Asian-Aust. J. Anim. Sci.
Vol. 22, No. 2 : 260 - 266
February 2009

www.ajas.info

Effect of β-Glucosidase as a Feed Supplementary on the Growth Performance, Digestive Enzymes and Physiology of Broilers*

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ABSTRACT : The effects of β-glucosidase on the overall growth performance and a set of physiological parameters of broilers were investigated. 240 male, one-day old Avine broiler chickswere randomly allocated to four treatment groups and fed with a corn-soybean meal supplemented with 0% (control), 0.2%, 0.4% and 0.6% β-glucosidase. The 0.2% β-glucosidase group, but not the 0.4% and 0.6% β-glucosidase groups, showed a significantly increased average daily weight gain ($p<0.05$) over that of the control. All three β-glucosidase feed groups showed significantly higher feed conversion ratios than the control group ($p<0.05$). Feed supplementation of 0.2% β-glucosidase significantly raised the contents of serum isoflavone aglycones as shown by decreases of genistin and daizin ($p<0.01$) and an increase of daidzein ($p<0.01$). The 0.2% β-glucosidase feeding significantly increased the intestinal amylase activity while it had little effect on lipase and trypsin activities ($p>0.05$). 0.2% β-glucosidase feeding also significant elevated the levels of high-density lipoprotein cholesterol and malate dehydrogenase while lowering the level of low-density lipoprotein cholesterol (LDL-C). Finally, β-glucosidase improved the anti-oxidative activities of the animals; the 0.2% β-glucosidase feed group had higher activities of superoxide dismutase ($p<0.05$), glutathione peroxidase and glutathione reductase in the liver ($p<0.05$), and malondialdehyde level in the serum ($p<0.05$). (**Key Words :** β-Glucosidase, Growth Performance, Digestive Enzyme, Physiological Parameter, Broiler)

INTRODUCTION

β-Glucosidase (β-glucoside glucohydrolase; EC3.2.1.21) hydrolyzes alkyl- and aryl-β-glucosides, as well as diglucosides and oligosacharides, to release glucose and an aglycone (Reese, 1977). The enzyme is wildly distributed in microorganisms, animals and plants, with *Aspergillus niger* (Heather et al., 2005) being the major source. It also hydrolyzes isoflavonal glycoside conjugates into isoflavone aglycones such as genistein, daidzein, and glycitein. These aglycones hydrolyzed by β-glucosidases from intestinal microorganisms are readily absorbed across the villi of the intestine (Ismail et al., 2005), and possess greater bioavailability than the corresponding glycoside conjugates (Izumi et al., 2000) and wide range of biological properties such as oestrogenic, antioxidant and anti-tumor activities (Fritz et al., 1998; Brouns et al., 2002). β-Glucosidase has been extensively studied due to its important medical, agricultural, biotechnological and industrial applications (Ducret et al., 2002).

Isoflavone is one of the major compounds found in various plant feeds for animals including broilers. Particularly, soybeans and soy products are abundant sources of isoflavoens with approximately 0.2-1.6 mg of isoflavones/g dry weight (Kurzer et al., 1997). Many animal and human studies as well as epidemiological studies have suggested that increased consumption of soy-based food is associated with decreases in cardiovascular disease, and the protective effects on cancers and osteoporosis (Jenkins et al., 2000; Walker et al., 2001; Hutchins et al., 2005; Kawakami et al., 2005; Ma et al., 2008). Based on the bioactivities of the enzyme and the beneficial effects of soy meals, in this study, we evaluated the effects of β-glucosidase on feed usage, growth performance, activities of digestive enzyme and a set of physiological parameters in broilers.

MATERIALS AND METHODS

Animals and feeding conditions

All procedures involved in the use of animals were pre-

* This work was supported by the National High-Tech Research and Development Plan (2007AA100601) and by Science and Technology Department of Zhejinag Province of China (2006C12036 and 2008C22035).

