Effects of Phytase Supplementation of Diets with Two Tiers of Nutrient Specifications on Growth Performance and Protein Efficiency Ratios of Broiler Chickens

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ABSTRACT : In two feeding experiments male and mixed-sex broiler chicks were offered diets based on sorghum and a wheat-sorghum blend with two tiers of nutrient specifications, without and with microbial phytase (600 and 800 FTU/kg), from 7-25 and 1-42 days post-hatch, respectively. The nutrient specifications for protein, amino acids, energy density and phosphorus (P) of standard diets were reduced to formulate the modified diets on a least-cost basis. Calculated differences in nutrient specifications between standard and modified diets ranged from 14.3 to 17.1 g/kg crude protein, 0.24 to 0.40 MJ/kg apparent metabolisable energy (AME) and 1.06 to 1.20 g/kg available P. In both experiments, reduced nutrient specifications had a negative impact on growth rates and feed efficiency and phytase supplementation had a positive influence on growth performance and protein efficiency ratios (PER). Phytase addition to the less expensive, modified diets either partially or entirely compensated for reduced growth performance and, consequently, feed costs per kg of live weight gain were reduced. In Experiment 1, phytase increased ($p<0.001$) nitrogen-corrected AME (AMEn) from 15.39 to 15.89 MJ/kg dry matter. For nitrogen (N) retention there was an interaction ($p<0.05$) between diet type and phytase as the effects of phytase on N retention were more pronounced in the modified diets, with an increase from 0.512 to 0.561. These results demonstrate the positive effects of phytase on protein and energy utilisation, in addition to its established liberation of phytate-bound P and illustrate the feasibility of assigning nutrient replacement values to the feed enzyme for consideration in least-cost ration formulations. Further work is, however, required to define the most appropriate reductions in nutrient specifications in association with phytase supplementation.
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Key Words : Broilers, Growth Performance, Protein Efficiency Ratio, Phytase

INTRODUCTION

It is becoming increasingly evident that, in addition to reducing the availability of P and several biologically important minerals, phytate may exert a negative influence on protein and energy utilization, which is ameliorated by phytase (Selle et al., 2000; Kies et al., 2001). It was initially proposed by Kies et al. (1997) that nutrient replacement values for P, calcium, protein/amino acids and energy density may be assigned to phytase so that the feed enzyme could be considered as a dietary component in least-cost ration formulations. However, the proposed protein and energy replacement values appear to be conservative when compared to the magnitude of improvements reported in ileal digestibility of amino acids and utilisation of protein and energy generated by added phytase (Cabahug et al., 1999; Ravindran et al., 1999, 2000, 2001).

In the two experiments reported herein broilers were fed diets with two tiers of nutrient specifications without and with phytase. In both experiments, the differences in specifications between the standard and modified diets are considerably more robust than those initially proposed by Kies et al. (1997). The primary aim was to determine if phytase supplementation could compensate for these reductions in nutrient specifications in terms of growth performance. In addition, the effects of treatments on apparent N retention, nitrogen-corrected apparent metabolisable energy (AME_n) and toe ash were determined in the first experiment and on protein efficiency ratios (PER) in both experiments.

MATERIALS AND METHODS

Feed ingredients were procured from a commercial feed mill (Weston Animal Nutrition, Merrylands, NSW) and relevant samples were submitted to a laboratory (BRI Australia Limited, North Ryde, NSW) for analyses of crude protein, calcium, total P and phytate-P contents and phytase activities. Based on this information, standard and modified diets were formulated on a least-cost basis. The average reductions in nutrient specifications adopted to formulate the modified diets are listed in Table 1 and include reductions of 0.36 MJ/kg for energy, 15.52 g/kg for crude protein and 1.45 g/kg for nonphytate-P.

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Table 1. Average reductions in nutrient specifications in the modified diets (Experiments 1 and 2)

Item	Average reduction[a]
Calculated	
Metabolisable energy	0.360
Protein	15.518
Available lysine	0.603
Methionine	0.283
Methionine+cystine	0.263
Threonine	0.538
Tryptophan	0.110
Isoleucine	0.528
Calcium	1.503
Total P	0.620
Nonphytate-P	1.143
Analysed	
Protein	16.868
Calcium	1.388
Total P	0.888
Nonphytate P	1.453

[a] Reductions in the modified diets compared to standard diet; average of the diet used in Experiment 1 and the three diets in Experiment 2.

