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Manipulation of plasma myo-inositol in broiler chickens: effect on growth performance, dietary energy, nutrient availability and hepatic function

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ABSTRACT

The aim of this study was to investigate the effects of graded levels of *myo*-inositol (INS) in diets containing two levels of available P, on growth performance, nutrient retention, liver N, fat and vitamin E contents, inositol and ALP concentrations in blood plasma. 120 male Ross 308 broilers were allocated to 60 small floor pens each holding 2 birds. Two basal diets were formulated to be nutritionally adequate for chicks at that age, as one of them was designed to have the recommended available P content (RP) (4.8 g/kg non-phytate P), and the other diet was relatively low available P content (LP) (2.5 g/kg non-phytate P). The two basal diets were then split in three batches, and each batch was supplemented with myo-inositol at 0.0, 3.0 and 30 g/kg diet, respectively to give six experimental diets. Each diet was offered, in mash form, *ad libitum* to birds in 10 pens in a randomized block design. The experiment lasted for 14 days from 7 to 21d age. Feeding RP diets improved (P<0.001) the birds' growth performance variables, mineral availability, and blood plasma alkaline phosphatase (ALP). However, feeding RP diets reduced (P<0.001) apparent metabolisable energy (AME), total tract dry matter and fat retention coefficients, blood plasma INS and hepatic vitamin E. Dietary INS did not (P>0.05) influence birds growth, dietary AME or nutrient retention coefficients. Feeding INS linearly increased (P<0.05) liver weight and hepatic N content, but linearly reduced (P<0.05) hepatic fat concentration. It also linearly increased (P<0.001) the inositol concentration in blood plasma, but did not influence (P>0.05) the SA concentration in excreta. Dietary inositol did not influence (P>0.05) the hepatic vitamin E concentration but increased (P<0.001) the ALP in the blood of birds fed 30 g/kg inositol. This experiment has confirmed expected biological effects of diets that differ in available P and INS contents. No available P x inositol interactions were observed in any of the variables studied.

Key words: *myo*-inositol, phytate, phytase, antioxidants, alkaline phosphatase

INTRODUCTION

The beneficial effects of dietary phytases (PHY) when fed to poultry are well documented (Selle and Ravindran, 2007). Phytase not only releases more available energy and nutrients to the birds, but also hydrolyses dietary phytate. Phytate is considered as an anti-nutritional factor binding minerals and proteins into indigestible complexes (Cowieson et al., 2004; Pirgozliev et al., 2007). Due to the conditions in bird's gastrointestinal tract (GIT), as well as the catalytic properties of supplementary microbial PHY, it is unlikely that phytate is completely dephosphorylated into free *myo*-inositol (inositol) and inorganic phosphate (Wyss et al., 1999). Recent studies in pigs (Kühn et al., 2016) and poultry (Cowieson et al., 2015; Beeson et al., 2017; Sommerfeld et al., 2017) have demonstrated that feeding supra doses of third generation *E. coli* PHY increases inositol concentrations in the digesta and excreta of

animals. These results indicate potential further phytate hydrolysis to free inositol, consequently released in the digestive tract of broiler chickens. The biological importance of inositol is well documented, with the involvement in cell survivability and growth, lipid metabolism, and insulin sensitivity the most relevant for poultry (Lee and Bedford, 2016; Huber, 2016).

Although a number of studies on the effects of supplementary inositol in broilers are published, the results for nutrient availability and performance are inconsistent. Some authors found an increase in growth performance in response to dietary inositol (Żyła et al., 2013; Pirgozliev et al., 2017; Sommerfeld et al., 2017), while others (Pearce, 1975; Farhadi et al., 2017) did not. In addition, Cowieson et al. (2013) reported an interaction between inositol and exogenous PHY as addition of inositol to either the normal or low P diet improved feed efficiency only in the presence of PHY (Cowieson et al., 2013). Farhadi et al. (2017) and Sommerfeld et al. (2017) reported no impact of supplementary inositol on dietary P digestibility and bone mineralisation in poultry. However, Pirgozliev et al. (2017) observed an interaction as the increase of dietary inositol content had no effect on P digestibility in the absence of PHY but it depressed P digestibility in the diets containing PHY.

