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## Original Research Article

## Effect of phytase superdosing, myo-inositol and available phosphorus concentrations on performance and bone mineralisation in broilers



Sophie A. Lee <sup>a,\*</sup>, Devanaboyina Nagalakshmi <sup>b</sup>, Mantina V.L.N. Raju <sup>c</sup>,  
Savaram V. Rama Rao <sup>c</sup>, Michael R. Bedford <sup>a</sup>

<sup>a</sup> AB Vista, Marlborough, Wiltshire SN8 4AN, United Kingdom

<sup>b</sup> Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad 500030, India

<sup>c</sup> Directorate of Poultry Research, Rajendranagar, Hyderabad 500030, India

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## ABSTRACT

A total of 2,376 one-day-old Ross broiler chickens were used to investigate the effect of myo-inositol (MYO) and phytase supplementation on performance and bone mineralization variables in broilers fed diets formulated to have varying concentrations of available phosphorus (P). The trial was designed as a  $2 \times 2 \times 3$  factorial; with and without phytase superdosing (0 or 1,500 FTU/kg), MYO (0 or 3 g/kg), and dietary P (low, moderate or high). At 21 d, dietary phytase and MYO had no consistent benefit on bone mineralization variables. Bone ash reduced by 4.7% from the medium to low P diet ( $P < 0.01$ ), with no effect of phytase supplementation. Superdosing improved bone P content by 6% in birds fed the low P diet, signifying an interaction between dietary P concentrations and phytase ( $P < 0.05$ ). Dietary MYO addition resulted in a numerical reduction in bone ash and a significant reduction in bone strength ( $P < 0.05$ ). At 42 d, the beneficial effect of phytase superdosing on feed intake and body weight gain was evident in the low P diet. Superdosing reduced feed conversion rate (FCR) at all P levels ( $P < 0.05$ ), although this effect was more pronounced on the low P diet, suggesting that sufficient P being released from the phytase itself to re-phosphorylate MYO and hence improve FCR. The significant improvement in FCR was greater with superdosing than with MYO alone, and the combination led to no further improvement in FCR compared with superdosing alone, signifying a phytase and MYO interaction ( $P < 0.05$ ). From these results, it can be estimated that MYO is providing around 30% to 35% of the total response to superdosing.

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## 1. Introduction

Exogenous phytases are one of the most routinely used feed additives in poultry diets. More recently, the concept of superdosing (Cowieson et al., 2011) has demonstrated further improvements to weight gain and feed conversion rate (FCR) compared

with standard doses, due to phytate destruction rather than phosphorus (P) provision. It is possible that a large part of the superdosing benefit of phytase is brought about through the production of myo-inositol (MYO) which is subsequently absorbed and utilised in a number of biological functions within the animal (Lee and Bedford, 2016). Although most animals are capable of synthesising MYO *de novo* from D-glucose-6-phosphate in a number of tissues, its importance for cell survival and growth is evident (Eagle et al., 1956; Holub, 1986). Specific re-phosphorylation of MYO within tissues determines its role within the body, potentially providing many of the benefits which we ascribe to superdosing.

Improvements in body weight gain (BWG) have already been demonstrated with MYO supplementation of low P diets. Zyla et al. (2004) reported improvements in BWG and FCR in broilers fed P-deficient diets supplemented with 0.1% MYO from 1 to 21 d. Since the re-phosphorylation of absorbed MYO is required for

\* Corresponding author.

E-mail address: [Sophie.Lee@abvista.com](mailto:Sophie.Lee@abvista.com) (S.A. Lee).

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functionality, this might suggest that a greater supply of MYO will increase P requirement of the animal. However, Zyla et al. (2004) reported a reduction in P retention (%) with addition of MYO. The reasoning for this was unclear and therefore requires further investigation. Pirgozliev et al. (2007) also reported an improvement in feed intake and BWG with 0.25% MYO supplementation to a low P diet in broilers from 7 to 17 d. However, higher inclusion levels of 5.0% and 7.0% MYO did not show any significant effect on parameters compared to the non-supplemented diet. These results might suggest a deviation of energy expenditure from growth towards absorption and utilisation of excess MYO being delivered to the animal.