General management

Day-old Cobb broiler chicks were obtained from a commercial hatchery. The birds were initially housed in electrically heated brooder units (Petersime Incubator Co. Ohio, USA) and, at 18 days post-hatch, were transferred to wire-floored battery cages. The cages were housed in environmentally controlled rooms with temperatures gradually declining from 32°C during week 1 to 22°C by week 5. Continuous fluorescent lighting was provided and the birds had free access to feed and water. Mortalities were monitored on a daily basis in both experiments and used to correct feed intakes per pen to calculate feed conversion ratios.

Experiment 1

The standard and modified diets (Table 2) were offered to birds, without and with phytase (600 FTU/kg), from 7 to 25 days post-hatch. The phytase (Natuphos® Granulate: 5,000 FTU/g) was included in relevant diets in granular form at 120 g per tonne and the diets were cold-pelleted (65°C) and crumbled. On analysis, the supplemented diets contained an average of 470 FTU/kg microbial phytase activity (Table 2). One phytase unit (FTU) is defined as the amount of enzyme which liberates 1.00 micromole of inorganic phosphorus per minute from 0.0051 mole per litre sodium phytate at 37°C and pH 5.5.

The day-old chicks were initially offered a commercial starter diet and, on day 7, weighed and assigned to 24 pens (10 birds per pen) on the basis of body weight. Each dietary treatment was fed to six replicate pens and body weights and feed intakes were recorded at weekly intervals. During the last four days of the feeding period, AME_n and N retention values were determined using a total excreta collection method. Feed intake and total excreta output were measured quantitatively per pen over four consecutive days. The excreta were collected daily, dried overnight at

Table 2. Composition (g/kg) and specifications (g/kg) of standard and modified diets (Experiment 1)

Composition	Diets		Specification	Diets	
	Standard	Modified		Standard	Modified
			Calculated values		
Sorghum	695.0	670.8	ME (MJ/kg)	12.38	12.14
Millrun	-	60.0	Crude protein	227.2	210.1
Soybean meal	145.0	85.0	Available lysine	11.20	10.42
Canola meal	60.0	70.0	Methionine	6.42	6.29
Cottonseed meal	50.0	40.0	Methionine+cystine	8.99	8.79
Meat and bone meal	75.0	50.0	Threonine	6.65	6.10
Limestone	2.5	5.5	Tryptophan	2.01	1.88
Lysine HCl	4.2	4.8	Isoleucine	7.08	6.31
DL-Methionine	3.7	3.7	Calcium	9.19	7.92
Threonine	-	0.1	Total P	8.15	7.44
Choline chloride	0.3	0.3	Available P	5.02	3.86
Sodium bicarbonate	3.9	4.4	Analysed values		
Salt	0.6	0.5	Total P	6.95	6.55
Vitamin-mineral	5.0	5.0	Phytate-P[a]	2.80	3.40
Premix			Non-phytate-P[b]	4.15	3.15
			Calcium	10.60	9.25
			Crude protein (N×6.25)	208.8	194.8
Ingredient costs[f] (A$ per tonne)			Intrinsic phytase activity[c]	30	120
Non-supplemented	279.06	257.82	Total phytase activity[d]	550	540
Supplemented	282.06	260.82	Microbial phytase[e]	520	420

[a] Calculated from analysed phytate-P contents of individual ingredients. [b] By subtraction, total P minus phytate-P. [cde] Units are FTU/kg. [c] Intrinsic phytase activity of non-supplemented diets. [d] Total phytase activity of supplemented diets. [e] By subtraction, total less intrinsic phytase activities of non-supplemented diets. [f] A$1.00 = -US$0.55.

