Journal of Animal and Veterinary Advances 10 (15): 2004-2009, 2011
ISSN: 1680-5593

Effects of Chinese Herbal Medicine Additives on Antioxidant Status, Serum Biochemical Parameters and Digestive Enzymatic Activities in Weaned Piglets

Y.Y. Ding, C.H. Zhang, X.L. He, L. Huang and Z.J. Yin
College of Animal Science and Technology,
Anhui Agricultural University, AnHui, 230036 Hefei, P.R. China

Abstract: The study investigated the effects of Chinese Herbal Medicine Additives (CHMD) supplemented diets on weaned piglets antioxidant status, serum biochemical parameters, digesive enzymatic activities in a 3 weeks trial. Total of 144 crossbred (Duroc x Landrace x Yorkshire) weaning piglets (BW = 5.86±0.24 kg) from 18 L with an age of 21 days were selected and divided randomly into 4 groups balanced for sex, weight and litter origin. In each group, the piglets were divided randomly into 3 pens (replicates, 12 pigs per pen), a corn-soybean meal-expanded soybean basal diet without antibiotics or probiotics was used as control and the other 3 groups were fed the control diet supplemented with the CHMD at rations of 0.5, 1 and 1.5% (wt/wt). After completion of the feeding experiment, 3 piglets from each treatment were randomly selected to determine the antioxidant status, serum biochemical parameters, digesive enzymatic activities. In the 3 weeks trial, the data showed that the 1% CHMD group had higher protease activity in stomach digesta than the control and 0.5% CHMD group ($p<0.01$) than 1.5% CHMD group ($p<0.05$). The 1.5% CHMD group showed similar significant difference in protease activities of piglets compared with the control group ($p<0.05$). The 1% CHMD group had higher lipase activity than the control group in the stomach and jejunum digesta ($p<0.01$) in the duodenum and ileum digesta ($p<0.05$) and than the 0.5% CHMD group in the stomach and jejunum digesta ($p<0.05$). The 1% CHMD group had higher amylase activity than the control group in the jejunum digesta ($p<0.01$) in the duodenum and ileum digesta ($p<0.05$) and than the 0.5% CHMD group and 1.5% CHMD group in the jejunum digesta ($p<0.05$). The 1.5% CHMD group had higher amylase activity than the control group in the ileum digesta ($p<0.05$). The 1% CHMD group had higher T-AOC and CAT activities than the control group and 0.5% CHMD group ($p<0.01$), than the 1.5% CHMD group ($p<0.05$). The 1.5% CHMD group had higher T-AOC and CAT activities than the control group ($p<0.05$). The 1% CHMD group had higher GSH-PX and SOD activities than the control group ($p<0.05$). The 1% CHMD group ($p<0.01$) and the 1.5% CHMD group ($p<0.05$) had lower MDA content than the control group. Compared with the control group, dietary supplementation of 1% CHMD reduced the serum BUN ($p<0.05$) and increased the total serum protein ($p<0.05$), reduced the serum triglyceride and total cholesterol contents ($p<0.01$). The results show that the CHMD used in this study as a dietary additive could enhance the antioxidant status, serum biochemical parameters, digesive enzymatic activities in weaned piglets, additionaly imply that the dose of 1% CHMD supplement is the most ideal concentration to achieve the most beneficial effects.

Key words: Weaned piglets, Chinese herbal medicine additive, antioxidant status, serum biochemical parameters, digesive enzymatic activities, China

INTRODUCTION

Natural weaning of piglets is a gradual process that lasts over several weeks or months. However, in modern intensive pork-production systems, piglets are weaned early between 15 and 28 days of age, to maximize whole-herd production (Smith *et al*., 2008). After weaning, the gastrointestinal tract of piglets has to develop a certain level of maturity in response to new diets and nutritional demands (Salgado *et al*., 2002). Intestinal adaptation is associated with adjustment of digestive enzyme activities and morphological changes of the mucosa (Pluske *et al*., 1997). Piglets weaned between 15 and 28 days of age are known for having an immature digestive tract with a limited capacity for the biosynthesis of pancreatic and intestinal enzymes and exhibit an increase in the susceptibility to Gram-negative bacterial (such as *E. coli*) infections (Nabuurs, 1995). The