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Received July 28, 2008; Accepted October 24, 2008

Table 1. Ingredients and the nutrient composition of the basal diet

Ingredient	Amount (%)	
	0 to 3 wk	4 to 6 wk
Corn	60.2	66.9
Soybean meal[1]	29	26
Fish meal	4	2
Soybean oil	3	1.5
Limestone	0.8	0.8
Calcium phosphate	1.5	1.3
Vitamin-mineral premix[2]	1.5	1.5
Nutrient levels (%)[3]		
CP	20.3	18.16
ME (MJ/ kg)	12.49	12.32
Ca	1.06	0.86
P	0.71	0.61
Lys	1.21	1.02
Met	0.45	0.41

[1] Soybean meal contained the following components: 317.80 μg/g daidzin, 75.73 μg/g glycitin, 1031.11 μg/g genistin, 8.87 μg/g daidzein, 4.78 μg/g glycitein, 8.12 μg/g genistein. The contents of soybean isoflavones were determined according to Lee et al. (2007).

[2] The vitamin/mineral premix provided the following (per kg feed): 80 mg Fe, 8 mg Cu, 60 mg Mn, 40 mg Zn, 0.35 mg I, 0.15 mg Se, 11,000 IU vitamin A, 2,200 IU vitamin D_3, 10 IU vitamin E, 3.7 mg vitamin K_3, 2.2 mg vitamin B_1, 6.6 mg vitamin $_{B2}$, 4.5 mg vitamin B_6, 0.02 mg vitamin B_{12}, 44 mg niacin, 13.5 mg D-pantothenic acid calcium, 1.1 mg folacin, 0.2 mg biotin.

[3] All nutrient levels were analytical values except that of ME.

approved by the Institutional Animal Care and Use Committee of Zhejiang University. 240 one day old male Avine broilers were randomly allocated to four treatment groups, each with four pens of 15 chicks, and were fed with the same basal diet of a corn-soybean meal with β-glucosidase added at the amount of 0% (as the control group), 0.2%, 0.4%, and 0.6%, respectively. β-Glucosidase was provided by Animal Science College, Zhejiang University with an specific activity of 300 U/g. β-Glucosidase activity in the basal diet was determined to be 0.05 U/g feed by the method of Alcantara et al. (1999). Starter diets were offered to the birds from 0 to 3 weeks, and grower diets from 4 to 6 weeks of age. Nutrient levels of the diets (Table 1) were based on the NRC (1994). The broilers were allowed free access to water and feed. Continuous lighting was provided and the temperature was set at 33°C for the first week and then reduced by a 3°C weekly rate until reaching 26-27°C.

Evaluation of growth performance

Feed consumption was recorded weekly. Chicks were scaled at 0 week and 6 weeks of age and their weights were recorded; average body weight for each group was calculated at the beginning and the end of each feeding trial. Growth performance was evaluated by values of average daily gain (ADG), average daily feed intake (ADFI), and feed conversion rate (FCR).

Sample collections

Since only the 0.2% β-glucosidase group showed significant increase of average daily gain over the control group, eight chicks of this group and eight from the control groups were selected for further analysis at the end of 6 weeks. Blood samples were collected via vena puncture into a container, and allowed to clot naturally. The sera were first poured into 10 ml tubes and centrifuged for 10 min at 5,000×g, then the cleared serum samples were aspirated by a pipette into 1.5-ml Eppendorf tubes and stored at -70°C until analysis. The broilers were then sacrificed by cervical dislocation and immediately eviscerated for collection of the intestinal digesta and the liver. The intestinal digesta were sampled by the method of Jin et al. (2000). Digesta from the small intestines were collected from the segment at the distal end of the duodenum to the ileo-caecal junction, and were messaged from both ends of the tract to obtain homogenous samples. The digesta samples were immediately stored at -20°C until used. Samples were freeze-dried, and extracted with 1 m Mol of HCL (50 mg lyophilized digesta in 1 ml 1 m Mol HCL) for 1 h at 4°C followed by centrifugation (3,000×g). The supernatants were then collected for analysis of trypsin, amylase and lipase activities. The liver samples were homogenized in nine volumes of ice-cold 0.05 M NaCl and the homogenates were stored at -20°C for future assays.

