Research Note: The effect of sequential displacement of dietary dextrose with myo-inositol on broiler chicken growth performance, bone characteristics, ileal nutrient digestibility, and total tract nutrient retention

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ABSTRACT A total of 480 male Cobb 500 broiler chicks were assigned to one of 6 dietary treatments to explore the energy equivalence of myo-inositol compared with dextrose. The 6 dietary treatments included a corn and soy-based control ration formulated with 5% anhydrous dextrose and 5 further diets that were generated by the sequential displacement of increments of 1% dextrose with myo-inositol. Each diet was fed to 8 replicate cages of 10 chicks per cage from day 8 to day 18 after hatch. The BW gain, feed intake, and feed conversion ratio (**FCR**) were measured, and on day 15 to day 17, excreta were collected to estimate the total tract nutrient retention. Ileal digestibility of nutrients and tibia mineral content was assessed on day 18. The displacement of dextrose with myo-inositol generated a significant linear reduction in the FCR that did not reach a plateau at 5% dietary inclusion of myo-inositol. There was no effect of the displacement of dextrose with myo-inositol on bone mineral concentration. However, supplemental myo-inositol linearly reduced ileal digestibility of DM, calcium, and ileal digestible energy. Myo-inositol addition resulted in a significant linear increase in the total tract retention of CP. It can be concluded that myo-inositol has an energy equivalence equal to approximately 78% of that of dextrose for young broiler chicks but exerts a range of extra caloric effects that improve feed efficiency and may influence nitrogen (**N**) retention and the uric acid cycle. Future work should focus on the role of phytase and myo-inositol on uric acid, creatine kinase, and other metabolites involved in renal function and biochemical flows of N in urine and feces in nonruminants.

Key words: broiler, nutrition, myo-inositol, energy, dextrose

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INTRODUCTION

Extra beneficial effects of phytases on trace minerals, amino acids, energy, calcium, and sodium in addition to phosphorus (**P**) release have emerged, and these have been connected to the capacity of phytase to reduce the electronegativity of phytic acid and the associated antinutritional consequences of its ingestion (Cowieson et al., 2009). In addition, the possible involvement of myo-inositol in the so-called 'extraphosphoric' effects of phytase has been suggested as phytic acid is approximately 30% myo-inositol by weight (Cowieson et al., 2011).

Myo-inositol is a cyclical sugar alcohol and is the nucleus of the phytic acid molecule. Cowieson et al. (2013) noted significant improvement in broiler performance in the finisher phase when diets were supplemented with 0.15% myo-inositol. Importantly, plasma myo-inositol increases in response to phytase supplementation of diets in poultry (Cowieson et al., 2014). Myo-inositol has also been detected in the gizzard in broiler chicks fed a phytase-containing diet, and this was correlated with improvements in growth performance (Walk et al., 2014).

Despite evidence that direct addition of myo-inositol or phytase-mediated myo-inositol genesis may have beneficial effects on the growth performance of nonruminants, the mechanisms involved in this remain unclear. Increases in plasma myo-inositol and upregulated pathways involved in protein accretion with the addition of

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phytase to a broiler diet are relevant (Schmeisser et al., 2017). However, the direct energy equivalence of myo-inositol for poultry remains unclear, and so, the objective of the experiment reported herein was to examine the consequences of the displacement of dextrose with myo-inositol in growing broiler chickens.

MATERIALS AND METHODS

The animal protocol for this research was approved by the Animal Welfare Committee of DSM (China) Animal Nutrition Research Center.

Animals

On day 8 after hatch, 480 birds (227 ± 1.3 g; mean ± standard error of mean) were selected and sorted by the BW and assigned to 48 cages (95 cm × 80 cm × 80 cm) with 10 birds per cage such that the average initial BW was similar across cages. Birds had ad libitum access to water and experimental diets from day 8 to 18 after hatch. The room temperature was set at 32°C at the outset of the trial and reduced by 2°C per week thereafter. The lighting cycle was 20-hour light and 4-hour dark.

Experimental Diets

The basal diet was based on 51.2 corn, 36% soybean meal, 3% soybean oil, and 5% dextrose (99%; Sacred Snow Co. Ltd., Shi Jia Zhuang, P. R. China), which met the birds' requirement for all nutrients but was reduced by 4.5% in ME relative to the recommendation prescribed by the NRC (1994). Myo-inositol (97%; Hao Tian Co. Ltd., Zhu Cheng, P. R. China) was included in the basal diet at 0, 1, 2, 3, 4, or 5% at the expense of dextrose to generate 6 experimental diets, which were pelleted at 75°C. Titanium was included at 3 g/kg feed as an indigestible marker.

The 48 cages were divided into 8 blocks according to their spatial distribution with 6 adjacent cages for each block. The experimental diets were randomly assigned to the cages in the blocks.