Table 3. Composition (g/kg) of standard and modified starter, grower and finisher diets (Experiment 2)

Ingredient[1]	Starter		Grower		Finisher	
	Standard	Modified	Standard	Modified	Standard	Modified
Wheat[a]	300.0	300.0	327.5	289.5	285.5	282.5
Sorghum[b]	343.0	345.0	320.0	290.0	285.0	280.0
Millrun[c]	33.0	50.0	-	120.8	120.0	123.0
Soybean meal[d]	157.5	106.0	175.5	123.0	88.0	98.0
Canola meal[e]	-	70.0	33.0	70.0	44.0	70.0
Cottonseed meal[f]	22.5	30.0	43.0	11.0	30.0	70.0
Meat and bone meal	85.0	62.5	50.0	50.0	71.0	-
Blood meal	15.0	4.0	-	-	12.7	-
Tallow	10.0	-	20.0	20.0	40.0	40.0
Vegetable oil	7.0	6.0	5.5	5.5	4.7	-
Dicalcium phosphate	-	-	6.0	-	-	12.2
Limestone	5.0	2.5	4.3	4.5	2.2	6.9
Lysine HCl	3.0	4.0	2.9	3.3	3.1	4.1
D,L-Methionine	3.6	2.9	3.0	3.0	2.7	2.7
Threonine	-	-	-	-	0.3	0.4
Choline chloride	0.4	0.1	0.1	0.1	-	-
Sodium bicarbonate	3.5	4.0	3.0	0.3	3.4	4.2
Potassium bicarbonate	6.0	7.5	-	-	1.5	-
Salt	0.7	0.5	1.2	1.0	1.0	1.2
Vitamin-mineral premix	5.0	5.0	5.0	5.0	5.0	5.0
Ingredient costs (A$ per tonne)						
Non-supplemented	261.16	240.66	223.93	201.46	193.05	190.06
Supplemented	265.16	244.66	227.93	205.46	197.05	194.06

[1]Analysed total P and phytate-P contents (g/kg) of the ingredients used in formulations: [a] 3.40 & 2.50, [b] 2.95 & 2.40, [c] 8.40 & 7.90, [d] 6.50 & 4.55, [e] 9.45 & 7.45, [f] 11.90 & 10.50.

80°C in a force-draft oven and collections from each pen were pooled for analysis. Gross energy of the diet and excreta samples was determined using an adiabatic bomb calorimeter (Gallenkamp Model 16CB110) standardised with benzoic acid. The N contents of diets and excreta samples were analysed using a 428 nitrogen determinator (LECO Corporation, St Joseph, MI USA) as described by Sweeney (1986). The N retention and AME_n values of the diets were calculated using the following formula:

$$\text{N retention (\%)} = \frac{(\text{Feed intake} \times \text{N content}_{diet}) - (\text{Excreta output} \times \text{N content}_{excreta})}{\text{Feed intake} \times \text{N content}_{diet}} \times 100$$

$$\text{AME (MJ/kg)} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

Appropriate corrections were made for differences in moisture contents. The AME_n values were calculated by correcting for nitrogen equilibrium (zero retention) by using a factor of 36.52 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958).

The PER values were calculated as follows from analysed crude protein (N×6.25) contents of the diets.

$$\text{PER} = \frac{\text{Weight gain (g/bird)}}{\text{Feed intake (g/bird)} \times \text{Protein content}_{feed}}$$

On Day 25, all birds were euthanased and toes removed for the determination of ash content following the procedure outlined by Potter (1988).

Experiment 2

The standard and modified starter, grower and finisher diets (Table 3), without and with phytase (800 FTU/kg), were offered to birds from 1-14, 15-28 and 29-42 days post-hatch, respectively. The specifications and analysed composition of these diets are presented in Table 4. The diets were cold-pelleted (65-70°C) and crumbled and relevant diets were sprayed with liquid phytase (Natuphos® Liquid: 5,000 FTU/g) at the rate of 160 g per tonne of feed in a horizontal mixer. On analysis, the supplemented diets contained an average of 782 FTU/kg microbial phytase activity (Table 4).

A total of 240 mixed-sex day old birds were wing-banded and allocated among the four dietary treatments on the basis of body weight, with ten pens of six birds per treatment. Birds were weighed at 14, 28 and 42 days post-hatch and feed intakes for the three feeding phases were recorded. The PER values were calculated as described above.

