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Effect of Exogenous Phytase on the Phosphorus and Zinc Metabolism of Fattening Bulls

Laura Winter

Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI)

Federal Research Institute for Animal Health, Braunschweig, Germany

Tel: 49-531-596-3151 E-mail: laura.winter@fli.bund.de

Ulrich Meyer (Corresponding author)

Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI)

Federal Research Institute for Animal Health, Braunschweig, Germany

Tel: 49-531-596-3137 E-mail: Ulrich.meyer@fli.bund.de

Liane Hüther

Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI)

Federal Research Institute for Animal Health, Braunschweig, Germany

Tel: 49-531-596-3128 E-mail: Liane.huether@fli.bund.de

Peter Lebzien

Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI)

Federal Research Institute for Animal Health, Braunschweig, Germany

E-mail: Peter.lebzien@fli.bund.de

Sven Dänicke

Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI)

Federal Research Institute for Animal Health, Braunschweig, Germany

Tel: 49- 531-596-01 E-mail: Sven.daenicke@fli.bund.de

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Abstract

This study investigated the effects of exogenous phytase, different dietary phosphorus (P) and zinc (Zn) levels on the P- and Zn-metabolism of fattening bulls.

48 German Holstein bulls (average initial live weight 312 ± 49 kg) were used for a feeding trial and allocated to four dietary treatments, P/Zn, P+MIN/Zn, P/Zn+PHY and P/Zn+MIN. All bulls received a diet of 80 % corn silage and 20% concentrate on a dry matter (DM) basis. The corn silage intake was for ad libitum and the concentrate intake restricted.

The concentrate of P+MIN/Zn was supplemented with dicalcium phosphate. The concentrate of the P/Zn+PHY group was supplemented with an exogenous phytase (0.1 g/kg DM in the diet, 50 000 FTU/g) and the concentrate of the P/Zn+MIN group was supplemented with Zn. The P- and Zn-concentration in the diet of the P/Zn-, P+MIN/Zn-, P/Zn+PHY- and P/Zn+MIN-groups were 2.41, 2.99, 2.48 and 2.41 g P/kg DM and 33.2, 33.6, 34.0 and 38.8 mg Zn/kg DM, respectively.

No differences in P- and Zn-concentration of faeces, liver, testes and performance were observed between the treatments. In the *Os metacarpale*, P- and phytase supplementation resulted in a slightly higher P-concentration, while the Zn addition led to the lowest value (P=0.062). Overall, it becomes clear that the microbial phytase of the rumen is sufficient enough to make the indigestible phytate-P and the hardly digestible Zn digestible for ruminants. The supplemented dietary phytase has no influence on the bioavailability of the mineral P and the trace element Zn.

Keywords: Phytase, Ruminants, P-metabolism, Zn-metabolism

1. Introduction

Phytase releases phosphorus (P) from inositol phosphate (InsP) by hydrolysis (Suttle, 2010). P from phytate is suggested to be highly available to ruminants because of the microbial phytase activity in the rumen (Clark et al., 1986; Morse et al., 1992). However, ruminal P-excretion with faeces seems to be linear to P-concentration in the diet of ruminants (Call et al., 1987; Suttle, 2010). Although ruminants generally have the ability to use phytin-bound phosphorus through ruminal hydrolysis, different studies showed that the dietary supplementation of exogenous phytase leads to reduced faecal excretion and an increased P-concentration in bones. However, not only P is bound to phytin but also trace elements such as zinc (Zn) are known to be released by the action of exogenously added phytase in monogastric animals (Garikipati & Kincaid, 2004). If such effects occur in ruminants is not known. Additionally phytase mediated release of these trace elements might increase their bioavailability.

Zn deficiency in the animals may occur due to different reasons. The first one is the primary deficiency because of an inadequate content in the diet of the animal. The secondary deficiency is caused by other elements that might hamper the Zn-absorption (Adeola et al., 1995). Because

of this in the actual study it has to be taken into account that metal cations, such as Zn form insoluble complexes with phytate, which decrease the enzymatic rate of hydrolysis by phytase (Garikipati & Kincaid, 2004). P-supplementation might result in Zn-deficiency symptoms as high concentrations of phytate in diets may lead to insoluble complexes of phytic-acid and Zn which are poorly available (Kirchgessner et al., 1994). Kornegay (2001) also described the influence of phytase on the Zn bioavailability in pig, poultry and fish diets and emphasized that Zn is closely connected to the phytate complex. Another important aspect of Zn in the feeding is that Zn is indispensable for the fertility of cattle (Van Laar & Jongbloed, 2010). A study with rats showed that Zn is important for the spermatogenesis and that testes exhibit a high concentration of Zn. Feeding rats with a low Zn diet resulted in lower weight of testes, epididymis and dorsal prostate (Millar et al., 1958). Not only rats were effected by Zn-deficiency, but also male goats showed reductions in testicular size and loss of libido (Neathery et al., 1973). Previous studies (Barney et al., 1968; Millar et al., 1958; Neathery et al., 1973) comprise investigations in rats and goats but not in bulls.