In one experiment by Cowieson et al. (2013), inclusion of 0.15% MYO tended to increase BWG of broilers fed diets low in Ca<sup>2+</sup> and digestible P (dP), and reduce BWG of broilers fed adequate Ca<sup>2+</sup> and dP diets. However, irrespective of Ca and dP level, supplementation of MYO reduced 42 d FCR by 9 points. In the second experiment, inclusion of 0.15% MYO increased BWG and reduced FCR of 42-day-old birds, regardless of Ca and dP level. During the starter phase (d 1 to 10), authors report negative effects of MYO on BWG and FCR, with addition of phytase partially alleviating this effect, thereby suggesting a positive interaction between MYO and phytase. This interaction is potentially due to the available P requirement of MYO to support its re-phosphorylation post-absorption and thus formation of metabolically active MYO phosphate esters.

Although the benefits of phytase superdosing are becoming well-recognised in the feed industry, the mechanisms of underlying such benefits still need to be elucidated. The current study was therefore designed to explore the interaction of MYO and phytase superdosing on performance and bone mineralization variables of broilers fed diets varying in available P content. Application of 3 g/kg MYO was chosen to represent the amount of MYO that would be generated if all the phytic acid in a typical corn–soy diet was dephosphorylated.

## 2. Materials and methods

### 2.1. Animals, housing and diets

A total of 2,376 one-day-old Cobb 400 broiler male chicks were randomly allocated to 12 treatment groups. Experimental diets (Table 1) were fed in mash form from d 0 to 21 (starter) and d 22 to 42 (grower). The trial was designed as a 2 × 2 × 3 factorial; with and without phytase (0 or 1,500 FTU/kg), MYO (0 or 3 g/kg), and dietary P (low, moderate or high). The phytase used in this experiment was an enhanced *Escherichia coli* phytase (Quantum Blue; AB Vista, Marlborough, UK) with an expected activity of 5,000 FTU/g. Each experimental diet was randomly assigned to 9 replicate floor pens of 22 broiler chicks (Sri Ramadhootha Poultry Research Farm, Hyderabad, India). Water and feed was provided *ad libitum* throughout the trial. During the first growth phase (d 0 to 21), light was provided for 24 h, with 6 dark hours per day being established in the second phase (d 22 to 42). The temperature of housing unit was kept at 35 °C during the first week, followed by a progressive decrease in temperature reaching 26 °C by 21 d. From d 22 to 42, birds were maintained at room temperature (19.5 to 28 °C).

### 2.2. Traits measured

Body weight and feed intake were recorded on a weekly basis per pen, for determination of FCR (feed intake/weight gain). At 21 d, 5 birds from each treatment replicate were slaughtered by cervical dislocation following a 12 h fast to study bone mineral variables (tibia breaking strength, tibia ash, Ca and P concentrations in tibia ash). Soft tissue was removed from the tibiae prior to drying at

**Table 1**  
Composition of experimental diets (as-fed basis).

Item	Starter (0 to 21 d)			Finisher (22 to 42 d)		
	Low P	Medium P	High P	Low P	Medium P	High P
Ingredient, g/kg						
Corn	605	598	593	685	679	674
Soybean meal 48	338	339	339	262	263	264
Soy oil	18.0	20.2	21.8	21.0	23.2	24.9
NaCl (Salt)	3.7	3.7	3.7	3.7	3.7	3.7
Sodium bicarbonate	1.3	1.3	1.3	1.3	1.3	1.3
DL-methionine	3.0	3.0	3.0	2.1	2.1	2.1
Lysine HCl	2.0	2.0	2.0	1.4	1.4	1.4
Threonine	0.5	0.5	0.5	0.1	0.1	0.1
Limestone	12.6	7.2	3.1	10.9	5.5	1.4
Dicalcium phosphate	11.7	20.5	27.1	6.7	15.5	22.1
Coccidiostat	0.2	0.2	0.2	0.2	0.2	0.2
Premix <sup>1</sup>	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient level, g/kg						
Crude protein	212	212	212	180	180	180
ME, kcal/kg	3,060	3,060	3,060	3,160	3,160	3,160
Calcium	9.8	9.8	9.8	7.8	7.8	7.8
Phosphorus	6.2	8.0	9.4	4.9	6.7	8.1
Available phosphorus	3.2	4.8	6.0	2.2	3.8	5.0
Methionine	6.3	6.3	6.3	5.0	5.0	5.0
Cysteine	3.5	3.5	3.5	3.1	3.1	3.1
Methionine + Cysteine	9.8	9.8	9.8	8.1	8.1	8.1
Lysine	13.1	13.1	13.1	10.5	10.5	10.5

