Effects of pelleting temperature of phytase supplemented broiler feed on tibia mineralization, calcium and phosphorus content of serum and performance

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ABSTRACT: The present study was conducted to determine the effects of different pelleting temperature on tibia mineralization, calcium, phosphorus content of serum and growth performance of broilers fed phytase-supplemented diets. The basal experimental diet type was typical maize-soybean meal. The basal diet was supplemented with a microbial 6-phytase (Novo CT: coated thermostable granulate, from Novo Nordisk A/S, Denmark) at 500 Phytase Units (FTU) per kg of feed before pelleting. The diets were pelleted at 65, 75, or 85°C except the basal mash diet as the control. The experimental diets were used from 0 to 6 weeks of age of birds. Dry matter, crude ash, Ca, total P, Na, K and Mg, Zn, Fe, Mn, Cu contents of tibia were not affected by the pelleting temperature. No effects of pelleting temperatures on Ca content in the serum were found out. However, P content in the serum was increased by feeding the diet pelleted at 65°C as compared to the control and other treatments. Pelleting at 65 and 75°C had a significant positive effect on body weights and body weight gains of broilers. It was concluded that the best pelleting temperature was 65°C. It is to note that the temperature of 85°C damages enzymatic activity.

Keywords: pelleting temperature; broiler; phytase; tibia mineralization; growth

In recent years there has been a considerable interest in the use of microbial phytase to release phytate-bound P and to improve overall P availability in poultry diets. The following results demonstrate that supplemental phytase is effective in improving the availability of phytate-bound P for growth, bone mineralization, metabolizable energy and nutrient digestibility and nutrient retention in broiler diets (Kornegay et al., 1996; Cabahug et al., 1999; Ravindran et al., 2000; Zyla et al., 2001).

The structure of an enzyme is critical to its specific activity. The primary and secondary structure of enzymes can be altered by exposure to heat, extremes of pH or certain organic solvents so that the activity can be decreased or completely abolished (Spring et al., 1996). The same temperature rise supplied with steam heating results in rapid inactivation. At a pre-pelleting conditioning temperature of 65°C, the commercial enzyme formulation under discussion is completely stable. However, as the conditioning temperature increases, the enzyme becomes gradually more inactivated until at 75°C the residual activity is about 30% of the starting level (Cowan, 1993a).

Feeds used for broiler production are often (steam and/or double) pelleted and the ability of enzymes to withstand this heat treatment is questioned (Al Bustany, 1996). Controversy exits on the extent of the loss in enzyme activity and on the feed properties that can diminish these losses. The nature of phytase makes it very susceptible to temperature increments. This in fact is a property which needs to receive the attention of the industry since feed manufacturing thermal processes usually take place (Eeckhout, 1999). The intrinsic phytase in small grains is inactivated by steam pelleting at temperatures above 80°C. Hydrothermal processes, such as pelleting, extrusion and expansion, were

recognized as potentially destructive for phytase (Jongbloed and Kemme, 1990). Controversy exits as to the extent of these losses which appear to be affected by the type of enzyme preparation as well as by the methods of assessing pelleting temperature and enzyme recovery (Gadient et al., 1993).

The main problem is the loss in activity at high processing temperatures. While the enzyme is stable up to 60°C, temperatures beyond this value cause a dramatic loss in activity. The feed industry is thus faced with the problem of retaining the biological activity of enzymes applied to compound feed (Khan, 1995). Pelleting losses are affected by the pelleting conditions, the matrix of other ingredients and properties of the commercial enzyme formulation, including its resistance to denaturation and its capacity to re-nature into an active form during cooling. *In vitro* studies showed that steam pelleting at temperatures higher than 60°C strongly reduced the phytase activity of some enzyme preparations (Nunes, 1993) but other commercial enzyme formulations were more resistant (Simons et al., 1990). Wyss et al. (1998) observed that thermostability properties varied among different types of phytases and pointed out that characterization and formulation of thermostable enzymes were issues of current research.