Thus one aim of the present experiment was to elucidate the effects of exogenous phytase, Zn and P on P- and Zn-status of growing bulls. Therefore it is appropriate to examine the P- and Zn-levels in liver, testes and bones to consider this when the results are evaluated. A further important point is to investigate the effect on the P- and Zn-concentration of faeces of the bulls. Do the animals differ in their P- and Zn-concentration if reduced or higher amounts of minerals are fed and if there is an exogenous phytase in the feed?

For this reason, a P- and Zn-reduced diet should be compared to a full supplied diet and a P-and Zn-reduced diet added with phytase. Feeding these four different treatments, it becomes possible to figure out if a supplemented enzyme is able to compensate a deficiency by splitting up more indigestible P-and Zn-complexes than the rumen microbes do, and to figure out if phytase supplementation in combination with a reduced level of P and Zn-intake has effects on the performance and the P and Zn-concentration in faeces and bones.

2. Materials and Methods

2.1 Animals and Experimental Design

The experiment with 48 German Holstein bulls was carried out at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, in Braunschweig, Germany. The animals were randomly assigned based on age and body weight to one of four experimental groups of 12 animals each. At the beginning of the trial, the bulls had a mean body weight of 312 ± 49 kg. The bulls were housed in a thermally non-isolated stable and were kept in pens (8 animals per pen, each pen two animals per group) on slatted floor. The body weight was measured weekly with cattle weighing scales.

The experimental design included different P- and Zn-concentrations and phytase supplementation in the feed. The intended supply of P and Zn is related to the recommendations given by the GfE (1995). The feeding group P/Zn, P/Zn+PHY and P/Zn+MIN achieved 80%, P+MIN/Zn achieved 100% of the intended P-concentration given by GfE (1995). Feeding group P/Zn, P+MIN/Zn and P/Zn+PHY got 80%, P/Zn+MIN 100% of

the intended Zn-concentration given by GfE (1995). The diet of the experimental group P/Zn+PHY received additionally 0.1 g experimental Phytase per kg DM.

The experimental diets differed in the concentration of P, Zn and phytase. The P/Zn-group received a basal diet with a P-concentration of approximately 2.4 g/kg dry matter (DM) and a Zn-concentration of approximately 32 mg/kg DM to cover approximately 80% of the present P and Zn recommendations (GfE, 1995) without phytase supplementation. The P+MIN/Zn-group was fed the same diet added with P covering the P-demand of about 2.97 g/kg DM. The P/Zn+PHY-group obtained the control diet added with an experimental phytase. According to the manufacturer ́s specifications the phytase had an activity of minimum 50.000 FYT/g. The quantity of the enzyme added to the concentrate amounted to 100 g/t DM. The P/Zn+MIN-group received the control diet with Zn-supplementation according to the recommendation (40 mg/kg DM) (GfE, 1995).

Table 1. Experimental design and realized concentrations

	P/Zn[1]	P+MIN/Zn[2]	P/Zn+PHY[3]	P/Zn+MIN[4]
Intended concentration				
P (g/kg DM)	2.4	2.97	2.3	2.4
Zn (mg/kg DM)	32	32	32	40
Phytase (g/kg DM)	-	-	0.1*	-
Intended supply related to the recommendations given by the GFE (1995)				
P (%)	80	100	80	80
Zn (%)	80	80	80	100
Realised concentration				
P (g/kg DM)	2.41	2.99	2.48	2.41
Zn (mg/kg DM)	33.2	33.6	34.0	38.8
Phytase (g/kg DM)	-	-	0.1*	-
Realised percentage related to the recommendations given by the GFE (1995)				
P (%)	79	100	86	82
Zn (%)	83	84	85	97
* The phytase had an activity of min. 50 000 FYT/g according to the manufacturer ́s specifications.				
FYT: one FYT is the amount of enzymes that liberates 1 µmol of inorganic P per minute from an excess of Na phytate at pH 5.5 and 37° C				
[1] diet with native P and Zn content				
[2] diet with supplemental mineral P and native Zn content				
[3] diet with native P and Zn content, added with phytase				
[4] diet with native P content and supplemental mineral Zn				

The components of the concentrates used during the trial are shown in e.g. Table 2. Changes in mineral content were achieved by changes in the mineral premix in the concentrates.

