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Biological Availability of Phosphorus in Raw and Acidulated Sinda and Chilembwe Rock Phosphates in Broiler Chickens

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Abstract

A 14 day broiler chickens feeding trial was conducted to determine bio-availability and relative biological values (RBV) of phosphorus in raw and acidulated local rock phosphates (RPs) as potential replacements for imported and more expensive di-calcium phosphate (DCP). Treatments included Raw, Sulphuric acid and Phosphoric acid acidulated Sinda and Chilembwe RPs. Bio-availability was assessed based on feed intake, body weight gains, feed conversion ratios, phosphorus retention and mineralization of tibia bones and blood serum. RBVs were calculated on the same parameters using DCP as a reference standard. Phosphorus contents in local RPs were significantly ($P \geq 0.05$) lower than in DCP, but acidulating RPs with Phosphoric acid significantly increased phosphorus, but reduced calcium contents. Other minerals were comparable with what was in DCP. Acidulating RPs significantly ($P \geq 0.05$) increased body weight gains and feed conversion ratios, but not feed intake. Phosphorus retentions, weights and ash contents of tibia bones were significantly ($P \geq 0.05$) lower in chickens fed diets based on raw than that of those fed diets based on acidulated RPs and DCP; an indication that acidulating the RPs improved phosphorus bio-availability in broiler chickens. There were however; no statistical differences ($P \geq 0.05$) among treatments in calcium and phosphorus contents in both tibia bones and blood serum.

Keywords: Sinda; Chilembwe; Rock Phosphates; Phosphoric and Sulphuric Acids; Acidulation; Phosphorus Bio-Availability

Introduction

Practical poultry diets are largely made up of cereal grains and oilseed meals that are normally deficient in dietary phosphorus. This is because up to 80% of phosphorus in feed ingredients of plant origin is bound to phytic acid (Rama Rao et al. 1999 and Kies *et al.,* 2001) and only about 30% of this amount is assumed to be available to poultry and other non-ruminants (Nelson *et al.,* 1968) [1-3]. Thus, rather than depending on total dietary concentrations, phosphorus requirements in poultry and other non-ruminants are based on biological availabilities that estimates portion of dietary phosphorus that is absorbed and utilized by the animal (Shastak *et al.,* 2012; WPSA, 2013) [4,5]. A number of response criterion are employed for assessing phosphorus bio-availabilities including feed intake, body weight gains, feed conversion ratios and mineralization of bones and blood. Bone mineralization is usually based on tibia or toe bone weights, ash content and concentration of phosphorus and calcium in these tissues (Shastak *et al.,* 2012) [4]. Phosphorus bio-availability may also be evaluated based on amounts retained in animal body after subtracting excreted amounts from what was consumed in the feed (Godoy and Chicco, 2001) [6]. To avoid underestimating retained amounts, feacal samples are usually collected from the distal end of the ileum in order to exclude urinary phosphorus excretions (Sastak *et al,* 2012) [4]. Phosphorus bio-availabilities may also be assessed based on relative biological values (RBVs), whereby different phosphorus sources are evaluated by comparing performance of animals fed diets based on test phosphorus sources with that of animals fed diets based on a known reference standard that is usually assigned 100% availability (Lima *et al.,* 1997) [7].

In recent years, a number of studies have established that available phosphorus requirements in broiler chickens is far less than current National Research Council (NRC, 1994) recommendations (Applegate and Angel, 2014; Li *et al.,* 2016; Hamdi *et al.,* 2017) [8-11]. However, commercial broiler diets are still formulated to meet current NRC recommendations for safety margins that guarantee animals from showing deficiency symptoms (Li *et al.,* 2016; Applegate and Angel, 2014; Angel *et al.,* 2003) [9,10,12]. However, with such intakes, there is always the danger of over-supplementation that does not only result in higher feed costs but also increased phosphorus excretions leading to environmental pollutions (Shastak and Rodehutscord, 2015) [13]. The main sources of inorganic phosphorus in poultry and other non-ruminant diets are mono-calcium, di-calcium and tri-calcium phosphates that are derived from processed rock phosphates (Lima *et al.,* 1997; Fernandes *et al.,* 1999; Petersen *et al.*, 2011) [7,14,15]. In Zambia, processed phosphates are imported and because of limited foreign exchange earnings, they have become too expensive; while use of alternatives such as animal processing by-products is limited by variability in supply and content of essential nutrients including phosphorus (Waldroup, 1999) [16]. This has necessitated the need for exploitation of local RPs to replace imported phosphates. This research was conducted to evaluate use of raw and acidulated Sinda and Chilembwe RPs as alternative sources of inorganic phosphorus in broiler starter diets. Evaluations were based on mineral composition, phosphorus retention, feed intake, body weight gains and feed conversion ratios and mineralization of tibia bones and blood serum in broiler chickens fed diets based on raw and acidulated RPs using DCP as a control.