ME = metabolizable energy.

<sup>1</sup> Vitamin/mineral premix supplied per kilogram of diet: zinc, 60 mg; iron, 45 mg; copper, 8 mg; selenium, 0.18; manganese, 80 mg; vitamin A (retinol acetate), 2.75 mg; vitamin D<sub>3</sub>, 0.03 mg; vitamin E, 10 mg; vitamin B<sub>1</sub>, 1 mg; vitamin B<sub>2</sub>, 10 mg; vitamin B<sub>3</sub>, 15 mg; vitamin B<sub>5</sub>, 10 mg; vitamin B<sub>6</sub>, 2 mg; vitamin B<sub>12</sub>, 0.01 mg; vitamin K (menadiolone), 2 mg; biotin 0.08 mg; choline chloride, 650 mg.

100 °C for 3 h. Bone samples were defatted by soaking in petroleum ether for 48 h. The right tibia of each bird was used to determine the breaking strength, as measured using a three-point method with a universal testing machine (EZ Test, Shimadzu, Japan). The bone was rested on 2 points with a gap of 50 mm and pressure was applied with a pressure sensitive load cell (10 kg) at the center of the 2 points, which coincided with the center of the bone at a speed of 5 cm/min. Both tibiae were ashed together at 600 ± 20 °C for 12 h, and the Ca (Atomic Absorption Spectrophotometer, AAnalyst 400, PerkinElmer, Shelton, CT, USA) and P (Fiske and Subbarow, 1925) concentrations in the bone ash were measured.

### 2.3. Statistical analysis

All parameters were compared statistically by ANOVA using the fit model platform of JMP Pro 12.2.0 (SAS Institute Inc., Cary, NC). The statistical model included dietary P concentration, MYO, phytase and the interaction between these 3 factors. When differences were significant ( $P \leq 0.05$ ), least significant differences (LSD) were determined. In all instances, differences were reported as with actual *P*-values.

## 3. Results and discussion

The importance of MYO for growth in chickens was firstly reported by Hegsted et al. (1941). Inclusion of MYO to chick diet demonstrated BWG responses ranging from 18 to 52 g in 28-day-old birds. In the current study, effects of dietary treatment on broiler performance are shown in Table 2. Within the first 3 weeks, there was evidence of a developing interaction between phytase and dietary P level for all performance parameters. This interaction can be best described by the fact that phytase had increasingly beneficial effects on BWG and feed intake in the lower P diets. Feed

**Table 2**  
Effect of dietary phytase, inositol and phosphorus (P) content on broiler feed intake, weight gain and FCR from d 0 to 42.<sup>1</sup>