Table 2. Components, mean nutrient, fibre and energy of the concentrates and corn silage of the experimental diets

	Corn silage	C-P/Zn[1]	C-P+MIN/Zn[2]	C-P/Zn+PHY[3]	C-P/Zn+MIN[4]
Components of the concentrates (g/kg)					
Wheat gluten		100	100	100	100
Corn		300	300	300	300
Dried sugar beet pulp		483.7	483.7	483.7	483.7
Calcium carbonate		36	16	36	36
Dicalcium phosphate		-	20	-	-
Soybean oil		12	12	12	12
Urea		30	30	30	30
Premix Control[*]		8.3	-	-	-
Premix with P[†]		-	8.3	-	-
Premix with phytase [‡]		-	-	8.3	-
Premix with Zn[§]		-	-	-	8.3
Mineral premix [a] (without P and Zn)		30	30	30	30
Nutrient, fibre and energy content of the concentrates and corn silage					
Organic matter (g/kg DM)	961	949	950	949	949
Crude ash (g/kg DM)	39	51	50	51	51
Crude protein (g/kg DM)	82	118	115	119	117
Ether extract (g/kg DM)	38	33	34	33	34
Crude fibre (g/kg DM)	184	166	168	166	167
ADF (g/kg DM)	208	190	192	190	191
NDF (g/kg DM)	403	372	374	371	372
P (g/kg DM)	2.33	2.85	5.59	2.93	2.76
Zn (g/kg DM)	0.02	0.13	0.14	0.14	0.18
IP-6 (g/kg DM)[5]	#	0.67	0.47	0.67	0.68
IP-5 (g/kg DM)[5]	#	0.02	0.05	0.03	0.04
IP-1/2/3/4 (g/kg DM)[5]	#	#	#	#	#
ME (MJ/kg DM)	10.7	11.9	11.9	11.9	11.9

[1] concentrate with native P and Zn content
[2] concentrate with supplemental mineral P and native Zn content
[3] concentrate with native P and Zn content, added with phytase
[4] concentrate with native P content and supplemental mineral Zn
[5] Inositol Phosphate
[*/†] 98.76 % Corn and 1.24 % Zinc sulphate monohydrate (35% Zinc in Zinc sulphate monohydrate)
[‡] 93.97 % Corn, 1.23 % Zinc sulphate monohydrate and 4.8 %, Experimental phytase (phytase activity amounted to 50 000 FYT/g)
[§] 97.6 % Corn and 2.4 % Zinc sulphate monohydrate [#] out of detection limit
[a] Composition (per kg): 560.000 IU vitamin A (E672), 70.000 IU vitamin D3 (E671), 1.050 mg vitamin E (alpha tocopherolacetat), 3.000 mg manganese (manganese (II) sulphate, monohydrate E5, 700 mg copper, 50 mg iodine (calcium jodate, water-free E2), 25 mg cobalt (cobalt sulphate, monohydrate, E3), 30 mg selenium (sodium selenate E8)

The animals were fed with restricted amounts of concentrate (2 kg/d) and corn silage for *ad libitum* intake.

The concentrate was provided via feeding stations (Type AWS HF 2ST, manufacturer: Insentec, Marknesse, The Netherlands). Water was available for *ad libitum* intake during the whole experiment. Because corn silage and concentrates were fed separately the added phytase was not exposed to moisture and was therefore assumed to be inactive prior to feeding.

The diets were intended to cover the energy and protein requirements according to the recommendations of the Society of Nutrition Physiology (GfE 1995).

2.2 Measurements and Sampling Procedure

Individual silage and water intake were recorded continuously by an automatic feeding system (RIC, manufacturer Insentec B.V., Marknesse, The Netherlands). The body weight was measured weekly with cattle weighing scales. During the experiment, representative samples of the diets were collected regularly. Silage and concentrate samples were collected twice a week and weekly, respectively. After collecting, the samples were stored at -18 ℃ and pooled over a 4 weeks period.

Additionally, once during the experiment, samples of faeces were taken from every fourth bull of every treatment group for P-, Zn- and acid insoluble ash-analysis.

The bulls were slaughtered at an average live weight of 578 kg at the slaughtering house of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute, FLI, Braunschweig. At the day of slaughter samples of *Os metacarpale,* liver and testes were taken and stored frozen at -20 ℃ until further analysis.