The amount of inositol supplemented to diets in the aforementioned experiments was between 0.1 and 0.75%. This is similar or slightly higher than the theoretical range of inositol released in the gut of chickens after feeding a commercial level of PHY. However, different dietary sources would have different phytate contents with varying bioavailability, and different PHY may possess different abilities to hydrolyse dietary phytate, explaining the discrepancies in the data published.

In view of the above, the objectives of the present study were to quantify the response of bird growth performance, dietary metabolisable energy and nutrient digestibility as a result of feeding a “super dose” of supplementary inositol in diets that contain low (LP) or recommended (RP) levels of dietary P. Hepatic vitamin E content, liver composition, alkaline phosphatase (ALP) and inositol content in the blood of broiler chickens were also determined.

MATERIALS AND METHODS

Birds and Housing

The experiment has been conducted at the National Institute of Poultry Husbandry and approved by the Ethical Committee of Harper Adams University. A total of 130 male Ross 308 broilers were obtained from commercial hatchery (Cyril Bason Ltd, Craven Arms, UK) and allocated to a single floor pen and offered a proprietary broiler feed. At 7d age, 120 birds were allocated to 60 small floor pens each holding 2 birds. Each of the pens has a solid floor and equipped with individual feeder and drinker. Feed and water were offered *ad libitum* to birds throughout the experiment. Each diet was offered to birds in 10 pens in a randomized block design. The birds were fed the experimental diets from 7 to 21d age, when the experiment ended. Room temperature and lighting regime met commercial recommendations (Aviagen Ltd, Edinburgh, UK). For the last 4 d of the study, the solid floor of each pen was replaced with a wire mesh in order to enable excreta collection. The well-being of the birds was checked regularly every day.

Diets and Treatments

Six maize-soy-based diets were offered to the birds during the experiment. Two basal diets were formulated to be nutritionally adequate for chicks at that age (about 12.90 MJ/kg ME, 216 g/kg CP), as one of them was designed to have the recommended available P content (4.8 g/kg non-phytate P), and the other diet was relatively low available P content (2.5 g/kg non-phytate P) (Table 1). The two basal diets were then split in three batches each, and each batch was supplemented with *myo*-inositol (Sigma-Aldrich, Inc., St. Louis, MO 63103, USA) (Table 2) at 0.0, 3.0 and 30 g/kg diet, respectively to give a total of six experimental diets. All diets were fed as a mash.

Sampling and Measurements

Birds were weighed on d1, in order to obtain information on the average birds weight at the start of the study. Birds and feed were then weighed on d7, and d21 in order to determine daily feed intake (FI), weight gain (WG) and to calculate the gain:feed ratio (G:F) on a pen basis. Excreta were quantitatively collected for the last four days (daily collection to avoid evaporation losses), immediately dried at 60◦C and then milled. At the end of the study, one bird per pen was electrically stunned and blood was obtained in heparin tubes from the jugular vein. The liver from the same birds was collected immediately after that, weighed, freeze dried and then milled.

Chemical Analysis

Dry matter (DM) in feed and excreta samples was determined by drying of samples in a forced draft oven at 105◦C to a constant weight (AOAC, 2000; method 934.01). Crude protein (6.25 × N) in samples was determined by the combustion method (AOAC, 2000; method 990.03) using a Leco (FP-528 N, Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC, 2000; method 945.16) using a Soxtec system (Foss UK Ltd.). The gross energy (GE) value of feed and excreta samples was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL) with benzoic acid used as the standard. Phosphorus and Ca in feed and excreta samples were determined by inductively coupled plasma emission spectrometry, ICP (Optima 4300 DV Dual View ICP-OE spectrometer, Perkin Elmer, Beaconsfield, UK) (Tanner et al., 2002).

