



# Phosphorus Utilisation and Growth Performance of Broiler Chicken Fed Diets Containing Graded Levels of Supplementary Myo-Inositol with and Without Exogenous Phytase

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## ABSTRACT

A total of 80 male Ross 308 broiler chickens were used in this study to quantify the response and inter-relationship of bird growth performance, dietary nitrogen corrected apparent metabolisable energy (AMEn), and nutrient digestibility as a result of feeding graded levels of supplementary myo-inositol (MYO) with and without exogenous phytase (PHY). Supplementation of diet that was formulated to be insufficient in available Phosphorus (P) with graded levels of MYO improved daily weigh gain (WG) and AMEn intake ( $P<0.05$ ; quadratic) and linearly reduced ( $P<0.05$ ) the concentration and the secretion of sialic acid (SA) in excreta. Supplementation with PHY improved ( $P<0.05$ ) dietary dry matter (DMD) and nitrogen (ND) digestibility coefficients. Dietary phosphorus digestibility (PD) increased with PHY addition as expected but the effect was much more pronounced in the low MYO group compared with high MYO diets as described by the interaction ( $P<0.05$ ). The interaction showed that increasing MYO content had no effect in the absence of PHY but it depressed P digestibility in the diets containing PHY. It can be concluded that dietary MYO improves bird growth and possibly intestinal health of broiler chickens. Dietary supplementation with either MYO or PHY may improve growth of chickens although these effects may not always be additive.

**Key words:** Phytase, Myo-inositol, Broiler, Nutrition

## INTRODUCTION

The beneficial effect of dietary phytases (PHY) is not only providing the phosphorus but also the destruction of phytate which is considered as anti-nutrient due to its negative effect on digestion and absorption of other minerals, amino acids and energy, besides acting as a gut irritant leading to increased endogenous losses (Selle and

Ravindran, 2007). Although the possibility of total conversion of dietary phytate by PHY to myo-inositol (MYO) has not been completely ruled out (Józefiak et al., 2010; Cowieson et al., 2013), the conditions in bird's gastrointestinal tract (GIT) as well as the catalytic properties of supplementary microbial phytases does not permit phytate molecules to be totally converted into free MYO and inorganic phosphate (Zyla et al., 2013). Myo-

inositol is a structural component in living tissues and normally synthesised in the body of birds and mammals (Lee and Bedford, 2016). Although the NRC (1994) does not stipulate that poultry have a requirement for inositol, dietary inositol deficiency has been demonstrated in most aquatic animals (Mai et al., 2001). Feeding low dietary MYO to fish species has been associated with low growth rates (Boonyaratpalin and Wanakowat, 1993; Waagbø et al., 1998). In some cases, however, *de novo* MYO synthesis by intestinal bacteria in fish is sufficient such that dietary MYO supplementation does not make any difference (Burtle and Lovell, 1989). Rats and gerbils have also been shown to respond to dietary MYO under certain conditions (Karasawa, 1972; Hegsted et al., 1974).

One of the first reports on the biosynthesis of MYO was in rat and chicken embryos (Daughaday et al., 1955). It has subsequently been shown that glucose is the precursor for MYO synthesis (Chu and Geyer, 1983; Charalampous and Chen, 1966) but the efficiency of conversion differs from species to species. Although the biological importance of inositol is well documented (Lee and Bedford, 2016), the impact of supplementary MYO on nutrient availability and performance in poultry has been inconsistent. Some authors found an increase in growth performance in response to dietary MYO (Żyla et al., 2013), while as others (Pearce, 1975) didn't. In addition, Cowieson et al. (2013) reported an interaction between MYO and exogenous PHY as addition of MYO to either the normal or low P diet improved feed efficiency only in the presence of PHY (Cowieson et al., 2013).

In view of the above facts, the objective of the present study was to quantify the response and inter-relationship of bird growth performance, dietary metabolisable energy and nutrient digestibility as a result of feeding graded levels of supplementary MYO with and without exogenous PHY. The impact on endogenous losses (mucin), measured as marker Sialic Acid (SA) was also determined.

