Journal of Animal and Veterinary Advances 12 (17): 1409-1414, 2013
ISSN: 1680-5593

Effect of Xylanase on Performance, Serum IGF-1 and Glucose of Broilers Fed Wheat Corn Soybean Diet

[1,2]A.Y. Abdullahi, [1]J.J. Zuo, [3]H.Z. Tan, [1]W.G. Xia, [3]S. Wei, [1]J.H. Liu, [1]Q. Ai, [1]S.Y. Zhao, [1]M.H. Xia, [1]Y.H. Liu, [1]C.D. Xia, [1]F. Wu, [1]J.P. Yu and [1]D.Y. Feng
[1]College of Animal Science, South China Agricultural University, 510542 Guangzhou, China
[2]Department of Animal Science, Kano University of Science and Technology Wudil, Kano, Nigeria
[3]Production Technology Department of WENs Group, Yunfu, China

Abstract: Six hundred and forty, 1 day old Yellow feather broiler chicks were fed wheat-corn soybean diet plus xylanase ENOLZYME™ 100 g ton^{-1} (enzyme activity = 1256.244 U mL^{-1}) to study additive effect of the enzyme on serum glucose and IGF-1 concentration relative to performance. The birds were randomly divided into 2 treatment groups with 64 birds/pen in 5 replicates. For the periods of 63 days body weight, feed efficiency and daily feed intake were not differed at 21 and 42 days. However, they were all differ significantly at 63 days ($p<0.05$); particularly feed efficiency from 1-63 days was highly significant ($p<0.01$). Carcass characteristics were not differed in all three stages but at finishing stage breast and thigh muscles, heart, gizzard and abdominal fat weights were numerically increased. Mortality rate was <0.02%. Serum glucose concentration levels at three feeding phases were not differed among groups. However, serum IGF-1 differ significantly at starter phase (21 days) ($p<0.05$). Correlation between treatments and feeding periods existed at growing (42 days) and finishing (63 days) stages ($p<0.05$). The present findings on supplementation of xylanase to corn wheat soy bean broiler diet hasincreased market weight cost value by 1.33% at finishing stage (43-63 days). The serum glucose and IGF-1 concentration have correlated positively on treatments offered, feeding periods and performance. Further research is needed on IGF-1 and GH (Growth Hormone) at molecular level to understand their influence in broiler's growth and development.

Key words: Xylanase, serum IGF-1, glucose, growth hormone, performance

INTRODUCTION

A high global inflation of feedstuff especially corn and wheat in one hand and soybean on the other, is the most challenge of commercial poultry production. Feeding enzymes to poultry is one of the major nutritional advances in the last 50 years (Khattak *et al*., 2006). Commercially, xylanase based products have gained wide acceptance, based on cost-efficacy and response predictability (Graham and Bedford, 2007).

Enzymes are biological catalysts that execute essential functions in living organism. They are naturally present in living organisms and can be mass produced via the aerobic and anaerobic cultures of bacteria and fungi (Anjum and Chaudhry, 2010). Their presence even in small quantities can accelerate the rate of reaction that transforms dietary substrates into products of biological significance for broiler growth and production (Taylor-Pickard and Spring, 2008). Xylanase is an enzyme that catalyzes the hydrolysis of 1, 4-beta-D-xylosidic linkages in xylans that are constituents of hemicellulose, a structural component of plant cell walls. Arabinoxylans (also known as pentosans) are highly branched xylans that occur in wheat and rye flour.