Measurement and Sampling

Growth performance was measured for birds from day 8 to 18 after hatch. Excreta were collected daily on day 15 through 17 after hatch by placing a tray under each cage. The collected excreta were pooled and then mixed to homogeneity before a subsample was taken. On day 18 after hatch, all the birds were slaughtered by cervical dislocation for collection of digesta in the distal two-thirds of the ileum. Ileal digesta were pooled by cage. Two birds from each cage were chosen for collection of right tibias. All the samples were stored at −20°C.

Chemical Analyses

The excreta and digesta samples were freeze-dried to a constant weight and then ground to pass through a 0.5-mm screen before analysis. The samples were dried at 105°C in an oven for 4 h for dry matter determination (method 934.01; AOAC International, 2006). Gross energy (**GE**) was determined using an adiabatic bomb calorimeter with benzoic acid as standard (C 2000 basic, IKA, Königswinter, Germany). The nitrogen content was determined by the Dumas method (method 992.23; AOAC International, 2006) with a nitrogen analyzer (FP-528, LECO Corp., St Joseph, MI). Titanium, Ca, and P were measured by inductively coupled plasma-optical emission spectrometry (Optima 8000, PerkinElmer, Shelton, CT) after microwave digestion (method 985.01; AOAC International, 2006). Myo-inositol was analyzed by HPLC/MS according to the method of Leung et al. (2011). Before the analysis, myo-inositol was extracted from the feed by the addition of water. After homogenization, centrifugation, and filtration, an aliquot spiked with deuterated internal standard was injected in Acquity UPLC/TQD system (Waters, Milford, MA). The detection of the specific fragment ions was performed using the multiple reaction monitoring mode. To assess the daily and long-term laboratory performance of the method, dedicated standard and quality-control samples were analyzed with unknown samples to ensure the accuracy and precision of the method. Data acquisition, integration, and quantification were performed by MassLynx software (Waters, Milford, MA). The tibias were extracted for lipids in ethanol and petroleum ether before drying and analyses of ash, Ca, and P. The tibias were extracted for lipids in ethanol and petroleum ether before drying and ashing (method 972.15; AOAC, 1990).

Calculations and Statistical Analyses

Schematic box plots were used to identify outliers. Observations falling either below quantile 1-1.5*interquartile range or above quantile 3 + 1.5* interquartile range were considered as outliers.

All the data were analyzed by the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) using a statistical model including dietary treatment as the only fixed effect. Orthogonal polynomial contrasts were constructed to test the linear and quadratic effects of the supplementation of *myo*-inositol. The statistical significance was defined at $P < 0.05$. The least square means are presented.

Digestibility or retention coefficients were calculated using the index method. The ileal digestible energy (**IDE**) or AME of the diet (kcal/kg diet) was calculated by multiplying the coefficients and the dietary energy concentration. The AME was corrected to zero nitrogen (**N**) retention with a factor of 8.22 kcal/g retained N for AME_n.

RESULTS AND DISCUSSION

It has been unequivocally demonstrated that the addition of exogenous phytase to the diets of nonruminant livestock generates appreciable concentrations of myo-inositol in the plasma and intestinal contents (Cowieson et al., 2014; Walk et al., 2014). However, beyond the obvious parallel release of phosphate, the utility of this additional myo-inositol for pigs and poultry is not clear. Zyla et al. (2004) added 0.1% myo-inositol to a corn-based diet and observed a reduction in the FCR from 1.57 to 1.47 but a simultaneous reduction in P retention from 56 to 50% for reasons that are not readily explainable. Cowieson et al. (2013) added myo-inositol to broiler diets in 2 separate experiments at a concentration (0.15%) intended to represent the concentration that would be generated by exogenous phytase in a diet with approximately 0.8% phytic acid. These authors noted that myo-inositol reduced the FCR (1–42 d) by 6-12 points with beneficial effects being exaggerated in diets with low available P and in the finisher period of the trial. Interestingly, when diets contained exogenous phytase, the beneficial effect of myo-inositol was diminished, which may indicate that the mechanisms of effect are saturable or overlapped. Zyla et al. (2013) added 0.1% myo-inositol to broiler diets that had been formulated to be deficient in available P and noted a reduction in the FCR from 2.19 to 1.98. Thus, there is some evidence from the literature that myo-inositol addition to broiler diets is capable of reducing the FCR, and this is in agreement with the results presented herein where a linear reduction ($P < 0.001$) in the FCR was observed when dextrose was sequentially displaced (Table 1). It should be noted however that the inclusion concentrations used in the present study are substantial, which was intentional to allow a comparison with the nutritional contribution of dextrose, but are not directly comparable to previous work.