## MATERIALS AND METHODS

### Diet formulation

Eight maize-soy-based diets were offered to male Ross 308 broiler chickens from 7 to 17 days of age (Table 1). A basal diet was formulated to be nutritionally adequate for chicks at that age (12.79 MJ/kg ME, 230 g/kg CP) but designed to have a relatively low available P content (2.5 g/kg non-phytate P). The basal diet was then split in two batches and one of them was supplemented with 500 units/kg of an *Escherichia coli*-derived phytase (Quantum<sup>TM</sup>, EC 3.1.3.26; AB Vista Feed Ingredients,

Marlborough, UK). The two batches (with and without phytase) were then split in four equal parts each and the parts were supplemented with MYO (Sigma-Aldrich, Inc., St. Louis, MO 63103, USA) at 0.0, 2.5, 5.0 and 7.5 g/kg diet, respectively to give a total of eight experimental diets. The MYO and the phytase were added on the top of the diets in powder form and mixed in a ribbon mixer for about 5 minutes. All diets were fed as a mash. Each treatment was replicated five times in a completely randomised block design.

**Table 1.** Ingredient composition (g/kg) of the control experimental diet fed to broiler chicken from 7 to 17 days of age.

Ingredients	g/kg
Maize	600
Soybean meal 48	300
Maize gluten meal	40
Vegetable oil	20
Salt	3.6
DL Methionine	4.2
Lysine HCl	3.0
Limestone	17.2
Dicalcium Phosphate	7.0
Vitamin Mineral premix <sup>2</sup>	5.0
	1000
<b>Calculated values (as fed)</b>	
Crude protein (Nx6.25), g/kg	231
ME, MJ/kg	12.79
Calcium, g/kg	8.6
Phosphorus, g/kg	5.2
Phytate phosphorus, g/kg	2.7
Available phosphorus, g/kg	2.5
<b>Determined values (as fed)</b>	
Dry matter, g/kg	867
Gross energy, MJ/kg	16.75
Crude protein (Nx6.25), g/kg	222
Phytate phosphorus, g/kg	2.9

<sup>1</sup>The inositol and the phytase were added over and above this formulation.

<sup>2</sup>The Vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). All the experimental diets were designed to be low in P. The premix provided (units/kg diet): retinol 3600 µg, cholecalciferol 125 µg, α-tocopherol 34 mg, menadione 3 mg, thiamine 2 mg, riboflavin 7 mg, pyridoxine 5 mg, cobalamin 15 µg, nicotinic acid 50 mg, pantothenic acid 15 mg, folic acid 1 mg, biotin 200 µg, iron 80 mg, copper 10 mg, manganese 100 mg, cobalt 0.5 mg, zinc 80 mg, iodine 1 mg, selenium 0.2 mg and molybdenum 0.5 mg.

## **Broiler growth performance and AME determination**

Day-old male Ross 308 broiler chickens were obtained from a commercial hatchery (Grampian Ltd., Whitburn, UK). They were placed in a single floor pen at 32°C and fed a proprietary broiler starter feed until 7 d age. On 7th day, the chicks were individually weighed and the heaviest and lightest birds discarded, leaving 80 birds. These were sorted into 5 weight blocks of 16 birds. The birds were randomised to treatment within weight block. Two birds were placed in each metabolism cage (0.35 x 0.35 m floor area) arranged in two tier levels within a controlled environment room. Each of the experimental diets was allocated at random to the cages. All the cages were equipped with metal feeders and cup drinkers. There was a metal tray under each cage for the collection of excreta. At 7d age the temperature was 30°C and it was then gradually reduced by 1°C on alternate days until room temperature reached 24°C. The light regime was 23 h light and 1 h dark throughout experimental period. Access to feed and water was *ad libitum*. The experiment ended when the birds were 17 d of age after a 10 day feeding period.

The nitrogen corrected apparent metabolisable energy (AMEn) of each diet was determined using the total collection procedure (Hill and Anderson, 1958) after recording the *ad libitum* feed intake and the total excreta produced for the last four days of the experimental period, between 13 and 17 d of age. The excreta then were freeze dried, weighed and milled. The feed intake and the weight of the birds were also measured on day 7 and 17 to assess weight gain and feed conversion efficiency on an individual bird basis.

Nutrient digestibility coefficients were determined as the difference between the intake and the output of the nutrient concerned divided by the intake of that nutrient. The daily secretion of sialic acid was obtained after multiplying the dry daily excreta voided by the concentration of sialic acid in excreta.

### **Ethical approval**

The present study was approved by the Animal Experimental Committee of Scottish Agricultural College, United Kingdom.