Simons et al. (1990) showed that a high energy input meant that the damage during the pelleting process was high. Under these conditions many plant cells are crushed, which is favourable for the digestion process in animals. The effect of the damage on the phytase activity appears to be small; it is mainly the high temperature which results from the high energy input which will inactivate the enzyme.

The present study was conducted to determine the effects of pelleting at different temperatures on tibia mineralization, calcium, phosphorus of serum and growth performance in broilers fed diets supplemented with phytase.

MATERIAL AND METHODS

Diets

The basal experimental diet was based on maize-soybean meal. The basal diet was supplemented with a commercial 6-phytase (Novo CT: coated thermostable granulate, from Novo Nordisk A/S, Denmark) at 500 FTU per kg of feed before pelleting. The diets were pelleted at 65, 75, or 85°C except the basal mash diet as the control. The basal experimental diet is described in Table 1.

Feeds were pelleted with a 150 KWh Robinson pellet mill using typical commercial pelleting: mixed mash travelled through a 150 cm auger conditioner

Table 1. The composition of experimental diets (g/kg) at 0 to 6 weeks

Ingredient (kg)		Analyzed composition (g/kg)		Calculated composition (g/kg)	
Yellow maize	580	Dry matter	935.2	AME (MJ/kg)	13.13
Soybean cake	310	Crude protein	216.5	Lysine	12.73
Fish meal	47	Crude fat	69.3	Methionine	5.95
Vegetable oil	40	Crude fibre	31.7	Methionine + cysteine	7.35
Ground limestone	18	Starch	407.5	Available phosphorus	2.35
Vitamin-mineral mixture[1,2]	2.5	Sugar	45.8		
D,L-Methionine	2.0	Crude ash	57.3		
Coccidiostatica[3]	0.5	Calcium	13.8		
		Phosphorus	4.53		

[1]supplied by the diet (mg/kg): retinol acetate 5.16; cholecalciferol 0.0375; tocopherol 20; menadione 5; thiamine 3; riboflavin 6; niacin 25; calcium D-pantothenate 12; pyridoxine 5; cyanocobalamin 0.03; folic acid 1; D-biotin 0.05; choline chloride 400; Carophyll yellow 25

[2]supplied by the diet (mg/kg): manganese 80; iron 60; zinc 60; copper 5; cobalt 0.2; iodine 1; selenium 0.15; calcium carbonate 447

[3]supplied by the diet (mg/kg): Lasalocid sodium (Avatec, Roche) 45

where 85°C steam was injected at 3 bar. After 15 s of conditioning the mash contained about 200 g/kg moisture and was then pelleted at 65, 75, and 85°C in a 0.475 cm die. Pellets were transferred to a vertical cooler for 10 min to remove heat and moisture. The pellets were 3 mm in diameter.

Experimental feed was ground through a 1 mm screen when it was prepared for chemical analyses. Dry matter content was determined by oven-drying at 105°C for 16 h. Crude protein was determined by the Kjeldahl method (AOAC, 1980). Ether extract content was obtained by the Soxhlet extraction using anhydrous diethyl ether. Crude fibre content was determined using 12.5% H_2SO_4 and 12.5% NaOH solutions (Nauman and Bassler, 1993). The samples were analyzed for starch, sugar, ash, calcium and phosphorus according to the procedures of the AOAC (1980). Estimates of ME were based on protein, ether extract, starch and sugar levels determined in the experimental feeds (Rose, 1997).

Experimental design and traits measured

A total of four hundred day-old male and female Arbor Acres broiler chicks were individually weighed, wing banded and distributed into 4 floor pens with 100 chicks per pen. Males and females were allocated to each of the pens randomly. Each floor pen was furnished with wood shavings litter, two tube feeders and a bell type drinker. Birds were weighed individually at 3 and 6 weeks of age. Body weight gain was calculated from 0 to 3 and 3 to 6 weeks. Feed and water were consumed *ad libitum*. Temperature and relative humidity were maintained within the optimum range. Lighting was 23 h light and 1 h darkness. Total feed intake was measured per cage at 3 and 6 weeks of age. Mortality was recorded daily. Feed intake and feed efficiency ratio were adjusted for mortality. The experiment lasted 42 days. At the end of the experimental period, 10 male birds were randomly selected from each pen. In all, 40 male birds were sacrificed, right and left tibia and blood samples were removed. Tibia dry matter content was determined by oven-drying at 105°C for 16 h. Ash content was determined after heating in a muffle furnace at 550°C for 16 hours. Ca, P, Na and K contents of tibia were determined using an Eppendorf flame photometer. Fe, Cu, Mg, Mn and Zn contents of tibia were determined with Perkin Elmer atomic absorption spectrophotometer. Serum calcium and phosphorus were determined using a Dacos XL auto-analyzer.

