Nutritional Management to Control Environmental Impact in the Sustainable Animal Production

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Abstract

The animal industry must be environmentally sound to insure its long-term sustainable growth. 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 can be reduced by using a low crude protein diet along with the supplementation of probiotics products. Excretion of minerals, which are 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 diets of broilers, layers and/or pigs.

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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 these plants 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 that contribute to the accumulation of greenhouse gas. Volatilization of ammonia is the cause of the acid rain that resulted 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 odors that 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 and zinc in the pig diets (Paik, 2001) can cause accumulation of these minerals in the soil. Major efforts are required to adopt all the 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. The present paper reports the results of experiments conducted at the author's laboratory regarding the nutritional management to control environment pollution from animal production.

Experiment

1.Phosphorus Control

1) Microbial Phytase

Layer experiments

It has been reported that hens consuming the low nonphytate P (NPP) diet with supplementary phytase performed as well as the hens fed with diets containing higher levels of NPP without supplementary phytase (Gordon and Roland, 1997;Van der Klis and Versteegh, 1996). 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 eggshell 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. The response to the level of supplementary phytase was quadratic. Supplementation of 250 U of phytase/kg diet in laying hens was equivalent to 0.8g of P from monocalcium phosphate (MCP) (Van der Klis et al, 1994) while supplementation of 500 U to a corn-soybean meal diet was equivalent to 1g of P (Peter and Jeroch, 1993). Simons et al. (1992) reported that supplementation of diets with 250 U of phytase/kg resulted in the degradation of 62% and 56% of the phytate-P at low and high Ca levels, respectively. Increasing phytase from 250 to 500 U/kg of diet had a further effect on degradation, increasing it by 16% and 11% at respective Ca level. Three layer experiments were conducted to determine if microbial phytase supplementation can reduce non-phytate phosphorus (NPP) level in a practical laying diet and result in concomitant reductions in P excretion. Following in Table 1 are abstracts of layer feeding trials conducted at the author's laboratory.

In Layer Experiment 1, supplementation of the microbial phytase to normal corn-soybean diet improved egg production and can reduce TCP level in the diet without affecting egg production and egg quality. Significant reduction of P excretion can also be achieved. (Um and Paik, 1999)

In layer Experiment 2, the NPP concentration in the diet of Brown layers consuming about 130g/d of feed can be safely lowered from 0.27% (0.55% total P) to 0.16% (0.45% total P) and excretion of P was also reduced by the inclusion of 250 U phytase/kg of diet. (Um et al, 1999)

In Layer Experiment 3, supplementation of microbial phytase at a level of 300U per kg diet of laying hens can improve egg production, decrease broken and soft egg production rate and P excretion. The level of Ca and NPP significantly modifies the effects of phytase supplementation. (Lim et al, 2002)

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., 1999) or do not require supplementary inorganic P sources with 500 U phytase supplemented (Um and Paik, 1999). 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. 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.

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
Layer-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
Layer-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
Layer-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

[1]Nonphytate phosphorus.

Broiler experiments

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 with 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 with 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 broilers up to 500 units of phytase per kg feed, 250 units phytase was equivalent for P absorption with 0.5 g of P from MCP per kg feed. The following Table 2 presents abstracts of broiler feeding trials conducted at author's laboratory.

In Broiler Experiment 1, NPP level of corn-soy broiler diets can be safely lowered by approximately 0.2% by supplementing 600 U of microbial phytase/kg diet. With the adjusted level of NPP and phytase supplementation, P excretion could be reduced by 50%. (Um et al, 2000).

In Broiler Experiment 2, lowering NPP level in the broiler diet significantly depressed the performance. Supplementation of crude phytase preparation produced from Aspergillus ficuum could partially recover the depression. (Paik et al., 2000)

In Broiler Experiment 3, dietary phytase could reduce P excretion and alleviate adverse affects caused by feeding low dietary NPP. Effects of phytase supplementation were greater in the lower NPP diets. (Lim et al, 2001)

In broiler Experiment 4, supplementation of the crude phytase, produced from the broth of *Aspergillus ficuum* culture, to broiler diets containing low NPP level improved growth performance and mineral availability and reduced fecal P excretion. (Lee et al, 2000a).