Several growth factors have been identified as candidate to modulate muscle growth at each stage of development. Insulin-like growth factor-1 is a key regulator of muscle development and metabolism in birds and other vertebrate species. Insulin-like Growth Factors (IGF-1 and IGF-2) exert a general effect on overall body growth (Jones and Clemmons, 1995) and both genes are expressed in the muscle tissue together with specific receptors, suggesting a paracrine mode of action. IGF-1 and GH are polypeptide hormones, small proteins that are vital for normal bone and tissue growth and development. GH is secreted into the bloodstream in pulses throughout the day and night with peaks that occur mostly during the night. IGF-1 is produced by the liver and skeletal muscle as well as many other tissues in response to GH stimulation. The IGFs have been shown to stimulate the proliferation, the differentiation and the metabolism of a number of myogenic cell lines from different species as

Corresponding Author: D.Y. Feng, College of Animal Science, South China Agricultural University, 510542 Guangzhou, China

well as the anabolism of differentiated myotubes or muscle fibers (Florini *et al*., 1996). In his research, Duclas, 2005 suggested that IGF-1 has the potential to be a key regulator of chicken growth and body composition both endocrine and paracrine IGF-1 are likely to contribute. IGF-1 mediates many of the actions of GH, stimulating the growth of bones and other tissues and promoting the production of lean muscle mass. IGF-1 mirrors GH excesses and deficiencies but its level is stable throughout the day, making it a useful indicator of average GH levels.

Blood glucose level is the amount of glucose (sugar) present in the blood of a human or animal. The body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis. Glucose is the primary source of energy for the body's cells and blood lipids (in the form of fats and oils) which are primarily a compact energy store. Glucose is transported from the intestines or liver to body cells via the bloodstream and is made available for cell absorption via the hormone insulin, produced by the body primarily in the pancreas. Plasma glucose level and glucose turnover rate were studied in chicks (*Gallus domesticus*) fed isocaloric diets containing glucose, fructose or soybean oil as the main energy supplement. Plasma glucose level and body weight gain were not affected by glucose intake (Riesenfeld *et al*., 1982). Thus, demonstrated the non-essentiality of any dietary glucose for normal growth and feed efficiency of chickens (Renner, 1964; Brambila and Hill, 1966). The gradual elimination of glucose from the diet through substitution with either fructose or neutral fat also did not result in any changes in plasma glucose concentration, apparently due to most efficient homeostatic mechanisms in the bird (Sarkar, 1971; Davison and Langslow, 1975; Belo *et al*., 1976) thus also demonstrated in the rat (Goldberg, 1971). However, it should be noted that Riesenfeld *et al*. (1982) result describes birds that maintain a steady state in flow of nutrients due to the provision of constant artificial light in the environment. Possibly, dietary glucose could affect plasma glucose concentration when fed dis-continually. Literatures have recorded novel aspect of IGF-1 and other growth factors, this research had analyzed serum IGF-1 and glucose from xylanase added to wheat corn soybean diet fed to Yellow feathered broilers (popular Chinese breed) to evaluate their effects and performance.

MATERIALS AND METHODS

Experimental design: Six hundred and forty, 1 day old Yellow feather broiler chicks were obtained from a WENS hatchery. The birds were randomly divided into 2 treatment groups with 64 birds/pen in 5 replicates. Diet formulation was made according to China Standard of Yellow feathered broiler requirements.

Table 1: Feed composition and calculated nutrients

Compositions	Starter (1~21 days)	Grower (22~42 days)	Finisher (43~63 days)
Ingredients			
Corn	43.75	36.23	44.35
Wheat	15.00	30.00	30.00
Soybean meal	31.30	23.10	14.80
Puffing whole soybean	4.00	4.00	4.00
Soybean oil	1.47	2.40	2.84
Threonine	0.00	0.04	0.05
Lysine (70%)	0.15	0.29	0.36
DL-Methionine (98%)	0.23	0.22	0.17
Limestone	1.18	1.08	1.03
$CaHPO_4$	1.83	1.56	1.31
NaCl	0.19	0.17	0.18
Choline chloride (50%)	0.10	0.10	0.10
Sodium bicarbonate	0.20	0.20	0.20
Premix[1] (0.6%)	0.60	0.60	0.60
Total	100.00	99.99	99.99
Analysed nutrients			
DM	85.86	84.94	84.28
DM	85.86	84.94	84.28
CP	21.00	19.01	16.01
Ca	1.00	0.90	0.80
ME	2900.00	3000.00	3099.00
Lys	1.16	1.04	0.88
DLys-P	1.05	0.95	0.80