### **Analysis of diets and excreta**

Diets and freeze-dried excreta were ground (Retsch GmbH, 42781 Haan, Germany) to pass through a 0.75 mm sieve and then analysed for DM (24 h in a force draft oven at 105°C, Baird & Tatlock London Ltd., Chadwell Heath,

Essex, UK), crude protein by the method of Sweeney (1989) using a LECO FP-200 Analyser (St. Joseph, Michigan, USA) and gross energy using an isoperibol bomb calorimeter (Parr-6200, Parr Instrument Company, Moline, IL). The content of dietary phytate P was determined using the method of Haugh and Lantzsch (1983). Total P concentrations in feed and excreta were assayed photometrically after wet digestion of samples using a nitric acid-perchloric acid mixture (AOAC, 1990). The crude fat (as ether extract) in the feed and excreta samples was extracted with diethyl ether using a Soxtec system (Foss UK Ltd.) (AOAC, 1990).

The concentration of excreta sialic acid (SA) was determined by the periodate-resorcinol method as described by Jourdian et al. (1971). The method involves conversion of free and glycosidically bound sialic acid to chromogenic substances by treatment with periodic acid followed by resorcinol. The colour of the samples was stabilized by 2-methyl-2-propanol, and after centrifugation the absorbance of the supernatant was determined spectrophotometrically at 630 nm (Spectrone 301, Milton Roy Company, USA). This procedure detects total, free and glycosidically bound N-acetyl neuraminic (sialic) acid.

### **Statistical analysis**

Statistical analysis was performed using the Genstat 16 statistical software package (IACR Rothamsted, Hertfordshire, England). A randomised block analysis of variance was performed and a 2 x 4 factorial structure was used to investigate the main treatment factors (PHY x MYO inclusion levels) and their interaction. An orthogonal partitioning of the dietary MYO inclusion level was used to quantitatively compare the linear and quadratic regression effects. Differences were reported as significant at P < 0.05 and trends were noted when the P value was near to 0.1.

## **RESULTS**

The analysed chemical composition of the basal diet is shown in table 1. All birds remained healthy throughout the study period and survived the experiment. Results relating to growth performance and dietary metabolisable energy are presented in table 2. Feed intake, body weight gain and AMEn intake were influenced only by dietary inositol content, and in all cases the effects were deemed to be quadratic, with the optimum appearing to be at approximately 2.5 g/kg. Phytase addition tended to increase weight gain (P=0.055) No treatment effects were noted on FCR or AMEn.

**Table 2.** The effect of supplementary inositol and phytase on growth performance, dietary AMEn, AMEn intake and AMEn: GE of broiler chickens from 7 to 17 day age (for AMEn data based on collection period from 13 to 17 day age).

Inositol (g/kg)	FI (g/b/d)	WG (g/b/d)	FCE	AMEn (MJ/kg DM)	AMEn int (MJ/d)	AMEn: GE
0.0	39	30	0.770	14.03	0.55	0.835
2.5	43	33	0.773	14.21	0.62	0.848
5.0	41	31	0.774	14.13	0.57	0.845
7.5	40	31	0.765	13.92	0.56	0.830
SEM	1.1	0.9	0.0199	0.147	0.016	0.0088
Phytase (500 FTU/kg)						
-	40	31	0.758	13.97	0.56	0.833
+	41	32	0.784	14.17	0.59	0.846
SEM	0.7	0.6	0.0141	0.104	0.011	0.0062
Inositol X Phytase						
0.0	38	28	0.746	13.66	0.52	0.813
2.5	44	33	0.754	14.26	0.63	0.851
5.0	39	30	0.756	13.95	0.55	0.834
7.5	40	31	0.775	14.00	0.56	0.834
0.0 +	41	32	0.795	14.40	0.58	0.857
2.5 +	42	33	0.792	14.15	0.60	0.845
5.0 +	42	33	0.792	14.31	0.60	0.855
7.5 +	41	31	0.756	13.83	0.56	0.826
SEM	1.5	1.2	0.0281	0.208	0.023	0.0124
Statistical probabilities of treatment differences						
Inositol						
Linear	NS	NS	NS	NS	NS	NS
Quadratic	0.056	0.040	NS	NS	0.024	NS
Phytase	NS	0.055	NS	NS	NS	NS
Inositol X Phytase	NS	NS	NS	NS	NS	NS

FI – daily feed intake; WG – daily weight gain; FCE – feed conversion efficiency; AMEn – nitrogen corrected apparent metabolisable energy; GE – gross energy; AMEn:GE – metabolisability of gross energy; NS – not significant ( $P>0.05$ ). SEM - standard errors of means. There were 5 observations per treatment.