Hepatic vitamin E was analysed at the IDEXX BioResearch Vet Med Labor GmbH (Ludwigsburg, Germany). Vitamin E in liver was determined by means of high performance liquid chromatography (Hess et al., 1991). The ALP in blood plasma was analysed at the APHA Laboratory (Shrewsbury, UK) following standard procedure by using a Randox Immolite Analyser and the associated Randox kit (Recommendations of the German Society for Clinical Chemistry).

The concentration of excreta sialic acid (SA) was determined by the periodate-resorcinol method as described by Jourdian et al. (1971). The procedure detects total, free and glycosidically bound N-acety neuraminic (sialic) acid.

For analysis of inositol, samples of milled feed (0.1g) were extracted in 5 mL of 20 mM EDTA, 100 mM NaF, pH 10, on a rotary shaker for 15 min followed by sonication in a bath sonicator for 15 min. The samples were held at 4◦C for 2 h before centrifugation at 14,000 x *g* for 15 min. The supernatant was filtered through a 13 mm x 45 µm pore PTFE filter (Kinesis Ltd, UK) and diluted 50-fold in 18.2 MOhm.cm water. Inositol was determined by HPLC pulsed amperometry (HPLC-PAD) on a gold electrode at 30◦C after separation by 2-dimensional HPLC (Dionex DX-600 HPLC System). Samples (20 µL) were injected onto a 4 mm x 50 mm CarboPac PA1 column (Dionex, UK) arranged in series with a 4 mm x 250 mm CarboPac MA1 column with 4 mm x 50 mm guard column of the same material. The

flow rate of the 150 mM NaOH eluent was 0.4 mL min^{-1}. After 1.5 min, the flow through the CarboPac PA1 column was switched to 750 mM NaOH, at 0.4 mL min^{-1} to elute more strongly retained sugars to waste. Eluent from the CarboPac MA1 column (150 mM NaOH) was directed to an ED50 electrochemical detector (Dionex) configured with a gold electrode and operating a standard Dionex carbohydrate waveform. After 11.5 min, the CarboPac PA1 column was switched back into the 150 mM NaOH flow (in series with the CarboPac MA1 column) to condition the column for a further 8.5 min, before initiation of the next injection sequence.

Inositol eluted at approximately 10.5 min. Injection of inositol standards (0.01-0.2 nmole in 20 µL) and integration of the peak yielded a linear calibration curve typically with $r^2 > 0.995$ and slope of approximately 100 nC.min $nmol^{-1}$.

For inositol measurement in plasma, plasma samples were mixed with 2 volumes of ice-cold 5% w/v perchloric acid and held on ice for 20 min to precipitate protein. The samples were centrifuged at 16,000 x *g* for 15 min at 4◦C and the supernatant diluted 50-fold in 18.2 MOhm.cm water before analysis (20 µL injection) by HPLC-PAD.

Calculations and Statistical Analysis

Daily FI, WG and G:F ratio were calculated for the experimental period from d7 to d21 on a pen basis using information and then divided by the number of remaining animals per pen. The AME of the diets was calculated following total collection technique. The total tract digestibility coefficient of each of the studied nutrients was calculated as the difference between the intake and the excretion of the nutrient divided by their respective intake based on data obtained for the last 4 days during collection period as previously described (Whiting et al., 2018). The quantity of P and Ca retained in the body (g/bird/d) was obtained by multiplying of P and Ca intake and their total tract digestibility coefficients.

Statistical analysis was performed using the Genstat 19 statistical software package (IACR Rothamstead, Hertfordshire, England). A randomised block analysis of variance was performed and a 2 × 3 factorial structure was used to investigate the main treatment factors (P × INS inclusion levels) and their interaction. When there were statistically significant differences in INS, the treatment sum of squares were partitioned to test the linear effects. Differences were reported as significant at $P < 0.05$ and trends were noted when the P value was near to 0.1.

