,150 U/g xylanase. This multi-enzyme product was tested with layers fed a barley-based diet (Brufau et al., 1994) and a wheat-based diet (Um et al., 1998). The results showed that barley and wheat can replace corn as an energy source in layer diets if the enzyme is properly supplemented.

For the better utilization of enzymes in feed industry, commercial enzyme preparations should be customized depending on the animal species, age of animals and major feed ingredients. Enzyme products which contain b-glucanase and xylanase in different proportion were produced from *Trichoderma longibrachiatum* and *Bacillus subtilis*. They were used with different diets (wheat-based or barley-based) in different animal species of different ages (poultry, starting pigs or growing-finishing pigs). Enzyme products supplemented to the respective diets reduced the viscosity caused by non-starch polysaccharides and increased amino acid availability as well as energy and P availability (Creswell, 1994). Low and Longland (1990) reported that N retention of pigs was slightly increased by enzyme supplementation.

Contents of moisture and N were lower in the litter of birds given diets supplemented with β-glucanase. Measurement of ammonia release from the litter indicated that when a second flock of birds was raised on the same litter, the presence of a glucanase in the diet reduced the level of ammonia release by 80% (Williams and Kelly, 1994).

An experiment was conducted to test the possible interaction of an enzyme complex and feed antibiotics on growth and metabolic parameters of broilers. The basal diet contained barley at a level of 40%. Both supplements, when added together in the diet, had almost an additive effect on growth parameters, and energy, fat and N utilization (Vukic Vranjes and Wenk, 1993).

Overall nutritional management can result in the reduction of manure output. A proven and more direct method is enzyme supplementation. Reducing the DM content of the digesta in the intestinal tract with supplemental feed enzymes has a marked impact on excreta volume and composition. In a trial offering wheat or wheat/barley-based diets to broilers, excreta weight was reduced by 17 - 28% in fresh or 12 - 15% in DM by supplementation of a multi-carbohydrases enzyme product (table 7). The direct production benefits of lower excreta output and reduced fecal DM are seen in some broiler trials where observations on the frequency of hock lesions and breast blisters are recorded. Reductions in manure output and water content will improve litter quality, and possibly decrease carcass downgrade.

An layer experiment was conducted to evaluate the effect of a microbial enzyme (Roxazyme-G), a multi-carbohydrases preparation, supplementation to the wheat-based layer diets. Diets were formulated to include different levels of wheat replacing yellow corn on isocaloric and isonitrogenous basis. The energy value of wheat in the enzyme supplemented diets was adjusted (spec-modified) to have 5% more ME than the wheat in diets without enzyme. A total of 864 Hy-Line brown layers were assigned to 4 dietary treatments: 10% wheat (T1), 25% wheat (T2), 25%

wheat (spec-modified) + 0.01% Roxazyme-G (T3), and all wheat (spec-modified) + 0.01% Roxazyme-G (T4). Overall performances are shown in Table 8. Hen-day egg productions of T1 and T4 were significantly ($p<0.05$) greater than that of T2 but not different from T3. Hen-housed egg production of T4 was significantly ($p<0.01$) greater than those of T1 and T3 but not different from T2. Egg weights of T1 and T2 were significantly ($p<0.01$) greater than that of T4. Feed consumption of T2 was significantly ($p<0.01$) lower than other treatments. Feed conversion ratio (feed/egg mass) was not significantly different among treatments. Eggshell thickness of T1 was significantly ($p<0.01$) greater than other treatments but ratio of broken eggs was not significantly different among treatments. Haugh unit of T4 was significantly greater ($p<0.05$) than that of T2. Egg yolk color was significantly ($p<0.01$) influenced by treatments in which enzyme treatment potentiated the yolk pigmentation. It was concluded that a multi-carbohydrases supplementation enables complete replacement of yellow corn with wheat without loss of productivity and major egg quality parameters.

Ammonia control

Ammonia release from animal manure should be controlled to avoid air pollution and conserve N in the manure for use as fertilizer. The smell of pig slurry has four times the intensity of cattle, broiler and poultry manure (Pain, 1990). In terms of odour control, ammonia reduction may only play a contributory role since Schaefer (1977) correlated odour intensity with the concentrations of volatile fatty acids (C2-C5), phenol, p-cresol, indole, skatole and ammonia, the highest correlations were obtained with *p*-cresol. Conservation of N in manure is important because P or K usually limit use of poultry manure for crop production and other sources of N are needed when the manure application is limited to needs for fertilizer elements. Ammonia release from manure can be limited by using additives, by drying and by acidic conditions.