**Table 3.** The effect of supplementary inositol and phytase on digestibility of dietary dry matter, nitrogen, fat, phosphorus, excreta sialic acid concentration and sialic acid secretion of broiler chickens (data based on collection period from 13 to 17 day age).

Inositol (g/kg)	DMD	ND	FD	PD	SAC (µg/g)	SA (µg/96h)
0.0	0.673	0.596	0.842	0.550	0.65	20.57
2.5	0.686	0.609	0.840	0.579	0.57	18.98
5.0	0.686	0.615	0.853	0.573	0.56	17.85
7.5	0.670	0.605	0.845	0.539	0.48	15.02
SEM	0.0078	0.0081	0.01146	0.0197	0.055	1.662
Phytase (500 FTU/kg)						
-	0.670	0.597	0.840	0.475	0.57	17.81
+	0.687	0.615	0.851	0.645	0.56	18.40
SEM	0.0056	0.0058	0.0081	0.0139	0.039	1.175
Inositol X Phytase						
0.0	0.652	0.579	0.828	0.437	0.58	18.29
2.5	0.684	0.607	0.832	0.476	0.57	18.92
5.0	0.676	0.606	0.856	0.499	0.57	17.39
7.5	0.669	0.597	0.843	0.489	0.55	16.63
0.0 +	0.693	0.613	0.857	0.663	0.72	22.86
2.5 +	0.689	0.611	0.849	0.683	0.56	19.03
5.0 +	0.696	0.624	0.850	0.646	0.55	18.31
7.5 +	0.671	0.614	0.847	0.589	0.41	13.40
SEM	0.0111	0.01152	0.0162	0.0278	0.078	2.350
Statistical probabilities of treatment differences						
Inositol						
Linear	NS	NS	NS	NS	0.048	0.026
Quadratic	0.076	NS	NS	NS	NS	NS
Phytase	0.040	0.036	NS	<0.001	NS	NS
Inositol X Phytase	NS	NS	NS	0.020	NS	NS

DMD – dry matter digestibility; FD – fat digestibility; PD – phosphorus digestibility; SAC – concentration of sialic acid in excreta; SA – total sialic acid excreted; NS – not significant ( $P>0.05$ ). SEM - standard errors of means. There were 5 observations per treatment.

Table 3 reports the nutrient digestibility data and the endogenous losses measured as SA. Dry matter digestibility increased in a quadratic fashion with supplementary MYO ( $P=0.076$ ) and increased with PHY addition. Nitrogen digestibility increased with PHY addition but was not influenced by MYO supplementation. Fat digestibility did not change with treatment whereas phosphorus digestibility increased with PHY addition as expected but the effect was much more pronounced in the low MYO compared with high MYO diets as described by the interaction. Indeed the interaction noted that increasing inositol content had no effect in the absence of PHY but it depressed P digestibility in the diets containing PHY. The SA content of the excreta and the total amount excreted, decreased ( $P<0.05$ ) with increasing MYO supplementation in a linear pattern. Increasing dietary MYO content by 1g/kg reduced SA concentration in the excreta by 0.02 µg (SAc = 0.64 (SE 0.019) – 0.02 (SE 0.004) \* MYO) and the total amount of SA excreted per day by 0.71 µg (SA = 20.77 (SE 0.465) – 0.71 (SE 0.099) \* MYO).

## DISCUSSION

The analysed dietary protein and phytate P content differed marginally from the calculated values, which could probably be due to the difference between the composition of the actual ingredients that were used in the present study and the values given by the National Research Council (NRC, 1994) for the same ingredients.

The experimental diets were formulated to be marginally deficient in P such that in the presence of the PHY the P requirements would be met. Indeed PHY addition did improve weight gain as was expected but failed to increase feed intake. This design allowed determination of the response to MYO in diets which are marginally deficient or adequate in P. In the present study, the addition of MYO resulted in improved feed intake and weight gain regardless of the presence or absence of PHY which is in agreement with previous reports (Żyła et al., 2004, 2013). It thus suggests that the growth-stimulating effect of MYO is not dependent upon adequate phosphorus levels in the diet for growing broilers. The superior growth rates of birds fed diets containing 2.5 g/kg added MYO correlated with concomitant increased daily AME intake and DMD, although the AMEn intake effect was a direct result of MYO increasing feed intake rather than any effect on AMEn per se. The literature investigating the effects of supplemental MYO on broiler performance is equivocal (Lee and Bedford, 2016). Żyła et al. (2013) demonstrated that supplementation with as little as 1g/kg MYO in wheat- and maize-based diets containing