Statistical analysis

Data were subjected to ANOVA using General Linear Models (SAS, 1986). The model included source of pelleting temperature and sex and in-

Table 2. Mean (± s.e.) tibia dry matter (DM) (g/kg), ash (g/kg) and minerals (mg/kg, expressed on a DM basis) of diets differing in pelleting temperatures

	Pelleting temperature				Probabilities
	–	65°C	75°C	85°C	
DM	421.29 ± 11.12	419.73 ± 11.12	419.49 ± 11.12	429.52 ± 11.12	0.9953
Ash	396.68 ± 9.88	397.19 ± 9.88	395.39 ± 9.88	398.98 ± 9.88	0.9081
Ca	332.07 ± 7.60	334.52 ± 7.63	344.22 ± 7.64	345.05 ± 7.46	0.2340
P	168.76 ± 3.75	174.17 ± 3.77	174.49 ± 3.77	173.69 ± 3.68	0.5000
Na	9.59 ± 0.24	9.65 ± 0.24	9.73 ± 0.24	9.06 ± 0.23	0.6079
K	7.43 ± 0.40	6.91 ± 0.41	6.96 ± 0.41	6.61 ± 0.40	0.6784
Mg	7.14 ± 0.24	7.08 ± 0.24	7.29 ± 0.24	6.66 ± 0.23	0.5254
Zn	0.64 ± 0.070	0.52 ± 0.070	0.64 ± 0.070	0.70 ± 0.070	0.3987
Fe	0.38 ± 0.024	0.33 ± 0.024	0.33 ± 0.024	0.33 ± 0.024	0.0926
Mn	0.14 ± 0.076	0.14 ± 0.076	0.15 ± 0.077	0.14 ± 0.076	0.7599
Cu	0.02 ± 0.002	0.02 ± 0.002	0.02 ± 0.002	0.02 ± 0.002	0.3643

teractions between pelleting temperature and sex. Data on tibia dry matter, ash and mineralization were analyzed using the same model across the sexes. Data on feed intake and feed conversion efficiency were not analyzed statistically.

RESULTS

The effects of different pelleting temperatures on dry matter, crude ash and mineral contents of tibia are presented in Table 2. Differences in dry matter, crude ash, Ca, P, Na, K and Mg, Zn, Fe, Mn, Cu contents of tibia due to the pelleting temperature were not significant ($P > 0.05$). Dry matter, crude ash, Ca, P, Na, K and Mg, Zn, Fe, Mn, Cu contents of tibia were not affected by the pelleting temperature.

No effects ($P > 0.05$) of pelleting temperatures on Ca content of serum were found (Table 3). However, P content of serum was increased by feeding the diet pelleted at 65°C as compared to the control and other treatments.

The average livability value was 98.5 ± 0.47 for the experiment and there were no treatment differences. Body weights of broilers at 3 and 6 weeks were affected by the pelleting temperature ($P < 0.05$) (Table 4). Body weights were increased by feeding the diet pelleted at 65°C (which indicates that phytase can release organic phytate-bound nutrients from vegetable feedstuffs) as compared to the control and other treatments ($P < 0.05$). Body weights were increased by the pelleting temperature at 75°C as compared to the control. No significant effects ($P > 0.05$) of pelleting temperature at 85°C were found for body weights as compared to the control. Pelleted feed had a significantly higher effect on body weight gains of broilers at 0 to 3 and 3 to 6 weeks and 0 to 6 weeks. Body weight gains were increased by feeding the diet pelleted at 65°C as compared to the control and other treatments ($P < 0.05$). Body weight gains were increased by the pelleting temperature of 75°C as compared to the control. No effects ($P > 0.05$) of pelleting temperature at 85°C were found for body weight gains as compared to the control, at 0 to 3 and 0 to 6 weeks.