Corresponding Author: Yin Zongjun, Department of Animal Science, College of Animal Science and Technology, AnHui Agricultural University, 230036 Hefei, P.R. China

inhibition of enzymatic digestion of dietary proteins in the digestive tract could result in poor total tract and ileal digestibility, digestive disturbances and reduced animal performance (Lizard *et al*., 1995). Antibiotics have traditionally been used as the main method for preventing and treating diseases induced by weaning stress (Kong *et al*., 2009). However, this therapy has side effects, now there is a worldwide attempt to reduce antibiotic use in animal production because increased microbial resistance to antibiotics and residues in animal products can be harmful to consumers (Jin *et al*., 1998). Recently, researchers have been trying to find alternatives to antibiotics in livestock production as potential antimicrobial alternatives, traditional Chinese herbal medicines or their extracts may suppress the growth of bacteria in early-weaned piglets (Tang *et al*., 2005; Deng *et al*., 2007) and enhance both cellular and humoral immunity in early weaned piglets.

Reactive Oxygen Species (ROS) are a family of oxygen derivatives in cluding superoxide, hydroxyl radical, hydrogen peroxide and nitric oxide. Excessive ROS *in vivo* may oxidize lipids, proteins, DNA or carbohydrates and cause a variety of impairments to tissue (Zhao and Shen, 2005). Measuring serum biochemical parameters of farm animals can provide important information on health and metabolism (Friendship and Henry, 1992).

On the basis of the recent finding that dietary supplementation with one complex additive of Chinese herbal medicines enhanced both indicators of gastrointestinal health and growth performance in early weaned piglets, we hypothesized that such a treatment could improve antioxidant status, serum biochemical parameters, digesive enzymatic activities in the neonates.

MATERIALS AND METHODS

The experiment was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol (Yin *et al*., 2004).

Composition of the Chinese herbal medicines additive: The Chinese Herbal Medicines additive (CHMD) used in this study is consisted of seven dried Chinese herbs including Astragalus Membranaceus, scutellaria, malt, Glycyrrhiza uralensis, Codonopsis pilosula, Poria cocos, Atractylodes macrocephala, the rate is 2:1:1:1:2:2:2. Before inclusion in the feed, they were mixed according the aforesaid ratio and crumbled to an ultra-fine powder with an average granule diameter of 30 μm, packed in hermetical plastic bags and stored at room temperature. All the medicinal herbs were purchased from WeiMing pharmacy in Hefei city of China.

Animals, diets and experimental design: At weaning, at 21 days of age, 144 crossbred (Duroc x Landrace x Yorkshire) piglets (72 females and 72 males) with a body weight of 5.86±0.24 kg were selected from 18 L (among the original 21 L) that were healthy and had not been treated by any antibiotics and were divided randomly into 4 groups balanced for sex, weight and litter origin. In each group, the piglets were divided randomly into 3 pens (12 animals per pen) and each group was fed one of 4 diets for 3 weeks.

The pens had concrete floors with no litter and each pen was equipped with a feeder and nipple drinker. The nursery had a temperature of 27.0°C in the 1st week after weaning. From week 2 until the end of the nursery, the temperature was decreased weekly by 0.5°C. The photoperiod was controlled to provide 12 h of light and 12 h of dark in the stall. The ventilation also was provided to ensure good air quality. All piglets were vaccinated against pasteurellosis, parathyphoid, asthma and hog cholera.

A basal diet without antibiotics or probiotics was used as control and was fed to one group (C). The other 3 groups were fed the control diet supplemented with 0.5, 1 and 1.5% (wt/wt) CHMD. The basal diet mainly contained maize, soyabean meal, expanded soybean, milk replacer, whey powder, soybean oil and a premix of vitamins and minerals and the nutrient contents met or exceeded nutrient requirements recommended by National Research Council (1998). The piglets were fed *ad libitum* and had free access to water. The diets were fed in meal form and the CHMD (Sealed, placed in the cool dry place in separate bags) was mixed into the basal diet every day.