RESULTS

Broiler Growth Performance, Dietary Metabolizable Energy and Nutrient Digestibility

Variation in growth performance, nutrient and mineral availability, and metabolisable energy were in the expected range for study involving broiler chickens at this age and fed similar diet formulation (Abdullah et al., 2016; Whiting et al., 2017) (Table 3). Coefficients of variation (CV%) of feed intake, weight gain, and feed efficiency were 6.9%, 9.2%, and 7.3%, respectively. The variation in dietary AME was relatively low (CV = 2.5%), ranging from 14.32 to 14.68 MJ/kg DM and was not affected by dietary inositol content ($P > 0.05$) (Table 4). Feeding diets low in available P improved ($P < 0.001$) dietary AME by 0.36 MJ/kg DM (by 2.5%). Daily intake of dietary AME was improved ($P<0.001$) by feeding RP diets, but was not influenced ($P>0.05$) by inositol supplementation.

Nutrient retention and digestibility coefficients were not affected ($P > 0.05$) by dietary INS content (Table 3). The CV in DMR, NR and FD coefficients were 3.0%, 7.8% and 4.0%, respectively. Feeding LP diets improved ($P < 0.001$) DMR and FD, but did not influence ($P>0.05$) the NR coefficient (Table 4).

Digestibility coefficients and daily retention of P and Ca in broilers were not affected ($P>0.05$) by inositol supplementation (Table 5). The CV of digestibility and retention data varied from 10.0 to 15.0%. Feeding LP diets improved the Ca digestibility coefficient ($P<0.001$) but did not affect P digestibility ($P>0.05$). Feeding RP diets improved ($P<0.001$) daily retention of P and Ca.

Hepatic vitamin E, fat and N contents, SA secretion, inositol and alkaline phosphatase in blood plasma

The CV in the variables presented in Table 6 varied between 2.2% for hepatic N concentration to 22.9% for inositol in blood plasma, likely reflecting some lysis of red cells. Feeding RP diets reduced ($P < 0.001$) the relative weight of the liver of the birds, and relative hepatic fat concentration and content ($P<0.05$). However, it increased ($P<0.001$) the relative hepatic N concentration but did not influence ($P>0.05$) hepatic N retention. The concentration of secreted SA was not affected ($P>0.05$) by any of the dietary treatments. Feeding LP diets increased ($P < 0.001$) the hepatic vitamin E and inositol concentration in blood plasma. However, birds fed RP diets had an increased ($P<0.001$) ALP concentration in blood.

Feeding inositol-supplemented diets linearly increased (P<0.05) liver weight and hepatic N content, but linearly reduced (P<0.05) hepatic fat concentration (Table 6). It also linearly increased (P<0.001) the inositol concentration in blood plasma, but did not influence (P>0.05) the SA concentration in excreta (Table 7). Dietary inositol did not influence (P>0.05) the hepatic vitamin E concentration but increased (P<0.001) the ALP in the blood of birds fed 30 g/kg inositol (Table 7).

DISCUSSION

The aim of this study was to investigate the effects of three different levels of inositol in LP and RP diets, on growth performance, nutrient retention, liver N, fat and vitamin E contents, inositol and ALP concentrations in blood plasma. The low inositol supplemented diet contained 3 g/kg inositol, which is the expected dose released in response to a commercial dosage of PHY in the GIT of broilers. The high inositol supplemented diet contained 30 g/kg inositol and was designed to emphasise the impact of inositol on the studied variables. Previous studies on inositol supplementation of broiler diets have used doses from 0.1 to 0.75% (1 to 7g/kg) (Żyła et al., 2004; Cowieson et al., 2013; Pirgozliev et al., 2007), but "super dosing" of inositol has not yet been studied.