Research into minimizing air pollution from animal wastes is continuing and taking many different paths. In the Netherlands, for example, they have identified a microorganism (aerobic denitrifier) which, under aerobic conditions, converts the nitrogen of ammonia and other nitrogen containing compounds into nitrogen gas. Nitrogen gas can be released into the atmosphere without causing pollution problems. Adding such bacteria to manure would reduce the emission of ammonia and reduce the nitrogen content of the manure. They are looking at the possibility of adding these bacteria to the feed (Holthuijzen, 1993).

The ammonia-binding properties of the Yucca extract have been widely studied. The earlier reports on the action of a Yucca extract to prevent the accumulation of ammonia erroneously attributed its

Table 6. Content of non-starch polysaccharides (NSP) in feedstuffs and digestibility of NSP in young chickens

Feedstuffs	NSP, % DM	Digestibility, %
Barley	15	14
Wheat	10	12
Soybean	20	0
Pea	22	18
Bean	23	19
Rapeseed	24	7
Wheat bran	34	9
Sunflower seed	28	17
Rice bran	25	3
Grass	28	5
Corn gluten feed (20%)	31	17

(Charlton, 1996)

Table 7. Excreta output of broilers fed wheat or wheat/barley-based diets with and without multi-enzyme (carbohydrases) supplementation at 19-21 days of age

	Wheat control	Wheat+ enzyme	Wheat /barley control	Wheat /barley +enzyme
Fresh excreta, g	221	184	258	185
Excreta DM, %	42.4	45.2	39.8	47.3
Dry excreta, g	94.0	83.0	102	87.0

(Wyatt, 1995)

Table 8. Overall performance of laying hens fed experimental diets during 20 to 40 wk of age

Parameters	Treatments[1]				SEM
	T1	T2	T3	T4	
Egg production (%, hen-day)	72.7	72.0	71.8	73.4	2.04
Egg production (%, hen-housed)	67.1	67.7	66.2	68.7	2.69
Egg weight, g	59.8	59.7	59.5	59.2	0.58
Feed consumption (g/hen/day)	127.2	125.0	126.9	128.0	1.72
Feed conversion (feed/egg mass)	2.93	2.92	2.97	2.95	0.067
Mortality (%)	7.84	6.05	7.84	6.38	1.979

[1] T1: 10% wheat, T2: 25% wheat+5 ppm Carophyll Red, T3: 25% spec-modified wheat+0.01% Roxazyme-G+5 ppm Carophyll Red, T4: spec-modified wheat (no-restriction)+ 0.01% Roxazyme-G+5 ppm Carophyll Red+25 ppm Carophyll Yellow.

action to an inhibition of urease by its component three steroid saponins, i.e. sarsapogenin, smilagenin and hecogenin. But Headon et al. (1991) reported that the Yucca extract does not inhibit urease activity and that saponin-free De-Odorase had an ammonia-binding capacity similar to that of the unfractionated De-Odorase. Recent work by Headon and Power (unpublished, cited by Leek, 1993) demonstrated that the binding agents in the Yucca extract are glycocomponents. Because the ammonia-binding action starts to decline slowly from fourth day onwards, atmospheric ammonia levels within the houses can be significantly reduced by including this product in the diet.

Zeolite products have been used at a level of 1 to 2% of the diet to improve pelleting quality. It is also believed that zeolite may improve the litter condition and environment of the barn. Due to a high ion-exchange capacity, it is expected that zeolite may bind ammonium ion in the litter (Moon et al., 1991). However, dietary supplementation of zeolite or top dressing of zeolite on the broiler litter did not significantly influence the level of ammonia produced from the broiler litter (Blair and Jacob, unpublished).

Table 9 and figure 3 are summaries of an experiment conducted to reduce ammonia level in the broiler barn. Diets were formulated to have different protein level with or without supplementary amino acids (arginine, threonine and tryptophan) and a probiotic product (*Bacillus subtilis* and Lactobacillus).