[1]The premix of 1~63 days provides the following per kg of diet: Mn: 80 mg, Zn: 60 mg, Fe: 80 mg, Cu: 8 mg, Se: 0.15 mg, I: 0.35 mg, VA: 5000 IU, VD_3: 1000 IU, VE: 10 IU, VK: 0.5 mg, VB_1: 1.8 mg, VB_2: 3.6 mg, VB_6: 3.6 mg, VB_5: 10 mg, VB_{12}: 0.01 mg, Biotin: 0.15 mg, Folic acid: 0.55 mg, Niacin: 35 mg at 1-21 days, 30 mg at 22-42 and 25 mg at 43-63

Experimental materials (feed): Wheat corn soy bean as basal broiler diet. Enzyme: (Xylanase: ENOLZYME™ 100 g ton^{-1}) EN Bio-Tech Limited Company Beijing-China (researchers analysed Enzyme activity = 1256.244 U m L^{-1}). Composition and nutrients of the diet were shown in Table 1. Parameters observed from 1-63 days (21, 42 and 63 days) include: growth performance, body weight gain, feed intake, feed conversion ratio and mortality rate. Carcass characteristics: weights of breast and thigh muscles, abdominal fat, heart, liver, spleen and gizzard were recorded.

Serum collection: At the beginning of the study, eighty 1 day old broiler chicks were killed and the blood samples were collected. Serum was also obtained from 5 m L blood samples of each two chicks randomly selected from each pen (10 chicken per replicate) at 21, 42 and 63 days, respectively. All the serums were harvested subsequently by centrifuging the whole blood samples at 3000 rmp for 15 min and kept in Eppendorf tube above -20°C until analysis. ELISA kits for IGF-1 (R&D System Inc., UK&USA) and Glucose kit (Shanghai Rongsheng Biological&Pharmaceutical Co., Ltd.) were used according to manufacturer's instructions to determine serum glucose and IGF-1 concentrations.

Statistical analysis: Random sampling technique was employed where 10 birds/treatment group. Data were analyzed using Microsoft Excel 2010 and application of computer packages SSPS 19.0; t-test was used for means and variance and Pearson Correlation analysis both at 5% level significant differences.

RESULTS AND DISCUSSION

Over the entire trail of 63 days, body weight, feed efficiency and daily feed intake were not significant at 21 and 42 days. However, they were all significant at 63 days ($p<0.05$); above all feed efficiency from 1-63 days was highly significant ($p<0.01$) (Table 2). Carcass characteristics records shown no significant differences in all three stages but at finishing stage breast and thigh muscles, heart, gizzard and abdominal fat weights were numerically increased (Table 3). Mortality rate was <0.02%. Growth performance and carcass characteristics results were summarized in Table 2 and 3. There were no significant differences among groups in serum glucose concentration levels at three phase feeding periods ($p>0.05$) (Fig. 1). However, serum IGF-1 differ significantly at starter phase (21 days) ($p<0.05$) (Fig. 2). Correlation between treatments and feeding periods existed at growing (42 days) and finishing (63 days) stages ($p<0.05$) but did not exist at starter period as shown in Table 4. On the other hand treatments, performance and feeding periods at all three phases were correlated (Table 5).