There are relatively few reported measurements of inositol in chicken plasma. Schmeisser and coworkers (Schmeisser et al 2013) recorded values of 0.199, 0.246 and 0.345 mM for birds fed PC, NC and PHY 1000-supplemented diets, while Sommerfield et al (2017) obtained values of 0.23 and 0.54 mM for birds fed control and (3.8 g/kg, starter; 3.5 g/kg grower) inositol-supplemented diets. The values obtained here for LP, and LP + 3 g/kg and 30 g/kg-supplemented diets were 0.583, 0.664 and 0.982 mM (Table 7). While these, perhaps, seem high the factors that control plasma inositol are poorly defined. The renal clearance of inositol in chickens is unknown, but in humans it has been shown that following intra-venous supply of inositol the renal clearance of inositol is exceeded at 3-4 mM plasma inositol, considerably higher than the values measured here (Doughaday and Larner, 1953). The elevations of inositol reported in our study coincided with a reduced concentration of hepatic fat content in birds fed inositol. Early reports support this observation and found that dietary inositol supplementation is effective at depressing liver fat synthesis (Bull, 1968; Cough, 1968). A beneficial effect of supplementing diets with inositol to treat fatty liver syndrome in layers was also reported by Parker and Deacon (1968). Holub (1986) describes the metabolism and the function of inositol and inositol phospholipids in animals. A close

negative correlation has been found between the activity of the enzymes fatty acid synthetase and acetyi-CoA carboxylase and levels of dietary inositol, thus further supporting the observed results in this study. The total hepatic N content increased primarily due to increased liver weight.

Cowieson et al. (2015) and Sommerfeld et al. (2017) reported higher inositol in blood plasma of PHY fed chickens compared to birds fed control diets. In the present study, broilers fed LP diets had higher blood plasma inositol concentration, compared to birds fed RP diets. In agreement with our findings, higher inositol concentrations in blood plasma (Cowieson et al., 2015) and excreta (Beeson et al., 2017) has been reported in chicks fed LP diets compared to birds fed RP diets. It has been demonstrated that a reduced intake of dietary minerals, especially of P and Zn, could increase the activity of intestinal alkaline phosphatase and PHY (Davies et al., 1970; Bitar & Reinolds, 1972). This enhanced the digestibility of P in a subsequently fed diet (Moore & Veum, 1982, 1983). In addition, Maenz & Classen (1998) demonstrated *in vitro*, that high mineral concentration markedly decreased the activity of brush border phytase in chicks. McComb et al. (1979) reported that many microbes adapt to low P concentrations by increasing the synthesis of alkaline phosphatase. This finding suggests that the intestinal microflora might be involved in the enhanced dietary P availability due to feeding LP diets. Although not determined in this study, it seems likely that the activity of the intestinal alkaline phosphatase and PHY in the gut of broilers fed LP diets is elevated and thus these birds exhibited an improved phytate degradation compared to the birds fed RP diets. The improved AME, DMR and FD of LP diets observed in the present study further support this hypothesis.

The apparent total tract Ca and P digestibility coefficients were in the expected range (Olukosi and Fru-Nji, 2014; Sommerfeld et al., 2017). The lack of difference between P digestibility in the LP and RP diets was expected and can be explained by the same bioavailability of the added dietary dicalcium phosphate. Similar to Olukosi and Fru-Nji (2014), the LP diet exhibited lower Ca digestibility compared to RP diets. When LP diets are fed the Ca:P ratio in the gut is relatively wide compared to the same ratio in RP diets. However, the widening of Ca:P ratio is known to reduce Ca utilisation in broilers (Olukosi and Fru-Nji, 2014; Farhadi et al., 2017). It seems that the decreased Ca utilization in diets with wide Ca:P can thus be explained by the presence of a greater amount of Ca in the intestine than can be used by the birds, leading to excessive Ca excretion or reduced efficiency of Ca absorption.

The hepatic vitamin E concentration was in line within with previous observations (Karadas et al., 2010, 2014; Pirgozliev et al., 2015). Although the reduction in vitamin E concentration in RP fed birds was not expected, the values were in the expected range for chickens at this age. The increased vitamin E concentration in the liver of LP fed birds coincided with reduced growth performance variables, thus suggesting that the relatively low feed intake of these birds reduced the oxidative stress, thereby preventing vitamin E reserves from depletion.