Materials and Methods

Study area and processing of rock phosphates

This study was conducted in the School of Agricultural Sciences at the University of Zambia in Lusaka. The RPs was obtained from Sinda and Chilembwe rock phosphate deposits in Sinda and Petauke Districts of Zambia's Eastern Province. RPs from each source was processed by crushing with a Jaw Stone Crusher (Denver Equipment Pvt. Limited) for particles to pass through a 5 mm screen followed by grinding with Fristsch Rotary Ball Mill through a 0.2 mm sieve. Ground RPs from each source were then divided into three portions of 10 kg each, with the first portion being left intact as raw, while the second and third were each acidulated with 2 litres per kg of concentrated Sulphuric acid (98% Analytical grade) and 84% Phosphoric acid: respectively. After acidulation, the RPs were washed with distilled water and left to dry in a forced air drying oven. This resulted in six treatments consisting of raw, Sulphuric acid and Phosphoric acid acidulated Sinda and Chilembwe RPs. Commercial DCP was included as a control for the study. Samples of prepared RPs were first analysed in triplicate for Calcium (Ca), Phosphorus (P), Sodium (Na), Zinc (Zn) and Lead (Pb) using standard procedures of the Association of Official Analytical Chemists (AOAC, 1998) [17].

Determination of phosphorus bio-availability and relative biological values of phosphates based on broiler chickens feeding trial.

After mineral composition analysis, prepared RPs were incorporated into starter rations for a 14-day feeding trial to determine phosphorus bio-availability based on performance of broiler chickens using DCP as a control treatment (Table 1). The trial used 252 ten-day old unsexed Hubbard broiler chickens that were initially fed a commercial broiler starter diet (Novatek Feeds Limited).

INGREDIENTS	Treatment Diets						
	DCP	Sinda Raw	Chilembwe Raw	Sinda Sulphuric	Chilembwe Sulphuric	Sinda Phosphoric	Chilembwe Phosphoric
Maize meal	55.6	55.6	55.6	55.6	55.6	55.6	55.6
Soybean meal	35.7	35.7	35.7	35.7	35.7	35.7	35.7
Fish meal	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Soya bean oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Limestone	0.6	0.6	0.6	0.6	0.6	0.6	0.6
P. source*	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Salt (NaCl)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-Lysine HCL	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mineral premix	0.3	0.3	0.3	0.3	0.3	0.3	0.3
TOTAL	100	100	100	100	100	100	100
Nutrient concentration – calculated analysis							
TME,kcal/kg	2989	2989	2989	2989	2989	2989	2989
CP %	22.1	22.1	22.1	22.1	22.1	22.1	22.1
Total Lys,%	1.21	1.21	1.21	1.21	1.21	1.21	1.21
Methionine%	0.48	0.48	0.48	0.48	0.48	0.49	0.49
Calculated Ca, %	0.86	0.78	0.74	0.82	0.79	0.74	0.86
Analyzed Ca, %	0.87	0.80	0.85	0.77	0.75	0.75	0.75
Calculated P %	0.73	0.60	0.63	0.56	0.56	0.71	0.72
Analyzed P %	0.71	0.63	0.60	0.59	0.61	0.72	0.71
Na, %	0.2	0.2	0.2	0.2	0.2	0.2	0.2

TME: True Metabolizable Energy; CP: Crude Protein; Ca: Calcium; P: Phosphorus; The amounts of P and Ca were differentiated between calculated in ration formulation and amounts analyzed by proximate analysis

Table 1: Composition (%) of broiler starter diets used for evaluating bio-availability of phosphorus in raw and acidulated Sinda and Chilembwe rock phosphates (% of diet)

For the trial, 6 chicks of relatively similar body weights (231.7±7.81g) were randomly allocated to experimental diets and placed in battery cages that were equipped with feeders and drinkers. Each treatment was replicated in 6 battery cages. The chicks were housed in a temperature controlled room where they were exposed to 23 hours of lighting at a temperature of 28 °C each day. The temperature was reduced to 24 °C after one week of the study. Feed and water were provided *ad libitum* throughout the experimental period.

Performance of chickens during the trial was assessed based on daily feed intake and weekly body weight gains that were used to calculate feed conversion ratios. Retention of phosphorus was determined by analyzing for phosphorus content in excreta to assess portions of consumed amounts that were retained for utilization by the chickens. The excreta was collected during the last 4 days of the trial and after each collection, the collected amounts were frozen at -20 °C and kept until the end of the trial. After the trial, all excreta samples from each cage were thawed, dried at 60 °C for 48hrs and weighed after cooling to room temperature. The dried samples were then pooled and analyzed for phosphorus to determine amounts retained by subtracting excreted amounts from what was consumed as described by Lima *et al.* (1995) and Coon *et al.* (2007) [18,19].

On day 20, three birds from each cage were randomly selected to collect blood from the wing web vein using plain vacutainer tubes for determination of phosphorus and calcium in blood serum as described by Lima *et al.* (1997) [7]. On day 24, there was another random selection of three birds from each cage that were slaughtered to harvest left legs from each chicken. The legs were then boiled for 10 minutes, after which the tibia bones were cleared of attaching muscles and ligaments and defatted before being dried at 50 °C for 48 hours (Driver *et al.,* 2006) [20]. The dried bones were then weighed after cooling to room temperature to determine tibia bone weights before being incinerated for 24 hours at 600 °C to determine ash content that was later analysed for calcium and phosphorus contents. The analysed parameters were then used to determine phosphorus bio-availabilities and to calculate relative biological values (RBV) of phosphorus in raw and acidulated local RPs using DCP as a reference standard.