There was no significant effect of replacement of dextrose with myo-inositol on bone ash or bone Ca or P concentration in the current experiment (Table 1). This is in agreement with Zyla et al. (2013) who noted no change in broiler toe ash when 0.1% myo-inositol was added to a broiler diet with low available P content. This may be partially dependent on dietary Ca and P supply because Zyla et al. (2004) observed that 0.1% myo-inositol addition increased toe ash in diets with adequate P and Ca but caused a reduction in toe ash in diets with low available P and Ca, effects that were reflected in P retention.

Table 1. Growth performance, bone parameters, and digestibility and retention coefficient of gross energy and nutrients.

	Myo-inositol, %								
Measurements	0	1	2	3	4	5	SD	Linear	Quadratic
No. of replicates	7	8	8	7	8	8			
Growth performance									
Initial BW, g/bird	227	227	228	228	227	228	1.3	0.417	0.602
Final BW, g/bird	748	748	753	778	767	766	13.5	<0.001[1]	0.119
Weight gain, g/bird	521	521	526	550	540	538	13.6	<0.001[2]	0.134
Feed intake, g/day/bird	615	618	617	625	610	601	17.8	0.131	0.051
FCR	1.18	1.19	1.17	1.14	1.13	1.12	0.02	<0.001[3]	0.293
Bone									
Ash, %	53.6	53.7	53.7	53.6	53.2	53.4	0.87	0.380	0.677
Calcium, %	37.3	37.0	37.0	36.9	38.1	36.8	1.17	0.945	0.998
Phosphorus, %	17.9	17.8	17.8	17.8	18.3	17.6	0.58	0.833	0.732
Ileal digestibility									
DM	0.711	0.713	0.701	0.698	0.678	0.695	0.015	<0.001[4]	0.455
Gross energy	0.729	0.733	0.724	0.718	0.701	0.717	0.016	<0.01[5]	0.676
CP	0.744	0.775	0.758	0.756	0.736	0.746	0.030	0.248	0.240
Calcium	0.580	0.588	0.574	0.562	0.560	0.548	0.024	<0.001[6]	0.561
Phosphorus	0.573	0.599	0.573	0.578	0.564	0.570	0.022	0.094	0.511
IDE, kcal/kg	3,047	3,058	3,051	3,005	2,936	3,002	69	<0.01[7]	0.904
Retention									
DM	0.727	0.732	0.716	0.731	0.713	0.729	0.010	0.292	0.165
Gross energy	0.757	0.763	0.747	0.760	0.742	0.757	0.009	0.066	0.182
CP	0.626	0.649	0.620	0.664	0.668	0.666	0.032	<0.01[8]	0.928
Calcium	0.621	0.609	0.586	0.608	0.610	0.605	0.022	0.414	0.081
Phosphorus	0.579	0.576	0.555	0.587	0.587	0.586	0.026	0.203	0.293
AME, kcal/kg	3,167	3,181	3,147	3,177	3,110	3,170	37	0.143	0.427
AME_n, kcal/kg	2,981	2,986	2,959	2,980	2,914	2,975	32	<0.05[9]	0.256

Abbreviation: IDE, ileal digestible energy; FCR, feed conversion ratio.
[1]y = 747.7 + 4.8*x, R^2 = 0.24 where y is the response parameter and x is the supplemented myo-inositol (%).
[2]y = 520.4 + 4.7*x, R^2 = 0.24.
[3]y = 1.192 – 0.015*x, R^2 = 0.60.
[4]y = 0.713 – 0.005*x, R^2 = 0.25.
[5]y = 0.732 – 0.005*x, R^2 = 0.18.
[6]y = 0.588 – 0.007*x, R^2 = 0.24.
[7]y = 3,063 – 18*x, R^2 = 0.17.
[8]y = 0.627 + 0.009*x, R^2 = 0.17.
[9]y = 2,982 – 7*x, R^2 = 0.08.

In the present study, the effect of replacement of dextrose with myo-inositol on nutrient digestibility and nutrient retention resulted in a linear reduction in the ileal digestibility of DM, GE, and Ca ($P < 0.01$) and a concomitant reduction in IDE ($P = 0.01$; Table 1). These effects were not replicated on a total tract level, and there was no effect of myo-inositol on AME and a linear increase in total tract retention of CP ($P < 0.01$). It should be pointed out that myo-inositol was included in the diet at the expense of dextrose, which has a digestible energy concentration of around 3,600 kcal/kg (Potter, 1979), and so, removal of 5% dextrose would be expected to generate a reduction in dietary IDE of around 180 kcal/kg if myo-inositol made no energetic contribution to the diet. However, the displacement of 5% dextrose with myo-inositol resulted in a decrease in IDE of around 45 kcal/kg, suggesting that myo-inositol has an energy equivalence of around 78% that of dextrose (approx. 2,800 kcal/kg). If dextrose is absorbed more proximally in the intestine than myo-inositol, then its displacement with myo-inositol may cause a reduction in ileal digestibility of DM and energy. Importantly, in the measurement of GE disappearance, the small intestine does not necessarily reflect the energy status of the animal as energy partitioning, maintenance energy, heat production, and so on are not captured in digestibility studies. Thus, the effect of myo-inositol on net energy and nutrient requirements of poultry is a potentially interesting area for further study, especially given the significant linear decrease in the FCR in the present work and the disconnect between this and ileal digestibility responses.

