ENERGY DIGESTIBILITY IN BROILER CHICKENS FED COTTONSEED MEAL BASED-DIETS SUPPLEMENTED WITH EXOGENOUS PHYTASE

$*Ilaboya, I. I., $Imouokhome, J. I. and &Iyayi, E. A.
$Department of Animal Science, Benson Idahosa University, Benin City, Edo State.
&Department of Animal Science, University of Ibadan, Ibadan
**Corresponding Author's Email: iilaboya@biu.edu.ng*

ABSTRACT

A total of 2881-day-old Arbor acres broiler chicks (BC) fed broiler starter for 20 days were used to investigate the effect of phytase on the digestibility of energy. On day 21, the chicks were weighed and allotted to 6 treatments with 6 replicates of 8 birds each using a 3x2 factorial arrangement in a randomized complete block design. Between days 25 and 27 post-hatch, samples of fresh excreta were collected once daily bulked and stored at -4°C. On day 28, the birds were euthanized with carbon (IV) oxide asphyxiation and dissected to obtain digesta from the distal two-third of the ileum. Six semi-purified diets with 150, 300 and 450g/kg rice husk (RH) and 0 or 1000 units of phytase were formulated. Ileal and excreta energy of the birds was significantly (P<0.05) reduced by feeding graded levels of cottonseed meal (CSM), while interaction of phytase and energy had no significant (P>0.05) effect on the ileal energy (IE) but significantly reduced (P< 0.05) excreta energy (EE). Apparent energy digestibility (AED) increased (P<0.05) by feeding graded levels of CSM while retained energy digestibility (RED) also increased (P<0.05) with the interaction of phytase and CSM. Increasing dietary concentration of energy intake from CSM, addition of phytase and the interaction, influenced digested and retained energy significantly (P<0.05). Therefore, digestibility of energy by broiler chickens fed CSM-based diets supplemented with phytase improved digested and retained energy in broiler chickens.

Keywords: Arbor acres, digesta, ilea, excreta, energy digestibility, phytase

INTRODUCTION

Nutritionally efficient and economically viable diets for broilers are formulated on the basis of digestibility results, since the energy density of a diet directly interferes with animal performance (Brumano *et al*., 2006). Soybean meal is the most commonly used protein ingredient in poultry feeding; however, its price fluctuates largely due to climatic variables affecting its productivity and because it is also widely consumed by humans. For this reason, researchers are constantly investigating feedstuffs considered alternatives to poultry. One of such is cottonseed meal (CSM), a by-product of the cotton industry obtained after the oil-extraction step which contains 41% crude protein (CP) and considerable amount of energy (NRC, 1994). However, the use of CSM in poultry diets is limited because of the presence of gossypol, a toxic polyphenolic compound found in the cottonseed (Dalle Zotte *et al.,* 2013) that reduces lysine utilization by broilers (Nagalakshmi *et al.,* 2007). One of the ways to maximize the utilization of these alternative ingredients by broilers is the addition of exogenous enzymes to their diets, which improve the utilization of nutrients, allowing reducing levels of metabolizable energy of the diets without negatively affecting animal performance (Alcilene, 2019). An accurate determination of alternative ingredients' nutrients and energy value will help to formulate a proper diet and satisfy animal requirements (Bolarinwa and Adeola, 2012). Furthermore, they allow for lesser environmental pollution due to the decreased excretion of nitrogen and phosphorus (P), since 77% of the total P in CSM is in phytate-P form (Selle and Ravindran, 2007).

Due to the recent challenges in the poultry industry of feed costs and the potential benefits of using alternative ingredients in broiler chicken diets, more data on the ileal digestible energy (IDE), retained energy (RE) and apparent digestible energy (ADE) are needed to optimize diet formulation. Therefore, the objective of the current study was to determine the IDE, RE, and ADE of CSM for broiler chickens using the regression method.

MATERIALS AND METHODS

A total of 288, one-day-old Abor acres broiler chicks were fed a starter diet till day 20. Vaccination and other routine management practices were carried out. On day 21, the chicks were individually weighed and distributed into 36 cages in a randomized complete block design with 6 replicate cages per treatment and 8 birds per cage using Experimental Animal Allotment Programme (EAAP). Six semi-purified diets of 3 levels (150, 300 and 450g/kg) of CSM, without and with phytase (1000FTU/kg) respectively, obtained by the gradual replacement of cassava starch were formulated (Table 1). Titanium dioxide was added as an indigestible marker at the rate of 5g/kg of diet. On days 23, 24 and 25, fresh excreta samples were collected from trays placed beneath each cage at 24-hourly intervals and dried at 55°C using a force draught oven. The experiment lasted 28 days. On day 26, the birds were weighed, slaughtered and digesta from the last 2/3rd of the ileum (Rodehutscord *et al.*, 2012) was collected, and pooled according to cage, frozen and dried.

