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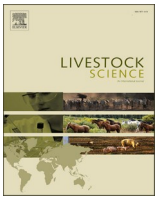

The effect of reduced feed pH, phytase addition and their interaction on mineral utilization in pigs

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HIGHLIGHTS

- Effect of formic acid and phytase was studied in pigs fed a high phytate-P diet.
- Phytase improved total tract digestibility of Ca and P and bone mineralization.
- There was no interaction effect between phytase and pH reduction in the stomach.
- Formic acid addition improved growth, FCR and total digestibility of Ca, Fe and Mg.

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ABSTRACT

An experiment with a 2 × 2-factorial arrangement of treatments was carried out to assess the effect of reduced feed pH and addition of phytase and their interactions on performance, mineral retention (Ca, P, Mg, Zn, Fe and Cu) and bone mineralization. A wheat-based diet with a high phytate-P content and no inorganic P added, or the same diet with either 1.4 % formic acid, 500 FTU *C. braakii*-derived phytase or both added, were used. Thirty-two piglets, with a mean weight of 21.06 ± 0.83 kg were distributed in eight pens. Individual feed intake was recorded, and all treatments were represented in all pens. The experimental period was 28 days. Performance was recorded, pH in stomach, jejunum and ileum was measured and content from jejunum and ileum was collected for assessment of P digestibility. In addition, feces were collected for total digestibility measurements and left third and fourth metacarpal were analyzed for bone mineralization. No interaction effects between phytase and acid addition were found. Phytase addition increased growth ($P=0.046$), jejunal P digestibility ($P=0.004$), total tract digestibility of P ($P<0.001$) and Ca ($P<0.001$) and bone mineralization ($P<0.001$). Acid addition improved growth ($P=0.002$) and FCR ($P=0.033$) in addition to total tract digestibility of Mg ($P=0.04$), Fe ($P<0.001$) and Ca ($P=0.001$).

The experiment confirmed that phytase addition improved P digestibility. However, no increased phytase efficacy was seen with acid addition.

1. Introduction

Phosphorus (P) from plant sources in pig diets occurs primarily in the form of phytate, and the pig have a limited ability to degrade phytate. To reduce the amount of P in feces and hence environmental pollution, exogenous phytase is routinely added to pig diets (Wilcock and Walk, 2016). However, phytase does not degrade the phytate completely. Phytase addition increased the P digestibility from 39 % to of 65 % (Rosenfelder-Kuon et al., 2020).

As for most enzymes, phytase has an optimal pH range. For most new-generation phytases, this optimum pH is between 3.0 and 5.0 (Menezes-Blackburn et al., 2015; Vieira et al., 2018). The main site for exogenous phytase activity in pigs is the stomach (Selle and Ravindran, 2008). However, the pH in stomach varies, and is reported to be between 2.7 and 4.8 (Dersjant-Li et al., 2001; Omogbenigun et al., 2003; Eberhard et al., 2007; Lee et al., 2018). With piglets having a pH in the higher range of this due to low endogenous acid secretion (Suiryanrayna and Ramana, 2015). A pH in the lower range of this interval will be beneficial to promote phytase activity and efficacy for the phytases that have a low pH optimum. The pH of the stomach may be lowered with addition

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of organic acid to the feed (Suiryanrayna and Ramana, 2015). A reduced pH in the stomach could also reduce the stomach emptying rate (Van der Aar et al., 2017). An increased degradation of phytate could be seen because of the increased retention time in the favorable low-pH environment (Blaabjerg et al., 2011). Organic acids may improve growth and feed conversion ratio (FCR) (Partanen and Mroz, 1999). This is the main reason, together with the beneficial anti-microbial effect (Suiryanrayna and Ramana, 2015), why organic acid is routinely added to pig feed today (Tugnoli et al., 2020).

Many minerals like calcium (Ca), zinc (Zn), iron (Fe), copper (Cu) and magnesium (Mg) are bound to phytate in mineral-phytate complexes. This reduces the phytate degradation, thus decreasing both mineral digestibility and phytate-P availability (Maenz et al., 1999). The effect of phytase addition on mineral digestibility is not consistent. Arredondo et al. (2019) found an increased apparent total tract digestibility (ATTD) of Mg and Zn with phytase addition, but no improvement in ATTD of Cu, Fe and Mn. The affinity of the minerals to phytate is dependent on pH level, so that a reduction in the inhibitory effects of minerals is seen when pH is lowered (Maenz et al., 1999). Therefore, organic acids are expected to increase mineral absorption by lowering stomach pH and reducing the binding of these minerals to phytate in addition to the increased phytase efficacy by lowered pH (Jongbloed et al., 2000).