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

Experiment	Level of NPP[1], %		Supplemental	Performance index		
	Starter	Finisher	phytase, unit	Gain	Feed/gain	P excretion
Broiler Exp.1	0.45	0.40	0	100	100	100
	0.34	0.31	600	101.0	98.7	76.2
	0.23	0.22	600	99.3	101.3	54.8
	0.12	0.13	600	96.7	103.3	40.5
Broiler Exp.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
Broiler Exp. 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
Broiler Exp.4	0.45	0.35	0	100	100	100
	0.45	0.35	600, Phyt-B[3]	100.6	100	92.7
	0.35	0.25	0	92.5	103.0	78.6
	0.35	0.25	600, Phyt-B[3]	100.5	101.2	72.9

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

2) 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 widely from 12% in tapioca to 83% in wheat bran. Natural phytase content in the feedstuffs also varies widely from almost none in corn to 2395U in wheat bran (Lee et al, 1999). Such differences should be considered in calculating available P content of diets. Three experiments have been conducted in the author's laboratory to study the characteristics of plant phytase and its application to feeding of broilers. The following are abstracts of experiments.

Plant Phytase Experiment 1

An experiment was conducted to measure the contents of phytate-P, total-P and phytase activity of cereals and cereal by-products. The effects of pH and temperature on the activity of wheat and microbial phytase were compared. Phytate-P content was higher in most cereal by-products than in cereal. Rice bran had the highest phytate-P (1,201mg/100g) followed by defatted rice bran (1,077mg/100g), corn gluten feed (896 mg/100g), wheat bran (742mg/100g) and rapeseed meal (535 mg/100g). The phytate-P contents of other ingredients were lower than 500 mg/100g. Total-P content was high in defatted rice bran (1,899 mg/100g), rice bran (1,889 mg/100g), and rapeseed meal (1,016 mg/100g) compared to other ingredients. Wheat and wheat bran had the highest phytase activity (1,121.9 and 2,935.1U/kg) among ingredients tested (Table 3).

Characteristics of wheat phytase and microbial phytase were compared. Both of them showed similar characteristics at varying pH and temperature. Maximum activities were achieved around pH5.5 and 50∞C (Figure 1 and 2). Considering these characteristics, plant phytase may be as effective as microbial phytase to the animals.

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

Ingredients	Phytate-P /100g	Total-P /100g	Phytate-P % of total P	Phytase activity, U/
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

(Lee et al., 1999)