Table 9. Feed intake, weight gain, feed/gain and mortality in broiler chickens fed different protein level diets for 35 d

Diets	Feed intake	Weight gain	Feed/ gain	Mortality
T1 (21.5% CP)	2869.8	1636.0[a]	1.76[b]	3.60
T2 (21.5% CP+BIO-211)	2879.9	1626.8[a]	1.77[b]	2.00
T3 (18.5% CP+AA2)	2794.5	1518.6[b]	1.85[a]	0.40
T4 (18.5% CP+AA+BIO-21)	2859.3	1511.6[b]	1.88[a]	2.00
SEM	32.74	12.09	0.02	1.31
	----------- Main effect -----------			
CP				
21.5 %	2874.9	1631.4[a]	1.76[b]	2.80
18.5 %	2826.9	1515.1[b]	1.87[a]	1.20
BIO-21(SP)				
0 %	2832.1	1573.8	1.80	2.00
0.1 %	2869.6	1572.7	1.83	2.00

[a,b] Values with different superscripts in the same column are significantly different ($p<0.05$).

[1] BIO-21 is a commercial probiotics containing *B. subtilis*, Lactobacillus and yeast.

[2] AA: amino acids supplement of arginine, threonine and tryptophan.

MINERAL CONTROL

Chelated minerals

Some micromineral supplements are produced in the form of protected forms. Metal amino acid chelate (Ashmead, 1992), metal proteinate and metal polysaccharide complex are protected minerals. The protected minerals may be more available and not react with digesta due to both their chemical (electrically neutral, ligand and metal make up) and physical structures (size and ligand source). If this is the case, we could use less to achieve the same result. This would be excellent as potentially it would save world resources and reduce pollution (Lowe, 1993).

High levels of copper sulphate have been widely used as growth promotants in pigs and broilers. Copper polysaccharide complex (sequestered Cu) at a level of 62.5 ppm of Cu was as effective as 200 ppm Cu in the form of copper sulphate in weanling pigs and broilers (Paik and Kim, 1993). The performance enhancing effect of methionine-Cu complex at a level of 100 ppm Cu was greater than that of copper sulphate at a level of 200 ppm Cu in broilers. The excretion of copper was significantly less in the

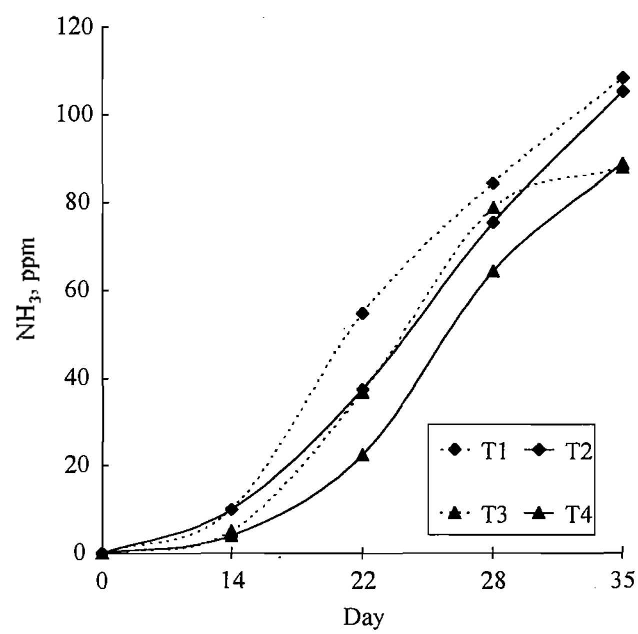

Figure 3. Ammonia level at the litter of broiler barn, determined in the air collected for 60 sec using trapping box of 36 cm L×27 cm W×18 cm H. T1; 21.5% CP, T2; 21.5% CP+BIO-21, T3; 18.5% CP+AA, T4; 18.5% CP+AA+BIO-21

methionine-Cu treatment than in the copper sulphate treatment (Min et al., 1993, 1994).

Table 10 shows summary of feeding trials conducted in author's laboratory to test effects of chelated copper sources on the performance of broiler chickens and pigs.