Several experiments have shown that organic acids have a positive effect on phytase efficacy (Kemme et al., 1999; Jongbloed et al., 2000). However, other experiments have shown no interaction between organic acid and phytase (Radcliffe et al., 1998; Omogbenigun et al., 2003). As organic acid is routinely added to piglet feed and conflicting results on the effect on phytase efficacy have been reported, it is interesting to investigate the effect of phytase, acid addition and their interaction effects. Therefore the hypothesis that acidification of the feed in combination with addition of microbial phytase will increase phytase efficacy was tested. In addition, the effect on other phytate-bound minerals was examined.

2. Material and methods

All the animals were handled in accordance with the applicable laws and regulations controlling experiments with live animals in Norway (the Animal Welfare Act of 28th of December 2009 and the local legislation derived from the directive 2010/63 EU of the European Parliament and Council of 22nd September 2010 on the protection of animals used for scientific purposes). The experiment was performed at the Center for Livestock Production, Norwegian University of Life Sciences, Ås, Norway, from April to May 2019, and lasted for twenty-eight days.

Thirty-two piglets were fed a diet with or without phytase and with or without formic acid in a 2 × 2 factorial design, with eight piglets per treatment combination. Seven sows (Norwegian Landrace × Yorkshire) inseminated with Duroc semen provided the piglets for this experiment. From six of the litters four piglets were selected, and from one litter eight piglets were selected. The piglets were weaned at approximately 5 weeks of age. At 52 days of age, and an average initial body weight of 21.06 kg ± 0.80 standard deviations, the piglets were equally distributed by weight and randomly assigned to one of the four dietary treatments with three barrows and five gilts on each diet. All piglets in one pen were from the same litter, and one litter was divided into two different pens.

The piglets were distributed in groups of four and kept in eight 3.35 m × 2.25 m concrete-floored, partially slatted pens, with individual feeding stations of 0.37 m × 1.35 m. A rubber mat of approximately 90 cm × 100 cm and wood shavings was used on the pen floor. The pens were equipped with activity enrichment toys. The room temperature was 18°C, with 11 h of light and 13 h dark. During the dark hours, only a night light was used.

The individual feeding stations enabled recording of individual feed intake and all four dietary treatments to be represented in all pens. The piglets were fed equal amounts twice daily at 08:00 and 14:00 in the individual feeding stalls for approximately 30 min, and leftovers were recorded. The piglets were fixed in the feeding stations during the 30 minutes and any leftovers were removed before the piglets were released. Feed was provided during these periods based on an estimated feed intake of 3 % of the live body weight, which was assumed to be close to *ad libitum*. Water was accessible *ad libitum* via nipple drinkers. All piglets were healthy at the start of the experiment and the clinical health status of the piglets was monitored daily.

The feed was wheat based, with a high content of phytate-P and no inorganic P added (Table 1). The feed was produced in two batches in a commercial feed plant (Felleskjøpet Rogaland Agder, Stavanger, Norway) with raw materials from the same batch to lessen the variation in raw material quality, and contained 5.0 g/kg titanium dioxide (TiO_2) as a digestibility marker. The feed was pelleted with a minimum heat treatment of 81°C in the production. One batch was produced without added formic acid, and the second batch was produced with 1.4 % formic acid (85 %) (ADDCON Nordic, Porsgrunn, Norway) added. The amount of formic acid needed to achieve the wanted pH level of 4.5 was determined by gradually adding formic acid to 1.0 g (±0.01 g) of the non-acidified diet mixed with 5.0 ml deionized water until the wanted

Table 1
Composition and nutrient content of the experimental diets (g/kg as fed unless otherwise stated).

Ingredient	Without acid[1]	With acid[2]
Wheat	424	420
Wheat bran	199	196
Pea starch	100	97
Rape seed meal (CP 34.4%)	80	79
Soy protein (CP 56%)	93	92
Soy oil	20	20
Pea protein (CP 48.6%)	18	17
Corn gluten (CP 60.6%)	11	10
Animal fat	20	20
Limestone	7	7
Sodium chloride	5.7	5.9
L-lysine HCl	5.5	5.3
Mineral and vitamin premix[3]	5.6	5.6
L-threonine	2	2
Methionine analogue	2	2
Choline chloride	0.8	0.8
L-Tryptophan	0.6	0.6
L-valine	0.4	0.4
Enzyme[4]	0.2	0.2
Taste enhancer[5]	0.2	0.2
Titanium dioxide	5	5
Formic acid	-	14
Nutrient composition		
Calculated MJ/kg DM[6]	10.9	10.8
Calculated Phytate P	3.2	3.2
Analyzed CP	187	180
Analyzed Starch	366	330
Analyzed Fat	64.8	65.1
Analyzed Total P	5.0	4.6
Analyzed Ca	5.38	5.07
Analyzed Mg	2.18	2.14
Analyzed Cu	0.02	0.03
Analyzed Fe	0.26	0.30
Analyzed Zn	0.17	0.14

