Influence of L-lactic acid on the efficacy of microbial phytase in broiler chickens

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ABSTRACT: Two growth trials and a short-term metabolism trial were conducted in broiler chickens in the period of 22 to 42 days of age in order to evaluate the effects of two dietary levels of L-lactic acid (1.03 or 2.06 g/kg) and microbial 6-phytase (750 U/kg), added either separately or in combination, on growth rate, feed conversion, dressing percentage and utilization of selected nutrients. In the first growth trial, six different dietary treatments were added to a basal grower diet containing 19.4% crude protein and a reduced level of dietary phosphorus (P) (5.9 g total and 2.9 g non-phytate P per kg). Single administration of L-lactic acid did not show any positive effect on the growth rate or feed conversion. In contrast, phytase addition to a low-P grower diet resulted in the increased final weight of birds and higher feed conversion. This beneficial effect was markedly stronger when the microbial phytase was added to the diets containing L-lactic acid. Based on two-factor analysis of variance, microbial phytase significantly increased the mean final weight by 6.5% ($P < 0.01$) and significantly improved feed conversion from 1.877 to 1.829 ($P < 0.05$). In the second growth trial, the same six dietary treatments were added to a basal diet containing a standard level of dietary P (6.7 g total and 4.0 g non-phytate P per kg), but the level of crude protein was reduced to 17.0%. L-lactic acid alone did not show any positive effects on performance. Phytase supplementation alone resulted in numerical improvement of the final weight (+1.1–2.4%), but a higher effect was observed in the diets containing L-lactic acid. In agreement with the reduced final weights of broilers fed the low-protein diets, markedly higher values of feed/gain ratio were noted. In the metabolism trial, selected dietary treatments were involved to evaluate the effects of L-lactic acid and microbial phytase, added either separately or in combination, on the digestibility of nitrogen (N) and fat as well as on the retention and excretion of N and P. Apparent digestibility of N and fat in the low-P diets was not affected by dietary treatments. Retention and utilization of N were numerically higher in all treatments fed low-P diets when compared to the treatment fed a standard diet, but the differences were not significant. Retention of P was numerically higher in all treatments fed low-P diets. When compared to the standard diet, the combination of phytase and L-lactic acid increased daily P retention by 37.6%. P excretion was significantly ($P < 0.05$) reduced in all treatments fed low-P diets supplemented by both test products, either separately or in combination. A numerical decrease in N excretion was noted in both treatments fed low-protein diets.

Keywords: phytase; L-lactic acid; P and N excretion; nutrient digestibility; performance; broiler chickens

Poultry diets contain mainly feeds of vegetable origin, such as cereal grains, extracted oilseed meals and cereal by-products. These feed ingredients provide a high proportion of total phosphorus (P), but only 20 to 30% of this P are available to the birds, because it is bound in phytate form. Such non-utilized phytate P is transferred with poultry excreta to soil and water and after the release it causes adverse effects on the environment. Enzymatic hydrolysis of phytate by the specific enzyme, phytase, results in release and improved utilization of phytate-bound P in the digestive tract, thus leading to a reduced P

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excretion. In an experiment with broiler chickens Schőner *et al.* (1993) studied the effects of graded doses of microbial phytase and different dietary levels of P from monocalcium phosphate on performance and retention of calcium (Ca) and P. Additions of phytase or inorganic P resulted in dose-dependent increases of weight gain, feed consumption and retentions of both minerals. Utilization of total and phytate-P was significantly improved by microbial phytase. The efficiency of microbial phytase in diets for broiler chickens and ducklings was evaluated by Farrell *et al.* (1993), using growth rate, feed intake, phosphorus retention and tibia ash percentage as main response parameters. In both experiments, microbial phytase showed positive effects on the used experimental parameters. Effects of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low-phosphorus diet without addition of inorganic phosphates were studied by Broz *et al.* (1994). The enhancement of chick performance was related to an improved utilization of dietary P, as confirmed by significantly elevated plasma concentrations of inorganic P and increased tibia ash percentage in birds receiving phytase-supplemented diets. Denbow *et al.* (1995) demonstrated that the phosphorus availability in soybean meal for broilers could be improved by supplemental phytase. A possibility to reduce phosphorus content in excreta by the application of microbial phytase in broiler diets was confirmed by Zobač *et al.* (1995).