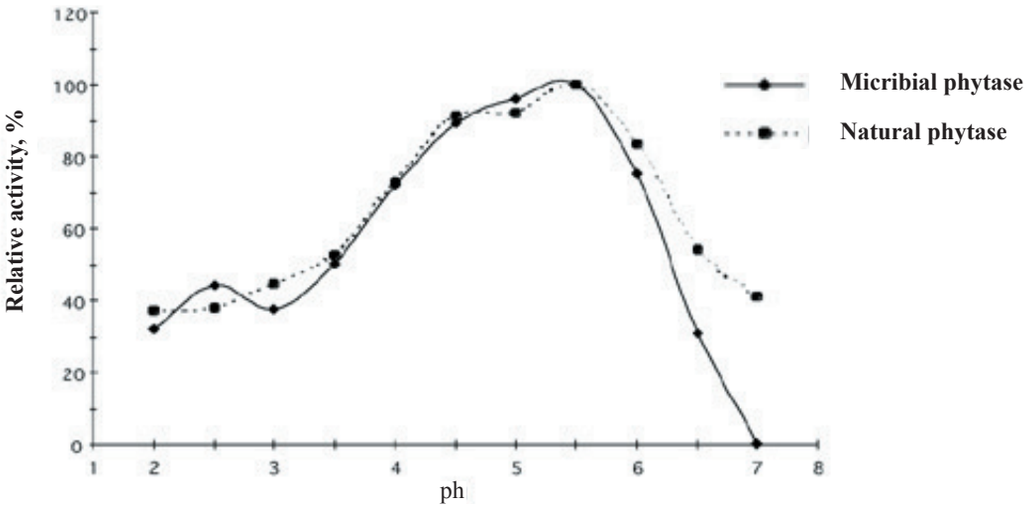


Fig 1. Activity of microbial and plant origin natural phytase at different pH

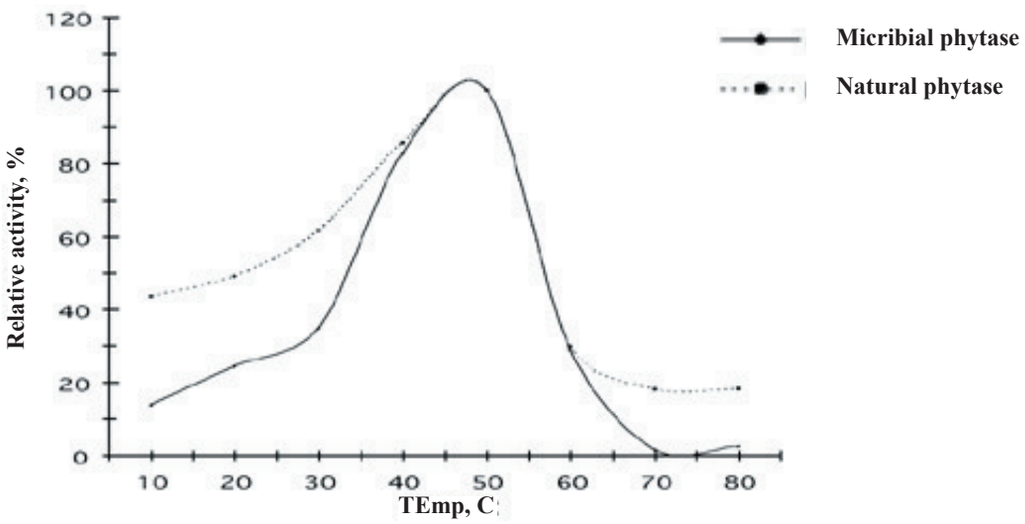


Fig 2. Activity of microbial and plant origin natural phytase at different temperature

Plant Phytase Experiment 2

This study was conducted to evaluate the efficacy of wheat and wheat bran as the source of phytase in a 5-week broiler feeding trial. One thousand day-old broiler chickens (Ross®) were divided into 20 pens of 50 broilers(25 male and 25 female) each. Four pens were randomly arranged to one of the five dietary treatments: T1, control diet containing normal NPP level ; T2, T1- 0.1% NPP; T3, T2£´600IU microbial phytase(NOVO®) per kg diet; T4, T2 £´600IU plant phytase from wheat and wheat bran; and T5, T2£´600IU plant phytase from wheat and hydrothermally treated wheat bran. Table 4 shows details of the feed formula of starter diets. Reduction of NPP level by 0.1% (T2) reduced weight gain and feed intake but plant phytase treatments (T4 and T5) recovered the lost performance. Plant phytase treatments showed better performance than the microbial phytase treatment (T3). There was no difference between regular wheat bran treatment (T4) and hydrothermally treated wheat bran treatment(T5). Mortality was highest in low NPP diet (T2) (Table 4). Availability of ether extract and crude ash of grower diet was highest in normal wheat bran diet (T4). Availability of Ca and P of grower diet was highest in T4 followed by T3 and T5. Availability of Mg, Fe and Zn was more drastically improved by phytase treatments (T3, T4 and T5) (Table 5). Excretion of Ca, P, Mg, Fe and Zn was lowest in microbial phytase treatment (T3). Serum level of Ca and Mg was highest in the low NPP treatment (T2). Tibial ash content of T2 and T3 was lower than that of T1, T4 and T5. However, tibial Ca content was higher in T1 and T2 than other treatments. Tibial P and Mg contents were highest in T1. It was concluded that plant phytase from wheat and wheat bran can be effectively used to improve P utilization of the broilers fed low NPP diets. Plant phytase improved the availability of crude ash and minerals such as Ca, P, Mg, Zn and Fe of the diets. Hydrothermal treatment of wheat bran prior to inclusion in the diet had no beneficial effects (Kim et al, 2002).