2.3 Analyses

Feedstuff samples were dried at 60° C for 72 hours and ground to pass a 1-mm screen. The

samples of *Os metacarpale*, testes and liver were freeze dried for the determination of P and Zn. After freeze drying, the samples were ground in mortars and after that passed through a spiral screw mincer.

Samples of feedstuff and faeces were analyzed according to the methods of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA 1997). Analysis of acid and neutral detergent fibre (ADF resp. NDF) was conducted following methods of VDLUFA (1997). P and Zn in feedstuff, bones, testes liver and faeces were analyzed by an optical emissions spectrometer with inductive coupled plasma (ICP-OES) according to VDLUFA (1997).

The digestibility of Zn and P was estimated by acid insoluble ash (AIA) as marker (Sunvold & Cochran, 1991). AIA in feed and faeces was analyzed with an adapted 4N HCl-method based on the method described by Wünsche et al (1984) and Mccarthy et al. (1974). A total of 2 to 5 g of freeze dried faeces or feed were ashed. The ignition of the samples lasted 5 hours at a temperature of 550 ℃. The ashes were boiled for 15 minutes with 4N HCl and the residues were filtered through an ashless filter paper. After drying the filters with the residues, they were ashed again to obtain the amount of AIA. Inositol-P was analysed in all feedstuff samples using high-performance ion chromatography according to the method of Brejnholt et al. (2011).

2.4 Calculations

The apparent total tract digestibility (AD) of P and Zn was estimated by acid insoluble ash as a marker occurring naturally in the diet as follows:

$$AD\ [\%] = ([(M_{diet}/AIA_{diet})-(M_{faeces}/AIA_{faeces})]/M_{diet}/AIA_{diet}) * 100,$$

where M_{diet} is the mineral content (P, Zn) in g/kg DM and AIA_{diet} is the AIA content in g/kg DM in the feed. M_{faeces} is the mineral content (P, Zn) in g/kg DM and AIA_{faeces} the AIA content (g/kg DM) in faeces.

The energy content of the diets was calculated based on table values given by the DLG (1997).

The phytase activity for the corn silage is calculated according to tabulated values of Eeckhout and De Paepe (1994). This results in a phytase activity for corn silage of 12 FYT/kg DM.

Empty Body weight is defined as the body weight without the content of the rumen, intestine, gall and urinary bladder. Dressing percentage was calculated as quotient of warm carcass weight and body weight.

2.5 Statistical Analysis

The statistical analysis was carried out with the SAS-software package Version 9.1.3 using the MIXED-procedure (SAS Institute, Cary, NC, USA 2004).

Treatment group was assumed to be the fixed effect. The fact that each bull was used for frequent measurements was considered using a "REPEATED" statement for the individual animal effect. The "PDIFF" option was used to determine significant effects between the least square means and "TUKEY-KRAMER" test was applied for post-hoc analysis. The results of the trial are presented in form of least square means (LS means) and standard error

(SE) of the mean. Effects are considered as significant with a $p < 0.05$.

3. Results

3.1 Chemical Composition of the Feedstuffs

The chemical composition of the concentrates is shown in e.g. Table 2. As intended the P and Zn-concentration of the groups manifested variations.

The proportion of phytate-P (IP-6/5/4/3/2/1) of the total P of the concentrates amounted to 24%, 24% and 26% phytate-P in the P/Zn-, P/Zn+PHY- and P/Zn+MIN-group. Because of the supplementation of inorganic P in the P+MIN/Zn-group, the percentage of phytate-P was lower and amounted to 9%.

The concentrated feed of group P/Zn, P+MIN/Zn and P/Zn+MIN showed no detectable phytase activity, while the feed of the P/Zn+PHY group showed a phytase activity of 5859 ±15 FYT/kg.

3.2 Performance

Corn silage intake amounted on average to 6.65 kg DM/d. No differences were observed in corn silage intake across the treatments. The mean concentrate intake was 1.75 ±0.03 kg DM/d and ranged from 1.72 to 1.78 kg DM/d e.g. Table 3. As intended the mean P-intake was significantly higher in the P+MIN/Zn-group with 26.2 g/d and the mean Zn-intake was significantly higher in the P/Zn+MIN-group with 0.32 g/d, as compared to the other groups.