Assays for digestive enzyme activities

Amylase (EC 3.2.1.1) activity was measured by the method of Somogy (1960). One unit of amylase activity was defined as the amount of enzyme in each milligram of proteins in the intestinal digesta that reduces 1 mg of glucose in 30 min at 38°C.

Lipase (EC 3.1.1.3) activity was assayed using olive oil as substrate with the method described by Tietz and Firereck (1966). One unit of lipase activity was defined by the volume of 0.05 M NaOH required to neutralize the fatty acid liberated from each milligram of intestinal proteins during 6-h incubation with 3 ml of lipase substrate at 38°C.

Trypin (EC 3.4.4.4) activity was determined by the method of Engberg et al. (2004) and Yuan et al. (2008). Trypsinogen in the intestinal homogenate was first converted into trypsin by Enterokinase. Trypsin activity was then measured using benzoyl DL-arginine p-nitroanilide as a substrate according to procedures described by Laine et al. (1993). One unit of enzyme activity was defined as the amount of enzyme in each milligram of intestinal digesta proteins to catalyze the hydrolysis of 1 μmol substrate in 1 min.

Assays for serum isoflavones

Serum levels of isoflavones, including daizin, glycitin,

Table 2. Effect of β-glucosidase on growth performance of male broiler chicks

Parameter	β-Glucosidase content (%)				SEM[1]
	0	0.2	0.4	0.6	
ADG (g/d)	43.24[a]	46.81[b]	45.20[a]	46.00[a]	0.61
ADFI (g/d)	96.86[a]	98.77[a]	98.53[a]	100.74[a]	1.09
FCR	2.24[a]	2.11[b]	2.18[c]	2.19[c]	0.01

[a, b, c] Indicate mean values significantly different from others within the same row ($p<0.05$). [1] SEM = Standard error of mean.

Table 3. Effect of β-glucosidase on digestive enzyme activities in male broiler chicks

Enzyme	β-Glucosidase content (%)		SEM
	0	0.2	
Amylase	16.08[a]	21.04[b]	2.78
Lipase	15.76[a]	17.72[a]	2.20
Trypsin	16.59[a]	26.21[a]	3.23

[a, b] Indicate mean values significantly different from others within the same row ($p<0.05$).

Table 4. Effect of 0.2% β-glucosidase feed on the contents of the serum isoflavones

Parameter	β-Glucosidase content (%)		SEM
	0	0.2	
Genistin (mg/L)	78.60[a]	29.37[b]	8.22
Daizin (mg/L)	13.23	ND	ND
Daidzein (mg/L)	1.90[a]	3.07[b]	0.20

[a, b] Indicate mean values significantly different from others within the same row ($p<0.05$). "ND" indicates values "not detected".

genistin, daidzein, glycitein, and genistein were analyzed by HPLC according to the method of Franke (1995).

Assays for the lipid metabolites

Concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyeride (TG), nonesterified fatty acids (NEFA), and the activities of lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) in the serum were measured with a biochemistry autoanalyzer according to the manufacturer's instruction (Beckman Instruments, Inc. Scientific Instruments Division, Fullerton, CA).

Assays for anti-oxidative molecules

Superoxide dismutase (SOD) activity was measured by the rate of the enzyme's inhibition to the reduction of nitroblue tetrazolium (NBT) (Asada et al., 1974). Malondialdehyde (MDA) was measured via the thiobarbituric acid colorimetric reaction for malondialdehyde by the method of Will (1966). Glutathione peroxidase (GSH-Px) activity was determined according to the method of Flohe and Grunzler (1984). Lutathione reductase (GR) activity was determined according to the method of Carlberg and Mannervik (1985).