Table 3. Mean (± s.e.) serum Ca and P of diets differing in pelleting temperatures

Pelleting	Ca (mg/dl)	P (mg/dl)
–	9.96 ± 0.34	8.00 ± 0.47^{b}
65°C	10.90 ± 0.34	9.60 ± 0.47^{a}
75°C	10.72 ± 0.32	8.22 ± 0.44^{b}
85°C	11.04 ± 0.37	7.77 ± 0.50^{b}
Probabilities	0.1332	0.0492

^{a,b}column means with common superscripts do not differ ($P > 0.05$)

Table 4. Mean (± s.e.) body weight (g) and body weight gain (g) of diets differing in pelleting temperatures

Pelleting	Body weights			Body weight gains		
	0 week	3 weeks	6 weeks	0–3 weeks	3–6 weeks	0–6 weeks
–	43.60 ± 0.44	586.2 ± 8.61^{c}	1 590 ± 23.38^{c}	542.6 ± 8.56^{c}	1 003 ± 20.15^{c}	1 546 ± 23.35^{c}
65°C	42.46 ± 0.42	673.4 ± 8.20^{a}	1 828 ± 22.26^{a}	631.0 ± 8.15^{a}	1 154 ± 19.19^{a}	1 785 ± 22.23^{a}
75°C	43.14 ± 0.43	607.9 ± 8.57^{b}	1 710 ± 23.28^{b}	564.8 ± 8.52^{b}	1 102 ± 20.07^{b}	1 667 ± 23.25^{b}
85°C	42.92 ± 0.41	574.0 ± 8.05^{c}	1 627 ± 21.85^{c}	533.1 ± 8.00^{c}	1 053 ± 18.84^{b}	1 584 ± 21.83^{bc}
Sex						
Male	42.74 ± 0.32	621.5 ± 6.26^{a}	1 812 ± 17.01^{a}	578.8 ± 6.23^{a}	1 190 ± 14.66^{a}	1 769 ± 16.99^{a}
Female	42.32 ± 0.28	599.3 ± 5.54^{b}	1 566 ± 15.04^{b}	557.0 ± 5.51^{b}	966.7 ± 12.96^{b}	1 524 ± 15.02^{b}
Source of variation probabilities						
Pelleting	0.3585	0.0001	0.0001	0.0001	0.0001	0.0001
Sex	0.3393	0.0083	0.0001	0.0131	0.0001	0.0001
Pelleting × sex	0.3377	0.1442	0.059	0.1487	0.0640	0.0540

^{a,b}column means with common superscripts do not differ ($P > 0.05$)

Table 5. Mean feed intake and feed efficiency ratio of diets differing in pelleting temperatures

Pelleting	Feed intake (g)			Feed efficiency ratio (feed intake/weight gain)		
	0–3 weeks	3–6 weeks	0–6 weeks	0–3 weeks	3–6 weeks	0–6 weeks
–	931.66	1 751.40	2 643.06	1.72	1.75	1.74
65°C	843.90	1 993.50	2 837.40	1.34	1.73	1.59
75°C	980.10	1 694.23	2 674.33	1.74	1.54	1.60
85°C	1 114.45	1 593.46	2 707.91	2.09	1.51	1.71

Feed intake and feed conversion ratio tended to be improved by the pelleting temperature at 65°C (Table 5).