Item <sup>2</sup>	Inositol, g/kg	Phytase, FTU/kg	Week 1 (0 to 7 d)			Week 3 (0 to 21 d)			Week 6 (0 to 42 d)		
			Intake, g	Gain, g	FCR, g/g	Intake, g	Gain, g	FCR, g/g	Intake, g	Gain, g	FCR, g/g
P level											
Low	0	0	101	131	0.77	1,009	730	1.38	3,934	2,134	1.84
Low	3	0	105	130	0.81	955	720	1.33	3,957	2,191	1.81
Low	0	1,500	105	140	0.75	1,044	813	1.28	4,065	2,304	1.76
Low	3	1,500	108	143	0.75	1,085	837	1.30	4,134	2,354	1.76
Medium	0	0	103	135	0.76	1,056	797	1.33	4,203	2,325	1.81
Medium	3	0	104	136	0.76	1,067	805	1.32	4,082	2,293	1.78
Medium	0	1,500	109	142	0.77	1,072	819	1.31	4,066	2,313	1.76
Medium	3	1,500	110	143	0.77	1,053	811	1.30	4,067	2,290	1.78
High	0	0	105	133	0.79	1,059	789	1.34	4,143	2,315	1.79
High	3	0	104	135	0.77	1,056	797	1.32	4,082	2,289	1.78
High	0	1,500	100	141	0.71	1,054	813	1.30	4,057	2,322	1.75
High	3	1,500	100	138	0.72	1,031	808	1.28	4,078	2,325	1.76
LSD 5%			11	10	0.043	46	31	0.03	74	41	0.017
P level × Inositol											
Low			105	136	0.77	1,023	775	1.32	4,023	2,246	1.79
Medium			107	139	0.77	1,062	808	1.31	4,104	2,305	1.78
High			102	137	0.75	1,050	802	1.31	4,090	2,313	1.77
LSD 5%			6	5	0.021	23	15	0.015	64	35	0.015
P level × Phytase											
	0		104	137	0.76	1,049	794	1.32	4,078	2,286	1.79
	3		105	137	0.76	1,041	796	1.31	4,067	2,291	1.78
LSD 5%			5	4	0.018	19	13	0.012	52	29	0.012
P level × Inositol × Phytase											
		0	104	133	0.78	1,033	773	1.34	4,067	2,258	1.80
		1,500	105	141	0.74	1,056	817	1.29	4,078	2,318	1.76
LSD 5%			5	4	0.018	19	13	0.012	52	29	0.012
P-value											
P level			0.264	0.403	0.063	0.004	0.000	0.237	0.029	0.001	0.009
Inositol			0.568	0.816	0.460	0.412	0.690	0.016	0.671	0.732	0.125
P level × Inositol			0.715	0.891	0.439	0.921	0.914	0.568	0.260	0.062	0.249
Phytase			0.479	0.000	0.001	0.016	0.000	0.000	0.686	0.000	0.000
P level × Phytase			0.144	0.486	0.008	0.000	0.000	0.017	0.001	0.000	0.029
Inositol × Phytase			0.918	0.974	0.915	0.427	0.913	0.126	0.116	0.730	0.014
Inositol × Phytase × P level			0.960	0.609	0.197	0.013	0.208	0.019	0.843	0.885	0.547

FCR = feed conversion ratio (feed intake: body weight gain); LSD = least significant difference.

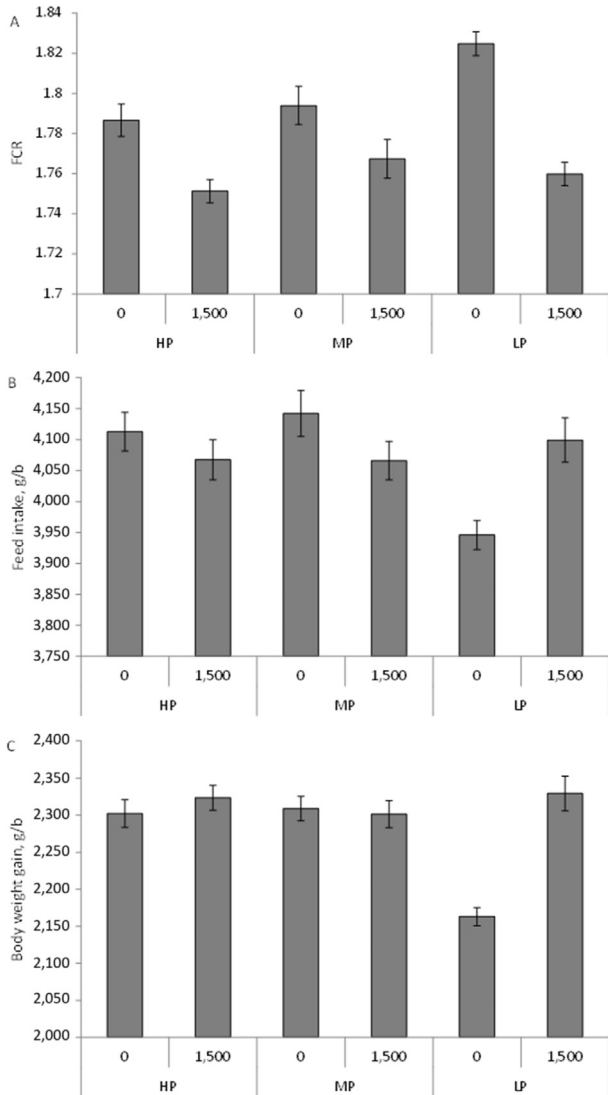
<sup>1</sup> Means represent the average response of 9 replicate pens (198 chicks) per treatment.