Statistical analysis

One-way ANOVA was performed using the General Linear Model (GLM) procedure of SAS software (SAS Institute, 1988). Differences among means were tested using Duncan's multiple range tests. Comparisons were considered significantly different if $p<0.05$.

RESULTS

Growth performance

The values for average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (FCR) of broilers are presented in Table 2. Compared with the control broilers, although all β-glucosidase feed groups showed the trends of increasing values of ADG and ADFI, only the 0.2% β-glucosidase group had an significant increase of ADG by 8.26% ($p<0.05$) while the increases of ADG in both the 0.4% and 0.6% β-glucosidase feed groups were not statistically significant. The changes in the ADFI values were not significant among all groups. At the same time, 0.2%, 0.4%, 0.6% β-glucosidase feed groups decreased FCR by 5.80% ($p<0.01$), 2.68% ($p<0.01$) and 2.23% ($p<0.05$), respectively. The FCR was the lowest in the 0.2% β-glucosidase group, and decreased by 3.21% ($p<0.01$) and 3.65% ($p<0.01$) in the 0.4% and 0.6% groups, respectively. There was no significant difference between the 0.4% and 0.6% β-glucosidase feed groups.

Activities of digestive enzymes in the intestine

Activities of digestive enzymes in the intestinal contents are shown in Table 3. Compared with the control group, the 0.2% β-glucosidase group significantly improved the activity of amylase by 30.85% ($p<0.05$). However, β-glucosidase addition did not affect the activity of lipase or trypsin.

Contents of the isoflavones in the serum

Table 4 shows the serum levels of isoflavones in the experimental or the control group. Genistin constituted the majority of the isoflavones detected in the serum of the control broilers, and its content decreased by 62.63% ($p<0.01$) after chicks were fed with 0.2% β-glucosidase diets. The serum level of daizin in the control group was the second highest; however, it reduced to an undetected level in chicks fed with 0.2% β-glucosidase. On the other hand, the serum content of daidzein increased by 61.58% ($p<0.01$) in the 0.2% β-glucosidase group. Other

Table 5. Effect of β-glucosidase on lipid metabolic parameters of male broiler chicks

Parameter	β-Glucosidase content (%)		SEM
	0	0.2	
TG3 (m mol/L)	0.44	0.42	0.02
TC (m mol/L)	2.27	2.26	0.01
HDL-C (m mol/L)	2.45[a]	2.82[b]	0.10
LDL-C (m mol/L)	2.58[a]	1.64[b]	0.23
NEFA (m mol/L)	2.74	2.58	1.96
LDH (U/ml)	5.46	5.1	0.10
MDH (U/ml)	0.44[a]	1.06[b]	0.08

[a, b] Indicate mean values significantly different from others within the same row ($p<0.05$).

isoflavones such as glycitin, gycitein and genistein were not detected in chicks of all groups.

Levels of lipid metabolites in the serum

Effects of β-glucosidase on a set of lipid metabolic molecules measured in the serum are presented in Table 5. Feeding with 0.2% β-glucosidase significantly increased the serum HDL-C content by 15.10% ($p<0.05$) and decreased the serum LDL-C content by 36.43% ($p<0.01$). MDH is a key enzyme in the lipid metabolism. Its activity in the 0.2% β-glucosidase group increased by 140.91% ($p<0.05$). However, 0.2% β-Glucosidase did not significantly change all other lipid metabolism parameters including TG3, TC, NEFA and LDH.

Levels of anti-oxidative molecules in the serum and the liver

The anti-oxidative properties of the serum and liver samples are shown in Table 6. 0.2% β-Glucosidase increased the activity of SOD by 16.45% ($p<0.05$) and decreased the content of MDA by 18.16% ($p<0.05$) from serum samples, and increased activities of GSH-Px and GR by 33.00% ($p<0.05$) and 14.88% ($p<0.05$), respectively, in liver samples.