DISCUSSION

The results of the present study indicate that dry matter, crude ash, Ca, P, Na, K and Mg, Zn, Fe, Mn, Cu contents of tibia were not affected by the pelleting temperature. No effects of pelleting temperatures on Ca content of serum were found. However, P content of serum was increased by feeding the diet pelleted at 65°C as compared to the control and other treatments. Simons et al. (1990) reported that many plant cells were crushed, which was favourable for the digestion process in animals under pelleting conditions. The effect of the shear on the phytase activity appears to be small; it is mainly the high temperature which results from the high energy input which will inactivate the enzyme. Furthermore the improved utilization of phytate P from a maize-soybean diet, as a result of steam pelleting, was reported (Summers et al., 1967). Two other studies (Bayley and Thomson, 1969; Bayley et al., 1975) reported small increases in the intestinal absorption of P from maize-soybean diets by swine when the diets were steam pelleted. But Takemasa and Hijikuro (1983) and Edwards et al. (1999) showed that the steam pelleting of maize-soybean diets did not have any effect on the availability of phytate P to chickens. Body weights of broilers at 3 and 6 weeks were affected by the pelleting temperature. Body weight and body weight gain were increased by the pelleting temperatures of 65 and 75°C. Pelleting feed at 85°C had an insignificant effect on body weight and body weight gain as compared to the control mash diet. These results agree with the finding of Ribeiro et al. (2003). Ribeiro et al. (2003) concluded that the pelleting of feeds at 85°C with supplemented phytase (280 FTU/kg) did not have any significant effect on body weight gain, feed intake and feed conversion ratio. Pelleting is associated with positive effects on both feed handling and bird performance, including increased feed utilization and better growth rate (Leeson and Summers, 1991; Gibson, 1995). Simons et al. (1990) reported that the thermal stability of microbial enzyme was good. The enzyme would remain stable if the proper pelleting conditions were chosen. But Nunes (1993) reported that steam pelleting at temperatures higher than 60°C strongly reduced the phytase activity. This was particularly marked for temperatures higher than 75°C. When pelleting at 80°C, the recovered phytase activity represented about 50% demonstrating the inactivation of enzymatic activities. Samarasinghe et al. (2000) reported that the activity of the enzyme cellulase was unaffected at 60 and 75°C, but it was reduced by 73% in feed processed at 90°C. Birds consumed 6% more feed and grew 9% faster when the pelleting temperature was increased from 60 to 75°C. The high pelleting temperature reduced energy metabolizability and nitrogen utilization but the enzyme activity almost completely compensated for this loss. No interaction could be detected between the pelleting temperatures and enzyme activity. Simons et al. (1990) reported that the pelleting experiments with feed to which microbial phytase had been added showed the significant inactivation of phytase activity when the temperature of pellets after pelleting exceeded 84°C.

Vukic Vranges et al. (1994) detected a positive effect of added enzymes on broiler performance after extruding feed at 110°C. Spring et al. (1996) reported that measurements on soluble substrates indicated that cellulase, fungal amylase, and pentosanase could be pelleted at temperatures up to 80°C at least and bacterial amylase up to 90°C without a considerable loss in the analyzed activity.

Feed composition also plays an important role in phytase stability. The feed composition has an influence on friction heat and in some way on the residence time in the die, so the influence of the fat or fibre content on the activity loss cannot be overlooked (Eeckhout, 1999).

As feed enzymes are normally inactivated in a buffer at temperatures above 65°C, it is reasonable to assume that some inactivation of enzymes can take place in feed processing despite the substrate stabilization that will occur when the enzyme is in the presence of its substrate (Cowan, 1993b).

Israelsen et al. (1995) reported that a low residual phytase activity was expected at high temperatures. The low intrinsic phytase activity was left in the expandate after pressure conditioning at high material temperatures above 80°C, which is also the case of steam pelleting.

In conclusion, the pelleting properties of the ingredients may be improved by complete absorption of fat and other liquids, softening of fibres and formation of a binding matrix by gelatinisation of starch. Recently, feed enzyme suppliers have been expected to indicate and guarantee what amount of heat processing their products can tolerate so that the user may choose a product that can continue to function in his manufacturing system. It is clear from these results that pelleted feeds have a substantial nutritional value for poultry, but the phytase activity from commercial feed enzyme formulations and nutritional quality of the feed may vary greatly. Further, the phytase activity of pelleted feeds needs to be considered when used in broiler feeds.

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