<sup>2</sup> Low, medium and high P levels were set as 3.2, 4.8 and 6.0 g/kg for starter, and 2.2, 3.8 and 5.0 g/kg for finisher phases, respectively.

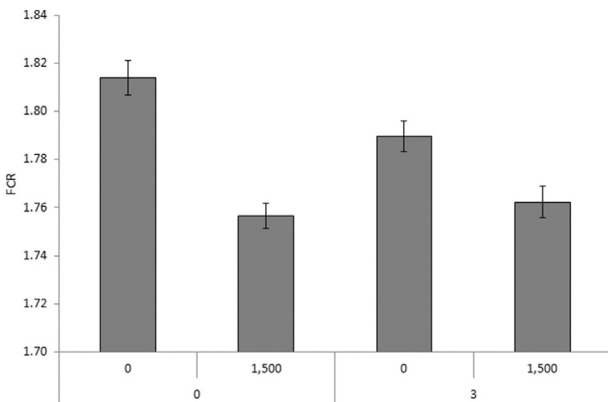
conversion ratio on the other hand, was improved on the low and high P diets with addition of phytase but not on the medium P diet ( $P < 0.05$ ). This interaction remained significant for almost every week and for each parameter but evolved such that the benefit of superdosing phytase on FCR became evident by the end of the trial with all P levels but was most evident on the low P diet (Fig. 1A). Nevertheless, the benefit of superdosing on both the medium and high P diets remained constant with a reduction in FCR of around 4 points. For intake (Fig. 1B) and gain (Fig. 1C), the picture remained that the benefit of the high phytase dose was most evident in the low P diets ( $P < 0.05$ ) and disappeared with medium or high P diets. This is in support of previous studies that have reported performance improvements with high phytase doses (1,500 FTU/kg) in P-deficient diets (dos Santos et al., 2013; Tang et al., 2012; Karimi et al., 2013). Such a finding indicates that the parameter which is most sensitive to superdosing is FCR regardless of P concentration of the diet. Given this finding, it is interesting to note the appearance of an interaction ( $P < 0.05$ ) between MYO and phytase at 6 weeks of age. The improvement in FCR with superdosing is reduced in the presence of MYO, but not entirely (Fig. 2). The alternate view is that the provision of MYO results in a significant 2.5 point improvement in FCR which disappears when the phytase is superdosed. This suggests that some but not all of the benefit of superdosing is driven by the generation of MYO. Given that superdosing yielded a 6 point reduction in FCR, while MYO decreased FCR by 2 points which was further reduced with additional superdosing, it can be estimated that MYO provides around 30% to 35% of the total response to superdosing.