The ALP concentration in blood was in agreement with the expected reference limits (Meluzzi et al., 1992). The levels of ALP for birds fed RP diets were higher than those fed LP diets possibly due to increased growth rates and osteoblastic activity of chickens (Bell and Freeman, 1971). ALP belongs to a group of enzymes with a low substrate specificity and catalyses the hydrolysis of phosphate esters in a basic environment. This suggests that dietary inositol may reform inositol phosphate isomers, provoking ALP synthesis, thus explaining the results for the "super dosed" inositol diet. There was a correlation between ALP activity and the rates of bone formation in mice (Dimai et al., 1998), suggesting that decreased bone reabsorption and an improved bone density might be a reason for elevated ALP in the blood of birds fed the "super dosed" inositol diets. Study by Cowieson et al. (2015) showed positive correlations between bone ash and plasma inositol after feeding phytase to LP diets. Conversely, there was no link between plasma inositol levels and bone ash contents in birds fed diets supplemented with either inositol or PHY (Sommerfeld et al., 2017).

Here, no effects of inositol supplementation on growth performance, energy, nutrient and mineral utilisation were observed. The literature investigating the effects of supplemental inositol on broiler performance is ambiguous. Żyła et al. (2013) demonstrated that supplementation with as little as 1g/kg inositol in wheat- and maize-based diets containing 1.5 g/kg of available P improved growth of broilers of a similar age. Pirgozliev et al. (2017) reported an optimised dietary AME and broiler growth at approximately 2.5 g/kg inositol when feeding maize-based diets containing 2.5 g/kg available P. Sommerfeld et al. (2017) found that supplementing 3.5 g/kg inositol to P sufficient wheat-based diets improved feed efficiency in broilers during the starter phase. However, Cowieson et al. (2013) found that the addition of inositol 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 inositol reduced feed intake (Cowieson et al., 2013) which is in contrast to the current work. Furthermore, Cowieson et al. (2013) reported an interaction

between inositol and exogenous PHY, whereby the addition of inositol to either a RP or LP diet improved feed efficiency in older birds only in the presence of PHY. Finally, Pearce (1975), Żyła et al. (2004), and Farhadi et al. (2017), did not find any advantage in broiler growth rates when fed inositol supplemented RP or LP diets.

Regardless of dietary P content, in the present experiment inositol did not influence studied mineral availability in agreement with previous reports (Farhadi et al., 2017; Sommerfeld et al., 2017). However, comparing diets with and without PHY supplementation, Pirgozliev et al. (2017) observed an interaction as the increase in dietary inositol content had no effect on P digestibility in the absence of PHY but it depressed P digestibility in the diets containing PHY. In addition, increasing dietary inositol content did not change SA concentration, which is contradictory to previous findings (Pirgozliev et al., 2017).

Dietary PHY supplementation in RP diets has been shown to improve inositol concentration in blood but not overall growth performance (Cowieson et al., 2015). Conversely, in the same study the dietary PHY supplementation in LP diets did not affect plasma inositol concentration but did improve overall growth performance variables. Similarly, Beeson et al. (2017) reported overall improvement in growth performances of PHY fed birds, although there was PHY by available P interaction for inositol in excreta. Thus, there was no apparent correlation between feed efficiency and inositol and inositol phosphate esters concentration in excreta. In addition, feeding LP diet resulted in a lower feed efficiency but higher inositol concentration in ileal digesta. Sommerfeld et al. (2017) reported highest inositol concentration in blood plasma of birds fed inositol supplemented diets, approximately 35% higher than the inositol in blood plasma of PHY only fed birds, but there was no difference between the overall feed efficiency between these diets.