Digesta collection and digestive enzymatic activities analysis: For enzymatic analysis at the end of the 3 weeks study period, 1 pig selected at random from each pen was held under halothane general anesthesia and killed by an intra-cardiac injection of sodium pentobarbital in order to study the effect of CHMD with different concentrations on digestive enzyme activities of weaned piglets. The piglets were then immediately eviscerated for collection of stomach, duodenum, jejunum, ileum samples. The digesta samples were stored immediately at -70°C (Forma 702, Thermo, USA) until used. The digesta samples were diluted 10x, based on the sample weight with icecold phosphate buffered saline (PBS, pH 7.0), homogenized using a hand held glass homogenizer, respectively. The

homogenate was then centrifuged at 18,000 g for 20 min at 4°C. The supernatants were divided into small portions and stored at 4°C prior to analysis. All enzymatic assays were conducted within 24 h after extraction.

Protein concentration was evaluated according to Bradford (1976) using bovine serum albumin as a standard protein. Protease activity was evaluated according to Lowry *et al.* (1951) using Folin-phenol reagent and amylase activity was measured by dinitrosalicylic acid according to Bernfeld (1951). Lipase activity was determined based on measurement of fatty acids release due to enzymatic hydrolysis of triglycerides in stabilized emulsion of olive oil using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) (Jin, 1995). Enzyme activities including lipase and amylase were both expressed as specific activity (U g^{-1} protein) and protease activity was expressed as U mg^{-1} protein.

Assay of antioxidant indexes in serum: After the feeding trial, three piglets from each treatment (one pig per pen) were slaughtered under general anaesthesia. Blood samples (10 mL per pig) were drawn in collection tubes by venipuncture of the anterior vena cava of pigs before slaughter and allowed to clot at 37°C for 2 h prior to collect serum by centrifugation at 1000 g at 4°C for 10 min then serum was removed and stored in Eppendorf tubes.

Assay kits for protein, MDA, SOD, GSH-Px, CAT and T-AOC were obtained from Nanjing Jiancheng Bioengineering Institute. Serum GSH-Px activity was determined by the coupled method of Paglia and Valentine (1967), SOD activity was determined using the system of xanthine-xanthine oxidase and nitroblue tetrazolium (Sun *et al*., 1988), CAT activity was determined by Aebi's method (Aebi, 1974) and the concentration of MDA was determined using the thiobarbituric acid technique (Wong *et al*., 1987), T-AOC was measured by the method of ferric reducing/antioxidant power assay (Benzie and Strain, 1996). The prepared serum were then subjected to the measurement of MDA, SOD, GSH-Px, CAT and T-AOC levels by spectrophotometric methods (Spectrophotometer: Spectronic Instruments Inc., NY, USA) as described earlier. The activity of the enzyme was expressed as units per mL serum.

Measurements of serum biochemical indices: After the feeding trial, three piglets from each treatment (one pig per pen) were slaughtered under general anaesthesia. Blood samples (5 mL per pig) from each of the sacrificed pigs were collected and chilled immediately, allowed to clot and centrifuged at 3000×g for 10 min at 4°C for obtaining serum samples and the processed serum samples were stored at 20°C until analysis for various biochemical indices. The determination of levels of Blood Urea Nitrogen (BUN), Total Serum Protein (TP), Triglyceride (TG) and Total Cholesterol (TC) contents were performed by colorimetric methods using a CX-4 Auto-Blood Biochemical Analyzer (Beckman Inc., USA) according to the manufacturer's instructions (Beijing Leadman Biochemistry Technology Co. Ltd., Beijing, China).

Statistical analyses: Data are presented as arithmetic means with standard deviation of the mean (Mean±SD). Differences among groups were compared by SPSS 18.0 statistics software using one-way ANOVA and LSD method test. $p<0.05$ was selected as significant standard, $p<0.01$ was selected as remarkably significant standard.