In recent years, some researchers reported that microbial phytase had a beneficial effect on the utilization of nutrients other than phosphorus, such as amino acids (Yi *et al*., 1996; Namkung and Leeson, 1999; Ravindran *et al*., 1999, 2000). Furthermore, several studies showed that added phytase improved ileal amino acid digestibility in individual feed ingredients (Ravindran *et al*., 1999; Rutherfurd *et al*., 2002) and in complete diets (Yi *et al*., 1996; Namkung and Leeson, 1999; Ravindran *et al*., 2000). However, this topic remains still controversial because in some other experiments only marginal or even no effects were observed (Sebastian *et al*., 1997; Biehl and Baker, 1997; Zhang *et al*., 1999; Peter *et al*., 2000). Another problem is that in the majority of published trials, basal diets contained reduced levels of total and non-phytate P. There arose a question if the supplemental microbial phytase would produce similar positive effects when added to phosphorus-adequate broiler diets. As reported by Ravindran *et al*. (2001), microbial phytase improved ileal amino acid digestibility of broilers fed a lysine-deficient, but P-adequate diet.

Studies examining the effects of microbial phytase in the digestive tract of monogastric animals revealed that its maximum activity could be reached at lower pH values. In this connection, some authors evaluated potential beneficial effects of citric acid on the efficacy of microbial phytase preparations in pigs and broiler chickens (Li *et al*., 1998; Radcliffe *et al*., 1998; Brenes *et al*., 2003). However, their results are not conclusive with regard to potential synergistic effects.

The objective of our experiments was to evaluate a potential synergistic effect between L-lactic acid and 6-phytase derived from *Peniophora lycii,* which has an optimum efficacy at pH 4.5. The opportunity to utilize a well defined combination of L-lactic acid and microbial phytase has not been evaluated in broiler nutrition yet.

MATERIAL AND METHODS

Two growth trials and one metabolism trial were conducted. A total of 480 male broiler chickens (hybrid Ross 308) were used in growth trials. The birds were obtained from a commercial hatchery and kept in wire-floored cages until the age of 21 days. During this pre-test period, all birds received a starter diet based on cereals and soybean meal, which was formulated to contain 12.6 MJ of metabolizable energy, 207 g crude protein and 4.3 g non-phytate P per kg. Both growth trials were conducted during the subsequent growing period, from day 21 to day 42 of age. A 2 × 3 factorial design was applied in each trial, consisting of two levels of phytase addition (0 or 750 U/kg feed) and three levels of lactic acid (0, 1.03, or 2.06 g/kg feed). In the first experiment, all grower diets were formulated with a reduced level of non-phytate P (2.9 g/kg feed). In contrast, the level of non-phytate P was maintained at 4.0 g per kg in all the diets used in the second trial, but the dietary level of crude protein was reduced to 170 g/kg feed. The composition of all experimental diets and calculated nutrient contents are presented in Table 1. The same feed ingredients were used for the production of experimental diets.

As a source of microbial phytase, 6-phytase derived from *Peniophora lycii* (RONOZYME P, CT) was used. The used granulated product contained 3 604 U/g. Chemically pure L-lactic acid 80% was used, which was manufactured by Lachema Brno.

Microbial phytase was supplemented to the diets as a part of the vitamin premix, lactic acid was mixed into ground wheat.

Each growth trial involved six dietary treatments and each of them was assigned to four replicate groups of 10 birds. Broilers were weighed individually at 21 and 42 days of age. Feed consumption was monitored per replicate group by weighing the remaining feed.