Data analysis

Experimental data was subjected to analysis of variance using a general linear models procedure (SPSS Inc. Chicago, IL). The feed cost per kg of live weight gain was calculated

Table 4. Specifications (g/kg) of standard and modified starter, grower and finisher diets (Experiment 2)

Item (g/kg)	Starter		Grower		Finisher	
	Standard	Modified	Standard	Modified	Standard	Modified
Calculated values						
ME (MJ/kg)	12.30	11.90	12.40	12.00	12.40	12.00
Crude protein	220.2	205.9	214.0	199.2	202.3	186.4
Available lysine	11.75	11.13	11.07	10.46	10.53	10.13
Methionine	6.50	5.82	6.00	5.82	5.50	5.36
Methionine + cystine	9.40	8.84	9.10	8.80	8.35	8.36
Threonine	7.40	6.79	7.10	6.60	7.00	6.51
Tryptophan	2.34	2.19	2.38	2.19	2.15	2.18
Isoleucine	7.41	7.03	7.76	7.07	6.60	6.63
Calcium	10.50	8.19	9.00	7.80	8.60	7.40
Total P	7.84	7.28	7.58	6.91	7.69	7.15
Available P	5.04	3.98	4.50	3.35	4.30	3.10
Analysed values						
Total P	8.65	8.30	7.05	6.60	10.10	7.75
Phytate-P[a]	2.78	3.29	3.08	3.56	3.38	4.05
Non-phytate-P[b]	5.87	5.01	3.97	3.04	6.72	3.70
Calcium	12.60	10.90	9.60	7.80	8.10	7.40
Crude protein (N×6.25)	223.8	209.4	228.1	205.0	209.0	193.0
Intrinsic phytase activity (FTU/kg)[c]	290	290	230	570	400	440
Total phytase activity (FTU/kg)[d]	830	850	1,060	1,260	1,470	1,440
Microbial phytase activity (FTU/kg)[e]	540	560	830	690	1,070	1,000

[a] Calculated from analysed phytate-P contents of individual ingredients. [b] By subtraction, total P minus phytate-P. [c] Intrinsic phytase activity of non-supplemented diets. [d] Total phytase activity of supplemented diets. [e] By subtraction, total less intrinsic phytase activities of non-supplemented diets.

Table 5. Effects of diet modification and phytase supplementation on growth performance, protein efficiency ratio (PER), N retention, N-corrected AME, toe ash and feed cost per kg live weight gain of broilers from 7-25 days post-hatch (Experiment 1)

Parameter	Standard		Modified diets		Pooled SEM	Significance (p=)		
	Nil	Phytase	Nil	Phytase		Diet	Phytase	Interaction
Weight gain (g/bird)	880	918	824	887	14.60	0.007	0.002	0.412
Feed intake (g/bird)	1,335	1,409	1,392	1,427	17.93	0.048	0.006	0.298
Feed:gain (g/g)	1.52	1.54	1.69	1.61	0.018	0.000	0.078	0.011
PER	3.16	3.12	3.04	3.19	0.034	0.460	0.109	0.014
N retention (%)	58.3	57.7	51.2	56.1	1.263	0.003	0.105	0.047
AMEn (MJ/kg DM)	15.62	15.98	15.16	15.79	0.108	0.007	0.000	0.244
Toe ash (%)	12.76	12.76	12.53	12.92	0.133	0.819	0.167	0.160
Feed cost/ kg gain (A$)	0.424	0.433	0.436	0.420	-	-	-	-

to assess the economic feasibility of enzyme supplementation.

RESULTS

Experiment 1

The results of this experiment are shown in Table 5. Reduction in nutrient specifications in the modified diet increased feed intake ($p<0.05$), but depressed growth ($p<0.01$), feed efficiency ($p<0.001$), N retention ($p<0.005$), and AMEn ($p<0.05$). Microbial phytase (600 FTU/kg) increased growth ($p<0.005$), feed intake ($p<0.01$) and AMEn ($p<0.001$). As main effects, microbial phytase increased growth rates by 6.0% (903 versus 852 g/bird) and AMEn by 0.50 MJ (15.39 versus 15.89 MJ/kg DM). There were interactions between diet type and phytase supplementation for feed efficiency ($p<0.02$), protein efficiency ratios ($p<0.02$) and N retention ($p<0.05$), because the effects of phytase were more pronounced with the modified diets. Addition of phytase to modified diets increased feed efficiency by 4.7% (1.69 versus 1.61), PER by 4.9% (3.04 versus 3.19) and N retention by 9.6% (0.512 versus 0.561). There were no significant enzyme effects on toe ash, indicating that the responses observed were not related to phytase effects on available dietary P.