Supporting the work of Zyla et al. (2004), supplementing P-deficient diets with MYO significantly reduced ( $P = 0.016$ ) FCR of broilers at d 0 to 21. However, the negative effects of MYO during the starter period, as seen by Cowieson et al. (2013), were not evident in this study. Upon reflection of the author's data, it is clear that there is a misinterpretation of statistical results. Myo-inositol had no significant effect on BWG or FCR during the starter period. The combination of MYO and phytase gave the best performance results, which may account for the MYO × phytase response, as this effect appeared to be additive. However, it should also be noted that all birds performed poorly during this starter period, with FCR being approximately 45 points above Ross 308 breed standards, while at the end of the 42-day experiment FCR was only marginally higher than breed standards. Therefore, performance variability seen in younger birds could have masked any real effects of MYO under more normal circumstances during this phase, with the true value of the MYO and phytase response being shown in later growth stages when performance was no longer hindered.

A number of studies support the beneficial effect of phytase on bone mineralisation (Onyango et al., 2004; Viveros et al., 2002). However, in the current study no consistent effects of note were seen in bone data at 21 d (Table 3). Ash content decreased by 4.7% from the medium to low P diet ( $P < 0.01$ ), but no effect of phytase was noted on this parameter ( $P > 0.05$ ). Previous work (Rama Rao et al., 1999) has also reported no influence of phytase on tibia ash content. In contrast to previous findings (Ptak et al., 2013), phytase was shown to depress bone Ca ( $P < 0.01$ ). This was unexpected since microbial phytase has been shown to improve Ca retention



**Fig. 1.** Effect of P level and phytase on broiler performance at 42 d. Data show (A) FCR, (B) feed intake and (C) body weight gain of broilers fed high (HP), medium (MP) or low (LP) dietary P with phytase supplemented at 0 or 1,500 FTU/kg.



**Fig. 2.** Effect of inositol and phytase on FCR of broilers at 42 d. Diets were supplemented with inositol at a level of 0 or 3 g/kg, and phytase at 0 or 1,500 FTU/kg.

**Table 3**

Influence of dietary phytase, inositol and phosphorus (P) content on bone parameters of broilers at 21 d.<sup>1</sup>

Item <sup>2</sup>	Inositol, g/kg	Phytase, FTU/kg	Strength, kg	Ash, %	Ca, %	P, %
P level						
Low	0	0	3.57	48.23	34.70	16.59
Low	3	0	3.16	48.90	34.67	16.56
Low	0	1,500	3.89	50.50	33.10	17.72
Low	3	1,500	3.98	48.57	32.28	17.56
Medium	0	0	3.88	51.28	34.97	16.89
Medium	3	0	3.63	50.54	34.48	17.72
Medium	0	1,500	3.59	52.02	32.51	17.02
Medium	3	1,500	3.02	51.99	32.77	17.51
High	0	0	3.30	52.47	34.70	17.02
High	3	0	3.16	51.99	32.06	17.84
High	0	1,500	3.64	50.88	30.04	16.99
High	3	1,500	3.27	50.65	31.49	17.28
LSD 5%			0.40	2.57	2.89	0.78
Low			3.65	49.05	33.69	17.11
Medium			3.53	51.46	33.68	17.28
High			3.34	51.50	32.07	17.28
LSD 5%			0.20	1.28	1.44	0.39
0			3.64	50.90	33.34	17.04
3			3.37	50.44	32.96	17.41
LSD 5%			0.16	1.05	1.18	0.32
0			3.45	50.57	34.26	17.10
1,500			3.56	50.77	32.03	17.35
LSD 5%			0.16	1.05	1.18	0.32
P-value						
P level			0.089	0.009	0.174	0.750
Inositol			0.018	0.524	0.638	0.092
P level × Inositol			0.640	0.985	0.969	0.314
Phytase			0.322	0.780	0.007	0.266
P level × Phytase			0.002	0.262	0.942	0.032
Inositol × Phytase			0.941	0.705	0.403	0.441
Inositol × Phytase × P level			0.268	0.593	0.447	0.924

LSD = least significant difference.