Table 1. Gross composition of experimental diets (g/kg) (as-fed basis)

	0 FTU/Kg (Phytase)			1000FTU/Kg (Phytase)		
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Cottonseed Meal	150.00	300.00	450.00	150.00	300.00	450.00
Cassava Starch	495.50	343.75	192.00	485.50	333.75	182.00
Wheat gluten	200.00	200.00	200.00	200.00	200.00	200.00
Soya oil	10.00	10.00	10.00	10.00	10.00	10.00
Dextrose	102.25	102.25	102.25	102.25	102.25	102.25
Methionine	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	10.25	12.00	13.75	10.25	12.00	13.75
Vitamin-Premix	2.50	2.50	2.50	2.50	2.50	2.50
Common salt	2.50	2.50	2.50	2.50	2.50	2.50
Phytase Enzyme	0.00	0.00	0.00	10.00	10.00	10.00
Titanium dioxide Premix	25.00	25.00	25.00	25.00	25.00	25.00
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**
Analysed Nutrients						
GE Kcal/kg	4080	4051	4170	4243	4218	3951
ME Kcal/Kg	3836.06	3666.45	3496.84	3801.16	3631.55	3461.94
CP (g/kg)	236.50	298.60	360.70	236.50	298.60	360.70
Ca (g/kg)	5.34	6.23	7.12	5.34	6.23	7.12
Total P (g/Kg)	3.46	4.91	6.37	3.46	4.91	6.37
Ca : Total P ratio	1.55	1.27	1.12	1.55	1.27	1.12

[1]Composition of vitamin premix per kg of diet: vitamin A, 12500 I.U; vitamin E, 40mg; vitamin K_3, 2mg; vitamin B_1, 3mg; vitamin B_2, 5.5mg; niacin, 5.5mg; calcium pantothenate, 11.5mg; vitamin B_6, 5mg; vitamin B_{12}, 0.025mg;choline chloride, 500mg, folic acid,1mg; biotin, 0.08mg; manganese, 120mg;iron 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg;selenium, 0.12mg, anti-oxidant, 120mg, [2]Phytase premix prepared by mixing phytase with maize. [3]Titanium dioxide premix prepared by mixing 1g of titanium dioxide with 4g of maize

Chemical and Statistical Analyses

Samples of CSM, experimental diets, digesta and excreta were analysed for energy according to the methods of AOAC (2000). Titanium concentration was determined using a colorimetric assay (Short *et al*., 1996). Data were analysed using the GLM procedure of SAS (SAS Institute, 2012). Orthogonal polynomial contrast was used to determine linear and quadratic effects of energy level, phytase and their interaction on all response criteria with α level of 0.05 considered as significant.

Total E_{output} (E_O) = E_i × $Ti_{diet}/Ti_{excreta\ or\ digesta}$, AED, % = 100 - [($Ti_{diet}$ /$Ti_{digesta\ or\ excreta}$) x ($E_{digesta\ or}$

$_{\text{excreta}}/E_{\text{diet}}$) x 100]. Where, E_i = Energy intake, Ti_{diet} =Titanium in diet, $Ti_{\text{excreta or digesta}}$ = Titanium in excreta or digesta, AED = Apparent energy digestibility, $E_{\text{digesta or excreta}}/E_{\text{diet}}$ = Energy in excreta or digesta.

RESULTS AND DISCUSSION

Table 2 shows the results for energy digestibilities. Ileal energy of the birds were ($P<0.05$) significantly affected due to increasing E intake from graded CSM levels. It was observed that phytase and energy×phytase had no effect ($P<0.05$) on ileal energy. Ileal output of birds fed 150g CSM/kg differed ($P<0.05$) significantly when compared to ileal output values obtained for birds fed 300 and 450g CSM/kg fed diets that were not supplemented with phytase. No observable difference was noticed in the supplemented phytase diets. Values for excreta energy outputs of birds were lower than their corresponding precaecal values. This observation could be attributed to the influence of hindgut microbes on the digesta at caeca section of gastrointestinal tract. At the total tract section, effects of energy and energy×phytase interaction had significant ($P<0.05$) influence on excreta energy voided. The results observed in the digested energy and apparent energy digestibility of the birds is as a result of feeding increasing dietary E concentration from CSM. Digested energy of birds linearly ($P<0.05$) increased (150 and 300g/kg of diet) in response to increase in dietary E intake from graded level of CSM. Apparent ileal energy digestibility increased significantly ($P<0.05$) with the inclusion of CSM while apparent energy retention was observed to be affected by both increase in CSM and its interaction with phytase (E*Phy).