Table 3. Mean feed and nutrient intakes during the experimental period (LS-means, SE)

	P/Zn[1]	P+MIN/Zn[2]	P/Zn+PHY[3]	P/Zn+MIN[4]	*SE	*P*-value
Animals/group	12	12	12	12		
DM intake (kg/d)	8.1	8.8	8.2	8.3	0.27	0.072
Corn silage (kg DM/d)	6.4	7.1	6.5	6.6	0.32	0.442
Concentrate (kg DM/d)	1.74	1.74	1.78	1.72	0.02	0.060
Crude protein (kg/d)	0.95	1.01	0.98	0.97	0.03	0.421
Ether extract (kg/d)	0.27	0.29	0.28	0.28	0.01	0.449
ADF (kg/d)	1.53	1.68	1.57	1.58	0.07	0.432
NDF (kg/d)	3.00	3.28	3.05	3.08	0.13	0.423
P (g/d)	19.6[b]	26.2[a]	20.3[b]	20.1[b]	0.65	<0.001
Zn (mg/d)	270[a]	294[b]	280[ab]	321[c]	6.5	<0.001
ME intake (MJ/d)	89.1	93.7	90.6	89.9	3.4	0.791
[a,b,c] Different letters in one row show significant differences (P<0.05)						
[*] Standard Error						
[1] diet with native P and Zn content						
[2] diet with supplemental mineral P and native Zn content						

[3] diet with native P and Zn content, added with phytase
[4] diet with native P content and supplemental mineral Zn

The average live weight gain did not differ between the treatments e.g. Table 4. The average live weight gain during the whole experiment was 1360±84 g/d. In the P/Zn- and P/Zn+PHY-group the daily live weight gain was numerically higher at the beginning of the experiment, while the bulls of the P+MIN/Zn- and P/Zn+MIN-group had a numerically higher daily live weight gain from day 101 until the end of the experiment. The average live weight gain of all groups was for the period from day 0-100 1381±64 g/d. It was 1345±107 g/d for day 101 to the end of trial. Mean feed per gain ratio (kg DM/kg LWG) was 6.03±0.15 kg DM/kg LWG, until day 101 and 6.78±0.24 kg DM/kg LWG from day 101 until the end. For the whole period mean feed per gain was 6.30±0.19 kg DM/kg LWG. Overall means for energy intake per gain were 66±2 MJ ME/kg LWG for day 1-100, 74±3 MJ ME/kg LWG for day 100-end and for the whole period 69±2 MJ ME/kg LWG.

Table 4. Live weight gain (LWG) and feed efficiency subjected to the feeding groups (n=12) (LS-means, SE)

	P/Zn[1]	P+MIN/Zn[2]	P/Zn+PHY[3]	P/Zn+MIN[4]	*SE	*P*-value
Animals/group	12	12	12	12		
Daily live weight gain (g/d)						
day 1-100	1455	1413	1321	1334	165	0.824
day 101-end	1284	1474	1234	1387	154	0.417
Total period	1379	1438	1275	1348	83	0.265
Feed per gain (kg DM/kg LWG)						
day 1-100	5.87	5.93	6.20	6.11	0.5	0.914
day 101-end	6.74	6.70	7.11	6.55	0.6	0.840
Total period	6.14	6.25	6.57	6.23	0.3	0.611
Energy intake per gain (MJ ME/kg LWG)						
day 1-100	64.8	63.4	68.3	66.3	5.7	0.844
day 101-end	74.4	71.7	78.3	71.2	6.9	0.713
Total period	67.8	66.9	72.4	67.7	3.5	0.376
* Standard Error						
[1] diet with native P and Zn content						
[2] diet with supplemental mineral P and native Zn content						
[3] diet with native P and Zn content, added with phytase						
[4] diet with native P content and supplemental mineral Zn						

Twenty four out of the 48 bulls were slaughtered. Six bulls out of every feeding group were sampled. Mean body weight of the slaughtered bulls was of 581±8 kg, while the dressing percentage amounted to 56±1 %. Considering the treatments, body weight, empty body weight,

retroperitoneal fat and dressing did not show any significant differences e.g. Table 5.

Table 5. Body weights of the bulls at slaughter and carcass characteristics (n=6) (LS-means, SE)

	P/Zn[1]	P+MIN/Zn[2]	P/Zn+PHY[3]	P/Zn+MIN[4]	*SE	*P*-value
Animals/group	6	6	6	6		
Body weight (kg)	577	584	577	584	24	0.984
EBW[†] (kg)	514	525	516	519	22	0.959
Fat of pelvic cavity and kidneys (g)	2365	2052	2371	2087	238	0.391
Dressing[‡] (%)	55.5	56.4	55.6	55.9	0.90	0.760
[*] Standard Error						
[†] Empty Body weight is defined as the body weight less the content of the rumen, intestine, gall and urinary bladder						
[‡] Dressing is calculated as carcass weight as percentage of body weight at slaughter						
[1] diet with native P and Zn content						
[2] diet with supplemental mineral P and native Zn content						
[3] diet with native P and Zn content, added with phytase						
[4] diet with native P content and supplemental mineral Zn						

The weight of the spleen tended to be lower in the P/Zn- and P/Zn+PHY-group than in the P/Zn+MIN-group and the weight of the heart tended to be lower in the P/Zn+PHY- as compared to the P/Zn+MIN-group e.g. Table 6. The treatments had no influence on the weight of lung, liver, kidneys, testes, pancreas, prostate and thyroid gland.