The decrease in the ileal digestibility of Ca when myo-inositol replaced dextrose is difficult to explain and does not appear to be related to dietary Ca concentration or bone Ca content. Schmeisser et al. (2017) noted that phytase addition to a diet with a low concentration of available P generated a significant increase in plasma myo-inositol in broiler chickens and also significantly upregulated calcium/calmodulin-dependent kinase, which is involved in skeletal myogenesis. Whether this effect is associated with myo-inositol specifically or perhaps related to other characteristics of exogenous phytase is not clear. However, it is plausible that myo-inositol influences Ca homeostasis.

The increase in total tract retention of CP associated with myo-inositol is interesting, especially as this effect was not apparent at the level of the ileum. This suggests that the increase in CP retention may be associated with nonprotein N rather than changes to the digestibility or retention of amino acids (which would have been reflected in ileal digestibility values). Phytase addition to a broiler diet has been previously observed to increase plasma myo-inositol, upregulate the phosphatidylinositol-3-kinase signaling pathway, which is involved in protein accretion (Schmeisser et al., 2017), and generate increases in breast meat yield. It is possible that myo-inositol may influence the N cycle in birds, improving N efficiency by partitioning more dietary N to lean gain, reducing urinary N losses.

It can be concluded that myo-inositol has the capacity to significantly improve the growth performance of broiler chickens when administered orally. These growth performance advantages appear unrelated to improvements in the ileal digestibility of nutrients or a direct energetic yield from myo-inositol. The direct caloric value of myo-inositol is estimated to be approximately 2,800 kcal/kg, or approximately 78% of the energy density of dextrose. Myo-inositol may play a role in renal function, and so, the effect of phytase and myo-inositol on uric acid, creatine kinase, and other metabolites involved in the uric acid cycle should be explored. These effects may be relevant not only for animal health and welfare but also in managing waste streams from nonruminant animal production systems.

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DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

AOAC. 1990. Official Methods of Analysis of the AOAC. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

AOAC. 2006. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Gaithersburgs, MD.

Cowieson, A. J., R. Aureli, P. Guggenbuhl, and F. Fru-Nji. 2014. Possible involvement of myo-inositol in the physiological response of broilers to high doses of microbial phytase. Anim. Prod. Sci. 55:710–719.

Cowieson, A. J., M. R. Bedford, P. H. Selle, and V. Ravindran. 2009. Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. Worlds Poult. Sci. J. 65:401–408.

Cowieson, A. J., A. Ptak, P. Mackowiak, M. Sassek, E. Pruszynska-Oszmalek, K. Zyla, S. Swiatkiewicz, S. Kaczmarek, and D. Jozefiak. 2013. The effect of microbial phytase and myo-inositol on performance and blood biochemistry of broiler chickens fed wheat/corn-based diets. Poult. Sci. 92:2124–2134.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. Worlds Poult. Sci. J. 67:225–236.

Leung, K. Y., K. Mills, K. A. Burren, A. J. Copp, and N. D. Greene. 2011. Quantitative analysis of myo-inositol in urine, blood and nutritional supplements by high-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. 879:2759–2763.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Potter, L. M. 1979. Metabolisable energy and digestible nutrient content of brewers grains and glucose for the young Turkey. Br. Poult. Sci. 20:265–271.

Schmeisser, J., A. A.-. Seon, R. Aureli, P. Guggenbuhl, S. Duval, A. J. Cowieson, and F. Fru-Nji. 2017. Exploratory transcriptomic analysis in muscle tissue of broilers fed a phytase-supplemented diet. J. Anim. Physiol. Anim. Nutr. 101:563–575.

Walk, C. L., T. T. Santos, and M. R. Bedford. 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash and gizzard phytate and inositol in young broilers. Poult. Sci. 93:1172–1177.

Zyla, K., R. Dulinksi, M. Pierzchalska, M. Grabacka, D. Jozefiak, and S. Swiatkiewicz. 2013. Phytases and myo-inositol modulate performance, bone mineralization and alter lipid fractions in the serum of broilers. J. Anim. Feed Sci. 22:56–62.

Zyla, K., M. Mika, B. Stodolak, A. Wikiera, J. Koreleski, and S. Swiatkiewicz. 2004. Towards complete dephosphorylation and total conversion of phytates in poultry feeds. Poult. Sci. 83:1175–1186.