At the end of each growth trial, selected chickens were slaughtered after previous stunning. In these birds, dressing percentage (DP) was determined, with main emphasis on the ratio between valuable parts of the carcass, slaughter by-products and the amount of abdominal fat.

$$DP = \frac{\text{Live weight in g} - (a + b + c + d + e + f)}{\text{Live weight in g}} \times 100$$

where: a = weight of blood (g)
b = weight of feathers (g)
c = weight of crop, oesophagus and proventriculus (g)
d = weight of gizzard (g)
e = weight of intestines, including digesta (g)
f = weight of shanks (g)

Table 1. Composition and calculated nutrient content (in %) of the used grower diets

Feed ingredient	Standard diet*	Trial 1	Trial 2
Wheat	20.00	20.00	20.00
Maize	45.09	45.57	53.90
Soybean extracted meal	28.18	28.07	20.77
L-lysine.HCl (98%)	0.13	0.14	0.19
L-threonine (98%)	0.07	0.07	0.08
Limestone	0.81	1.18	0.85
Salt	0.32	0.32	0.33
Dicalcium phosphate	1.63	1.04	1.63
Aminovitan BR 2 Plus[1]	0.50	0.50	0.50
Rapeseed oil	3.27	3.11	1.75
Metabolizable energy (MJ/kg)	12.60	12.60	12.60
Crude protein (g/kg)	193.85	193.85	170.00
Crude fibre (g/kg)	34.06	34.10	31.40
Lysine (g/kg)	10.57	10.57	9.25
Methionine (g/kg)	4.67	4.67	4.37
Met + cys (g/kg)	8.03	8.03	7.38
Threonine (g/kg)	7.66	7.65	6.71
Tryptophan (g/kg)	2.32	2.32	1.93
Arginine (g/kg)	12.06	12.04	10.02
Linoleic acid (g/kg)	18.79	18.30	18.30
Calcium (g/kg)	7.95	7.95	7.95
P total (g/kg)	6.96	5.90	6.74
P non-phytate (g/kg)	3.97	2.90	3.97
Sodium (g/kg)	1.46	1.45	1.45

*this diet was used in the metabolism trial only

[1]Premix Aminovitan BR2 Plus supplied per 1 kg of feed: vitamin A 12 000 IU, vitamin D_3 2 500 IU, vitamin E 35 mg, vitamin K_3 2.5 mg, vitamin B_1 2 mg, vitamin B_2 5 mg, vitamin B_6 3 mg, vitamin B_{12} 0.02 mg, niacin 25 mg, calcium pantothenate 10 mg, choline 200 mg, antioxidant (Endox) 100 mg, DL-methionine 1 800 mg, Maduramycin 5 mg, cobalt 0.33 mg, copper 7 mg, iron 34 mg, iodine 0.55 mg, manganese 72 mg, zinc 50 mg, selenium 0.15 mg

Subsequent to the growth trials, a metabolism trial was conducted in order to evaluate the influence of microbial phytase and L-lactic acid on nutrient digestibility as well as on retention and excretion of phosphorus and nitrogen. Selected dietary treatments were included into this trial, four treatments from Trial 1 (treatments 1, 3, 4, 6) with the reduced level of dietary P and two treatments from Trial 2 (1 and 4) with the reduced level of crude protein. In addition, two treatments were included receiving a standard grower diet (containing 194 g crude protein and 4.0 g non-phytate P per kg), either without or with microbial phytase at 750 U/kg feed. The composition of this standard diet is presented in Table 1. Further details about the eight treatments evaluated in this trial are given in Table 4. Each dietary treatment was assigned to 6 replicate groups of 4 broiler chickens each. The birds were 22 days old and were kept in balance cages.

The metabolism trial lasted for a period of one week and consisted of 3 days of the adaptation period and 4 days of the collection period. All excreta were collected daily and rests of feathers and feed were removed before their storage in the refrigerator. After concluding the balance period, the excreta from each replication were pooled, freeze-dried and finely ground for chemical analyses. Determinations of nitrogen and P were conducted according to the AOAC methods (AOAC, 1984). In order to determine apparent N digestibility, the non-protein nitrogen (uric acid) in broiler excreta was determined as the difference between total and protein N in excreta, after transferring uric acid into soluble allantoin (Ekman *et al.*, 1949). All chemical analyses were conducted in three replications.

The experimental data were subjected to analysis of variance. Scheffe's test was used to determine significant differences between dietary treatments (Snedecor and Cochran, 1969).