The effects of copper sources were compared with the results of non-supplemented control groups. In broiler chickens, supplemental copper sulfate at the level of 200 ppm was effective for increasing weight gain in Exp 1 and 5 but not in Exp 3, 4 and 8. Supplementation of chelated coppers (SQM-Cu and Met-Cu) had always positive effect on broiler performance. Supplementation of copper sources had detrimental effect on rats. Met-Cu, SQM-Cu, and copper sulfate had better effect on weight gain in pig than in broiler chickens. The effect of Met-Cu on the performance of broiler chicks was high compared to those of fish meal-Cu (FM-Cu) or sodium alginate-Cu (SA-Cu). Considering the results of experiments 6 (pig) and 8 (broiler chickens), it appears that dietary level of 100~125 ppm copper in chelated form is enough to improve the performance of pigs and chickens. This will result in reduction of fecal Cu excretion to the environment. Growth-stimulating action of dietary Cu has been attributed to its antimicrobial actions (Fuller et al., 1960; Vogt et al., 1981). However, the antimicrobial hypothesis alone can not fully explain the effects of Cu. It has been demonstrated that intravenous injection of Cu stimulates the growth of weanling pigs (Zhou et al., 1994). The results of this experiment indicated that Cu acts systemically to influence the growth regulatory system in many ways. Kratzer and Vohra (1986) reported that metal ion chelated with low molecules of peptide such as amino acid or organisms have more stable, neutral electrocity and therefore, chelated minerals is easier to pass through intestinal wall than natural minerals.

Table 10. Effects of supplementary copper chelates

Experiment	Animals	Source and level of Cu, ppm	Difference from the control, %		
			Gain	Feed intake	Feed /gain
1	Broilers	CuSO4, 200	3.8	-0.2	-4.0
		SQM-Cu, 63.5	2.6	0.6	-3.5
		SQM-Cu, 127	3.5	-0.2	-4.0
		SQM-Cu, 191	3.8	1.7	-2.0
2	Pigs	CuSO4, 200	6.4	4.0	-3.1
		SQM-Cu, 63.5	4.1	0.9	-3.5
		SQM-Cu, 127	7.5	3.1	-4.8
3	Broilers	CuSO4, 200	-0.9	-0.6	0.0
		Met-Cu, 200	2.5	-0.5	-3.3
		SA-Cu, 200	1.1	-0.3	-1.6
4	Broilers	CuSO4, 200	0.3	-2.0	-2.2
		Met-Cu, 200	2.1	-0.5	-2.2
		FM-Cu, 200	0.2	-1.4	-1.7
	Rats	CuSO4, 200	-7.0	-5.3	1.9
		Met-Cu, 200	-7.5	-3.7	3.8
		FM-Cu, 200	-6.3	-3.0	3.4
5	Broilers	CuSO4, 200	4.3	-1.4	-5.7
		Met-Cu, 200	6.3	3.1	-3.2
		Met-Cu-Zn-Fe, 200	2.2	0.0	-1.9
		FM-Cu, 200	2.0	1.2	-0.6
6	Pigs	CuSO4, 200	7.5	-5.9	-3.8
		Met-Cu, 100	10.1	0.4	-1.9
		Met-Cu, 200	8.2	9.6	0.0
7	Pigs	CuSO4, 200	-3.8	2.6	-4.6
		Met-Cu, 200	-1.9	-7.2	-9.8
		FM-Cu, 200	0.0	-16.4	-4.6
8	Broilers	CuSO4, 250	-1.5	-2.2	-0.6
		Met-Cu, 125	4.6	2.3	-2.5
		Met-Cu, 250	4.0	2.4	-1.8

Table 11 shows the effects of supplemental chelated zinc in weanling pigs. Chelated zinc had a similar solubility with chelated copper. In experiment 1, dietary levels of methionine chelated zinc at 100 and 200 ppm and zinc oxide at 100 ppm in zinc increased weight gain and feed intake compared to the control (100 ppm of Zn in ZnO) but only 200 ppm of zinc in the form of chelated zinc improved feed to gain ratio compared to the control. In experiment 2, 1,000 or 2,000 ppm of dietary zinc in zinc oxide increased weight gain and feed intake but high dietary level of Zn (1,000 and 2,000 ppm) in the form of methionine chelate had diminishing effect on weight gain and feed intake compared to 100 ppm of Zn in the form of methionine chelate. One hundred ppm of Zn in the form of methionine chelate showed highest