Table 4. Body weight gain, feed intake, feed/gain and mortality of broiler chickens fed experimental diets from 1 to 35 days

Item	Age (day)	Treatments[1]					SEM
		T1	T2	T3	T4	T5	
Weight gain, g/bird	1-21	703.8[a]	577.1[c]	654.5[b]	683.6[a]	688.0[a]	8.89
	22-35	906.9[a]	712.3[c]	797.0[b]	880.7[a]	904.1[a]	18.06
	1-35	1610.7[a]	1289.5[c]	1451.5[b]	1564.3[a]	1592.1[a]	23.44
Feed intake, g/bird	1-21	973.6[a]	814.8[c]	919.5[b]	951.7[a]	965.6[a]	9.42
	22-35	1674.6[a]	13545.0[c]	1487.0[b]	1642.2[a]	1707.2[a]	35.69
	1-35	2648.2[a]	2160.6[c]	2407.4[b]	2594.9[a]	2672.8[a]	44.01
Feed/gain (g/g)	1-21	973.6[a]	814.8[c]	919.5[b]	951.7[a]	965.6[a]	9.42
	22-35	1674.6[a]	13545.0[c]	1487.0[b]	1642.2[a]	1707.2[a]	35.69
	1-35	2648.2[a]	2160.6[c]	2407.4[b]	2594.9[a]	2672.8[a]	44.01
Mortality, %	1-21	2.00	3.50	3.00	1.50	3.50	0.97
	22-35	0.00	3.62	2.06	0.00	1.04	0.92
	1-35	2.00[b]	7.00[a]	5.00[ab]	1.50[b]	4.50[ab]	1.27

[1]T1 = control diet containing normal NPP level, T2 = control diet - 0.1% NPP, T3 = control diet – 0.1% NPP + 600IU microbial phytase, T4 = control diet - 0.1% NPP + 600IU natural phytase(wheat +wheat bran), T5 = control diet - 0.1% NPP + 600IU natural phytase(wheat + soaked wheat bran)

[a-c] Values with different superscripts in the same row are significantly different ($p<0.05$).

Table 5. Availability of Ca, P, Mg, Fe and Zn of broiler grower diets

Item(%)	Treatments[1]					SEM
	T1	T2	T3	T4	T5	
Calcium	26.0[d]	26.7[d]	46.1[b]	57.2[a]	38.9[c]	1.93
Phosphorus	33.5[b]	35.1[b]	47.5[a]	49.9[a]	37.0[b]	2.08
Magnesium	8.2[b]	13.9[b]	31.1[a]	30.7[a]	26.3[a]	2.10
Iron	3.36[d]	3.69[d]	15.98[c]	37.64[a]	23.04[b]	1.80
Zinc	17.6[c]	8.0[d]	29.0[b]	42.7[a]	21.3[b]	1.92

[1]T1 = control diet containing normal NPP level, T2 = control diet - 0.1% NPP, T3 = control diet – 0.1% NPP + 600IU microbial phytase, T4 = control diet - 0.1% NPP + 600IU natural phytase(wheat +wheat bran), T5 = control diet - 0.1% NPP + 600IU natural phytase(wheat + soaked wheat bran)

[a-d] Values with different superscripts in the same row are significantly different ($p<0.05$).