RESULTS AND DISCUSSION

The results of Trial 1 (see Table 2) showed that the application of L-lactic acid alone did not show any significant effect on the final weight of broiler chickens at day 42. In contrast, phytase supplementation to the low-P grower diet resulted in the increased final weight of birds and this effect was markedly higher when the microbial phytase was added to the diets containing L-lactic acid. Based on two-factor analysis of variance, microbial phytase significantly improved the mean final weight by 6.5% ($P < 0.01$). Combined application of microbial phytase and L-lactic acid in treatments 4 and 6 also improved feed conversion by 3.9 and 2.8%, when compared to the diets containing L-lactic acid alone. Phytase supplementation resulted in the overall significant improvement ($P < 0.05$) of feed conversion during the grower period from 1.877 to 1.829 (see Table 2).

The second growth trial was designed in a similar way, but the effects of both test compounds were evaluated in the grower diet with the reduced level of crude protein, containing 170 g CP, 6.7 total P and 4.0 g non-phytate P per kg. The results of Trial 2 are summarized in Table 3. Broiler chickens had lower

Table 2. Effects of L-lactic acid and phytase on performance and dressing percentage of broiler chickens fed grower diets with a reduced level of non-phytate P (Trial 1)

Treatment	1	2	3	4	5	6	Overall effects				
Lactic acid (%)	0	0	0.103	0.103	0.206	0.206	0	0.103	0.206		
Phytase (U/kg feed)	0	750	0	750	0	750				0	750
Initial weight (g) day 22	718	707	702	729	706	738	713	716	722	709	725
Final weight (g) day 42	2 197	2 280	2 173	2 359	2 202	2 358	2 238	2 266	2 280	2 190^A	2 332^B
Total weight gain (g) day 22–42	1 479	1 573	1 471	1 630	1 496	1 620	1 525	1 550	1 558	1 481^A	1 607^B
Feed/gain ratio day 22–42	1.855	1.835	1.888	1.814	1.888	1.835	1.845	1.851	1.862	1.877^a	1.829^b
Dressing percentage (%)	74.42	76.03	76.75	76.27	76.14	76.21	75.22	76.51	76.17	75.77	76.17

^{A,B}mean values with different superscripts are significantly different at $P < 0.01$
^{a,b}mean values with different superscripts are significantly different at $P < 0.05$

Table 3. Effects of L-lactic acid and phytase on performance and dressing percentage of broiler chickens fed grower diets with a reduced level of crude protein (Trial 2)

Treatment	1	2	3	4	5	6	Overall effects				
Lactic acid (%)	0	0	0.103	0.103	0.206	0.206	0	0.103	0.206		
Phytase (U/kg feed)	0	750	0	750	0	750				0	750
Initial weight (g) day 22	722	731	711	717	741	701	727	714	721	725	716
Final weight (g) day 42	2 185	2 208	2 186	2 239	2 198	2 251	2 197	2 212	2 224	2 189	2 233
Total weight gain (g) day 22–42	1 463	1 477	1 475	1 522	1 457	1 550	1 470	1 498	1 503	1 464	1 517
Feed/gain ratio day 22–42	1.943	1.934	1.939	1.933	1.948	1.897	1.939	1.936	1.923	1.943	1.922
Dressing percentage (%)	76.86	76.79	76.26	76.82	77.79	76.38	76.82	76.54	77.08	76.97	76.66

final weights in this experiment and this observation is in line with the reduced level of dietary crude protein that was below the requirement standard. Like in the previous trial, dietary supplementation of L-lactic acid did not show any significant positive effects on performance, neither on final weight nor on feed conversion. In the case of microbial phytase, only numerical improvements of final weight by 1.1–2.4% were noted (see Table 3), but this effect was much higher in the diets containing L-lactic acid. In agreement with reduced final weights of broilers fed the diets containing the reduced crude protein level, markedly higher values of feed/gain ratio were noted in this trial. The overall effect of microbial phytase on feed conversion was not significant under these conditions, but some marginal numerical improvement was noted.

Enzyme supplementation of broiler grower diets containing either the reduced level of non-phytase P (Trial 1) or the reduced level of crude protein (Trial 2) showed no significant effects on dressing percentage.