[1] Feed without acid and with and without phytase
[2] Feed with acid added, with and without phytase
[3] Supplied per kilogram of diet: 8030 IU vitamin A, 1506 IU cholecalciferol, 188 mg tocopheryl acetate, 6.0 mg menadione, 105 mg ascorbic acid, 4.0 mg thiamine, 12.1 mg riboflavin, 60.25 mg niacin, 12.05 mg pyridoxine, 0.04 mg cyanocobalamin, 30.12 mg pantothenic acid, 3.2 mg folic acid, 0.4 mg biotin, 72.3 mg Mn ($MnSO_4$), 108.4 mg Zn (ZnO), 144.6 mg Fe (FeSO4), 26.5 mg Cu ($CuSO_4$), 0.45 mg Se (Na_2SeO_3), 0.72 mg I.
[4] Enzyme Rovabio® Excel LC 2, Adisseo, France, provided xylanase and β-glucanase obtained from a fermentation broth of *Penicillium funiculosum*.
[5] Maxarome Sweet RP 1516, Nutriad, England
[6] Digestible energy

pH level was obtained. For the two diets with phytase, 500 FYT phytase (RONOZYME® HiPhos, DSM, Denmark) was added per kg feed at the Center for Feed Technology (Ås, Norway). The phytase was mixed with 0.4% water to ensure even distribution and sprayed on the pellet in a twin-shaft paddle mixer-vacuum-coater (Dinnissen, Sevenum, The Netherlands) to ensure even distribution.

The analyzed phytase activity was 660 FYT/kg feed with phytase added and 692 FYT/g feed with both phytase and acid added, while phytase activity for the diets without phytase added was below detection level due to the heat treatment when pelleted. Samples taken from wheelbarrows used in feeding were used to measure feed pH. The pH in the control diet, diet with phytase added, diet with formic acid added and the diet with both formic acid and phytase added was 5.7, 5.9, 4.2 and 4.2, respectively.

2.1. Sampling

Body weight (BW) and feed intake (FI) were recorded weekly. However, the first two weeks of the experimental period was regarded as an adaption period, and therefore body weight gain (BWG), FCR and FI only from the last two weeks have been used to determine differences between treatments. However, performance results from the first period is also presented. Individual fecal samples were collected once per day from the floor immediately after defecation or from rectum at experiment days 24 to 27. The fecal samples were frozen at -20°C between each sampling and samples from each pig were pooled. Dissection was done on day 28 and 29. The feeding time of the pigs was adjusted between pens the day before dissection to ensure that all pigs had two hours from start of feeding to start of euthanizing. The animals were euthanized with a captive bolt pistol followed by exsanguination. Intestinal content from the last meter of jejunum and the last 1.5 meter of ileum was collected immediately after slaughter. The stomach content was emptied into a container and mixed well before pH was measured. All pH-values were measured by inserting the pH meter (pH 100, VWR International, Radnor, PA, USA) into the container with the samples. In addition, the left third and fourth metacarpals from each pig were collected for determination of bone ash. The digesta samples were immediately frozen in liquid nitrogen after pH measurements, and stored at -20°C.