RESULTS AND DISCUSSION

Specific enzyme activities for protease, amylase and lipase across all groups were shown in Table 1. In the 3 weeks trial, highest protease activity in stomach digesta was observed in 1% CHMD group and there was highly significant difference compared with the control and 0.5% CHMD group ($p<0.01$), significantly different compared

Table 1: Effect of CHMD supplemented diets on digestive enzymatic activities in early-weaned piglets

	CHMD dose in wt/wt (%)			
Items	0	0.5	1	1.5
Stomach				
Protease (U mg^{-1})	2.55±0.2700^{Bc}	2.79±0.2100^{Bbc}	3.57±0.2500^{Aa}	3.04±0.1800^{ABb}
Lipase (U g^{-1})	46.56±3.8300^{Bb}	54.92±6.2800^{ABb}	69.08±6.9900^{Aa}	58.03±8.9400^{ABab}
Duodenum				
Amylase (U g^{-1})	1272.9±155.400^{b}	1567.43±288.87^{ab}	1824.59±321.82^{a}	1573.45±233.82^{ab}
Lipase (U g^{-1})	65.11±8.2600^{b}	75.95±15.700^{ab}	90.31±13.810^{a}	75.38±13.160^{ab}
Jejunum				
Amylase (U g^{-1})	2679.67±241.16^{Bb}	2960.86±67.160^{ABb}	3414.33±165.46^{Aa}	2991.77±189.58^{ABb}
Lipase (U g^{-1})	76.26±10.490^{Bb}	87.17±8.5900^{ABb}	106.10±10.510^{Aa}	92.68±8.5600^{ABab}
Ileum				
Amylase (U g^{-1})	1704.25±308.26^{b}	2200.76±492.25^{ab}	2757.92±610.19^{a}	2234.10±576.06^{ab}
Lipase (U g^{-1})	58.48±12.610^{b}	77.91±17.470^{ab}	92.95±18.370^{a}	78.43±18.600^{a}

In the same row values with different small letter superscripts mean significant difference ($p = 0.05$) and with different capital letter superscripts mean extremely significant difference ($p<0.01$)

with 1.5% CHMD group ($p<0.05$). The 1.5% CHMD group showed similar significant difference in protease activities of piglets compared with the control group ($p<0.05$). The 1% CHMD group had higher lipase activity than the control group in the stomach and jejunum digesta ($p<0.01$) in the duodenum and ileum digesta ($p<0.05$) and than the 0.5% CHMD group in the stomach and jejunum digesta ($p<0.05$). The 1% CHMD group had higher amylase activity than the control group in the jejunum digesta ($p<0.01$) in the duodenum and ileum digesta ($p<0.05$) and than the 0.5% CHMD group and 1.5% CHMD group in the jejunum digesta ($p<0.05$). The 1.5% CHMD group had higher amylase activity than the control group in the ileum digesta ($p<0.05$). The protease and amylase played an important role in the fermentation of relative nutrient materials and ultimately in animal performance and health (Wang and Gu, 2008). The results in the present study demonstrated that stomach protease activity was significantly higher in 1% CHMD group and 1.5% CHMD group but that in 0.5% CHMD group was not affected. Thus, digestive utilisation of feed protein in diets was improved consequently. Supplementation of 1% CHMD to weaned piglets significantly increased the levels of amylase and lipase after 21 days of feeding. This result was similar to the finding of Ren-Jun *et al.* (2002) who reported that inclusion of another one compound of CHMD (mainly consisted of Astragalus Membranaceus, Poria cocos, Atractylodes macrocephala, Angelica) resulted in significantly higher amylase and lipase enzyme activities in the small intestine of weaned piglets. The better enzyme activities obtained with the supplemented diets suggested that the addition of CHMD improved diet digestibility including protein, starch and fattiness which might in turn explain the better growth performances and Feed efficiency (FCR) seen with the supplemented diets in the previous study.