Among alternatives to reduce poultry feed costs is replacing corn with wheat despite its negative features due to presence of Non-Starch Polysaccharides (NSP: glucan and xylan), lower metabolizable energy and variable nutrients than corn. Wheat is a promising source of energy in broiler diets because of its high starch and CP content and is often the only cereal in grower and finisher diets (Del-Alamo *et al*., 2008). Supplementation of enzyme fed a wheat diet to broiler chickens have reported improve growth performance and change nutritional status such as increased utilization of nutrients (e.g., fat and protein), improved AME values, increased growth rate, improved feed to gain ratio, decreased viscosity of intestinal digesta, reduced incidence of sticky excreta, improved litter conditions and reduced environmental pollution due to a decreased output of manure and gases such as ammonia (Broz and Ward, 2007; Costa *et al*., 2008). Commercially, xylanase based products have gained wide acceptance, based on cost-efficacy and response predictability (Graham and Bedford, 2007).

Table 2: The effect of xylanase on growth performance at 3 phase periods in Yellow feathered broilers

Parameters	Without xylanase	Xylanase	SEM	Significant
Performance				
1-21 days				
Weight gain (g/bird)	391.52	389.23	3.59	NS
Feed intake (g/d/bird)	27.97	28.06	0.25	NS
Feed gain ratio (g g^{-1})	1.65	1.66	0.17	NS
22-42 days				
Weight gain (g/bird)	1031.30	1030.30	1.11	NS
Feed intake (g/bird)	75.95	74.44	1.37	NS
Feed gain ratio (g g^{-1})	2.50	2.47	0.26	NS
43-63 days				
Weight gain (g/bird)	1853.70	1948.80	1.76	*
Feed intake (g/bird)	117.56	123.14	0.84	*
Feed gain ratio (g g^{-1})	3.01	2.82	0.30	*
1-63 days				
Weight gain (g/bird)	1853.70	1948.80	1.76	*
Feed intake (g/bird)	117.56	123.14	0.84	*
Feed gain ratio (g g^{-1})	3.01	2.82	0.30	**

NS: Not Significant, *p<0.05, **p<0.01

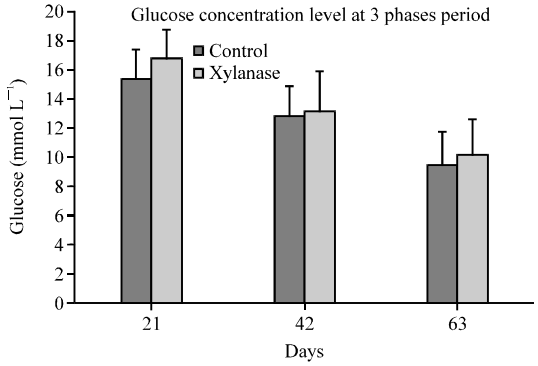

Fig. 1: Glucose concentration levels at stating, growing and finishing stages of Chinese Yellow feathered broiler fed wheat corn soybean diet plus xylanase

Table 3: The effect of carcass characteristics at 3 phase periods in yellow feathered broilers

Items	Without xylanase	Xylanase	SEM	Significant
Performance				
1-21 days				
Breast muscle (g)	17.43	15.36	0.33	NS
Thigh muscle (g)	24.50	23.63	0.67	NS
Heart (g)	2.61	2.55	0.11	NS
Liver (g)	12.24	12.30	0.61	NS
Spleen (g)	0.74	0.78	0.05	NS
Gizzard (g)	1031.3	1030.30	1.11	NS
22-42 days				
Breast muscle (g)	48.75	45.78	2.11	NS
Thigh muscle (g)	73.33	69.92	2.36	NS
Heart (g)	5.73	6.18	0.32	NS
Liver (g)	28.85	27.00	0.71	NS
Spleen (g)	2.09	2.36	0.17	NS
Gizzard (g)	18.97	18.34	1.83	NS
Proventriculus (g)	6.37	6.08	0.52	NS
Abdominal fat (g)	16.80	15.33	1.16	NS
43-63 days				
Breast muscle (g)	90.97	95.28	2.68	NS
Thigh muscle (g)	147.41	152.16	4.08	NS
Heart (g)	9.74	10.27	0.58	NS
Liver (g)	46.36	43.25	0.85	NS
Spleen (g)	4.24	4.06	0.28	NS
Gizzard (g)	24.02	27.16	2.44	NS
Proventriculus (g)	7.59	6.95	0.38	NS
Abdominal fat (g)	33.68	36.76	4.71	NS