2.2. Chemical analyses

The diets were analyzed in duplicate for dry matter (DM), starch, crude protein (CP), P, Ca, Cu, Mg, Zn and Fe. Fecal, jejunal and ileal samples were freeze-dried and homogenized. Jejunal samples were analyzed in duplicate for P and titanium. Ileal samples were analyzed in duplicate for P, titanium, starch, and CP. Fecal samples were analyzed for titanium, P, Ca, Cu, Mg, Zn and Fe. The DM used in calculations was the lyophilized DM content. Crude protein (Kjeldahl-nitrogen × 6.25) was determined with a Kjeltec 8400 (Foss, Denmark) according to the methods described in the European Commission Regulation (EC) (No 152/2009). Titanium content was determined by following the procedure described by Short et al. (1996). P analysis was done according to the method of FAO (2011). Briefly, HCl was added to the samples and the solutions were mineralized until they became colorless. Thereafter an ammonium molybdate solution was added, and the samples were read on a MaxMat PL II Multi-analyser (MaxMat, France) at 340 nm. Starch was hydrolyzed with α-amylase and amyloglucosidase-enzymes to glucose, and glucose concentration was determined using a spectrophotometer (MaxMat PL II Multianalyzer, France) as described by McCleary et al. (1994). Ca, Cu, Mg, Fe and Zn was analyzed according to the method described in European Commission Regulation (EC) (No 152/2009), with the modification that Application Note PRO-AG-02; Dried Plant Tissue (Milestone Srl) was used for decomposition, and analyzed spectrophotometric in a Microwave Plasma Atomic Emission Spectrometer, MP-AES 4200 (Agilent Technologies Inc, Santa Clara, USA). One unit (FYT) of phytase was defined as the activity that released 1 μmol inorganic phosphate from 5.0 mM phytate per minute at pH 5.5 and 37°C, and the phytase activity in the diets was determined by the ISO Standard 30024 (2009) method. For metacarpal analyses, soft tissues from the bones were removed by hand after boiling the pig's trotters. DM was determined by drying the bones for 16 h at 104°C, thereafter bones were ashed at 550°C for 16 h to determine and percentage.

2.3. Calculations

Feed conversion ratio (FCR) was calculated as feed intake/body weight gain.

Apparent digestibility of nutrients was calculated by the following formula:

Nutrient digestibility coefficient = 1 – [([TiO_2]diet/[TiO_2]digesta) × ([nutrient]digesta/[nutrient]diet)]

2.4. Statistical analyses

For statistical analyses the general linear model procedure in SAS software 9.4 (SAS Inst. Inc., Cary, NC, USA) was used with the Ryan–Einot–Gabriel–Welsh F-test to investigate differences ($P<0.05$) between the different treatment groups. P-values between 0.05 and 0.1 were considered tendencies. The square root of means square error ($\sqrt{MSE}$) was used as a measure of random variation. Performance, digestibility, and bone parameters were subjected to a two-way analysis with phytase and acid as effects. Pig was used as the experimental unit.

3. Results

The piglets were healthy, and no diarrhea or other illness were registered during the experimental period. No interaction effects between acid and phytase were found in the experiment. For the first two weeks in the experiment (adaption period), no difference in performance between treatments was found (data not shown). An increased weight gain in the last 14 days of experimental period was seen with phytase ($P=0.046$) and acid addition ($P=0.002$) (Table 2). In addition, acid reduced FCR ($P=0.033$).

Acid addition reduced pH in stomach ($P<0.001$), but did not influence pH in the jejunum or ileum ($P>0.05$) (Table 3). Ileal pH was reduced by phytase addition ($P=0.014$). Table 4 show that phytase addition significantly increased ($P=0.004$) the apparent jejunal digestibility (AJD) of P from 0.12 to 0.29 and increased ATTD of P with 70 % from 0.33 to 0.43 and Ca with 15 % to 0.75 ($P<0.001$). In addition, phytase tended to improve apparent ileal digestibility (AID) of P

Table 2
Effect of phytase and formic acid addition on feed intake (kg), body weight gain (kg) and FCR performance in growing piglets from experimental day 15 to 28.

Phytase	Acid	BWG[1]	FI[2]	FCR[3]
Phytase				
With		11.1[a]	18.9	1.71
Without		10.5[b]	17.9	1.72
Acid				
With		11.3[a]	18.7	1.66[b]
Without		10.3[b]	18.2	1.77[a]
√MSE[4]		0.84	1.86	0.138
p-value				
Phytase		0.046	0.130	0.977
Acid		0.002	0.410	0.033
Acid x phytase		0.444	0.950	0.399

[1] Body weight gain
[2] Total feed intake
[3] Feed conversion ratio, calculated as feed:gain
[4] Square root of means square error in the analysis of variance[a-d]Means within column without common letters are significantly different at $P<0.05$

Table 3
Effect of phytase and formic acid addition on pH in stomach, jejunum and ileum in growing piglets.

Phytase	Acid	Stomach pH	Jejunal pH	Ileal pH
Phytase				
With		4.44	6.46	7.12[a]
Without		4.42	6.28	6.98[b]
Acid				
With		4.06[b]	6.41	7.02
Without		4.80[a]	6.34	7.08
√MSE[1]		0.255	0.337	0.15
p-value				
Phytase		0.855	0.152	0.014
Acid		<0.001	0.535	0.249
Acid x phytase		0.286	0.206	0.991

[1] Square root of means square error in the analysis of variance[a-d]Means within column without common letters are significantly different at P<0.05.