Table 6. Weight of different organs at slaughter (g per 100 kg LWG) depending on the diet (n=6) (LS-means, SE)

	P/Zn[1]	P+MIN/Zn[2]	P/Zn+PHY[3]	P/Zn+MIN[4]	[*]SE	*P*-value
Animals/group	6	6	6	6		
Heart	420	425	406	392	9	0.082
Liver	1357	1360	1348	1314	30	0.689
Kidneys	225	202	222	202	9	0.138
Testes	174	169	176	175	10	0.950
Lung	650	663	620	743	38	0.156
Spleen	190	195	180	216	9	0.062
Pancreas	55	70	74	73	7	0.228
Prostate	59	70	58	70	10	0.746
Thyroidgland	6	7	6	6	1	0.855
[a, b] Different letters in one row show significant differences (P<0.05)						
[*] Standard Error						

[1] diet with native P and Zn content
[2] diet with supplemental mineral P and native Zn content
[3] diet with native P and Zn content, added with phytase
[4] diet with native P content and supplemental mineral Zn

3.3 P and Zn in Faeces

There were no significant differences between the treatments, but the P+MIN/Zn-group showed the numerically highest P-concentration e.g. Table 7.

All four groups together showed a mean P-concentration of faeces of 4.6±0.9 g/kg DM. The averaged Zn-concentration of the faeces samples was 102±8 mg/kg DM.

3.4 P and Zn-digestibility

The digestibility of P and Zn showed no significant differences between the treatments e.g. Table 7. All four groups together showed a mean P digestibility of 48 percent and a mean Zn digestibility of 16.3 percent.

Table 7. P- and Zn- concentration in faeces (g/kg DM), excretion with faeces (g/d) and -digestibility depending on the diet (n=3) (LS means, SE)

	P/Zn[1]	P+MIN/Zn[2]	P/Zn+PHY[3]	P/Zn+MIN[4]	*SE	*P*-value
Concentration (g/kg DM)						
P	4.1	5.6	3.9	4.5	0.60	0.218
Zn	0.1	0.1	0.1	0.1	0.01	0.452
Excretion (g/d)						
P	9.0	11.9	10.6	10.2	1.77	0.718
Zn	0.04	0.04	0.04	0.08	0.04	0.786
Digestibility (%)						
P	49.0	47.7	48.9	50.2	6	0.994
Zn	14.1	12.3	13.9	24.8	13	0.889
* Standard Error						
[1] diet with native P and Zn content						
[2] diet with supplemental mineral P and native Zn content						
[3] diet with native P and Zn content, added with phytase						
[4] diet with native P content and supplemental mineral Zn						

3.5 Analysis of Organs

There were no differences in P- and Zn-concentration in liver and testes e.g. **Table 8**. In *Os metacarpale* the P-concentration tended to be influenced by the treatments (*P*=0.062). The animals of the P+MIN/Zn-group showed the highest P-concentration in bones (95.9 g/kg DM) while the P/Zn+MIN-group had the numerically lowest value (90.0 g/kg DM) e.g. Table 8.

Table 8. P- and Zn-concentration of liver, testes and Os metacarpale (n=6) (LS-means and SE)

	P/Zn[1]	P+MIN/Zn[2]	P/Zn+PHY[3]	P/Zn+MIN[4]	*SE	*P*-value
Animals/group	6	6	6	6		
Liver						
P (g/kg DM)	10.7	10.5	10.3	10.1	0.3	0.181
Zn (mg/kg DM)	104	97	103	93	6	0.247
Testes						
P (g/kg DM)	6.1	5.5	6.2	5.4	0.9	0.756
Zn (mg/kg DM)	41.5	36.7	39.9	35.4	5	0.641
Os metacarpale						
P (g/kg DM)	92.6	95.9	95.1	90.0	2	0.062
Zn (mg/kg DM)	57.9	60.4	57.3	58.0	3	0.657