Experimental design and statistical analysis

The study was conducted as a Completely Randomized Design (CRD) consisting of six treatments and a control. The feeding trial used six birds in a cage for each treatment that served as an experimental unit that was replicated six times. All collected data were subjected to Analysis of Variance (ANOVA) according to General Linear Models (GLM) of Statistical Analysis System (SAS). An F-test was used to detect significant differences among treatment means, which were separated using least significant different (LSD) according to Tukey's test of significance at $P \geq 0.05$.

Results

Composition of minerals in raw and acidulated rock phosphates

Sinda and Chilembwe raw RPs contained similar amounts of phosphorus that were significantly ($P \geq 0.05$) lower than what was in DCP (Table 2). Acidulating the RPs with Sulphuric acid had no effect on phosphorus content, but phosphoric acid increased amounts closer to what was in DCP. The content of calcium in Sinda raw RPs was statistically similar to what was in DCP, while Chilembwe raw had significantly ($P \geq 0.05$) lower amounts. Acidulating RPs with either acid reduced calcium content, with the reduction being greater when phosphoric acid was used as demonstrated by significantly ($P \geq 0.05$) lower calcium levels in phosphoric acid acidulated RPs. Content of lead was significantly ($P \geq 0.05$) higher in raw RPs an indication that acidulating the RPs reduced its concentration as demonstrated by having no significant ($P \geq 0.05$) differences in lead content among acidulated RPs including DCP. The amount of zinc was significantly higher in Sinda than Chilembwe RPs, with DCP having the lowest amounts. The amounts of sodium in RPs were between 0.25 and 0.39% and did not seem to have been affected by acidulating the RPs. The sodium in DCP were statistically similar to what was in Sinda RPs that was significantly ($P \geq 0.05$) higher than what was in Chilembwe RPs.

Treatment Diets	Phosphorus (%)	Calcium (%)	Lead (mg/kg)	Zinc (mg/kg)	Sodium (%)
Di-calcium phosphate	18.09^{a}	23.90^{a}	23.6^{bc}	24.04^{e}	0.35^{ab}
Sinda Raw	8.97^{c}	24.50^{a}	27.7^{ab}	46.48^{a}	0.39^{a}
Chilembwe Raw	10.54^{c}	21.88^{b}	29.9^{a}	34.54^{c}	0.31^{bc}
Sinda Sulphuric	7.21^{c}	19.95^{c}	20.5^{c}	43.46^{b}	0.37^{ab}
Chielembwe Sulphuric	7.24^{c}	20.17^{c}	20.5^{c}	32.65^{c}	0.26^{c}
Sinda Phosphoric	15.52^{b}	17.15^{d}	21.6b^{c}	44.47^{ab}	0.37^{ab}
Chilembwe Phosphoric	16.58^{ab}	17.30^{d}	21.6b^{c}	28.33^{d}	0.25^{c}
SEM	1.04	0.60	0.82	1.81	0.01
***Allowed Critical toxic threshold value**	-	-	1 500.00	50 000.00	2.00

Means within a column with different superscript letters were significantly ($P \leq 0.05$) different from each other.
*Critical values for Pb and Zn are the maximum tolerable concentrations in the P source. **Critical value for Na is the maximum concentration (%) in complete diet for poultry (NRC, 1980). SEM – Standard Error of the Mean.

Table 2: Concentrations of Phosphorus, Calcium, Lead, Zinc (Zn) and Sodium (Na) in raw and acidulated rock phosphates used as sources of inorganic P in broiler chicken rations as compared with what was in DCP

Phosphorus bio-availability based on performance of broiler chickens fed diets based on raw and acidulated rock phosphates

Performance of broiler chickens fed diets based on raw and acidulated Sinda and Chilembwe RPs in comparison with that of the control group showed no significant differences ($P \geq 0.05$) in feed intake among all treatments (Table 3). There were however; significant differences ($P \geq 0.05$) in body weight gains (BWG) with birds fed diets based on DCP and acidulated Sinda and Chilembwe RPs having significantly ($P \geq 0.05$) higher weight gains than those fed diets based on raw RPs. The increase in body weight gains of chickens fed diets based on acidulated Sinda RPs was however; not significantly ($P \geq 0.05$) different from that of chickens fed diets based on raw Sinda RPs. Results on feed conversion ratios (FCR) reflected that of body weight gains and also showed that chickens fed diets based on acidulated RPs and the control had significantly ($P \geq 0.05$) better feed conversion ratios than that of chickens fed diets based on raw RPs.

	Broiler Performance			Relative Biological Values (%)		
Treatment	**Feed Intake (g)**	**Weight Gain (g)**	**FCR**	**FI**	**BWG**	**FCR**
DCP	672.72	451.97^{a}	1.49^{b}	100	100	100
Sinda raw	669.86	399.22^{b}	1.67^{a}	99.6	88.3	88.4
Chilembwe raw	663.88	399.83^{b}	1.66^{a}	98.7	88.5	89.4
Sinda sulphuric	659.73	432.66^{ab}	1.53^{b}	98.1	95.7	97.3
Chilembwe Sulphuric	676.03	441.25^{a}	1.53^{b}	100.5	97.6	97.9
Sinda Phosphoric	637.35	430.03^{ab}	1.48^{b}	94.7	95.2	100.1
Chilembwe Phosphoric	650.64	452.81^{a}	1.44^{b}	96.7	100.2	103.4
SEM	5.27	5.39	0.02	0.89	1.3	1.1