DISCUSSION

In this study, we showed that certain amount of β-glucosidase improved the growth performance and physical well-beings of male broilers. Since β-glucosidase can specially hydrolyze soybean isoflavonoid glucosides into aglycones (Reese, 1977), its effect is likely attributed to the superior bioavailability of aglycones (Watanabe et al., 1998; Izumi et al., 2000). Our experiment also showed that β-glucosidase (0.6 U/g feed) helped the chicks to gain more weights only at the amount of 0.2%, but not higher, indicating the growth-promoting effect is dose-sensitive. Since dietary soy and its isoflavones have been known to possess both beneficial and potentially adverse effects (Greiner et al., 2001), the amount of aglycones the chicks taken from their feeds may need to be well-balanced. β-Glucosidase increased the feed conversion rates significantly when added to the diet at all tested concentrations of 0.2%, 0.4%, or 0.6%, while no difference in the ADFI among all groups. This indicated a net improvement on feed efficiency by β-glucosidase.

Historically, the effects of soy isoflavones on weight gain, feed intake and feed conversion rate have been somehow variable largely dependent on the animal species, their genders and ages. From previous studies by Payne (2001), female rats fed with isoflavones increased weight gain, but male rats fed a similar diet showed decreases in the weight gain and the feed efficiency (Payne et al., 2001) while the feed efficiency remained unchanged. Payne et al. (2001) also reported that soy isoflavones increased the growth of fully growing pigs. Cook (1998) found that supplementing soy isoflavones (1,585 mg/kg) in the feed increased ADG in gilts from 6 to 30 kg BW, but the diet added together with genistein at a range of concentrations did not affect ADG in barrows from 5 to 28 kg BW. The same group also showed that a corn-soy protein concentrate diet supplemented with different amounts of soy isoflavones (Cook, 1998) decreased ADG and ADFI of Sprague-Dawley rats.

Effects of β-glucosidase or isoflavones on the activity of digestive enzymes in intestinal content have not been reported before. Previously, the exogenously added complex enzyme did not influence the activities of intestinal amylase and lipase in broilers fed with a corn-soybean meal (Tsvetanvo, 1984). However, it improved the activities of amylase and lipase when the births fed with a different diet composed of high non-starch polysaccharides (Almirall, 1995). Jiang et al. (2008) found that oral administration of exogenous amylase affected activities of intestinal enzymes in a dose-dependent manner in broilers. In our study, β-glucosidase increased the activity of amylase, but not the activities of lipase and trypsin. Trypsin is the main enzyme

Table 6. Effect of β-glucosidase on the antioxidative parameters of serum and liver

Sample parameters		β-Glucosidase content (%)		SEM
		0	0.2	
Serum	SOD (U/ml)	175.41[a]	204.26[b]	7.19
	MDA (n mol/ml)	10.3[a]	8.43[b]	0.49
Liver	GSH-Px (U/mgprot)	39.30[a]	52.27[b]	2.87
	GR (U/gprot)	8.20[a]	9.42[b]	0.25

[a, b] Indicate mean values significantly different from others within the same row ($p<0.05$).

to digest proteins in animals. Although β-glucosidase preparation has been shown to increase the intestinal trypsin activity (Dovgan et al., 1986), it did not change the intestinal trypsin activity in the broilers of this study. These different results indicate that exogenous enzymes may influent endogenous digestive enzymes differently, depending on different types of enzymes, the animal species and their complicated relationships.

Generally, isoflavonal glycoside conjugates are believed to be absorbed only as the free aglycones or as equol or O-DMA after their degradation by β-glucosidase produced from the intestinal bacteria (Setchell, 2002; Murota, 2002). Interestingly, Izumi (2002) has shown that soybean isoflavone conjugates can be absorbed directly inside the body (Izumi, 2002). After extensive studies on the serum metabolisms of both free and the conjugated isoflavones, Izumi showed that the amount of free aglycones reached the peak at 2 h but the conjugated form peaked at 4-6 h after feeding; the amount of free form declined linearly, and its level can be as low as or even lower than the amount of conjugated form after 24 h. These results showed the rapid catabolism of the free isoflavones; however, the conjugated form remained at a relatively stable level in the serum, indicating its very low bioavailability.