The determined results of antioxidant indexes in serum of weaned piglets are shown in Table 2. The 1% CHMD group had higher T-AOC and CAT activities than the control group and 0.5% CHMD group ($p<0.01$) than the 1.5% CHMD group ($p<0.05$). The 1.5% CHMD group had higher T-AOC and CAT activities than the control group ($p<0.05$). The 1% CHMD group had higher GSH-PX and SOD activities than the control group ($p<0.05$). The 1% CHMD group ($p<0.01$) and the 1.5% CHMD group ($p<0.05$) had lower MDA content than the control group. The antioxidant enzymes GSH-Px, SOD and CAT play important roles to eliminate reactive oxygen species and MDA is the oxidative stress end product that serves as an index of antioxidant status (Shi *et al.*, 2011). MDA is a main marker of endogenous lipid peroxidation and the activity levels of the antioxidant enzymes GSH-Px, SOD and CAT (Wang *et al.*, 2008). In the current study in creasing dietary CHMD levels decreased theMDA levels in serums and of piglets which indicated that CHMD could protect from lipid peroxidation of piglets. Although, a variety of mechanisms contribute to protection against ROS-mediated cell and tissue injury in tracellular AOEs in cluding SOD, GSH-Px and CAT are considered to play a major role. SOD converts superoxide to H_2O_2. GSH-Px and CAT modulate the conversion of H_2O_2 to H_2O. GSH-Px can also degrade lipid peroxides. In the present study, GSH-Px, SOD and CAT activities in serum substantially increased in piglets fed 1% CHMD treated diet. It is likely that the increase in the activity of GSH-Px, SOD and CAT are the main factors of decrease in lipid peroxidation. In this study, we also investigated the T-AOC of the experimental piglets which reflects the non-enzymatic antioxidant defense system. The increase in T-AOC of serum from piglets fed the additional 1% CHMD diets suggested that increase in the non-enzymatic antioxidant defense probably contributed to protect from endogenous lipid peroxidation and oxidation. Similar reports were obtained by Fu-Gui *et al.* (2007) and Yu-Fang *et al.* (2007) that Chinese herb feed additives (mainly consisted of Astragalus Membranaceus, Poria cocos, Atractylodes macrocephala) could improve antioxidant status of weaned piglet.

The changes of selected serum biochemical parameters of weaning piglets were shown in Table 3. Compared with the control group, dietary supplementation of 1% CHMD reduced ($p<0.05$) the serum BUN and increased ($p<0.05$) the total serum protein. Changes in serum BUN concentrations can reflect the whole body status of amino acid metabolism and

Table 2: Effects of CHMD on antioxidant indexes in serum of weaning piglets

	CHMD dose in wt/wt (%)			
Indexes	0	0.5	1	1.5
T-AOC (U mL^{-1})	2.77±0.140^{Bc}	3.01±0.220^{Bbc}	3.66±0.300^{Aa}	3.21±0.250^{ABb}
GSH-PX (U mL^{-1})	352.26±34.01^{b}	370.67±27.22^{ab}	389.46±31.79^{a}	372.84±23.08^{ab}
MDA (nmol mL^{-1})	3.48±0.210^{Aa}	2.90±0.360^{ABab}	2.59±0.130^{Bb}	2.79±0.440^{ABb}
SOD (U mL^{-1})	117.49±13.58^{b}	124.75±15.82^{ab}	137.81±9.700^{a}	129.95±16.43^{ab}
CAT (U mL^{-1})	14.49±0.740^{Bc}	15.45±0.890^{Bbc}	17.84±0.910^{Aa}	16.26±0.770^{ABb}

Table 3: Effects of CHMD on serum biochemical parameters of weaning piglets

	CHMD dose in wt/wt (%)			
Parameters	0	0.5	1	1.5
TP (g L^{-1})	44.79±3.4600^{b}	47.05±6.1200^{b}	56.35±4.5200^{a}	47.76±3.6800^{ab}
BUN (mmol L^{-1})	5.58±0.9600^{a}	4.55±0.2600^{ab}	3.83±0.4400^{b}	4.49±0.6900^{ab}
TG (mmol L^{-1})	0.458±0.055^{Aa}	0.415±0.024^{ABa}	0.308±0.041^{Bb}	0.385±0.039^{ABab}
TC (mmol L^{-1})	0.582±0.067^{Aa}	0.503±0.065^{ABab}	0.393±0.053^{Bb}	0.473±0.049^{ABab}