Plant Phytase Experiment 3

An in vitro test and a broiler feeding trial have been conducted to test the effect of hydrothermal treatment of wheat bran on phytate-P degradation and its feeding effect in broiler. Hydrothermal treatment of wheat bran was carried out at 55 ° with pH 5.5 buffer. Phytate-P content of wheat bran showed quadric decrease as the rate of wheat bran : buffer ratio increased from 1:0.5 to 1:5. Phytate-P degradation was not significantly affected by incubation time above 10 min., drying temperature (55°, 65° and 75°) or pH of buffer (5.5 and 7.0). Feeding trial was conducted with 240 sex separated day-old broiler chickens (Ross ®). Broilers were randomly housed to 24 cages of 10 birds each. Six cages (3 in each sex) were assigned to 4 treatments control; normal level of non-phytate-P (NPP), LP; low NPP treatment which has 0.1% lower NPP than the control, LPWB; LP with wheat bran, which provides 500IU of plant phytase per kg diet, LPHWB; LP with hydrothermally treated wheat bran. Results of feeding trial showed that broilers of LP treatment gained significantly lower than other treatments in starter period (1~21d) but only male broilers of LP gained significantly lower than the control in grower (22~35d) and overall period (Table 6). There were no significant differences among the birds of LPWB, LPHWB and control. Feed intake of overall period was not significantly different between LPWB and control but that of LP was lower than LPHWB and that of LPHWB was lower than control. Feed conversion ratio was significantly lower in LPHWB and LP than in control and LPWP. Mortality was highest in LPHWB. Utilizability of crude fat, crude ash and Ca was significantly lower but that of Fe was significantly higher in LP than other treatments. Utilizability of P, Mg and Zn was higher in LPWB and LPHWB than in control and LP. Excretion of P was significantly lower in low NPP treatments than in control (Table 7). Serum Ca level was highest but serum P level was lowest in LP. Tibial crude ash content was high in wheat bran treatments but tibial Ca content was high in control and LP. Tibial P content of LP and LPWB was lower than control. However, Tibial content of Fe was highest in LP. It was concluded that wheat bran, a source of plant phytase, can be used in low NPP broiler diet to prevent the depression of performance. Reduction of P excretion can be achieved concomitantly. Hydrothermal treatment of wheat bran was effective in improving utilizability of some minerals but was not effective in improving performance of broilers (Kim and Paik, 2002).

Table 6. Body weight gain of broiler chickens fed experimental diets from 1 to 35 days. (g/bird)

Age (day)	Sex	Treatments[1]				SEM
		T1	T2	T3	T4	
1-21	Male	773.3	704.0	772.6	766.9	23.36
	Female	719.6	678.4	709.7	729.7	17.27
	All	746.5[a]	691.2[b]	741.2[a]	748.3[a]	16.73
22-35	Male	874.8[a]	811.9[b]	863.8[ab]	866.8[ab]	18.04
	Female	782.7	764.9	773.7	790.2	19.64
	All	828.8	788.3	818.8	828.5	21.24
1-35	Male	1648.2[a]	1515.9[b]	1636.5[ab]	1633.7[ab]	36.69
	Female	1502.3	1443.6	1483.4	1502.3	23.57
	All	1575.2	1496.6	1559.9	1576.8	34.15

[1]T1 = control diet containing normal nonphytate P(NPP) level, T2 = control diet - 0.1% NPP, T3 = control diet - 0.1% NPP + 500IU plant phytase(wheat bran), T4 = control diet - 0.1% NPP + 500IU plant phytase(hydrothermally treated wheat bran)
[a,b] Values with different superscripts in the same row are different ($p<0.05$).

Table 7. Excretion of Ca, P, Mg, Fe and Zn of broilers fed grower diets

Item	Treatments[1]				SEM
	T1	T2	T3	T4	
	---------- g/bird/d ----------				
Calcium	0.396	0.363	0.346	0.348	0.018
Phosphorus	0.234[a]	0.174[b]	0.168[b]	0.172[b]	0.008
Magnesium	0.108	0.115	0.105	0.102	0.005
	---------- /bird/d ----------				
Iron	93.71	91.94	99.16	101.02	4.74
Zinc	48.67	46.52	48.24	48.42	2.12

[1]T1 = control diet containing normal nonphytate P(NPP) level, T2 = control diet - 0.1% NPP, T3 = control diet - 0.1% NPP + 500IU plant phytase(wheat bran), T4 = control diet - 0.1% NPP + 500IU plant phytase(hydrothermally treated wheat bran)
[a,b] Values with different superscripts in the same row are different ($p<0.05$).

2. Nitrogen Control

1) Amino Acids Supplementation and Protein Restriction

The effects of protein levels and enzyme supplementation to corn-soy diets on daily N output in broiler are shown in Table 8.. Broilers fed with 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 output (24% at 2 wk and 17% at 5 wk).

A similar experiment with laying hens indicated that reducing crude protein levels of corn-soy diets 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 9).