NS: Not Significant, *p<0.05, **p<0.01

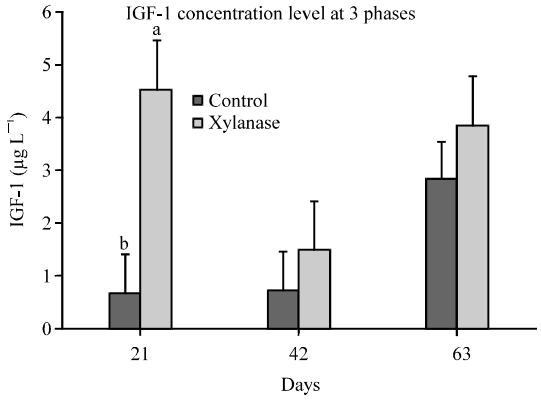

Fig. 2: IGF-1 concentration levels at stating, growing and finishing stages of Chinese Yellow feathered broiler fed wheat corn soybean diet plus xylanase

Table 4: Correlations between treatment and feeding phase periods

Correlation	Treatment	21 days	42 days	63 days
Pearson correlation				
Treatment	1.000	-0.790	-0.871	-0.826
21 days	-0.790	1.000	0.987	0.996
42 days	-0.871	0.987	1.000	0.996
63 days	-0.826	0.996	0.996	1.000
Sig. (1-tailed)				
Treatment	-	0.105	0.064	0.087
21 days	0.105	-	0.007	0.002
42 days	0.064	0.007	-	0.002
63 days	0.087	0.002	0.002	-
N				
Treatment	4	4	4	4
21 days	4	4	4	4
42 days	4	4	4	4
63 days	4	4	4	4

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

Table 5: Correlations between treatment performance and feeding phase periods

Correlation	Treatments	21 days	42 days	63 days
Pearson correlation				
Treatment	1.000	0.814	0.823	0.832
21 days	0.814	1.000	1.000	0.999
42 days	0.823	1.000	1.000	0.999
63 days	0.832	0.999	0.999	1.000
Sig. (1-tailed)				
Treatment	-	0.024	0.022	0.020
21 days	0.024	-	0.000	0.000
42 days	0.022	0.000	-	0.000
63 days	0.020	0.000	0.000	-
N				
Treatment	6	6	6	6
21 days	6	6	6	6
42 days	6	6	6	6
63 days	6	6	6	6

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

The present research results revealed that BW, FI and FE were all significant at finishing stage which is in agreement with the findings of Wang when added Versazyme significantly ($p<0.01$) improved 48 days BW 2.54% (from 2.36-2.42 kg, $p<0.01$), feed efficiency 1.62% (from 1.912-1.881, $p<0.01$). Similarly, Odetallah *et al.* (2005) supplemented his diets with Varsazyme improved BW and Feed Conversion Ratio (FCR) at 21 days of age and BW at 42 days of age. Zakaria *et al.* (2010) also recorded Body Weight (BW) and Body Weight Gain (BWG) were significantly higher ($p<0.05$) for birds fed corn diet at 42 days finishing phase.

A number of research studies with layers have reported positive responses to the addition of Avizyme in terms of egg weight (Gonzales *et al.*, 2001), FCR improvement; liveability and reduced diet cost Gonzales *et al.*, 2001). In broilers and ducks, improvements in FCR, body weight, uniformity and nutrient digestibility (Zanella *et al.*, 1999; Wyatt *et al.*, 1997) were reported.