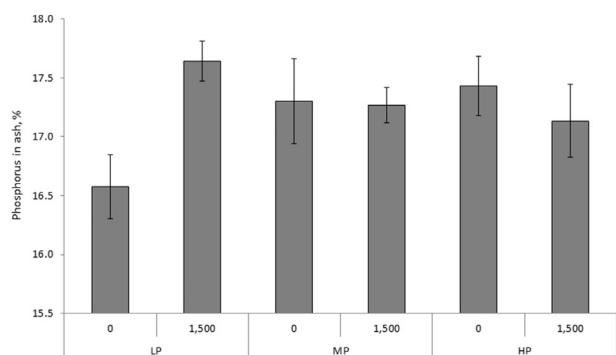
<sup>1</sup> Means represent the average response of 5 chicks per treatment.

<sup>2</sup> Low, medium and high P levels were set as 3.2, 4.8 and 6.0 g/kg for starter, and 2.2, 3.8 and 5.0 g/kg for finisher phases, respectively.

and bone mineralization (Rutherford et al., 2012; Chung et al., 2013).

Bone P content was the lowest in birds fed the low P diet ( $P < 0.05$ ). Phytase supplementation of the low P diet increased bone P content by 6%, indicating an interaction between dietary P level and phytase ( $P < 0.05$ ). Since P levels were lower in the low P diet, the total amount absorbed in milligram per day would be less and hence birds fed the lower P diet would expect to have poorer bone P content. Moreover, as dietary Ca level was kept consistent in diets despite changes in dietary P level, variations in Ca:P ratio may also have been responsible for this response. The high Ca:P ratio of the low P diet might cause greater interaction of Ca with inorganic P (Hurwitz and Bar, 1971; Rama Rao et al., 2006), as well as forming Ca-phytate complexes that impede phytate degradation (Tamim et al., 2004), thereby limiting P utilisation. Superdosing may reduce the potential for chelation, through degradation of not only phytase (IP6) but also the lower esters (IP5, IP4 and IP3) which also have an anti-nutritive effect in the animal. Since phytase did not have any additional benefit on bone P in birds fed diets with higher levels of P (Fig. 3), yet a positive FCR response was evident, this might suggest that this FCR response was an ‘extra-phosphoric’ effect of superdosing (Walk et al., 2013).

Dietary MYO addition caused a numerical reduction in bone ash and a significant reduction in bone strength ( $P < 0.05$ ). Since bone Ca and P concentrations were not significantly affected by MYO ( $P > 0.05$ ), it could be speculated that any P release from



**Fig. 3.** Effects of phytase and phosphate level on the percentage of P in bone ash of broilers at 21 d. Broilers were fed high (HP), medium (MP) or low (LP) dietary P, with phytase supplemented at 0 or 1,500 FTU/kg.

phytate destruction may be preferentially used to re-phosphorylate MYO rather than remodel bone. In accordance, Zyla et al. (2004) reported a reduction in P retention (%) with addition of MYO. This reinforces the concept that MYO alone cannot give full superdosing benefits, and that phytase is necessary to improve P retention and bone characteristics (Denbow et al., 1998; Onyango et al., 2004; Rutherford et al., 2004; Bougouin et al., 2014). Bone strength was also significantly lower ( $P < 0.05$ ) in birds fed the high P diet, compared to birds fed the moderate P diet and low P diet with phytase. Overall, these results demonstrate no interaction between bone strength and ash, Ca or P content ( $R^2 = < 0.01$ ).

#### 4. Conclusions

Superdosing of phytase resulted in a significant improvement in FCR compared with the negative control in the absence of MYO, and only a suboptimal improvement in its presence. This suggests that at least some part of the superdosing response, approximately 30% to 35%, is driven by MYO generation. The fact that the presence of phytase removed any benefit seen with inclusion of MYO suggests that the superdosing treatment is supplying enough MYO to remove the benefit of adding 3 g/kg MYO to the diets. Since the addition of phytase and MYO had no consistent benefit on bone parameters, FCR seems to be the parameter most sensitive to superdosing. The mechanisms underlying performance benefits with superdosing still need to be fully established, although MYO appears to play an important role.

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