Table 11. Effects of supplementary zinc oxide and Zn chelates in weanling pigs

Experiment	Source and level of Zn, ppm	Difference from the control (ZnO, 100 ppm), %		
		Gain	Feed intake	Feed/gain
1	ZnO, 200	8.3	9.6	0.5
	Met-Zn*, 100	3.0	3.0	0.5
	Met-Zn, 200	18.8	15.1	-3.8
2	ZnO, 1000	10.0	9.2	-1.4
	ZnO, 2000	11.0	12.8	1.4
	Met-Zn, 100	15.6	15.9	0.0
	Met-Zn, 1000	6.5	12.2	7.6
	Met-Zn, 2000	-2.0	9.4	11.7

* Met-Zn; methionine-Zn chelate.

Table 12. Result of laying performance of hens fed mineral-methionine chelate diets for 8 wks (96~103 wks of age)

	Treatment*					SEM
	Control	Zn-Met	Cu-Met	Zn-Met +Mn-Met	Zn-Met +Mn-Met +Cu-Met	
Hen-day eggproduction, %	69.97[ab]	67.13[c]	72.57[a]	68.37[bc]	71.67[a]	0.956
Hen-housed eggproduction, %	68.80[bc]	65.61[d]	72.57[a]	67.48[cd]	70.82[ab]	0.997
Egg weight, g	70.27[ab]	70.77[a]	69.33[c]	69.72[bc]	70.04[abc]	0.253
Feedintake, g/hen/day	127.9[ab]	127.3[ab]	129.2[ab]	126.9[b]	130.4[a]	1.048
Feed conversion ratio, g/100g egg mass	2.62[ab]	2.71[a]	2.58[b]	2.68[ab]	2.60[ab]	0.036

* Mineral-methionine chelate was supplemented at the level of 100 ppm of each mineral.

Table 13. Result of eggshell quality of hens fed mineral-methionine chelate diets for 8 wks (96~103 wks of age)

	Treatment*					SEM
	Control	Zn-Met	Cu-Met	Zn-Met +Mn-Met	Zn-Met +Mn-Met +Cu-Met	
Albumin hight, mm	6.305	6.259	6.375	6.296	6.382	0.0607
Haugh unit	74.96	74.55	75.46	74.63	75.77	0.5010
Specific gravity	1.0845[b]	1.0851[b]	1.0864[a]	1.0847[b]	1.0851[b]	0.0004
Eggshell strength, kg/cm^2	0.540[c]	0.546[bc]	0.573[a]	0.562[ab]	0.562[ab]	0.0065
Eggshell thickness, mm	0.366	0.375	0.374	0.376	0.369	0.0034
Soft egg production, %	0.59[ab]	0.78[ab]	0.31[b]	1.07[a]	0.60[ab]	0.1751
Broken eggproduction, %	3.81[c]	4.76[bc]	4.71[bc]	6.90[a]	5.59[ab]	0.5178

* Mineral-methionine chelate was supplemented at the level of 100 ppm of each mineral.

Table 14. Result of IgG, gizzard erosion index and mineral contents in liver of hens fed mineral-methionine chelate diets for 8 wks (96~103 wks of age)

	Treatment*					SEM
	Control	Zn-Met	Cu-Met	Zn-Met +Mn-Met	Zn-Met +Mn-Met +Cu-Met	
IgG, mg/ml	24.3	25.5	24.5	26.2	24.0	1.77
Gizzard erosion index	0.30[c]	0.50[bc]	1.25[a]	0.90[ab]	1.35[a]	0.18
Mineral contents in liver						
Zinc	99.50[ab]	104.53[a]	96.41[ab]	95.06[ab]	82.80[b]	6.33
Copper	12.57	12.32	14.86	12.75	14.11	2.18
Manganese	10.25	10.11	10.29	10.32	11.29	0.72

* Mineral-methionine chelate was supplemented at the level of 100 ppm of each mineral.

weight gain and feed intake.