Means within a column with different superscript letters were significantly different from each other at $P \geq 0.05$.
TFI: Total Feed Intake; BWG: Body Weight Gain; FCR: Feed Conversion Ratio; SEM: Standard Error of the Mean.
Table 3: Performance of broiler chickens and Relative Bio-availability of phosphorus in raw and acidulated Sinda and Chilembwe rock phosphates using DCP as reference standard

When the results on performance of broiler chickens were expressed as relative biological values (RBVs) of phosphorus using DCP based diet as a reference standard, feed intake figures ranged from 94.7 to 100.5% and also showed no major differences among treatments. The RBV figures on body weight gains ranged from 88.3 to 100.2% with chickens fed diets based on raw RPs having significantly inferior gains than that of chickens fed diets based on phosphoric acid acidulated RPs. This was also the case when RBVs were based on FCR where the figures ranged from 88.44 to 103.36% again demonstrating that diets based on raw RPs promoted inferior phosphorus utilizations compared with that of chickens fed diets based on acidulated RPs and DCP.

Bio-availability of phosphorus based on its retention in broiler chickens fed raw and acidulated Sinda and Chilembwe rock phosphates

The results on retention of phosphorus in broiler chickens showed the amounts of phosphorus retained in chickens fed diets based on raw Sinda and Chilembwe RPs to be significantly lower ($P \geq 0.05$) than that of chickens fed diets based on acidulated RPs and the control (Table 4). Only chickens fed diets based on Sulphuric acid acidulated Chilembwe RP had phosphorus retention figures that were statistically ($P \geq 0.05$) similar to what was recorded for raw RPs. When phosphorus retention results were expressed as RBVs using DCP as reference standard, they showed Sinda and Chilembwe raw RPs to have lower figures when compared with that of chickens fed diets based on acidulated RPs. Only chickens fed diets based on Sulphuric acid acidulated Chilembwe RPs had retention figures that were significantly lower than that of the control diet.

Treatment	Amount of P. retained (%)	Relative Bioavailability (%)
DCP	68.72^{a}	100
Sinda raw	60.27^{bc}	87.72
Chilembwe raw	56.04^{c}	81.56
Sinda Sulphuric	71.78^{a}	104.45
Chilembwe Sulphuric	60.48^{bc}	88.02
Sinda Phosphoric	72.12^{a}	104.95
Chilembwe Phosphoric	71.65^{a}	104.27
Pooled SEM	1.40	2.24

Means with different superscript letters within a column were statistically different from each other ($p \leq 0.05$).
SEM: Standard Error of the Mean
Table 4: Apparent availability and relative biological availability of phosphorus from inorganic phosphorus based on Retained Phosphorus in broiler chickens fed diets based on raw and acidulated rock phosphates

Bio-availability of phosphorus in rock phosphates based on tibia bone and blood mineralizations

The tibia bone weights of chickens fed diets based on the control diet were significantly heavier (P ≥ 0.05) than that of chickens fed on diets based on raw Sinda and Chilembwe RPs (Table 5). There were however; no significant differences (P ≥ 0.05) in tibia bone weights of chickens fed diets based on acidulated Sinda and Chilembwe RPs and that of chickens fed the control diet. However, the increase in tibia bone weights as a result of acidulating RPs was not enough to be statistically different (P ≥ 0.05) from that of chickens fed diets based on raw RPs. When results on tibia bone weights were expressed as RBVs using DCP as a reference standard, the figures for chickens fed diets based on raw RPs were about 15% points lower than that of the chickens fed diets based on the control. The results on tibia bone ash contents showed significant differences (P ≥ 0.05) among chickens fed diets based on raw and acidulated Sinda and Chilembwe RPs. There were however; no significant (P ≥ 0.05) differences in tibia bone ash contents between chickens fed diets based on the control and acidulated Sinda and Chilembwe RPs. When tibia bone ash contents were expressed as RBVs, the figures ranged from 99.76 to 102.44%, which was very close to 100% that was assigned for the reference standard. There were however, no significant differences (P ≥ 0.05) in calcium and phosphorus content in tibia bones of chickens fed diets based raw and acidulated RPs including that of the control diet.

Treatment	Weight (g)	Ash (%)	Calcium (%)	Phosphorus(%)	Relative bioavailability		
					Weight	Ash	P
DCP	10.26[a]	50.01[a]	12.85	6.11	100	100	100
Sinda raw	8.87[b]	45.45[b]	11.51	5.45	86.46	90.88	89.24
Chilembwe raw	8.78[b]	45.62[b]	11.18	5.28	85.58	91.22	86.42
Sinda Sulphuric	9.45[ab]	51.97[a]	13.39	5.98	92.15	103.91	97.87
Chilembwe Sulphuric	9.39[ab]	49.89[a]	11.85	5.94	91.25	99.76	97.82
Sinda Phosphoric	9.57[ab]	51.23[a]	13.49	6.40	92.72	102.44	104.75
Chilembwe Phosphoric	9.81[ab]	51.18[a]	11.60	5.65	95.64	102.34	92.41
SEM	0.12	0.58	0.56	0.18	1.33	1.14	3.23

Means with different superscript letters within a column were statistically different from each other (p≤0.05).
SEM: Standard Error of the Mean
Table 5: Biological availability of phosphorus in raw and acidulated Sinda and Chilembwe rock phosphates based on tibia bone parameters of broiler chickens fed various experimental diets

The concentration of phosphorus and calcium in blood serum was also not affected by the source of inorganic phosphorus as there were no significant (P ≥ 0.05) differences among treatments including that of the control (Table 6). The concentration of phosphorus in blood serum ranged from 2.06 to 2.55 mmol/litre while that of calcium was from 1.99 to 2.44 mmol/litre and both showed no particular trends in the concentration of the two minerals as a result of acidulating the two local RPs. The RBVs also revealed no differences (P ≥ 0.05) among treatments in the content and relative biological availability of calcium and phosphorus in blood serum.