* Standard Error

[1] diet with native P and Zn content

[2] diet with supplemental mineral P and native Zn content

[3] diet with native P and Zn content, added with phytase

[4] diet with native P content and supplemental mineral Zn

4. Discussion

The P- and Zn-reduced diet should be compared to P or Zn supplemented diets and a P- and Zn-reduced diet added with phytase. Feeding these four different diets, it should be possible to figure out if the supplemented enzyme is able to compensate the deficiency by splitting up more indigestible P- and Zn-complexes than the rumen microbes do. The intended P-concentrations in the diets of group P/Zn, P+MIN/Zn, P/Zn+PHY and P/Zn+MIN as well as the desired difference of 20% between the groups with or without P- and Zn-supplementation were almost achieved. The feed ingredients including corn silage, corn, wheat gluten and dried sugar beet pulp contributed to the comparatively low dietary P-concentration. The P-concentration of the corn silage was on average 2.33 g/kg DM and the mean P-concentration of the unsupplemented concentrates was 2.85 g/kg DM. With 24 to 26 % phytate P (sum of InsP-6/5/4/3/2/1) of total P of concentrates of the unsupplemented groups, the current study supports the data of Maenz (2001). Maenz (2001) investigated the occurrence of phytic acid in plants and found that cereals and grain legumes that are commonly used as feed ingredients all have similar phytate levels. The content of phytate P in the concentrate of the P-supplemented group was only 9%. This can be explained with the supplementation of inorganic P in the P+MIN/Zn-group, while the P content of the other groups is native.

The requirement of Zn is met when the diet contains 40 mg Zn/kg dry matter intake (DMI) for bulls (GfE, 1995). The P- and Zn-reduction of group P/Zn+PHY to 86 respectively 85% related to the recommendations given by the GfE (1995) should be compensated by the effect of the supplemented exogenous phytase. Brask-Pedersen et al. (2011) and Garikipati and Kincaid

(2004) found, that exogenous phytase can influence the availability of P positively. As Zn is probably the most vulnerable mineral to phytate complexation (Kornegay, 2001) it seems possible that exogenous phytase could decrease Zn-excretion with faeces, too.

But in the actual study, there are no differences visible between the P/Zn- and P/Zn+PHY-group. Phytase showed neither an effect on the P and Zn-excretion with faeces nor on the P and Zn digestibility of the animals.

Geisert et al. (2010) investigated the relationship between P requirement and excretion of finishing beef cattle fed different concentrations of P. Geisert et al. (2010) did not find differences in the DMI depending on P-intake. In contrast, in the present study there was a tendency towards a higher feed intake of the P+MIN/Zn group (P=0.072). The DMI of this group was 0.5 to 0.7 kg per day higher compared with the other groups. Like in the study of Geisert et al. (2010) there were no differences in DMI depending on P-intake. The observation that feed intake was not affected by Zn-treatment is in agreement with Khan (1978) and Mandal et al. (2007), where feed intake of growing calves and bulls was unaffected by an increase of dietary Zn-concentration of 2.5 mg Zn/kg DM. Supplementations of Zn methionine to a diet containing more than 25 mg Zn/kg DM did not affect feed intake in ewes (Salama Ahmed, 2003), goats (Puchala et al., 1999), growing lambs (Droke et al., 1998), beef steers (Duff et al., 2000) and bulls (Mandal et al., 2007). Mandal et al. (2007) investigated diets for growing bulls with a Zn-concentration of 32 and 35 mg Zn/kg DM, respectively. That complies with 80% and 87.5% of the recommendations given by the GfE (1995). The different Zn-concentrations did not affect the intake and balance of minerals like Ca and P in bulls. In accordance with Mandal et al. (2007) in the present study no effect of Zn-treatment on P-intake, P-excretion and P-digestibility was detected, while Zn-supplementation to feed results as desired in higher Zn-intake in group P/Zn+MIN. Contrary to Mandal et al. (2007), the current study showed no influence of Zn-supplementation on Zn-excretion with faeces and the Zn-digestibility.

Fattening bulls excrete the main part with faeces and only 1% of total P- excretion with urine (Klosch et al., 1994; Vitti et al., 2000). Klosch et al. (1994) investigated that the faecal P-excretion of fattening bulls is between 0.71 and 1.10 g/kg DMI. Calculating the faecal P-excretion of the animals during the current study considering these assumptions, the P-excretion should amount from 5.75 to 9.7 g/d. In the actual study the analyzed P-excretion with faeces ranges from 9.0 g to 11.9 g/d. Consequential the faecal P-excretion was between 1.0 and 1.6 g/kg DMI. While Geisert et al. (2010) observed a significant effect of P-intake on the faecal P-excretion in a study with steers, the P+MIN/Zn-group of the actual study showed a tendency towards a higher P-excretion compared with the other groups (P=0.085). Both results indicate that faecal losses were related to P-intake. This was also confirmed by studies with dairy cattle (Braithwaite, 1985; Khorasani et al., 1997; Scott & Buchan, 1985; Ternouth, 1989; Valk et al., 2002).