Tables 12, 13 and 14 show the effect of supplementary Zn, Cu and Mn chelated with methionine in laying hens. One hundred ppm Cu in the form of methionine chelate was most effective in improving egg production, feed efficiency and egg shell quality. Zn-Met and Mn-Met+Zn-Met did not improve laying performance and egg quality. Combination of Zn-Met+Mn-Met+Cu-Met did not have any additive effects in the performance of layers.

REFERENCES

Annison, G. and M. Choct. 1993. Enzymes in poultry diets. Enzymes in Animal Nutrition (Ed. C. Wenk and M. Boessinger). Proceedings of the 1st Symposium. Kartause Ittingen, Switzerland. pp. 61-68.

ApSimon, H. M., M. Kruse and J. N. B. Bell. 1987. Ammonia emissions and their role in acid deposition. Atmos. Enviro. 21:1939-1946.

Ashmead, H. D. 1992. The Roles of Amino Acid Chelates in Animal Nutrition. Albion Laboratories, Inc. Clearfield, Utah.

Brufau, J. R. Cos, A. Perez-Vendrell and E. Esteve-Garcia. 1994. Performance of laying hens as affected by the supplementation of a barley-based diet with a crude enzyme preparation from *Trichoderma viride*. Can. J. Anim. Sci. 74:129-133.

Charlton, P. 1996. Expanding enzyme applications: higher amino acid and energy values for vegetable proteins. In: Proceedings of the 12th Annual Symposium on Biotechnology in the Feed Industry (Ed. T. P. Lyons and K. A. Jacques). Nottinghan Unversity Press. pp. 349-354.

Christensen, S. 1983. Nitrous oxide emission from soil under permanent grass: seasonal and diurnal fluctuations as influenced by manuring and fertilization. Soil Biol. Biochem. 15:531-536.

Creswell, D. 1994. Use of enzymes with wheat or barley in broiler, layer and swine feeds. Proceedings of Seminar, Finnfeeds International Ltd. And Jeil Vet. Chem. Co. Ltd. Seoul, Korea.

Fuller, R., L. M. G. Newland, C. A. E. Briggs, R. Braude and K. G. Mitchell. 1960. The normal intestinal flora of the pigs. IV. The effect of dietary supplements of penicillin, chlorotetracycline or copper on the fecal flora. J. Appl. Bacteriol. 23:195.

Graham, H. 1992. Enzymes for wheat-based diets. Poultry International, May. pp. 72-77.

Headon, D. R., K. Buggle, A. Nelson and G. Killeen. 1991. Glycofractions of the Yucca plant and their role in ammonia control. In: Biotechnology in the Feed Industry (Ed. T. P. Lyons and K. Y. Nicholasville). Proceedings of Alltech's 7th Annual Symposium. pp. 95-108.

Holthuijzen, Y. A. 1993. Environmental effects of the microbial degradation of poultry manure. World's Poultry Sci. J. 49:173-174.

Jacob, J. P., S. Ibrahim, R. Blair, H. Namkung and I. K. Paik. 2000a. Using enzyme supplemented, reduced protein diets to decrease nitrogen and phosphorus excretion of broilers. Asian-Aus. J. Anim. Sci. 13(11): 1561-1567.

Jacob, J. P., S. Ibrahim, R. Blair, H. Namkung and I. K. Paik. 2000b. Using enzyme supplemented, reduced protein diets to decrease nitrogen and phosphorus excretion of White Leghorn hens. Asian-Aus. J. Anim. Sci. 13(12):1743-1749.

Kratzer, F. H., and P. Vohra. 1986. Chelates in Nutrition. CRC Press, Inc., Boca Ration, Florida.

Leek, B. F. 1993. The problem of nitrogen waste products in animal production: Investigations into the mode of action of certain glycocomponents capable of manipulating nitrogen. In: Biotechnology in the Feed Industry Ibid. pp. 307-330.

Low, A. G. and A. C. Longland. 1990. Carbohydrate and dietary fiber digestion in the pig and the possible influence of feed enzymes. Feed Compounder. 10:37-42.

Lowe, J. 1993. Protected minerals, an expensive luxury or a cost-effective necessity? Biotechnology in the Feed Industry. Ibid. pp. 61-70.

Lyons, T. P. and G. A. Walsh. 1993. Applications of enzymes in feed manufacturing. Enzymes in Animal Nutrition. Ibid. pp. 241-254.