The objective of the metabolism trial was to evaluate the effects of L-lactic acid and microbial phytase, fed either alone or in combination, on the digestibility and retention of selected nutrients and the excretion of phosphorus and nitrogen. The results of this trial are summarized in Table 4. Digestibility of nitrogen and fat in the diet with the reduced level of non-phytate P was not significantly affected by experimental treatments. When compared to the non-supplemented standard diet (treatment B1), retention and utilization of nitrogen were higher in all treatments fed low-P diets, but the differences were not statistically significant. In theory, phytate is able to bound proteins and amino acids (Cosgrove, 1980), thus decreasing their absorption and availability for monogastric animals. However, the results of digestibility studies in both chickens and pigs were controversial, some of them showing a positive effect for at least some amino acids (Ravindran *et al*., 1999; Zhang *et al*., 1999; Kemme *et al*., 1999) while others failed to demonstrate any beneficial effect (Sebastian *et al*., 1997; Traylor *et al*., 2001). It is interesting to note that even though the positive effect on protein or amino acid digestibility was demonstrated, growth rate or protein utilization remained mostly unaffected (Zhang *et al*., 1999; Peter and Baker, 2001; Boling-Frankenbach *et al*., 2001). Even though very high levels of phytase were used, thus releasing most of P from the phytate, protein utilization in chickens fed a practical corn-soybean diet remained unchanged (Augspurger and Baker, 2004). The lack of consistency in protein utilization response to phytase supplementation calls for caution when ascribing an "amino acid value" to phytase products (Adeola and Sands, 2003).

Retention of total phosphorus was numerically higher in all treatments fed low-P diets. Treatment B6, receiving a combination of L-lactic acid and phytase, resulted in relative improvements of daily P retention by 37.6%, when compared to the standard grower diet (treatment B1). Phosphorus excretion in treatments fed low-P grower diets, supplemented by either phytase or L-lactic acid, was significantly lower ($P < 0.05$) when compared to the standard diet. The highest reduction (–23%) was noted in treatment B6, receiving the combination of both supplements. Based on these results, it is obvious that the tested microbial phytase reveals its beneficial effects only in broiler diets with reduced levels of inorganic, non-phytate phosphorus. A ben-

Table 4. Effects of phytase and L-lactic acid on apparent digestibility of selected nutrients and on retention and excretion of nitrogen and phosphorus in broiler chickens (metabolism trial)

Dietary treatment	B1	B2	B3	B4	B5	B6	B7	B8
Crude protein (g/kg feed)	194	194	194	194	194	194	170	170
Non-phytate P (g/kg feed)	4.0	4.0	2.9	2.9	2.9	2.9	4.0	4.0
L-lactic acid (%)	0	0	0	0.206	0	0.206	0	0
Phytase (U/kg feed)	0	750	0	0	750	750		750
N digestibility (%)	83.05	84.31	85.22	83.40	84.80	85.20	85.47	84.36
N retention (% of intake)	57.72	60.61	63.49	59.59	62.76	63.79	63.46	62.18
Daily N retention (g)	6.03	6.95	7.09	6.56	7.41	7.19	5.98	5.84
Fat digestibility (%)	87.19	86.53	88.02	88.35	87.94	87.33	85.68	85.50
Daily P retention (g)	0.173	0.141	0.194	0.200	0.233	0.238	0.198	0.250
N excretion (g/kg of gain)	27.89	28.20	28.68	28.97	25.40	25.06	23.96	24.11
P excretion (g/kg of gain)	5.85[a]	6.41[a]	5.52[a,b]	4.79[b]	4.76[b]	4.52[b]	6.48[a,b]	7.49[b]

[a,b] mean values with different superscripts are significantly different at $P < 0.05$

eficial influence of L-lactic acid was noted again and the results suggest that lactic acid and phytase may have an additive effect in poultry. A similar conclusion was drawn by Boling *et al.* (2000), who studied the combination of phytase and citric acid in chicks fed a phosphorus-deficient diet. In pigs, Kemme *et al.* (1999) found that the digestibility of total P was increased by the combination of phytase and lactic acid to a greater extent than it was calculated as the sum of the effects of separate supplementations. Whether or not the positive effect of organic acids on phytate hydrolysis is due to the lower pH value remains unclear. Some other modes of action were proposed such as delayed gastric evacuation that prolongs the action of phytase in the stomach (Kemme *et al.*, 1999) or the Ca-complexing property of citric acid which makes the phytate molecule less stable and more susceptible to phytase (Boling *et al.*, 2000). Since the combination of phytase and organic acid(s) may present an efficient means of improving phytate-P utilization and decreasing P excretion, further research is needed to elucidate this problem in greater detail.