1.5 g/kg of available P improved growth of broilers of a similar age. However, Cowieson et al. (2013) found that the addition of MYO to a diet low in Ca and digestible P resulted in a negative effect on feed efficiency during the starter phase, although during the finisher phase the effect became positive. Moreover, feeding MYO reduced feed intake (Cowieson et al., 2013) which is in contrast to the current work. Furthermore, Cowieson et al. (2013) reported an interaction between MYO and exogenous phytase whereby addition of MYO to either the positive or negative control diet improved feed efficiency in older birds only in the presence of phytase. Finally, Pearce (1975) and Żyła et al. (2004) did not find any advantage in broiler growth rates when P sufficient diets were supplemented with MYO.

The results suggest that performance response to MYO may interact with dietary and husbandry factors yet to be identified such as diet formulation, age, rearing conditions and perhaps health status of the bird. Although marginal, the reported positive effect of PHY on broiler growth was in agreement with previous studies when a similar dosage of the same product (Cowieson et al., 2006; Pirgozliev and Bedford, 2013) was fed. The relatively low activity of exogenous PHY, i.e. 500 FTU only, may explain the lack of effect on AMEn and nutrient digestibility.

There was MYO by PHY interaction for P digestibility which was explained by the fact that MYO had no effect on P digestibility in the absence of PHY whereas in the presence of PHY it actually depressed this metric. Myo-inositol is the end product of dephosphorylation of phytate and perhaps it may act as an end product inhibitor although most phytase fail to dephosphorlate IP1 and as a result IP1 and not MYO is the end product. Nevertheless, P digestibility in the presence of PHY was always higher than in its absence, regardless of MYO content of the diet. The lack of correlation between performance and P digestibility may simply be because of the fact that in the presence of PHY, the digestible P content of the diet exceeded requirement even at the highest MYO concentration.

Regardless of PHY inclusion level, performance was optimised at approximately 2.5 g/kg MYO. Myo-inositol has been shown to influence multiple pathways of metabolism including increasing the activity of ATPase and improving nerve conduction velocity in rats (Greene and Lattimer, 1983; Yorek et al., 1993). Cowieson et al. (2013) reported an increase in blood glucose content when MYO or a combination of MYO and PHY were fed to birds, compared to a low Ca and P control diet, suggesting improved efficiency and/or rate of nutrient absorption

which facilitated the increased growth rate and feed efficiency.

The results of present study suggest that dietary supplementation with either MYO or PHY may enhance the growth of chickens although these effects may not always be additive. Cowieson et al. (2013) concluded that the presence of both, MYO and PHY in poultry diet may result in some antagonistic interactions mediated via competitive mechanisms.

Increasing dietary MYO content resulted in reduced SA content of the excreta suggesting that mucin excretion is reduced. However, the SA content of the excreta may also be affected by the GIT microflora (Varky, 1992) as the SA content varies with bacterial species. Myo-inositol is involved in the control of cell volume and osmolarity (Kane et al., 1992), and thus likely varies with the total microbial population and its species distribution, thereby contributes to variance in SA excretion from the GIT. Reduction in SA secretion have also been noted with inclusion of high doses of PHY (Cowieson et al., 2004), an effect which was attributed to improved intestinal integrity. In this study no effect of PHY on SA excretion was noted which may be a consequence of the lower dosage employed and thus less destruction of IP6 as compared with other studies. The lack of effect of the PHY on AMEn suggests such a limit on IP6 destruction may indeed have been the case in the present study.

## CONCLUSION

The present study demonstrated that dietary MYO improves bird growth and possibly intestinal health of broiler chickens. Further, the dietary supplementation with either MYO or PHY may improve growth of chickens, although these effects may not always be additive. The mechanism of action of dietary MYO in poultry needs further investigation. Moreover, the studies on the interaction between dietary minerals, exogenous PHY and MYO may bring more clarity on the mode of action of MYO.

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### Competing interests

The authors declare that they have no competing interests.

### Author's Contributions

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contributed to the planning of the study. V.P., M.R.B. and S.P.R. were involved in the design and execution of the study and also drafting of the manuscript. The rest of the authors were involved in the chemical and statistical analyses, design and revision of the manuscript.

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