In conclusion, this experiment has confirmed expected biological effects of diets that differ in available P contents. The experiment also confirmed expected biological effects of small increases in dietary inositol. We did not observe available P x inositol interactions in any of the variables studied. The principal finding that large dietary inositol significantly elevates plasma inositol with neither beneficial nor deleterious effect focuses attention on aspects of avian physiology concerned with renal clearance of inositol. The half-life of inositol in plasma is rarely reported, but values of approximately 2 h are evident for human adults in the data of Doughaday and Larner (1953) and 5 h in neonatal infants (Phelps et al. 2013). With regards to inositol measurement, the methods elaborated here have been tested by the authors

and found to be appropriate for chicken muscle, liver and kidney. They should enable metabolomic and transcriptomic study of tissue response to phytate-derived inositol, providing means can be found to distinguish between organ-specific de-novo synthesis of inositol (from glucose 6-phosphate) and inositol derived from dietary phytate. In this regard, the known refractory nature of proximal, but not distal, PI-3kinase signalling to insulin in chicken muscle (but not liver) remains a puzzle that would benefit from measurement of plasma, liver and muscle inositol and its metabolites.

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Table 1. Ingredient composition (g/kg 'as fed') of the control experimental diets[1]

Ingredients	g/kg	g/kg
Maize	604	600
Soybean meal 48	300	300
Maize gluten meal	40	40
Vegetable oil	20	20
Salt	3.6	3.6
DL Methionine	4.2	4.2
Lysine HCl	3.0	3.0
Limestone	15.2	6.7
Dicalcium Phosphate	6.0	18.5
Vitamin Mineral premix[2]	4.0	4.0
	1000	1000
Calculated values (as fed)		
Crude protein (Nx6.25), g/kg	216	216
Crude oil g/kg	47	47
ME, MJ/kg	12.87	12.82
Calcium, g/kg	9.4	9.2
Av Phosphorus, g/kg	2.5	4.8
Dig Lysine	11.7	11.7
Dig M+C	10.3	10.3
Dig Thr	7	7
Dig Try	1.9	1.9
Determined values		
DM	0.903	0.903
GE	16.87	16.80
Crude protein (Nx6.25), g/kg	213	200
Crude oil g/kg	41	42
Ca	9.5	10.2
P	4.5	7.4
Na	1.2	1.4

[1]The inositol was added on the top of this formulation.
[2]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, pantotenic 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 molybdenium 0.5 mg.

Table 2. Determined myo-inositol content in the experimental diets

Diets	nmol / g	g / Kg
LP	1377.6	0.248
LP + 3 g/kg INS	13779.4	2.482
LP + 30 g/kg INS	150173.2	27.055
RP	1552.9	0.280
RP + 3 g/kg INS	13464.3	2.426
RP + 30 g/kg INS	156319.9	28.163

Data are given as treatment means of two replicates per diet.

Table 3. Body weight (BW), feed intake (FI), weight gain (WG) and gain to feed ratio (G:F) of broiler chickens fed the experimental diets.

Treatment factor	BW kg/bird (21d age)	FI kg/bird (7-21d)	WG kg/bird (7-21d)	G:F kg/kg (7-21d)
av P (g/kg)				
2.5	0.594	0.766	0.461	0.601
4.8	0.727	0.890	0.594	0.668
SEM	0.0092	0.0105	0.0089	0.0085
INS (g/kg)				
0	0.656	0.817	0.522	0.636
3	0.662	0.838	0.530	0.628
30	0.664	0.829	0.530	0.639
SEM	0.0112	0.0128	0.0109	0.0104
p-Value				
av P	<0.001	<0.001	<0.001	<0.001
INS	NS	NS	NS	NS
av P x INS	NS	NS	NS	NS
CV%	7.6	6.9	9.2	7.3

av P, calculated dietary available P; INS, added dietary *myo*-inositol; CV%, coefficient of variation.
There were 10 observations per treatment.
Means within a column with no common superscript differ significantly.

Table 4. AME, DMD, NR, FD of broiler chickens fed the experimental diets.