An insignificant decrease in N excretion was noted in both treatments fed diets with the reduced level of crude protein (B7, B8), when compared to the standard diet. Phytase supplementation to the low-protein diet did not show any significant effect on experimental parameters, except P excretion, which was significantly increased by 28% ($P < 0.05$), when compared to the standard diet. This part of the metabolism trial showed that phytase supplementation of broiler diets in which the P requirement was fully met by addition of inorganic phosphates resulted in an increased phosphorus output in excreta.

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ABSTRAKT

Vliv kyseliny L-mléčné na účinnost mikrobiální fytázy v krmných směsích pro kuřecí brojlery

Ve dvou růstových a jednom bilančním pokusu na kuřatech ve věku 22–42 dnů byl studován vliv přídavků kyseliny mléčné (1,03 nebo 2,06 g/kg), fytázy (750 U/kg) a jejich kombinace na růst, konverzi krmiva, jateční hodnotu a využití živin. V prvním růstovém pokusu byly doplňky zařazeny do směsí obsahujících 19,4 % N-látek a redukovanou hladinu fosforu (5,9 g celkového a 2,9 g/kg nefytátového P). Kyselina mléčná bez fytázy neměla průkazný vliv na hmotnost kuřat ve 42. dni věku. Pozitivní efekt se projevil a to vysoce průkazně ($P < 0,01$) v přítomnosti enzymového preparátu, kdy konečná hmotnost kuřat skupin krmených směsmi s fytázou byla o 6,5 % vyšší oproti kontrole a statisticky významně ($P < 0,05$) se zlepšila z 1,877 na 1,829 i konverze krmiva. Druhý růstový pokus byl koncipován stejně jako první s tím rozdílem, že účinky obou látek byly studovány u směsí obsahujících 17,0 % N-látek, 6,7 g/kg celkového a 4,0 g/kg nefytátového fosforu. V tomto pokusu se efekt samostatně aplikované kyseliny L-mléčné na užitkovost neprojevil. Přídavek fytázy měl za následek neprůkazné zvýšení konečné hmotnosti (+1,1–2,4 %), silnější efekt byl však pozorován u diet obsahujících kyselinu L-mléčnou. V souladu s nižší hmotností kuřat krmených směsmi s nižší hladinou N-látek byla zaznamenána i vyšší spotřeba směsí. V bilančním pokusu u vybraných skupin kuřat byl studován vliv kyseliny mléčné, fytázy a jejich kombinace na stravitelnost živin, retenci živin a vylučování fosforu a dusíku. Stravitelnost dusíku a tuku ve směsích se sníženou hladinou fosforu nebyla žádným ze sledovaných zásahů významně ovlivněna. Retence a využití dusíku u skupin se sníženou hladinou fosforu byly ve srovnání s kontrolní skupinou vyšší, rozdíly však nebyly statisticky významné. Retence fosforu byla neprůkazně vyšší u skupin krmených směsmi se sníženou hladinou fosforu, přičemž největší rozdíl oproti kontrole (zvýšení denní retence P o 37,6 %) byl zaznamenán u skupiny s kombinovaným doplňkem fytázy a kyseliny mléčné. Vylučování fosforu bylo průkazně ($P < 0,05$) redukováno u všech skupin krmených směsmi se sníženým obsahem fosforu s doplňkem obou testovaných látek, jak jednotlivě, tak v kombinaci. Neprůkazný pokles exkrece dusíku byl zaznamenán u obou skupin kuřat krmených směsmi se sníženým obsahem N-látek.

Klíčová slova: fytáza; kyselina L-mléčná; vylučování fosforu a dusíku exkrementy; stravitelnost živin; užitkovost; kuřecí brojleři

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