Treatment	Ca (mmol/l)	P (mmol/l)	Relative bioavailability (%)	
			Ca	P
DCP	2.44	2.48	100	100
Sinda raw	2.05	2.24	84.09	90.32
Chilembwe raw	2.17	2.29	88.71	92.44
Sinda Sulphuric	2.07	2.07	84.84	83.38
Chilembwe Sulphuric	2.12	2.05	86.86	82.84
Sinda Phosphoric	2.29	2.53	94.02	101.84
Chilembwe Phosphoric	2.37	2.55	98.19	102.66
SEM	0.08	0.07	3.83	2.93

There were no statistical differences in blood serum content of calcium and phosphorus (P≤0.05). SEM: Standard Error of the Mean
Table 6: Content and Relative Biological availability of Ca and P in blood plasma as influenced by phosphate rocks as sources of inorganic phosphorus

Discussion

Composition of minerals in raw and acidulated Sinda and Chilembwe rock phosphates

The contents of calcium and phosphorus in commercial DCP used for the current study were as expected considering that the two minerals in DCP vary between 24.0 and 27.0% and 18.0 and 20.5% for calcium and phosphorus, respectively (Lima *et al.,* 1995) [19]. Recently phosphorus contents in DCP were reported to vary between 18.4 and 22.8% (Bikker *et al.,* 2016) [21]. The current results were however; lower than the 22.7% and 29.0% reported as phosphorus and calcium contents in DCP that was used as a control for the Venezuelan study (Godoy and Chicco, 2001) [6]. The reported figures for phosphorus in Sinda and Chilembwe RPs are an indication that Zambian RPs are generally low in phosphorus content and these results are consistent with earlier findings that classified Zambian RPs to be low grade phosphate deposits (Aydin *et al.,* 2009; Simukanga *et al,* 1994) [22,23]. The 9.87% phosphorus content in Sinda raw RP was comparable with 9.4% reported by Cheleshe *et al.* (2000) and Chirwa (2014) while the 10.54% in Chielembwe RPs was closer to the 12.0% reported by Appleton (2002) [24-26]. When compared with phosphates from other sources, the amounts of calcium and phosphorus in Sinda and Chilembwe raw RPs were within ranges of 10.5 to 12.3% for phosphorus, and 20.0 to 34.4% for calcium reported for Venezuelan RPs (Godoy and Chicco, 2001) [6]. The reduced calcium content noted in acidulated Sinda and Chilembwe RPs could have been due to formation of insoluble Calcium Carbonates whereas the increase in phosphorus content noted in phosphoric acid acidulated RPs was probably from the 15.9% soluble phosphorus contained in this acid (Zapata, 2004) [27].

The amounts of sodium in Sinda and Chilembwe RPs were higher than 0.03% recorded for DCP; an amount that was comparable with 0.028% reported for DCP by Hyghebeart *et al.* (1980) [28]. Other studies have reported sodium contents in RPs to vary between 0.18 and 0.44% (Luiz *et al.,* 2009); although Waldroup (1999) reported a narrower range of 0.10 to 0.17% [16,29]. Higher amounts of sodium are reported in sodium phosphates where they were found to average 5.28% in de-fluorinated sodium phosphates, 28.74% in disodium phosphates and 14.70% in monosodium phosphates (Hyghebeart *et al.,* 1980) [28]. This may be an indication that sodium contents in RPs tend to vary with the source, type and processing conditions to which the rock phosphates are exposed (Lima *et al.,* 1995; Abouzeid, 2008) [19,30]. The same could be said for the content of lead and zinc and it is unlikely that their contents in Sinda and Chilembwe RPs could have been affected by acidulation of RPs. It must however, be noted that the amounts of lead and zinc in Sinda and Chilembwe RPs were all below allowed maximum toxic thresholds for use in broiler chicken diets (NRC, 1980; AAFCO, 1999) [31,32].

Bio-availability of phosphorus based on performance of broiler chickens fed diets based on raw and acidulated rock phosphates