Table 8. 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

[1]The protein levels of control protein diet were 23% CP in starter and 21% CP in grower.
[2]The potein 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.
[a-d] Values with different superscripts in the same column are different ($p<0.05$).
(Jacob, et al. 2000a)

Table 9. 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

[1]13.5% CP diets were supplemented with synthetic amino acids to meet their requirements.
[a-c] Values with different superscripts in the same column are different ($p<0.05$).
(Jacob, et al. 2000b)

2) Carbohydrase Supplementation

Non-starch polysaccharides (NSP) in some feed grains (e.g. pentosans or arabinoxylans in wheat and rye, and b-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. 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 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 with a barley-based diet (Brufau et al., 1994) and a wheat-based diet (Um et al., 1998). The results showed that barley and wheat could 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 that contained β -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. 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.

A 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 10.

Table 10. 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)	74.65[a]	73.60[b]	74.03[ab]	74.78[a]	0.33
Egg production (%, hen-housed)	67.10[BC]	67.67[AB]	66.23[C]	68.72[A]	0.33
Egg weight, g	58.73[A]	58.65[A]	58.51[AB]	58.14[B]	0.119
Feed consumption (g/hen/day)	128.83[A]	126.35[B]	129.05[A]	129.23[A]	0.339
Feed conversion (feed/egg mass)	2.93	2.92	2.97	2.95	0.039
Mortality (%)	7.84	6.05	7.84	6.38	1.143

[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.
[a,b, A-C] Means with different superscript in the same row are significantly different at $p < 0.05$ (a,b) and $p < 0.01$(A-C).

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 (Um et al., 1998).

3) 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 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, levels of atmospheric ammonia within the houses can be significantly reduced by including this product in the diet. Zeolite products

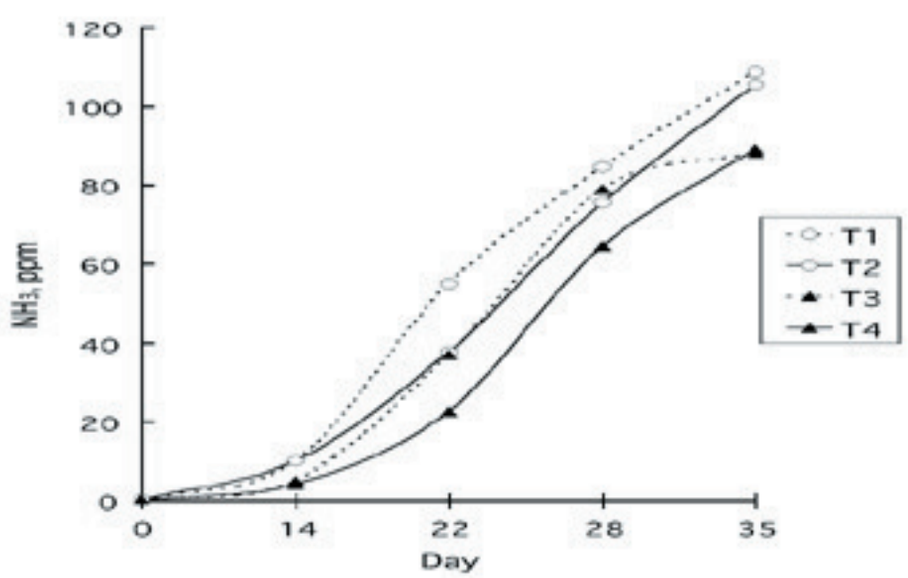
have been used at a level of 1 to 2% of the diet to improve pelleting quality. It is also believed that zeolite may improve the litter condition and environment of the barn. Due to a high ion-exchange capacity, it is expected that zeolite may bind ammonium ion in the litter (Moon et al., 1991). However, 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 11 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*) (Lee at al., 2000b).

Table 11. 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
	(g)		(g:g)	%
T1 (21.5% CP)	2869.8	1636.0a	1.76b	3.60
T2 (21.5% CP+BIO-21[1])	2879.9	1626.8a	1.77b	2.00
T3 (18.5% CP+AA[2])	2794.5	1518.6b	1.85a	0.40
T4 (18.5% CP+AA+BIO-21)	2859.3	1511.6b	1.88a	2.00
SEM	32.74	12.09	0.02	1.31
	Main effects			
CP				
21.5 %	2874.9	1631.4a	1.76b	2.80
18.5 %	2826.9	1515.1b	1.87a	1.20
BIO-21(SP)				
0 %	2832.1	1573.8	1.80	2.00
0.1 %	2869.6	1572.7	1.83	2.00

[1]BIO-21 is a commercial probiotics containing B. *subtilis, Lactobacillus* and yeast.
[2]AA: amino acids supplement of arginine, threonine and tryptophan.
a,b Values with different superscripts in the same column are significantly different(P<0.05).