utilization in animals (Eggum, 1970). Reduction of serum BUN concentration would suggest a potential enhancement of synthesis of protein in animals (Brown and Clinc, 1974). Therefore, dietary supplementation of 1% CHMD improved whole body protein anabolism in the early-weaned piglet. Liver is the primary source of serum proteins. Dietary amino acids are the preferred precursor for hepatic protein synthesis in animals (Stoll *et al*., 1998). Total serum protein content may also reflect hepatic protein metabolic status in responses to dietary treatments in the early-weaned piglet. Thus, dietary supplementation of 1% CHMD may have improved hepatic functions in the early-weaned piglet. Compared with the control group, dietary supplementation of 1% CHMD reduced the serum triglyceride ($p<0.01$) and total cholesterol contents ($p<0.01$), meant that supplementation of 1% CHMD in the diets could reduce serum fat and cholesterol concentrations in the weaned piglets, suggesting a role of CHMD for intestinal metabolism in lipid homeostasis in animals (Kong *et al*., 2007).

CONCLUSION

In the study, these findings suggest that CHMD as a dietary additive could enhance gastrointestinal health by regulating the digestive enzyme activities and enhance the antioxidant status in weaned piglets are effective in regulating the whole body protein anabolism and the metabolism of fat and cholesterol, thereby increasing the provision of energy for supporting cell function and tissue growth. These findings indicate that the CHMD is safe and effective in preventing the weaning-associated digestive disturbances and improving the growth performance in piglets.

ACKNOWLEDGEMENTS

This research was jointly supported by grants from the key scientific and technological project of AnHui province (Grand No. 08010301079), the school young scientists fund of AnHui Agriclutral University (Grand No. 2009zr01), the school initial fund for the newly-enrolled scholars of AnHui Agriclutral University (Grand No. yj2008-31).

REFERENCES

Aebi, H., 1974. Catalase. In: Methods in Enzymatic Analysis, Bergmeyer, H.V. (Ed.). Academic Press, New York, pp: 674-684.

Benzie, I.F.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239: 70-76.

Bernfeld, P., 1951. Enzyme of Starch Degradation and Synthesis. In: Advance in Enzymology, Nord, F.F. (Ed.). Interscience Publications Inc, New York, pp: 379-424.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Brown, J.A. and T.R. Clinc, 1974. Urea excretion in pigs: An indicator of quality and amino acid requirements. J. Nutr., 104: 542-553.

Deng, Z.Y., J.W. Zhang, G.Y. Wu, Y.L. Yin and Z. Ruan *et al*., 2007. Dietary supplementation with polysaccharides from semen cassiae enhances immunoglobulin production and interleukin gene expression in early-weaned piglets. J. Sci. Food Agric., 87: 1868-1873.

Eggum, B.O., 1970. Blood urea measurement as a technique for assessing protein quality. Br. J. Nutr., 24: 983-988.

Friendship, R.M. and S.C. Henry, 1992. Cardiovascular System, Hematology, and Clinical Chemistry. In: Diseases of Swine, Leman, A.D., B.E. Straw, W.L. Mengeling, S. D'Allaire and D.J. Taylor (Eds.). Iowa State University Press, Ames, IA., pp: 3-10.

Fu-Gui, Y., Y. Yu-Long, K. Xiang-Feng, H. Qing-Hua and L.J.T. Bie *et al*., 2007. Effects of Chinese herbal formula on blood totaland differential leucocytes and anti-oxidative capacity inweaned piglet. Jiangsu J. Sci., 23: 442-446.

Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin, 1998. Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diets containing *Lactobacillus cultures*. Poult. Sci., 77: 1259-1265.

Jin, Z.L., 1995. The Evaluation Principle and Method of Functional Food. Beijing Publishers, Beijing.

Kong, X.F., F.G. Yin , Q.H. He , H.J. Liu and T.J. Li *et al*., 2009. Acanthopanax senticosus extract as a dietary additive enhances the apparent ileal digestibility of amino acids in weaned piglets. Livest. Sci., 123: 261-267.