When the supplemented modified diet and the non-supplemented standard diet are compared, phytase entirely compensated for growth (880 versus 887 g/bird). Phytase partially compensated for feed efficiency (1.52 versus 1.61) as pair-wise comparisons showed that the difference in feed efficiency between the two diets was significant ($p<0.05$). Feed cost per kg of live weight gain with the supplemented, modified diet (A$ 0.420) was 0.94% less than the non-supplemented, standard diet (A$ 0.424).

Table 6. Effects of diet modification and phytase supplementation on growth performance, protein efficiency ratios and cost of feed ingredients per kg live weight gain of broilers from 1-42 days post-hatch (Experiment 2)

Parameter	Standard		Modified diets		Pooled SEM	Significance (p=)		
	Nil	Phytase	Nil	Phytase		Diet	Phytase	Interaction
1-14 days post-hatch								
Weight gain (g/bird)	322	344	298	315	5.78	0.000	0.002	0.681
Feed intake (g/bird)	444	463	427	445	9.26	0.062	0.054	0.953
Feed per gain (g/g)	1.38	1.35	1.43	1.41	0.017	0.002	0.158	0.775
PER	3.24	3.32	3.34	3.39	0.043	0.074	0.149	0.766
15-28 days post-hatch								
Weight gain (g/bird)	794	826	740	776	14.16	0.001	0.022	0.908
Feed intake (g/bird)	1,312	1,295	1,277	1,309	26.49	0.691	0.773	0.361
Feed per gain (g/g)	1.65	1.57	1.73	1.69	0.026	0.001	0.024	0.400
PER	2.65	2.81	2.83	2.89	0.045	0.007	0.022	0.303
29-42 days post-hatch								
Weight gain (g/bird)	1,114	1,108	1,118	1,189	18.59	0.031	0.091	0.046
Feed intake (g/bird)	2,289	2,265	2,354	2,408	39.48	0.012	0.704	0.325
Feed per gain (g/g)	2.06	2.04	2.11	2.03	0.030	0.558	0.131	0.271
PER	2.34	2.34	2.46	2.56	0.035	0.000	0.142	0.201
1-42 days post-hatch								
Weight gain (g/bird)	2,230	2,278	2,156	2,279	28.17	0.208	0.005	0.193
Feed intake (g/bird)	4,046	4,023	4,058	4,162	58.85	0.208	0.488	0.289
Feed per gain (g/g)	1.82	1.77	1.88	1.83	0.018	0.001	0.006	0.828
PER	2.55	2.61	2.68	2.76	0.026	0.000	0.008	0.792
Feed cost/ kg gain (A$/kg)	0.391	0.388	0.378	0.374	0.004	0.001	0.375	0.854

Experiment 2

The effects of dietary treatments on growth performance are summarised in Table 6. During the starter phase, reduced nutrient specifications depressed ($p<0.005$) growth rates and efficiency of feed conversion and phytase enhanced ($p<0.005$) weight gains. Also, in the grower phase, dietary modifications reduced ($p<0.005$) growth rates and efficiency of feed conversion; whereas, phytase addition increased ($p<0.03$) growth and conversion. Surprisingly during the finisher phase, birds offered the modified diets had greater weight gains ($p<0.05$) than those receiving standard diets and diet type did not alter feed efficiency. For weight gain, there was an interaction ($p<0.05$) between diet type and phytase addition, as birds on the supplemented modified diets grew 6.4% faster than their counterparts.

Over the entire feeding period, birds receiving modified diets, with reduced nutrient specifications, had significantly ($p<0.001$) higher PER than those receiving standard diets. Phytase inclusion increased ($p<0.005$) PER from 1-42 days post-hatch, with improvements of 2.4% in standard diets and 3.0% in modified diets or by 2.7% overall (2.615 versus 2.685).

When assessed by pair-wise comparisons, the performance of birds offered the supplemented, modified diets did not differ significantly from those offered non-supplemented standard diets over the entire feeding period. Feed cost per kg of live weight gain for the non-supplemented standard diet was A$ 0.391 as opposed to A$ 0.374 for the supplemented modified diet. Phytase supplementation entirely compensated for performance losses associated with reduced nutrient specifications and lowered feed ingredient costs per kg live weight gain by 4.3%.