Footnotes : T1; 21.5% CP, T2; 21.5% CP+BIO-21, T3; 18.5% CP+AA T4; 18.5% CP+AA+BIO-21

Fig 3. Ammonia level at the litter of broiler barn, determined in the air collected for 60 sec using trapping box of 36 L × 27 W × 18H.

3. Mineral Control

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).

1) Cu-Chelates

Growth performance- broilers and pigs

Summary of effects of copper sources on the growth performance of broilers and pigs is shown in Table 12. The effects of copper sources were compared with the results of non-supplemented control groups. In broiler experiments, supplemental copper sulfate at the level of 200ppm was effective for increasing weight gain in Cu-Exp 1 and 3 but not in Cu-Exp 2, 4 and 6. Supplementation of chelated copper products, especially SQM-Cu and Met-Cu, improved weight gain and feed conversion efficiency in broilers. In Cu-Exp 1, supplementation of 127ppm of Cu from SQM was as good as higher level of Cu (191ppm from SQM and 200ppm from $CuSO^4$) in broiler performance. In Cu-Exp 3, chelate mineral combination of Cu, Zn and Fe (Met-Cu-Zn-Fe), which are known to have strong interactions, did not perform any better than Met-Cu alone. In Cu-Exp 4, 125ppm of Cu in the form of Met-Cu performed as good as 200ppm of Cu of the same source. In Cu-Exp 5, the growth-promoting effect of 100ppm Cu from Met-Cu was better than those from chitosan chelate or yeast chelate. The effect of Met-Cu on the performance of broilers was high compared to those of sodium alginate-Cu (Cu-Exp.2) or fish meal-Cu (Cu-Exp.3). Supplementation of copper sources had a detrimental effect on rats (Cu-Exp.6), however. In Cu-Exp 8, weanling pigs fed diet supplemented with 100ppm of Cu in the form of Met-Cu performed as good as, if not better, those fed 200ppm of Cu from either Met–Cu or $CuSO_4$.

In general, feed conversion efficiency was consistently improved by Cu supplementation regardless of the sources of Cu. However, growth-promoting effect was variable depending on the sources of Cu, animal species and unidentified variables of experimental conditions. Met-Cu, SQM-Cu, and copper sulfate had a better growth promoting effect in pigs than in broilers

Considering the results of Cu-Exp 7 and 8 (pig), and 1,4 and 5 (broiler chickens), it appears that dietary level of 100~125ppm copper in the chelated form is enough to improve the performance of chickens and pigs. This will result in a reduction of fecal Cu excretion The 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. It is also known that copper regulates cholesterol biosynthesis by reducing hepatic glutathione concentration (Kim et al, 1992). Therefore, copper deficiency induces hypercholesterolemia in rats (Klevay, 1973). In Cu-Exp 4 and 5, copper supplementation tended to reduce serum and muscle total cholesterol and increase serum HDL cholesterol level in broilers (Paik et al, 1999). Kratzer and Vohra (1986) reported that metal ion chelated with low molecule weight peptides are more stable, neutral electronically and therefore, chelated minerals are better able to pass through intestinal wall than are ionic minerals.

Laying performance

Pharmacological level of copper is not commonly used in layer diets because it can cause gizzard erosion. However, supplementation of copper in the form of Met-Cu improved performance of laying birds. In Cu-Exp 11, 75ppm Cu in the form of Met-Cu showed best performance in egg production, egg weight, feed conversion efficiency and eggshell strength. In Cu-Exp 12, 100ppm treatment showed slightly better performance than 50ppm treatment. Supplementation of Met-Cu to layer diet is a subject of further study (Lim and Paik, 2001).