Kong, X.F., G.Y. Wu, Y.P. Liao, Z.P. Hou and H.J. Liu *et al*., 2007. Effects of Chinese herbal ultra-fine powder as a dietary additive on growth performance, serum metabolites and intestinal health in early-weaned piglets. Livest. Sci., 108: 272-275.

Lizard, R., J. Peiniau and A. Aumaitre, 1995. Effect of sorghum on performance, digestibility of dietary components and activities of pancreatic and intestinal enzymes in the weaned piglet. Anim. Feed Sci. Technol., 56: 67-82.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Nabuurs, M.J.A., 1995. Microbiological, structural and functional changes of the small intestine of pigs at weaning. Pig News Inf., 16: 93-97.

National Research Council, 1998. Nutrient Requirements of Swine. 10th Edn., National Academy Press, Washington, DC., USA.

Paglia, D.E. and W.N. Valentine, 1967. Studies on quantitative and qualitative characterization of erythrocyte gluthathione peroxidase. J. Lab. Clin. Med., 70: 158-169.

Pluske, J.R., D.J. Hampson and I.H. Williams, 1997. Factors influencing the structure and function of the small intestine in the weaned pig: A review. Livest. Prod. Sci., 51: 215-236.

Ren-Jun, Z., C. Zhi-Bing, T. Yun-Bo and G. Chang-Ron, 2002. Effect of Chinese herb feed additives on the activities of digestive tract enzymes in pig starter. J. Yunnan Agric. Univ., 17: 67-71.

Salgado, P., J.P.B. Freire, M. Mourato, F. Cabral, R. Toullec and J.P. Lalle's, 2002. Comparative effects of different legume protein sources in weaned piglets: Nutrient digestibility, intestinal morphology and digestive enzymes. Livest. Prod. Sci., 74: 191-202.

Shi, L., W. Xun, W. Yue, C. Zhang and Y. Ren *et al*., 2011. Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. Small Rumin. Res., 96: 49-52.

Smith, A.L., K.J. Stalder, T.V. Serenius, T.J. Baas and J.W. Mabry, 2008. Effect of weaning age on nursery pig and sow reproductive performance. J. Swine Health Prod., 16: 131-137.

Stoll, B., D.G. Burrin, J. Henry, H. Yu, F. Jahoor and P.J. Reeds, 1998. Dietary amino acids are the preferential source of hepatic protein synthesis in piglets. J. Nutr., 128: 1517-1524.

Sun, Y., L.W. Oberley and Y. Li, 1988. A simple method for clinical assay of superoxide dismutase. Clin. Chem., 34: 497-500.

Tang, Z.R., Y.L. Yin, C.M. Nyachoti, R.L. Huang and T.J. Li *et al*., 2005. Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. Domestic Anim. Endocrinol., 28: 430-441.

Wang, Y. and Q. Gu, 2008. Effect of probiotic on growth performance and digestive enzyme activity of arbor acres broilers. Res. Vet. Sci., 89: 163-167.

Wang, Y.Z., C.L. Xu, Z.H. An, J.X. Liu and J. Feng, 2008. Effect of dietary bovine lactoferrin on performance and antioxidant status of piglets. Anim. Feed Sci. Technol., 140: 326-336.

Wong, S.H., J.A. Knight, S.M. Hopfer, O. Zaharia, C.N. Jr. Leach and F.W. Jr. Sunderman, 1987. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clin. Chem., 33: 214-220.

Yin, Y.L., Z.Y. Deng, H.L. Huang, T.J. Li and H.Y. Zhong, 2004. The effect of arabinoxylanase and protease supplementation on nutritional value of diets containing wheat bran or rice bran in growing pig. J. Anim. Feed Sci., 13: 445-461.

Yu-Fang, M.A., Z. Zhong-Qiao, Y. Jin-Shu, Y. Dao-Jin, H. Xiao-Hong and H. Yi-Fan, 2007. Effect of Chinese herb additives on antioxidant status ofweaned piglets. J. Agric. Forestry Univ., 36: 77-81.

Zhao, R.Z. and G.X. Shen, 2005. Functional modulation of antioxidant enzymes in vascular endothelial cells by glycated LDL. Atherosclerosis, 179: 277-284.