However, in the results carcass characteristics were not differ which are in agreement with Mohamad and Hamza (1991), Vranjes and Wenk (1995), Saleh *et al.* (2004, 2005) and Zakaria *et al.* (2010) who reported no apparent effects on carcass yields when enzymes were added. Although, researchers observed that numerically breast and thigh muscles, heart, gizzard and abdominal fat increased at 63 days than control group while liver, spleen and proventriculus were reverse the case. Similar results were reported by Zakaria carcass characteristics showed no significant effects on whole carcass weight, dressing percent and weight of breast, thighs and wings. Buthe found enzyme supplemented diets significantly ($p<0.05$) increased liver percent in contrast to corn diet and he also reported no significant differences for heart, gizzard and abdominal fat pad. He added that enzyme supplementation had no significant effect on Feed Intake (FI) at 21 days and on Feed Conversion Ratio (FCR) thus is agreeable with the data for no difference on FI, FE in addition to BW at both 21 and 42 days.

The Insulin like Growth Factor-1 (IGF-1) test is an indirect measure of the average amount of Growth Hormone (GH) being produced by the body. IGF-1 and GH are polypeptide hormones, small proteins that are vital for normal bone and tissue growth and development. Gao *et al.* (2007) showed that xylanase supplementation of wheat based diets increased the concentration of blood insulin and IGF-1. Insulin and glucose levels are associated with IGF in broiler chickens, the role of IGF-1 being similar to that of mammals regulating the proliferation and differentiation of cells and participating in amino acid uptake and DNA synthesis (McMurtry *et al.*, 1997). The present study analyzed serum glucose and IGF-1, results indicated that there were no significant differences between treatment groups in serum glucose concentration levels at three phase feeding periods ($p>0.05$) (Fig. 1). It is in agreement with Lu *et al.*

(2007) who reported unchanged on blood glucose when phytase plus carbohydrase were added to control diet. This differ with result of Johnston *et al.* (2004) when supplemented pig diets with phytase results showed a greater ileal starch digestibility and increased plasma insulin and glucose concentrations. Hence, some researchers demonstrated the non-essentiality of any dietary glucose for normal growth and feed efficiency of chickens (Renner, 1964; Brambila and Hill, 1966).

On the other hand, the present study found serum IGF-1 differ significantly at starter phase (21 days) ($p<0.05$). The findings were similar to study of Tesseraud *et al.* (2003) and Beccavin *et al.* (2001). Tesseraud found in broiler chickens selected for increased breast yield and decreased fatness had a significantly higher circulating IGF-1 concentration than the control line. While Beccavin in chickens selected for high growth rate, significantly higher levels of liver IGF-1 and mRNA were observed when compared with a slower growing line. However, Jozefiak *et al.* (2010) reported that birds fed diets supplemented with phytase and a cocktail of carbohydrase enzymes had the greatest growth rate and significantly better FCR but expression of liver IGF-1 decreased ($p = 0.083$) by 32% when compared with the control treatment. Researchers speculate significant concentration of IGF-1 at starter stage in the study might be related to IGF-1 and GH relationship because IGF-1 mirrors GH excesses and deficiencies. Jones and Clemmons (1995) indicated that Insulin-like Growth Factors (IGF-1 and IGF-2) exert a general effect on overall body growth. Duclos (2005) suggested that IGF-1 has the potential to be a key regulator of chicken growth and body composition.

Besides, Jozefiak *et al.* (2010) stated that the relationship between IGF-1 and growth in modern broiler chicken strains remains unclear. Interestingly, in the findings there is positive relationship between treatments and feeding periods on serum glucose and IGF-1 concentrations at growing and finishing stages but none at starter phase. In addition, strong relationship was observed while compared treatment and feeding periods on serum glucose, IGF-1 concentrations and performance.