To get an impression of the P retention in body, the P-balance was calculated. The P-excretion with urine was assumed to be 1% of total P-excretion and consequently amounts to 0.1 g/d. Taking the P-excretion with faeces e.g. Table 7 and the calculated P-excretion with urine into

account, P-balances are as follows: 10.4, 14.2, 9.6 and 9.8 g/d for groups P/Zn, P+MIN/Zn, P/Zn+PHY and P/Zn+MIN. Winter et al. (2013) investigated the P-balance of lactating cows fed comparable diets. The study resulted in P-balances between 16.2 and 26.4 g/d. The difference between the studies can be explained with the gender and the age of the animals.

Compared to the P-excretion, the Zn-excretion showed a similar pattern. While Mandal et al. (2006) found significantly higher Zn-excretion with faeces and urine of Zn-supplemented groups, in the actual experiment with fattening bulls, these results could not be confirmed.

The skeleton is the main P-storage (Fernandez, 1995; Pfeffer et al., 2005). 80% of whole body P is stored in bones, while the remaining part is contained in tissues and fluids (Kirchgessner et al., 1994). Taking this fact and the P-balance in the actual study into account, a quantity of 8.3, 11.4, 7.7 and 7.8 g P/d is stored in the bones (groups P/Zn, P+MIN/Zn, P/Zn+PHY and P/Zn+MIN). The P-concentration of bones is different, depending on the particular type of bone. Results of investigations by Williams et al. (1991) indicate that chemical and physical properties of bovine bones are sensitive to dietary P. Erickson et al. (2002) studied the effects of dietary P-concentration on quantity and route of P-excretion and P-concentration in the bones of cattle feed finishing diets over 180 days. They concluded like Shupe et al. (1988), Ternouth (1990) and Geisert et al. (2010) that the metacarpal bone of cattle could be an indicator of mineral status. The ash of *Os metacarpale* showed an average P content of 17.3%. In the actual study, the phytase as well as the P-supplementation to a diet low in P tended to increase the P-concentration in *Os metacarpale* (P=0.062). Therefore, it can be ascertained that there seems to be a potential of the tested phytase to replace mineral P-supplementation under P deficient conditions.

An interesting aspect of the present study is the liver with its Zn binding protein, metallothioneins (MTs). MTs have the ability of releasing Zn, when necessary (Tapiero & Tew, 2003). For this reason the question arises if the Zn-concentration in the liver increases linear to the Zn-supplementation of the diet. Cao et al. (2000) analyzed the Zn-concentration of the liver of growing lambs depending on the dietary Zn-intake and ascertained that the Zn-concentration of the liver increased linearly with the Zn-concentration of the diet. Wright and Spears (2004) studied the effect of different Zn sources and different amounts of supplementation on the Zn-concentration in the liver and bones of calves. They found that the Zn-concentration in the liver and bones rose linearly with the Zn-intake. In contrast to these results, no relation between the dietary Zn-intake and Zn-concentration in the liver and bones were observed in the present study. An explanation for the differences between calves and fattening bulls could be a more effective homeostatic control mechanism of older animals which is responsible for the regulation of the Zn content of liver tissue (Kincaid et al., 1997).

In the actual study, the P- and Zn-concentration of the testes was not influenced by the dietary treatment. These results were in line with the studies of Wright et al. (2004) and Cao et al. (2000) who found similar effects. There was no effect of dietary treatment on the weight of testes, too. Foote et al. (1977) observed a positive relation between the number of sperms and the size of scrotum. Amstutz (1979) detected a positive relation between the size of scrotum and the motility of sperms in ejaculate of bulls. Furthermore density of ejaculate is sensitive to

external parameters like genetic and feed. In addition there is evidence that a Zn supplementation could have a positive influence on the fertility of bulls.

The logical effect of this study is that the microbial phytase of the rumen is sufficient enough to make the indigestible phytate-P and the hardly digestible Zn digestible for ruminants.

5. Conclusions

In the present study a supplementation of phytase has no influence on the feed intake, live weight gain, feed efficiency, slaughter characteristics and P and Zn digestibility. Only the P-concentration of *Os metacarpale* tended to be increased for animals fed a P deficient diet supplemented with phytase. Under the conditions of the present study the microbial phytase in the rumen appears to be sufficient to make the phytate bound P and the hardly digestible Zn available for the animals.

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