The lack of significant differences in feed intake among chickens fed different treatment diets was in concert with findings of Rama Rao and Reddy (2003) and Mello *et al.* (2012) who also reported no significant differences in feed intake among broiler chickens fed starter diets based on different sources of inorganic phosphorus [33,34]. In more recent years, a similar study also reported feed intake not to be affected by levels of available phosphorus in the diets, despite influencing body weight gains and feed conversion ratios (Zieai *et al.,* 2011) [35]. The lack of significant differences in feed intake among chickens fed different treatment diets in the current study could have been due to the fact that all treatments had adequate supply of available phosphorus that was above minimum requirements (Cardoso *et al.,* 2010) [36]. This is supported by findings of Bikker *et al.* (2016) who only noted variations in feed intake, body weight gains and feed conversion ratios among broiler chickens fed different treatment diets when retainable phosphorus levels were less than 2.7g/kg diet [21]. No significant differences were recorded in the performance of chickens when retainable phosphorus levels ranged from 0.27 to 6.0 g/kg diet. Similar observations were reported by Askari *et al.* (2015) when different dietary calcium and phosphorus levels could not affect feed intake, body weight gains and feed conversion ratios [37]. Hamdi *et al.* (2017) also observed no differences in the performance of broiler chickens on phosphorus availability among different mineral phosphorus sources [11]. A feeding trial with pigs also reported neither the source nor the amount of phosphorus in the diet to have any influence on feed intake, daily gain and the feed to gain ratios (Petersen *et al.* 2011) [15]. However, other studies have reported reduced feed intake, body weight gains and feed efficiency under higher calcium and low phosphorus intakes (Shastak *et al.,* 2012) [4]. In the current study, the reported inferior body weight gains and feed conversion ratios among chickens fed diets based on raw RPs could either have been due to reduced phosphorus bio-availability or intake of some toxic elements such as mercury, lead or fluorine in the RPs (Godoy and Chicco, 2011; Odongo *et al.,* 2002; Tahir *et al.,* 2011) [38-40]. Presence of toxic elements in Sinda and Chilembwe RPs may be ruled out on the basis that there was no mortality among chickens fed different treatment diets and the fact that all measured potentially toxic elements in the diets were below allowed maximum thresholds for toxicity (NRC, 1980) [31]. The calcium to Phosphorus ratios could also have affected the response of chickens to different treatment diets in the study (Leske and Coon, 2002; Yan and Waldroup, 2006, Mello *et al.,* 2012) [33,41,42].

Retention of phosphorus in raw and acidulated rock phosphates

Phosphorus bio-availability in non-ruminants may also be assessed as a percentage of the amounts consumed in feed that is retained in animal tissues or relative biological values based on comparison of performance of animals fed a test phosphorus source and that of others given diets based on a known reference standard that is assumed to have 100% availability (Lima *et al.,* 1997) [7]. Both methods were used in this study to evaluate bio-availability of phosphorus in raw and acidulated Sinda and Chilembwe RPs. The results on retainable phosphorus in this study were generally within range of several studies in literature (Bikker *et al.,* 2016; Li *et al.,* 2016; Shastak and Rodehutscord, 2015) [10,13,21]. The reported figures were generally low may be because of the method used in estimating retainable phosphorus that was based on measuring excretion from the entire gut, which sometimes is said to underestimate amounts as it does not account for urinary excretions (Shastak *et al.,* 2012; WPSA, 2013) [4,5]. The current results were however consistent with findings of De Groote and Huyghebeart (1997), who also reported apparent availabilities of phosphorus in broiler chickens fed mono calcium phosphate based diets to vary between 78.1 and 85.5% [43]. Other studies have reported phosphorus retention figures to vary between 55 and 92% when commercial RPs and animal processing by-products were used as sources of inorganic phosphorus (Van Der Kliss and Versteegh, 1996) [44]. Even pre-caecal digestibilities were found to range from 45 to 72% (Bikker *et al.,* 2016) and from 44.57 to 59.56% (Hamdi *et al.*, 2017) when different types of mono calcium phosphates used as sources of inorganic phosphorus in broiler chickens between 19 and 21 days of age [11,21]. It must be noted that there are considerable variations in literature on retained phosphorus among various studies, mostly as a result of variations in the nature of bio-assays, type and age of animals and assay conditions (Li *et al.,* 2016) [13]. The low retained phosphorus levels in chickens fed diets based on raw Sinda and Chilembwe RPs may be an indication of reduced phosphorus bio-availability in unprocessed RPs (Lima *et al.,* 1997) [7]. It could also have been influenced by many other factors including calcium levels and the calcium to phosphorus ratios in the diet, experimental techniques, chemical form of P, concentration of non phytate phosphorus, availability and interactions of various nutrients to name a few among many others (Li *et al.,* 2016; Ziaei *et al.,* 2011) [13,35]. High calcium and low phosphorus levels in the diet have been reported to reduce absorption and retention of phosphorus especially if it goes beyond 1.5:1 (Liu *et al.,* 1998) [45]. Thus; optimum calcium to phosphorus ratio is crucial for increased uptake and retention of phosphorus in broiler chickens.

Bio-availability of phosphorus in raw and acidulated Sinda and Chilembwe rock phosphates based on tibia bone and blood serum parameters

Increased dietary phosphorus bio-availability is documented to result in increased bone tissue synthesis and thickness (Petersen *et al.,* 2011) [15]. Thus, the increase in tibia bone weights and ash contents in chickens fed diets based on acidulated RPs and the control could be an indication of increased phosphorus deposition in bone tissue. This is supported by earlier findings that showed tibia bone weights, ash content and the concentration of Ca and P in bones to increase with increasing levels of available phosphorus in the diet (Petersen *et al.,* 2011; Shastak *et al.,* 2012) [4,15]. However, the current study failed to demonstrate a corresponding increase in calcium and phosphorus deposition in both tibia bones and blood serum of chickens fed diets based on raw and acidulated RPs. Similarly, Rama Rao and Reddy (2001) also failed to show any relationship in tibia bone ash, Ca and P contents with variations in dietary phosphorus from different inorganic phosphorus sources. This led to the conclusion that Ca and P levels in diets does not seem to affect performance of chickens including their blood and bone parameters [36,46]. However, when pigs were fed diets with marginal concentrations of inorganic phosphorus (0.07 to 0.14%), they showed a correspondingly increase in bone ash, calcium and phosphorus concentrations [15]. These findings are supported by Summers *et al.,* (1959) who concluded that body weight gains and feed conversion ratios are more sensitive measures of phosphorus bio-availability than bone and blood mineralization parameters [47]. This conclusion is consistent with more recent findings that have demonstrated that phosphorus concentrations in fat free bones may not be affected by dietary sources [4,34,37,46] . They however, contradict findings of Huyghebeart *et al.* (1980) and Lima *et al.* (1997) who reported tibia bone phosphorus contents to be a good indicator of its retention in animal tissues [19,28].