The present study also detected a larger amount of conjugated isoflavone in the serum of the boilers chicks with genistin being the most. This is consistent with the result of Izumi and confirmed that isoflavonal glycoside conjugates can be absorbed into the body though an unknown mechanism. Using β-glucosidase as feed supplementary can promote the degradation of conjugated soybean isoflavones and improve the absorption of free aglycones by intestines, therefore reducing the level of conjugated soybean isoflavone in the experimental group. These results suggest that β-glucosidase can degrade conjugated isoflavones *in vivo*, increase their bioavailability, and result in a series of beneficial physiological changes.

In small intestines, β-glucosidase hydrolyzes soybean isoflavonoid glucosides to aglycones, which can also affect lipid metabolism (Ali et al., 2005). Isoflavones in soy meals have been shown to reduce cholesterol levels and the incidence of atherosclerotic plaque in primates (Anthony et al., 1997) and also decrease LDL-C and increase HDL-C levels in the plasma of male New Zealand rabbits (Teixeira Damasceno et al., 2007). Thomas and Frank (2007) found that rats fed with soy protein concentrates had significantly a lower serum HDL-C and a higher TC/HDL-C ratio. In our experiment, the HDL-C level increased by 15.10% and the LDL-C level decreased by 36.43%, which was consistent with the previous findings in rats. MDH is an important enzyme in lipid and amino acid metabolism (Solomon et al., 2005). Its activity was increased to 140.91% by β-glucosidase in this experiment. These results indicate that β-glucosidase may affect the lipid metabolism by improving the bioavailability of isoflavones in the diet.

SOD, GSH-Px and GR are main antioxidant enzymes in the body that scavenge harmful free radicals such as $O_2^{\cdot}$, H_2O_2 and $ROO^{\cdot}$, and protect the affected tissues. On the other hand, MDA is a terminal product of lipid peroxidation used to estimate the extent of lipid peroxidation (Zhang et al., 2008). In this study, β-glucosidase significantly increased SOD, GSH-Px and GR activities and reduced MDA levels, suggesting the improvement of the antioxidant capacity of the body by β-glucosidase. These components of the antioxidant enzyme system play very important roles in the defense against oxidative stress (Ursini et al., 1995). Soybean isoflavones have been shown to protect skeletal muscle cells from the oxidative damage, which may be attributed to their antioxidant activity (Jiang et al., 2007). Although how β-glucosidase is correlated to the anti-oxidative molecules is not clear, given the well-known anti-oxidative effects of isoflavones and the enzyme's activity to convert isoflavones into aglycones, the anti-oxidative effect of β-glucosidase is most likely attributed to the superior bioavailability of free aglycones. In this study, all the broiler chicks fed with 0.2% β-glucosidase grew better than the controls, which may also be attributed by the effects of soybean isoflavone in the reduction of the oxidative stresses.

ACKNOWLEDGMENTS

This work was supported by the National High-Tech Research and Development Plan (2007AA100601) and by Science and Technology Department of Zhejinag Province of China (2006C12036 and 2008C22035).

REFERENCES

Alcantara, P. H. N., S. M. C. Dietrich and M. S. Buckeridge. 1999. Xyloglucan mobilisation and purification of a (XLLG/XLXG) specific β-glucosidase from Cotyledons of Copaifera langscloffii. Plant. Physi. Biochem. 37:653-663.

Ali, A. A., T. V. Manuel and T. H. Carl. 2005. Modulation of carbohydrate metabolism and peptide hormones by soybean isoflavones and probiotics in obesity and diabetes. J. Nutr. Biochem. 11:693-699.