Treatment factor	AME (MJ/kg DM)	AME:GE	AME int (MJ)	DMR	NR	FD
av P (g/kg)						
2.5	14.68	0.870	10.17	0.761	0.647	0.774
4.8	14.32	0.852	11.51	0.743	0.627	0.751
SEM	0.067	0.0040	0.161	0.0042	0.0090	0.0056
INS (g/kg)						
0	14.52	0.863	10.71	0.753	0.634	0.760
3	14.57	0.865	11.02	0.756	0.641	0.766
30	14.41	0.856	10.78	0.746	0.636	0.763
SEM	0.082	0.0049	0.198	0.0051	0.0111	0.0069
p-Value						
av P	<0.001	<0.05	<0.001	<0.05	NS	0.006
INS	NS	NS	NS	NS	NS	NS
av P x INS	NS	NS	NS	NS	NS	NS
CV%	2.5	2.5	8.2	3.0	7.8	4.0

av P, calculated dietary available P; INS, added dietary Ins-inositol; CV%, coefficient of variation.
There were 10 observations per treatment.
Means within a column with no common superscript differ significantly.

Table 5. Minerals digestibility and retention of dietary Ca and P.

	Digestibility		Retention (g/b/d)	
Treatment factor	Ca	P	Ca	P
av P (g/kg)				
2.5	0.396	0.503	2.89	1.72
4.8	0.549	0.524	4.99	3.47
SEM	0.0111	0.0094	0.109	0.059
INS (g/kg)				
0	0.473	0.507	3.89	2.53
3	0.470	0.510	3.98	2.61
30	0.475	0.524	3.94	2.64
SEM	0.0135	0.0116	0.133	0.072
p-Value				
av P	<0.001	NS	<0.001	<0.001
INS	NS	NS	NS	NS
av P x INS	NS	NS	NS	NS
CV%	12.8	10.1	15.1	12.4

av P, calculated dietary available P; INS, added dietary Ins-inositol; CV%, coefficient of variation.
There were 10 observations per treatment.
Means within a column with no common superscript differ significantly.

Table 6. Liver weight and concentration of hepatic fat and N of the experimental birds (subheading as in previous table).

Treatment factor	Weight		Concentration (g/kg)		Retention (g)	
	Liver (g)	Liver (% body weight)	Fat	N	Fat	N
av P (g/kg)						
2.5	19.3	3.1	11.8	12.2	0.22	0.24
4.8	19.4	2.6	10.1	12.4	0.20	0.24
SEM	0.40	0.08	0.34	0.05	0.007	0.005
INS (g/kg)						
0	18.4[a]	2.7	11.6	12.2	0.21	0.22[a]
3	19.7[ab]	2.8	10.9	12.3	0.21	0.24[b]
30	20.1[b]	2.9	10.2	12.4	0.20	0.25[b]
SEM	0.49	0.10	0.42	0.06	0.009	0.006
p-Value						
av P	NS	<0.001	<0.001	<0.001	0.008	NS
INS	0.048	NS	0.090	0.084	NS	0.028
av P x INS	NS	NS	NS	NS	NS	NS
CV%	11.4	15.6	17.3	2.2	18.2	12.2

av P, calculated dietary available P; INS, added dietary Ins-inositol; CV%, coefficient of variation.
There were 10 observations per treatment.
Means within a column with no common superscript differ significantly.

Table 7. Sialic acid (SD) concentration in excreta, ~~Ins~~*myo*-inositol (INS) and alkaline phosphatase (ALP) in blood, and hepatic vitamin E of chickens fed the experimental diets.

Treatment factor	SAc (mg/g)	INS (nmol / mL)	ALP (U/ml)	Vitamin E (µg/g)
av P (g/kg)				
2.5	0.756	834.0	5817	29
4.8	0.768	652.7	13446	22
SEM	0.0201	31.1	661.6	1.04
INS (g/kg)				
0	0.765	584.0[a]	8519[a]	25
3	0.743	663.0[a]	8620[a]	25
30	0.781	982.0[b]	11756[b]	26
SEM	0.0246	38.1	810.3	1.3
p-Value				
av P	NS	<0.001	<0.001	<0.001
INS	NS	<0.001	0.010	NS
av P x INS	NS	NS	NS	NS
CV%	14.4	22.9	37.6	22.6

av P, calculated dietary available P; INS, added dietary Ins-inositol; CV%, coefficient of variation.
There were 10 observations per treatment.
Means within a column with no common superscript differ significantly.

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