Table 2. Energy intake, E outputs and calculated response criteria of 28-day-old broilers fed cottonseed meal-based diets

	Without phytase g/kg of diet			With phytase g/kg of diet				P-value						
											Without Phytase		With Phytase	
Items	CSM 150	CSM 300	CSM 450	CSM 150	CSM 300	CSM 450	PSEM	Phy	E	PhyxE	L^2	Q^2	L^2	Q^2
IE(DMI)	2.23^{a}	$0. 61^{b}$	$0. 53^{b}$	0.28	0.54	0.38	0.04	0.6	0.003	0.44	0.34	0.02	0. 58	0.05
DE(DMI)	3.44^{b}	3.45^{a}	$3. 67^{ab}$	3.97^{b}	3.68^{ab}	3.40^{a}	0.04	0.29	<0.01	0.36	0.05	0.04	0.08	0.34
AED(%)	94.26^{b}	85.07^{a}	87.94^{ab}	93.5	87.16	90.97	0.99	0.56	0.002	0. 59	0.27	0.02	0.38	0.07
EE(DMI)	0.21^{a}	0.36^{a}	0.60^{b}	0.19^{a}	0.44^{b}	0.39^{b}	0.03	0.24	<0.01	0.01	0.02	<0.01	0.01	0.02
RE(DMI)	3.87^{b}	3.70^{a}	$3. 57^{a}$	4.06^{c}	3.77^{b}	3.56^{a}	0.03	0.04	<0.01	0.15	<0.08	0.01	<0.01	0. 57
AER(%)	94.19^{b}	91.22^{b}	85.70^{a}	95.58^{b}	89.49^{a}	90.18^{a}	0.72	0.25	<0.01	0.04	0.03	<0.01	0.08	0.04

$^{a\ b\ c}$ Means in a row with different superscripts are significantly different from each other ($P<0.05$)

CSM=Cottonseed meal, L^2 = Linear effect: Q^2 = Quadratic effect ($P=0.05$). PSEM=Pooled Standard Error of Mean, E=Energy, IE=Ileal Energy, DE=Digested Energy, AED=Apparent Energy digestibility, EE=Excreta Energy, RE=Retained Energy, AER=Apparent Energy Retention, DMI = dry matter intake.

No significant differences were observed in the ileal energy (IE), digested energy (DE), apparent energy digestibility (AED), excreta energy (EE) and apparent energy retention (AER) of broiler chickens fed phytase supplemented diets. But retained energy (RE) significantly improved with the addition of phytase. In this regard, it can be observed that the effect of enzyme, especially phytase, on the energy digestibility and utilization has been inconsistent, with highly conflicting information (Kong and Adeola, 2011). However, there are positive reports about the use of phytases on the digestibility of protein, amino acids, and phosphorus in broiler diets, such as those found by Zouaoui *et al.* (2018). According to Barbosa *et al.* (2014), enzyme supplementation in diets with adequate nutritional levels does not lead to increased energy digestibility. Nevertheless, when this supplementation is performed along with a reduction of dietary nutritional levels, digestibility improves. The supplementation strategy used in this study (without reductions in the nutritional levels of the diet) with the inclusion of phytase was possibly responsible for the lack of effects on the energy digestibility of the evaluated nutrients. Sequential increases of CSM in the diet were observed to significantly affect all the energy response criteria. As a result, there were linear responses in EE and RE with the increasing level of CSM and addition of phytase. There were also quadratic responses with IE and AER. One of the major reasons may be due to the high fiber content of CSM (Nagalakshmi *et al.,* 2007). When the inclusion of CSM increased, the increased fiber diets may result in a lower protein and energy digestibility (Hetland *et al*., 2004). Another reason may be due to the anti-nutrients from CSM, such as gossypol. As described above, those anti-nutrients may also negatively affect energy digestibility. The CSM used in current study is decorticated, which has a higher ME than undecorticated CSM (Sharma *et al.,* 1978). The decorticated CSM has ME ranging from 1,901 to 2,811 kcal/kg, mainly depending on the residual oil content (Nagalakshmi *et al*., 2007), and our results fell above this range. The ME in current study is more than 3000 kcal/kg higher than IED. One possible explanation is because of the comparatively high fiber contents in CSM, which resulted in the production of short-chain fatty acid in ceca, contributed partly to this energy increment (Annison *et al*., 1968). In addition, different studies have reported that phytase and xylanase supplementation improved AME (Wu *et al*., 2004). However, a feeding trial conducted by Munyaka *et al.* (2016) revealed that there was no influence of a xylanase and β-glucanase blend on the AME in meat chickens fed with wheat-and corn-based feeds. These corroborate this present study as the effect of phytase did not significantly influence energy digestibility.