Almirall, M., M. Francesch and A. M. Perez Vendrell. 1995. The differences in intestinal viscosity produced by barley and β-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. J. Nutr. 125: 947-955.

Anthony, M., T. Clarkson, B. Bullock and J. Wagner. 1997. Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atheroslerosis of male cynomolgus monkeys. Arter. Thromb. Vasc. Biol. 17:2524-2531.

Asada, K., M. Takahashi and M. Nagate. 1974. Assay and inhibitors of spinach suoeroxide dismutase. Agric. Biol. Chem.

38:471-473.
Brouns, F. 2002. Soya isoflavones: a new and promising ingredient for the health food sector. Food. Res. 35:187-193.
Carlberg, I. and B. Mannervik. 1985. Glutathione reductase. Methods Enzymol. 113:484-485.
Cook, D. R. 1998. The effect of dietary soybean isoflavones on the rate and efficiency of growth and carcass muscle content in pigs and rats. Ph. D. Thesis. Iowa State Univ. Ames.
Dovgan, N. Y. and A. A. Bramyuk. 1986. Dipeptidase activity and intensity of absorbtion of amino acid in the intestine of chickens fed on diets containing enzyme preparation. Poult. Abs. 12:2980.
Ducret, A., M. Trani and R. Lortie. 2002. Screening of various glycosides for the synthesis of octyl glucoside. Biotechnol. Bioeng. 77:752-757.
Engberg, R. M., M. S. Hedemann, S. Steenfeldt and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925-938.
Flohe, L. and W. A. Grunzler. 1984. Assays of glutathione peroxidase. Methods. Enzymol. 105:114-121.
Fritz, W., L. Coward, J. Wang and C. Lamartiniere. 1998. Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. Carcinogenesis. 19: 2151-2158.
Greiner, L. L., T. S. Stahly and T. J. Stable. 2001. The effect of dietary soy genistein on pig growth and viral replication during a viral chang. J. Anim. Sci. 79:1272-1279.
Heather, F. S. and E. Reuben. 2005. Transglucosidic reactions of the *Aspergillus niger* family 3β-glucosidase: qualitative and quantitative analyses and evidence that the transglucosidic rate is independent of pH. Archives of Biochemistry and Biophysics 436:254-264.
Hutchins, A. M., I. E. McIver and C. S. Johnston. 2005. Soy isoflavone and ascorbic acid supplementation alone or in combination minimally affect plasma lipid peroxides in healthy postmenopausal women. Journal of the American Dietetic Association 105:1134-1137.
Ismail, B. and K. Hayes. 2005. β-glycosidase activity toward different glycosidic forms of isoflavones. J. Agric. Food Chem. 53:4918-4924.
Izumi, T., M. K. Pisxula, S. Osawa, A. Obata, K. Tobe and K. Saito. 2000. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in human. J. Nutr. 130: 1695-1699.
Jenkins, D. J., C. W. Kendall and E. Vidgen. 2000. Effect of soy-based breakfast cereal on blood lipids and oxidized low-density lipoprotein. Metabolism. 49:1496-1500.
Jiang, S. Q., Z. Y. Jiang and T. X. Wu. 2007. Protective effects of a synthetic soybean isoflavone against oxidative damage in chick skeletal muscle cells. Food. Chem. 105:1086-1090.
Jiang, Z. Y., Y. M. Zhou, F. Z. Lu and T. Wang. 2008. Effects different levels of supplementary alpha-amylase on digestive enzyme activities and pancreatic amylase mRNA expression of Young broilers. Asian-Aust. J. Anim. Sci. 21:97-102.
Jin, J., Y. W. Ho, N. Abdullah and S. Jalaludin. 2000. Digestive and bacterial enzyme activities in broilers fed diets supplemented with lactobacillus cultures. Poult. Sci. 79:886-891.