CONCLUSION

Researchers conclusively observed that in present findings supplementation of xylanase to corn-wheat soy bean broiler diet has increased market weight cost value by 1.33% at finishing stage (43-63 days). The serum IGF-1 was high at starter stage and serum glucose and IGF-1 concentration have correlated positively on treatments offered, feeding periods and performance. Researchers suggest a further research to investigate IGF-1 and GH at molecular level to find out their contributions in growth and development in broiler chickens.

ACKNOWLEDGEMENTS

This research was jointly supported by grants from the Guangdong Natural Science Foundation (S2013010012040), the State Key Laboratory of Animal Nutrition open subject of China, the Key Programs for Science and Technology Development of Guangdong (2010B020306002). A.Y. Abdullahi and J.J. Zuo contributed equally.

REFERENCES

Anjum, M.S. and A.S. Chaudhry, 2010. Using enzymes and organic acids in broiler diets. J. Poul. Sci., 47: 97-105.

Beccavin, C., B. Chevalier, L.A. Cogburn, J. Simon and M.J. Duclos, 2001. Insulin-like growth factors and body growth in chickens divergently selected for high or low growth rate. J. Endocrinol., 168: 297-306.

Belo, P.S., D.R. Romsos and G.A. Leveille, 1976. Blood metabolites and glucose metabolism in the fed and fasted chicken. J. Nutr., 106: 1135-1143.

Brambila, S. and F.W. Hill, 1966. Comparison of neutral fat and free fatty acids in high lipid-low carbohydrate diets for the growing chicken. J. Nutr., 88: 84-92.

Broz, J. and N.E. Ward, 2007. The role of vitamins and feed enzymes in combating metabolic challenges and disorders. J. Applied Poult. Res., 16: 150-159.

Costa, F.G.P., C.C. Goulart, D.F. Figueiredo, C.F.S. Oliveira and J.H.V. Silva, 2008. Economic and environmental impact of using exogenous enzymes on poultry feeding. Int. J. Poult. Sci., 7: 311-314.

Davison, T.F. and D.R. Langslow, 1975. Changes in plasma glucose and liver glycogen following the administration of gluconeogenic precursors to the starving fowl. Comp. Biochem. Physiol. Part A: Physiol., 52: 645-649.

Del-Alamo, A.G., M.W.A. Verstegen, L.A. Den-Hartog, P.P. De-Ayala and M.J. Villamide, 2008. Effect of wheat cultivar and enzyme addition to broiler chicken diets on nutrient digestibility, performance and apparent metabolizable energy content. Poult. Sci., 87: 759-767.

Duclos, M.J., 2005. Insulin-like growth factor-I (IGF-1) mRNA levels and chicken muscle growth. J. Physiol. Pharmacol., 56: 25-35.

Florini, J.R., Ewton, D.Z. and S.A. Coolican, 1996. Growth hormone and the insulin-like growth factor system in myogenesis. Endocr. Rev., 17: 481-517.

Gao, F., Y. Jiang, G.H. Zhou and Z.K. Han, 2007. The effects of xylanase supplementation on growth, digestion, circulating hormone and metabolite levels, immunity and gut microflora in cockerels fed on wheat-based diets. Br. Poult. Sci., 48: 480-488.

Goldberg, A., 1971. Carbohydrate metabolism in rats fed carbohydrate-free diets. J. Nutr., 101: 693-697.

Gonzales, N.J., S. Cornejo, F. Jimenez and G. Lorenzoni, 2001. Effect of dietary supplementation with and enzyme complex (Avizyme 1500) containing amylase, xylanase and protease on performance and egg quality of commercial layers. Poult. Sci., 80: 151-151.

Graham, H. and M. Bedford, 2007. Using enzymes to improve energy utilization in animal feeds. Proceedings of the 15th Annual ASAIM Southeast Asian Feed Technology and Nutrition Workshop, May 27-30, 2007, Conrad Bali Resort, Indonesia.

Johnston, S.L., S.B. Williams, L.L. Southern, T.D. Bidner, L.D. Bunting, J.O. Matthews and B.M. Olcott, 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total tract nutrient digestibility in pigs. J. Anim. Sci., 82: 705-714.