CONCLUSION

Phytase supplementation does not affect the energy digestibility of cottonseed meal. However, phytase rather decreases excreta energy and increases retained and apparent energy retention. These being able to provide less excretion of these nutrients in the environment.

REFERENCES

Alcilene, M. A., Tavares, S., Wilson, M. D. J., Liliane, O.P, Cláudia, C. L , Carlos, B.R , Andrew , H. S. C. C. (2019). Determination of nutrient and energy values of cottonseed meal supplemented or not with phytase and protease for broiler chicks. *R. Bras. Zootec. 48: 1-9.*

Annison, E. F., Hill, K. J. and Kennworthy, R. (1968). Volatile fatty acids in the digestive tract of the fowl. *Br. J. Nutr. 22:207–216.*

AOAC (2000). Association of Official Analysis Chemists. Official methods of analysis. 17th ed. AOAC International, Arlington, VA, USA.

Barbosa, N. A. A., Bonato, M. A., Sakomura, N. K., Dourado, L. R. B., Fernandes, J. B. K. and Kawauchi, I. M. (2014). Digestibilidade ileal de frangos de corte alimentados com dietas suplementadas com enzimas exógenas. *Comunicata Scientiae 5:361-369.*

Bolarinwa, O. and Adeola, O. (2012). Energy value of wheat, barley, and wheat dried distillers grains with solubles for broiler chickens determined using the regression method. *Poult. Sci. 91:1928– 193.5*

Brumano, G., Gomes, P. C., Albino, L. F. T., Rostagno, H. S., Generoso, R. A. R. and Schmidt, M. (2006). Composição química e valores de energia metabolizável de alimentos protéicos determinados com frangos de corte em diferentes idades. *Revista Brasileira de Zootecnia 35:2297-2302.*

Dalle Zotte, A., Brand, T. S., Hoffman, L. C., Schoon, K., Cullere, M. and Swart, R. (2013). Effect of cottonseed oilcake inclusion on ostrich growth performance and meat chemical composition. *Meat Science 93:194-200.*

Hetland, H., Choct, M. and Svihus, B. (2004). Role of insoluble nonstarch polysaccharides in poultry nutrition. *World's Poult. Sci. J. 60:415–422.*

Kong, C. and Adeola, O. (2011). Protein utilization and amino acid digestibility of canola meal in response to phytase in broiler chickens. *Poult. Sci. 90:1508-1515.*

Munyaka, P.M., Nandha, N.K., Kiarie, E., Nyachoti, C.M., Khafipour, E. (2016). Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. *Poult Sci. 95:528-40.*

Nagalakshmi, D., Rao, S. V. R. Panda, A. K. and Sastry, V. R. B. (2007). Cottonseed meal in diets poultry: a review. *J. of Poult. Sci. 44:119-134.*

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

SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA

Selle, P. H. and Ravindran, V. (2007). Microbial phytase in poultry nutrition. *Anim. Feed Sci. and Technology 135:1-41.*

Sharma, N., Lodhi, G. and Ichhponani. J. (1978). Comparative feeding value of expeller-processed undecorticated and decorticated cottonseed cakes for growing chicks. *J. Agric. Sci. 91:531–541.*

Short, F.J., Gorton, P., Wiseman, J., Boorman, K. N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim Feed Sci Technol. 59:215-21.*

Wu, Y.B., Ravindran, V., Pierce, J. and Hendriks, W.H. (2004). Influence of three phytases preparations in broiler based diets on wheat or corn; *in vitro* measurements of nutrient release. *Intl. of Poult. Sci.7: 450-455.*

Zouaoui, M., Létourneau-Montminy, M. P. and Guay, F. (2018). Effect of phytase on amino acid digestibility in pig: A metaanalysis. *Anim. Feed Sci. and Tech 238:18-28.*