(P=0.086). Acid addition increased ATTD of Ca (P=0.001), Mg (P=0.040) and Fe (P<0.001) and reduced the ATTD of Zn (P<0.001). In addition, a tendency of interaction (P=0.062) between acid and phytase was seen on ATTD of Fe where acidification of the feed only improved Fe digestibility when no phytase was added. As shown in Table 5, phytase increased ash % and ash mg/g bone (P<0.001), however mg P/g bone ash was not increased with phytase addition (P=0.245).

4. Discussion

In this experiment, no interaction effects between phytase and acid addition was found, this is contradictory to previously experiments that have shown this interaction effect (Kemme et al., 1999; Jongbloed et al., 2000).

The increased growth with phytase addition is in accordance with previous experiments (Torres-Pitarch et al., 2017). However, the lack of any effect on feed intake and FCR is contrary to the conclusion in the same meta-analysis. Though it should be noted, that in 11 of 36 studies included in this meta-analysis, an unchanged or reduced FCR was observed. The increase in BWG and improved FCR with acidification during the last two weeks of the experimental period was in accordance with the results of Jongbloed et al. (2000). A prolonged retention time in the stomach is associated with an increased digestibility of protein and energy (Partanen and Mroz, 1999), and hence increase growth. Lowering of pH in stomach by adding organic acids to the feed is associated with a reduced gastric emptying rate (Van der Aar et al., 2017). However, in the current experiment no improvement in ileal digestibility of protein or starch was observed. Therefore, the increased weight gain and improved FCR with acid addition seems not to be caused by an improved ileal energy digestibility. A possible explanation for the improved growth could be other effects of the acid supplementation, such as acting as an energy source or an improved total tract digestibility of energy (Partanen and Mroz, 1999; Suiryanrayna and Ramana, 2015).

The more than doubled AJD of P with phytase addition compared to no phytase addition implies that the main site of exogenous phytase activity is in the anterior digestive system, as previously described (Selle and Ravindran, 2008). The difference in P digestibility between diets with phytase and diets without phytase was reduced from jejunum to ileum. The lack if increase in differences between treatments could be explained by the potential of phytate degradation by the phytase was already used before the feed reached jejunum. Another explanation for the lack of difference between treatments in the ileum could be that exogenous phytase may reduce the mucosal phytase activity (Selle and Ravindran, 2008), and hence there could be less total phytase activity in the ileum for the diets with phytase added. Previous studies where pigs with cannulas in the distal ileum where used, showed a significant higher AID of P with phytase addition (Lindberg et al., 2007; Zeng et al., 2011). The methodology in the current experiment where digesta from the last 1.5 meters of ileum were used to determine AID of P could be a possible explanation for the lack of finding a significant effect of phytase on AID of P and the difference between the ileal and fecal level on P digestibility. There was a clear effect of phytase addition on ATTD of P in the current experiment concurrent with previous knowledge (Selle and Ravindran, 2008; Torres-Pitarch et al., 2017). Phytase also improved bone ash content in accordance with previous results (Torres-Pitarch et al., 2017).

Acid addition reduced the pH in stomach in the current experiment

Table 5
Effect of phytase and formic acid addition on bone mineralization of the left third and fourth metacarpal in growing piglets.

Phytase	Acid	Ash %[1]	Ash mg/g bone	mg P/g ash
Phytase				
With		41.3[a]	260[a]	180
Without		37.34[b]	229[b]	178
Acid				
With		39.4	245	180
Without		39.3	243	179
√MSE[2]		1.78	14.1	3.5
p-value				
Phytase		<0.001	<.001	0.246
Acid		0.839	0.748	0.316
Acid x phytase		0.354	0.946	0.142

[1] ash % of bone DM
[2] Square root of means square error in the analysis of variance[a-b]Means within column without common letters are significantly different at P<0.05.

Table 4
Effect of phytase and formic acid addition on the apparent jejunal and ileal digestibility and apparent total tract digestibility in growing piglets.