Min, S. K., H. Namkung and I. K. Paik. 1993. Effects of supplementary copper complexes on the performance of broiler chickens. Korean J. Anim. Nutr. Feed. 17(5): 247-257.

Min, S. K., J. S. Um and I. K. Paik. 1994. Effects of supplementary methionine-copper and protein-copper complexes on the growth, mineral metabolism and intestinal microflora of broiler chickens and rats. Korean J. Anim. Nutr. Feed. 18(2):103-113.

Moon, Y. Y., H. Namkung, M. B. Chang and I. K. Paik. 1991. The effects of zeolite supplementation on the performance of broilers fed diets containing moldy corn. Korean J. Anim. Nutr. Feed. 15(4):170-175.

Paik, I. K. and Y. K. Kim. 1993. The effects of supplemental sources of copper on the performance of weanling pigs and broilers. Proceedings of VII World Conference on Animal Production, Edmonton, Alberta, Canada. 2:395-393(Abst.).

Pain, B. F. 1990. Don't kick up a stink. Reported in Stock Magazine (UK) 3(4):4.

Peter, W. and H. Jeroch. 1993. The effectiveness of microbial phytase addition to layer rations on maize and wheat basis. Enzymes in Animal Nutrition. Ibid. pp. 206-209.

Pettersson, D., H. Graham and P. Aman. 1991. The nutritive value for broiler chickens of pelleting and enzyme supplementation of a diet containing barley, wheat and rye. Anim. Feed Sci. Technol. 33:1-14.

Power, R. 1993. Phytase: The limitations to its universal use and how biotechnology is responding. In: Biotechnology in the Feed Industry. Ibid. pp. 355-368.

Schaefer, J. 1977. Sampling, characterization and analysis of malodours. Agric. Environ. 3:121-127.

Simons, P. C. M. and H. A. J. Versteegh. 1993. Role of phytase in poultry nutrition. In: Enzymes in Animal Nutrition (Ed. C. Wenk and M. Boessinger). Proceedings of the 1st Symposium. Kartause Ittingen. Switzerland. pp. 181-186.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. a. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Buedeker and G. J. Verchoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 66:525-540.

Um, J. S., S. H. Ahn and I. K. Paik. 1998. Effects of a microbial enzyme supplementation on the performance of laying hens fed diets containing different levels of wheat. Asian-Aus. J. Anim. Sci. 11(6):702-707.

Um, J. S., I. K. Paik M. B. Chang and B. H. Lee. 1999. Effects of microbial phytase supplementation to diets with low non-phytate phosphorus levels on the performance and bioavailability of nutrients in laying hens. Asian-Aus. J. Anim. Sci. 12(2):203-208.

Um, J. S. and I. K. Paik. 1999. Effects of Microbial Phytase Supplementation on Egg Production, Eggshell Quality, and Mineral Retention of Laying Hens Fed Different Levels of Phosphorus. Poultry Sci. 78:75-79.

Vogt, H., S. Matthes and S. Harnisch. 1981. Preservatives organic acids in broiler and laying rations. Conf. On Feed Additives, Budapest, Hungary.

Vukic Vranjes, M. and C. Wenk, 1993. Influence of dietary enzyme complex on broiler performance in diets with and without antibiotics supplementation. Enzymes in Animal Nutrition. Ibid. pp. 152- 155.

Williams, P. E. V. and J. M. Kelly. 1994. Animal production and pollution problems. In: Livestock Production for the 21st Century: Priorities and Research Needs (Ed. P. A. Thacker). pp. 159-186.

Wyatt, C. L. 1995. The use of feed enzymes in poultry diets to improve nutrient digestibility. Proceedings of 1995 Maryland Nutrition Conference for Feed Manufacturers. pp. 37-45.

Zhou, W., E. T. Kornegay, M. D. Lindemann, J. W. G. M. Swinkels, M. K. Welton and E. A. Wong. 1994. Stimulation of growth by intravenous injection of copper in weanling pigs. J. Anim. Sci. 72:2395-2403.

Zyla, K. and J. Koreleski. 1993. In-vitro and in-vivo dephosphorylation of rapeseed meal by means of phytate-depleting enzymes derived from *Aspergillus niger*. J. Sci. Food Agric. 61:1-6.