Jones, J.I. and D.R. Clemmons, 1995. Insulin-like growth factors and their binding proteins: Biological actions. Endocrinol. Rev., 16: 3-34.

Jozefiak, D., A. Ptak, S. Kaczmarek, P. Mackowiak, M. Sassek and B.A. Slominski, 2010. Multi-carbohydrase and phytase supplementation improves growth performance and liver insulin receptor sensitivity in broiler chickens fed diets containing full-fat rapeseed. Poult. Sci., 89: 1939-1946.

Khattak, F.M., T.N. Pasha, Z. Hayat and A. Mahmud, 2006. Enzymes in poultry nutrition. J. Anim. Plant Sci., 16: 1-7.

Lu, J.W., J.P. McMurtry and C.N. Coon, 2007. Developmental changes of Plasma insulin, Glucagon, insulin-like growth factors, thyroid hormones and glucose concentrations in chick embryos and hatched chicks. Poult. Sci., 86: 673-683.

McMurtry, J.P., G.L. Francis and Z. Upton, 1997. Insulin-like growth factors in poultry. Domest. Anim. Endocrinol., 14: 199-229.

Mohamad, M.A. and A.S. Hamza, 1991. Using enzymepreparation in corn-soybean meal broiler rations. Egypt J. Anim. Prod., 28: 245-254.

Odetallah, N.H., J.J. Wang, J.D. Garlich and J.C. Shih, 2005. Versazyme supplementation of broiler diets improves market growth performance. Poult. Sci., 84: 858-864.

Renner, R., 1964. Factors affecting the utilization of carbohydrate free diets for the growing chicken. J. Nutr., 84: 322-326.

Riesenfeld, G.A., A. Geva and S. Hurwitz, 1982. Glucose homeostasis in the chicken. J. Nutr., 112: 2261-2266.

Saleh, F., A. Ohtsuka, T. Tanaka and K. Hayashi, 2004. Carbohydrases are digested by proteases present in enzyme preparations during *in vitro* digestion. J. Poult. Sci., 41: 229-235.

Saleh, F., M. Tahir, A. Ohtsuka and K. Hayashi, 2005. A mixture of pure cellulose, hemicellulase and pectinase improves broiler performance. Br. Poult. Sci., 46: 602-606.

Sarkar, N.K., 1971. Gluconeogenesis and the factors that control the process in chickens. Life Sci., 10: 293-300.

Taylor-Pickard, J.A. and P. Spring, 2008. Gut Efficiency; the Key Ingredient in Pig and Poultry Production: Elevating Animal Performance and Health. Wageningen Academic Pub., Texas, USA., ISBN-13: 9789086860609.

Tesseraud, S., R.A. Pym, E. Le-Bihan-Duval and M.J. Duclos, 2003. Response of broilers selected on carcass quality to dietary protein supply: Live performance, muscle development and circulating insulin-like growth factors (IGF-I and -II). Poult. Sci., 82: 1011-1016.

Vranjes, M. and C. Wenk, 1995. Influence of dietary enzyme complex on the performance of broilers fed on diets with and without antibiotic supplementation. Br. Poult. Sci., 36: 265-275.

Wyatt, C.L., E. Moran and M.R. Bedford, 1997. Utilizing feed enzymes to enhance the nutritional value of corn-based broiler diets. Poult. Sci., 76: 39-39.

Zakaria, H.A.H., M.A.R. Jalal and M.A. Abu Ishmais, 2010. The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality traits of broiler chickens. Int. J. Poult. Sci., 9: 126-133.

Zanella, I., N.K. Sakomura, F.G. Silversides, A. Fiqueirdo and M. Pack, 1999. Effect of enzyme supplementation of broiler diets based on corn and soybeans. Poult. Sci., 78: 561-568.