Kawakami, Y., W. Tsurugasaki and S. Nakamura. 2005. Comparison of regulative functions between dietary soy isoflavones aglycone and glucoside on lipid metabolism in rats fed cholesterol. J. Nutr. Biochem. 16:205-212.
Kurzer, M. and X. Xu. 1997. Dietary phytoestrogens. Ann. Rev. Nutr. 17:353-381.
Laine, J., M. Beattie and D. Lebel. 1993. Simultaneous kinetic determinations of lipase, chymotrypsin, trypsin, elastae, and amylase on the same microtiter plate. Pancreas. 8:383-386.
Lee, Y. W., J. Z. Kim and J. Z. Zheng. 2007. Comparisons of isoflavones from Korean and Chinese soybean and processed product. Bioch. Engin. J. 36:49-53.
Ma, D. F., L. Q. Qin and P. Y. Wang. 2008. Soy isoflavone intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials. Eur. J. Clin. Nutr. 62:155-161.
NRC. 1994. Nutrient Requirements of Poultry .9th. Ed. National Academy Press. Washington, DC.
Payne, R. L., T. D. Bidner, L. L. Southern and J. P. Geaghan. 2001. Effects of dietary isoflavones on growth, carcass traits, and meat quality in growing-finishing pigs. J. Anim. Sci. 79:1230-1239.
Reese, E. T. 1977. Degradation of polymeric carbohydrates by microbial enzymes. Rec. Adv. Phytochem. 11:311-364.
SAS. 1988. SAS user's guide: statistical .SAS Inst. Inc., Cary, NC.
Solomon, W. Leung and C. K. L. James. 2005. Differential effects of anionic surfactants on activities of GDH, LDH, and MDH. Bioch. Engin. J. 25:79-88.
Somogy, M. 1960. Modificatihe on two methods for the assay of amylase. Clin. Chem. 6:23-27.
Teixeira Damasceno, N. R., D. S. P. Abdalla, E. Apolinario, F. Dias Flauzino and I. Fernandes. 2007. Soy isoflavones reduce electronegative low-density lipoprotein (LDL) and anti- LDL autoantibodies in experimental atherosclersis. Eur. J. Nutr. 46: 125-132.
Thomas, A. W., O. Frank and J. N. Robert. 2007. Soy protein concentrate lowers serum high-density lipoprotein cholesterol concentrations compared with casein in ovariectomized rats fed a low-fat, cholesterol-free diet. Nutr. Res. 27:417-422.
Tietz, Z. W. and E. A. Fiereck. 1966. A specific method for serum lipase determination. Clin. Chem. Acta. 13:352-355.
Tsvetanvo, I. 1984. The effects of some enzyme preparations in mixed forages for broiler chickens. Poul. Abs. 10:1480.
Ursini, F., M. Maiorino, R. Brigelius-Flohe', K. D. Aumann, A. Roveri and D. Schomburg. 1995. Diversity of glutathione perxidases. Methods. Enzymol. 252:38-114.
Walker, H. A., T. S. Dean and T. A. B. Sanders. 2001. The phytoestrogen genistein produces acute nitric oxide-dependent dilation of human forearm vasculature with similar potency to 17 beta-estradiol. Circulation 103:258-262.
Watanabe, S., M. Yamaguchi, T. Sobue, T. Takahashi, T. Miura, Y. Arai, W. Mazur, K. Wahala and H. Adlercreutz. 1998. Phaarmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). J. Nutr. 128:1710-1715.
Wills, E. D. 1966. Mechanisms of lipid peroxide formation in animal tissue. Biochem. J. 99:667-676.

Yuan, J., J. H. Yao, F. X. Yang and X. D. Yang. 2008. Effects of supplementing different levels of a commercial enzyme complex on performance, nutrient availability, enzyme activity and gut morphology of broilers. Asian-Aust. J. Anim. Sci. 21:692-700.

Zhang, H., J. Z. Wang and L. Ran. 2008. Attenuated effects of peptides derived from porcine plasma albumin on *in vitro* lipid peroxidation in the liver homogenate of mice. Food Chem. 111: 364-369.