Conclusion

The content of phosphorus in Sinda and Chilembwe RPs was significantly lower than in DCP and acidulating the two local RPs with phosphoric acid increased phosphorus contents to levels that were statistically similar to what was in DCP. However, acidulating the RPs with either acid reduced calcium contents. The content of other minerals was not affected by acidulation and were comparable with what was in DCP. Except for Sulphuric acid acidulated Chilembwe RP, acidulating both Sinda and Chilembwe RPs improved retention and bio-availability of phosphorus as demonstrated by improved body weight gains, feed conversion ratios and retention of phosphorus in broiler chickens. This was further supported by increased weights and ash contents in tibia bones of chickens fed diets based on acidulated RPs and DCP.

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References

1. Rama Rao SV, Reddy VR, Reddy VR (1999) Non-phytin phosphorus requirements of commercial broilers and white leghorn layers. Anim Feed Sci Technol 80: 1-10.

2. Kies AK, Van Hemert KHF, Sauer WC (2001) Effect of phytase on protein and amino acid digestibility and energy utilization, World Poult Sci Journ 57: 109-26.

3. Nelson TS, Ferrara LW, Storer NL (1968) Phytin content in feed ingredients derived from plants. Poult Sci 67: 1372-4.

4. Shastak Y, Witzig M, Hartung K, Bessei W, Rodehutscord M (2012) Comparison and evaluation of bone measurements for the assessment of mineral phosphorus sources in broilers. Poult Sci 91: 2210-20.

5. WPSA (World's Poultry Science Association) (2013) Determination of phosphorus availability in poultry. World's Poult Sci J 69: 687-98.

6. Godoy S, Chicco CF (2001) Relative bio-availability of phosphorus from Venezuelan raw rock phosphates for poultry. Anim Feed Sci Technol 94: 103-13.

7. Lima FR, Mendonça, CX, Alvarez JC, Garzillo JMF, Ghion E, et al. (1997) Biological evaluations of commercial di-calcium phosphates as sources of available phosphorus for broiler chicks. Poultry Sci 76: 1707-13.

8. NRC (1994) Nutrient requirements of poultry (9th Revised Edn) National Academy Press, Washington, DC, USA.

9. Applegate TJ, Angel R (2014) Nutrient requirements of poultry publication: History and need to update. J Appl Poult Res 23: 567-75.

10. Li X, Zang D, Yang TY, Bryden WL (2016) Phosphorus bioavailability: Aspect for conserving this critical animal feed resource with reference to broiler nutrition; Review. Agriculture 6: 25.

11. Hamdi M, Solá-Oriol D, Franco-Rosselló R, Allegué-Alemany R. Pérez JF (2017) Comparison how different phosphates affect performance, bone mineralization and phosphorus retention in broilers. Spanish J Agri Res 15: e0605.

12. Angel R, Applegate TJ, Christman M (2003) Non-phytate phosphorus requirements of broilers fed on a four phase feeding programme. Proc. Arkansas Nutrition Conference. University of Arkansas, USA.

13. Shastak Y, Rodehutscord M (2015) Recent developments in determination of available phosphorus in poultry. J Appl Poult Res 20: 283-92.

14. Fernandes JIM, Lima FR, Mendonca jr CX, Mabe I, Albuquerque R, et al. (1999) Relative bioavailability of phosphorus in feed and agricultural phosphates for poultry. Poult Sci 78: 1729-36.

15. Petersen GI, Pedersen C, Lindamann MD, Stein HH (2011) Relative bio-availability of phosphorus in inorganic phosphorus sources fed to growing pigs. J Anim Sci 89: 460-6.

16. Waldroup PW (1999) Nutritional approaches to reducing phosphorus excretion by poultry. Poult Sci 78: 683-91.

17. AOAC (1998) Association of Official Analytical Chemists. Official methods of analysis. (9th Edn) Arlington, Virginia, USA.

18. Coon CN, Seo S, Manangi MK (2007) The determination of retainable phosphorus, relative biological availability and relative biological value of phosphorus sources for broilers. Poult Sci 86: 857-68.

19. Lima, FR, Mendonça, CX, Alvarez JC, Ratti G, Lenharo SLR, et al. (1995) Chemical and physical evaluations of commercial dicalcium phosphates as sources of biologically available phosphorus for broiler chicks. Poult Sci 74: 1659-70.

20. Driver JP, Pesti GM, Bakalli RI and Edwards Jr HM (2006) The Effect of feeding calcium and phosphorus deficient diets to broiler chickens during the starting and growing-finishing phases on carcass quality. Poult Sci 85: 1939-46.