Phytase	Acid	AJD[1] P	AID[2] P	AID Starch	AID CP[3]	ATTD[4] P	ATTD Ca	ATTD Cu	ATTD Mg	ATTD Zn	ATTD Fe
Phytase											
With		0.29[a]	0.43	0.98	0.72	0.61[a]	0.75[a]	0.12	0.31	0.17	0.05
Without		0.12[b]	0.33	0.97	0.72	0.36[b]	0.65[b]	0.16	0.33	0.17	0.06
Acid											
With		0.19	0.39	0.98	0.69	0.50	0.74[a]	0.15	0.34[a]	0.10[b]	0.12[a]
Without		0.23	0.39	0.98	0.76	0.47	0.66[b]	0.13	0.30[b]	0.25[a]	-0.02[b]
√MSE[5]		0.130	0.153	0.025	0.118	0.063	0.061	0.054	0.061	0.073	0.082
p-value											
Phytase		0.004	0.086	0.587	0.967	<0.001	<0.001	0.060	0.369	0.912	0.735
Acid		0.654	0.849	0.791	0.141	0.202	0.001	0.168	0.040	<0.001	<0.001
Acid x phytase		0.573	0.987	0.277	0.288	0.163	0.648	0.115	0.961	0.548	0.062

[1] Apparent jejunal digestibility
[2] Apparent ileal digestibility
[3] Crude protein
[4] Apparent total tract digestibility
[5] Square root of means square error in the analysis of variance[a-d]Means within column without common letters are significantly different at P<0.05.

to 4.1. This reduction in pH was expected to increase phytate-P digestibility, as the maximum phytase activity for the phytase used was observed with pH 4.0 (Menezes-Blackburn et al., 2015). The surprising lack of interaction effect between acid addition and phytase may be due to a too small pH reduction in the stomach. However, an increased phytase effect with acid addition was not shown in the experiments of Radcliffe et al (1998) and Omogbenigun et al. (2003) which had a larger pH difference between the different treatments than in the current experiment. Another explanation for the unexpected lack of interaction effects between acid and phytase could be that there was no potential for increasing the phytase effect in the early segments of the digestive system. However, in other experiments with a higher effect of phytase alone, there was still an additional effect of organic acid addition on phytase efficacy (Kemme et al., 1999; Jongbloed et al., 2000). The origin and pH optimum of the phytase product is influencing these results, as the phytase product used in the other experiments was from *Aspergillus niger* and in the current experiment the phytase was a *C. braakii* derived phytase. In addition, the P digestibility was increased throughout the whole digestive system for both diets with phytase addition, indicating that there is phytase activity also in the intestine from exogenous phytase. Thus, the lack of an additional acid effect could not be explained by a high phytase efficacy without acid addition, and the reason for the lack of effect of acid addition remains unexplained.

Lowered stomach pH by organic acids is expected to increase mineral absorption by reducing the binding of minerals to phytate (Jongbloed et al., 2000). In the current experiment, all minerals except available P were formulated to meet the requirements of the animal. The ATTD of minerals that are majorly regulated by the absorption from the intestine, like Cu, Fe and Zn will not be increased more than the requirement of the animal (Windisch, 2002). A surplus of Mg and Ca could be absorbed despite regulatory mechanisms and the potential surplus in blood and body tissues is regulated by urine secretion (De Baaij et al., 2015; Sjaastad et al., 2016). Because of this regulation mechanism, it could be more likely to see an effect of phytase addition on ATTD of Ca and Mg. The digestibility of Ca was increased by both phytase and acid addition. Because of the regulation mechanism, and since Ca is the most abundant mineral in the diet of the minerals examined and thus most likely to be bound to phytate (Humer et al., 2015) this was expected. A surprisingly decreased digestibility of Zn with acid addition was found, However, Blank et al. (2012) also found a decrease in ATTD of Zn with formic acid addition. In the absorption Cu and Zn are antagonists (Bikker et al., 2012), and this could influence absorption of both Cu and Zn as the digestibility is dependent of the level of inclusion in the diet and solubility of the minerals.

In the current experiment, it was confirmed that phytase increases P and Ca digestibility in addition to improving bone mineralization. The addition of formic acid improved growth, FCR and ATTD of Ca, Fe and Mg. However, even though acid addition reduced stomach pH, no interaction between phytase and acidification was seen.

Author statement

Siril Kristoffersen: Conceptualization, Methodology, Investigation, Formal analysis, Writing- Original draft preparation Torger Gjefsen: Conceptualization, Methodology, Writing - review & editing Birger Svihus: Conceptualization, Methodology, Investigation, Writing - review & editing. Nils Petter Kjos: Conceptualization, Investigation, Writing - review & editing

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Declarations of Competing Interest

None.

Reference

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