Table 12. Effects of supplementary copper chelates on the performance of broilers and pigs

Copper Experiments	Animals	Source and level of Cu, ppm	Difference from the control, %		
			Gain	Feed intake	Feed/gain
1	Broiler	$CuSO^4$, 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	Broiler	$CuSO^4$, 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
3	Broiler	$CuSO^4$, 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
4	Broiler	$CuSO^4$, 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
5	Broiler	Met-Cu, 100	5.3	2.8	-2.3
		Chitosan-Cu, 100	2.3	4.4	2.3
		Yeast-Cu, 100	1.8	4.9	3.4
6	Broiler	$CuSO^4$, 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
	Rat	$CuSO^4$, 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
7	Pig	$CuSO^4$, 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
8	Pig	$CuSO^4$, 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
9	Pig	$CuSO^4$, 200	5.5	2.6	-4.6
		Met-Cu, 200	17.8	-7.2	-9.8
		FM-Cu, 200	0.0	-16.4	-4.6
10	Pig	Met-Cu, 100	0.3	-2.1	-2.4
		Chitosan-Cu, 100	1.7	-3.7	-1.0

Footnotes: $CuSO_4$; $CuSO_4 \sum 5H_2O$, SQM-Cu; sequestered mineral copper; Met-Cu; methionine-copper chelate, SA-Cu; sodium alginate-Cu complex, FM-Cu; fish meal digest-Cu complex, Met-Cu-Zn-Fe; methionine-copper, zinc and iron complex, Chitosan-Cu; chitosan-copper complex, Yeast-Cu; yeast-copper complex.

Table 13. Effects of supplementary methionine-copper chelate on the performance of laying birds

Copper Experiment	Level of Cu, ppm	Difference from the control, %				
		Egg production	Egg weight	Feed intake	Feed conversion	Eggshell strength
11	25	2.11	0.21	0.99	-1.30	1.63
	50	1.46	1.50	2.60	3.03	1.33
	75	3.84	2.16	3.74	-1.73	3.26
	100	3.66	1.85	4.01	-1.30	0.15
12	50	1.75	0.67	2.82	0.00	4.31
	100	2.33	0.98	1.13	-1.65	3.10

Conducted for 12 wks with 740 ISA Brown layers of 56wk old.
Conducted for 8 wks with 396 ISA Brown layers of 72wk old.

2) Zn-Chelates

The effects of supplemental zinc in weanling pigs are shown in Table 14. In the first experiment, 100 and 200ppm of Zn in the form of Met-Zn and 200ppm in the form of ZnO increased weight gain and feed intake compared to the control (100 ppm of Zn from ZnO). In the second experiment, 1,000 or 2,000ppm of Zn in the form of ZnO increased weight gain and feed intake. At the level of 100ppm in the form of Met-Zn improved weight gain and feed intake but high dietary level of Zn (1,000 and 2,000ppm) in this form had adverse effects on the performance. Zinc is an essential element being the prosthetic of many metalloenzymes, such as carbonic anhydrase, alcohol dehydrogenase, carboxy peptidase, alkaline phosphatase, thimidine kinase, RNA and DNA polymerase and so on. Zinc also plays a role in immune system (Miller et al, 1979; Beach et al., 1980; Dardenne and Bach, 1992) and reproduction system (Kirchgessner and Rothe, 1992). Kasahara and Anraku (1972; 1974) reported that Zn ion might be related to the inhibition of active transport system and respiratory chain of E. coli. Holm (1988) and Poulsen (1989) reported that supplementing high level of ZnO could control baby pig diarrhea caused by E. coli. In Zn-Exp 1 and 2, the activity of alkaline phophatase activity increased as the dietary level of Zn increased regardless of the source of Zn. Serum IgG concentration tended to be higher in Met-Zn treatments as compared to ZnO treatments at 100 and 200ppm Zn level. However, the opposite was true at 1,000 – 2,000ppm level of Zn. The results of the present experiments indicate the 100 - 200ppm of Zn in the form of methionine chelate may be adequate for the improvement of weanling pig performance (Ahn et al, 1998 a,b).

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Table 14. Effects of supplementary zinc oxide and methionine zinc chelate in weanling pigs

Zinc 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

Footnotes: Met-Zn; methionine-Zinc chelate

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