21. Bikker P, Sperk JW, Van Emous RA, Van Krimpen MM (2016) Precaecal phosphorus digestibility of inorganic phosphate sources in male broilers. British Poult Sci 57: 810-7.

22. Aydin I, Sefik I, Aydin F, Saydut A, Hamamci C (2009) Determination of mineral phosphate species in sedimentary phosphate rock in Mardin, SE Anatolia, Turkey by sequential extraction. Micro Chem J 91: 63-9.

23. Simukanga S, Nkonde GK, Shitumbanuma V (1994) Status of phosphate rock in Zambia - resources and use. In: Mathers S.J and A.J.G Notholt (eds.) Industrial minerals in developing countries. AGID Report Series Geosciences in International Development 18: 257-64.

24. Chileshe F, Nkonde GK, Simukanga S (2000) Zambian phosphate resources: Local benefits. Phosphorus and Potassium 226: 9-18.

25. Chirwa B, Chileshe F, Nkonde GK (2014) Upgrading phosphate content in acidulated Sinda phosphate rock. Copper Belt University, School of Mines and Mineral Sciences.

26. Appleton JD (2002) Local phosphate resources for sustainable development in sub-Sahara Africa. British Geological survey report, CR/02/121/N 112-3.

27. Zapata F (2004) Use of phosphate rocks for sustainable agriculture. FAO Fertilizer and Plant Nutrition Bulletin 13: 92-5.

28. Huyghebaert G, De Groote G, Keppers L (1980) The relative biological availability of phosphorus in feed phosphates for broilers. Ann Zootech (Paris) 29: 245-63.

29. Luiz Waldemar OS, Fernanda Marcussi T, Nedilse Helena S, Paulo Martins LA, Nelson HA (2009) Phosphorus availability of rock phosphates as compared with feed-grade phosphates for swine: J Anim Sci Brazil.

30. Abouzeid AZM (2008) Physical and thermal treatment of phosphate ores – an overview. Int J Miner Proc 85: 59-84.

31. NRC (1980) Mineral tolerance of domestic animals. National Research Council. National Academy Press. Washington DC, USA.

32. AAFCO (1999) Association of American Feed Control Officials, Washington, DC, USA.

33. Rama Rao SV, Reddy VR (2003) Relative bio-availability and utilization of phosphatic fertilizers as sources of phosphorus in broilers and layers. Poult Sci 44: 272-5.

34. Mello HHC, Gomes PC, Rostagno HS, Albino LFT, Da Rocha TC, et al. (2012) Dietary requirements of available phosphorus in growing broiler chickens at a constant calcium: available phosphorus ratio. Re Bra Zootec 41: 2323-8.

35. Ziaei N, Kermanshahi H, Mohammad P (2011) Effects of dietary crude protein and calcium/phosphorus content on growth, nitrogen and mineral retention in broiler chickens. African J Biotech 10: 13342-50.

36. Cardoso Jr A, Rodrigues PB, Bertechini AG, Freitas RTF, Lima RR, et al. (2010) Levels of available phosphorus and calcium for broilers from 8 to 35 days of age fed rations containing phytase. R Bras Zootec 39: 1237-45.

37. Askari M, Khatibjoo A, Taherpoor K, Fattahnia F, Souri H (2015) Effects of calcium, phosphorus and zinc in wheat based diets on broiler chickens performance, immunity and bone parameters. Iranian J Appl Anim Sci 5: 723-30.

38. Godoy S, Chicco CF (2011) Relative bio-availability of phosphorus from Venezuelan raw rock phosphates in pigs. Anim Feed Sci Technol 95: 109-20.

39. Odongo NE, Plaizier J, Van Straaten P, McBride B (2002) The effect of replacing dicalcium phosphate with basumbu rock phosphate on performance and the mechanical properties of bone in growing chicks. Trop Anim Health Prod 34: 349-58.

40. Tahir M, Lughmani AB, Pestiet GM (2011) Evaluation of an indigenous source of rock phosphate as a supplement for broiler chickens. J Poult Sci 90: 1983-91.

41. Leske K, Coon C (2002) The development of feedstuff retainable phosphorus values for broilers. Poult Sci 81: 1681-93.

42. Yan F, Waldroup PW (2006) Nonphytate phosphorus requirements and phosphorus excretion in broiler chicks fed diets composed of normal and high available phosphate corn as influenced by phytase supplementation and vitamin D source. Int J Poult Sci 5: 219-28.

43. De Groote G, Huyghebeart G (1997) The bio-availability of phosphorus from feed phosphates for broilers as influenced by bio-assay method, dietary calcium level and feed form. Anim Feed Sci Technol 69: 329-40.

44. Garnsworthy PC, Wiseman J, Haresign W (1996) Phosphorus nutrition in broilers In: Recent Advances in Animal Nutrition, Nottingham University Press, Nottingham, United Kingdom.

45. Liu BL, Rafiq A, Tzeng YM, Rob A (1998) The induction and characterization of phytase and beyond. Enzyme Microb Tech 22: 415-24.

46. Rama Rao SV, Reddy VR (2001) Relative bio-availability of different phosphorus supplements in broiler and layer chicken diets. J Anim Sci 14: 979-85.

47. Summers JD, Slinger SJ, Pepper WF, Motzok I, Ashton CC (1959) Availability of phosphorus in soft phosphate and phosphoric acid and the effect of acidulation of soft phosphate. Poult Sci 38: 1168-79.