DISCUSSION

In two feeding studies, on average, the modified diets contained analysed reductions of 16.9 g/kg crude protein, 1.45 g/kg nonphytate-P, 1.39 g/kg Ca and a calculated reduction of 0.36 MJ/kg AME in comparison to the standard diets. These reductions lowered bird performance and also the feed costs. In the first experiment, supplemental phytase compensated for reduced nutrient specifications in terms of weight gain and partially compensated for feed efficiency with an associated 1.2% reduction in feed cost per kg of live weight gain. In the second experiment, phytase entirely compensated for reduced nutrient specifications in terms of bird performance resulting in a 4.3% reduction in feed cost per kg live weight gain over the entire feeding period. These results indicate that, for broiler diets based on sorghum or wheat-sorghum blends, more substantial nutrient values may be assigned to phytase than those suggested by Kies et al. (1997).

During the finisher phase in Experiment 2, birds on the modified diet had greater feed intake and growth rates than birds on the standard diets with an interaction between diet type and enzyme supplementation for growth rates. It is not surprising that birds on diets with reduced specifications exhibited greater responses to phytase supplementation, but the better growth of birds fed modified diets was

unexpected. This finding may be due to differences in diet composition. The standard and modified finisher diets differed in that the major P sources were meat-and-bone meal (71 g/kg) and dicalcium phosphate (12.2 g/kg), respectively. It is suggested that diets with a more similar profile of feed ingredients should be used in future evaluations of this kind.

Arguably PER assays are not the specific indicators of the influence of a feed enzyme on protein utilisation as the name suggests. Logically, any improvement in energy utilitisation following enzyme supplementation would be reflected in increased feed efficiency and, in turn, enhanced PER. In experiment 1, phytase supplementation increased dietary AME values. Similar energy responses have been recently reported by Ravindran et al. (2001) in wheat/sorghum diets and Camden et al. (2001) in maize diets.

Phytase supplementation of modified broiler diets increased PER from 3.04 to 3.19 in experiment 1 (7-27 days post-hatch). Moreover, the main effect of phytase was to increase PER from 2.615 to 2.685 in experiment 2 (1-42 days post-hatch) and this effect was most evident in the grower phase. These findings contrast with some recent reports (Peter et al., 2000; Peter and Baker, 2001; Boling-Frankenbach et al., 2001), where phytase did not increase PER of broilers fed semi-purified diets containing individual feed ingredients, including soyabean meal. The discrepancy observed may be due to differences in the methodology employed or the dietary substrate levels. Conventional poultry diets typically contain from 2.50 to 4.00 g/kg phytate-P (Ravindran, 1995) and, in the present study, phytate-P concentrations ranged from 2.78 to 4.05 g/kg. Dietary phytate levels used in the above studies (Peter et al., 2000; Peter and Baker, 2001; Boling-Frankenbach et al., 2001) were probably considerably lower; for example, phytate-P levels in the semi-purified assay diet containing soyabean meal would have ranged from an estimated 0.42 to 1.21 g/kg. The substrate level may be critical as there are indications in pigs (Cadogan et al., 1997; Selle et al., 1997) and poultry (Cabahug et al., 1999; Ravindran et al., 2000) that dietary levels of phytate influence responses to phytase supplementation.

It has been argued that the *de novo* formation of binary protein-phytate complexes in the upper digestive tract, that are refractory to hydrolysis by pepsin, is central to the ‘protein effect’ of phytate and phytase (Selle et al., 2000). Since the relative levels of phytate and protein in feed ingredients are essentially constant, the discrepancy in PER results with phytase may stem from absolute differences in dietary levels of phytate.

In conclusion, this study suggests that relatively robust nutrient replacement values for protein/amino acids and energy density, in addition to P and Ca, may be assigned to this phytase feed enzyme for consideration in least-cost ration formulations of broiler diets based on wheat and/or sorghum. The decrease in the feed cost per kg live weight gain observed when supplemented, modified diets compared to non-supplemented, standard diets lends support to this strategy. Further work is required to define the most appropriate nutrient replacement values for phytase, which would be facilitated by a better understanding of the anti-nutritive properties of phytate and the ameliorative capacities of phytase in relation to protein and energy utilisation.

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