Effects of Dietary Soybean Meal Levels and -Mannanase on Serum Galactomannan Content, Biochemical Indices, and Intestinal Amino Acid Transporter Gene Expression in Weaned Piglets: Postprint

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Abstract

This experiment, based on the analysis of -galactomannan (-GM), -galactomannan (-GM), and galactomannan (GM) contents in 22 feed ingredients, investigated the effects of dietary soybean meal level and -mannanase (-MN) on serum -GM, -GM, and GM contents, serum biochemical indices, and the relative expression levels of solute carrier family 7 member 1 (SLC7A1), solute carrier family 7 member 11 (SLC7A11), and solute carrier family 38 member 2 (SLC38A2) genes in the intestine of weaned piglets. A 2×2 factorial design was adopted, and 24 healthy crossbred weaned piglets with similar initial body weight were randomly assigned to 4 groups (Groups , , , and), with 6 replicates per group and 1 pig per replicate. Group was fed a diet with 22% soybean meal level, Group was fed a diet with 22% soybean meal level supplemented with 0.02% -MN, Group was fed a diet with 37% soybean meal level, and Group was fed a diet with 37% soybean meal level supplemented with 0.02% -MN. The experimental period lasted for 30 days. On day 30, 10 mL of blood was collected from the carotid artery, mesenteric vein, and hepatic portal vein of the 24 piglets to determine serum -GM, -GM, and GM contents; 10 mL of blood was collected from the anterior vena cava to determine serum biochemical indices; and the anterior jejunum and posterior ileum were sampled to determine the relative expression levels of SLC7A1, SLC7A11, and SLC38A2 genes. The results showed that: compared with Group , Group exhibited significantly decreased average daily feed intake (ADFI) and relative expression level of ileal SLC7A1 gene in weaned piglets ($P<0.05$), while Group showed significantly increased average daily intake of -GM, serum -GM content in carotid artery, serum -GM and GM

contents in mesenteric vein, and serum alanine aminotransferase, aspartate aminotransferase activities, and urea nitrogen content in anterior vena cava (P<0.05); Group exhibited significantly decreased serum -GM content in mesenteric vein and serum -GM and GM contents in hepatic portal vein, and significantly increased serum glucose, calcium, and high-density lipoprotein contents in anterior vena cava in weaned piglets (P<0.05). Compared with Group , Group showed significantly decreased serum GM content in hepatic portal vein and significantly increased relative expression levels of SLC7A1, SLC7A11, and SLC38A2 genes in jejunum of weaned piglets (P<0.05). These results indicate that increased average daily intake of -GM in piglets can reduce ADFI in piglets; serum -GM, -GM, and GM contents in hepatic portal vein, carotid artery, and mesenteric vein increase with the increase of dietary -GM, -GM, and GM contents. When dietary -GM, -GM, and GM contents increase, supplementation of -MN can reduce serum GM content in hepatic portal vein and upregulate the relative expression levels of SLC7A1, SLC7A11, and SLC38A2 genes in jejunum.

Full Text

Effects of Dietary Soybean Meal Level and -Mannanase on Serum Galactomannan Content, Biochemical Indices, and Intestinal Amino Acid Transporter Gene Expression in Weaner Piglets

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Abstract

Based on the analysis of -galactomannan (-GM), -galactomannan (-GM), and galactomannan (GM) contents in 22 feed ingredients, this study investigated the effects of dietary soybean meal (SBM) level and -mannanase (-MN) supplementation on serum -GM, -GM, and GM concentrations, serum biochemical parameters, and the relative expression of intestinal solute carrier family 7 member 1 (SLC7A1), solute carrier family 7 member 11 (SLC7A11), and solute

carrier family 38 member 2 (SLC38A2) genes in weaner piglets. A 2×2 factorial design was employed, with twenty-four healthy dual-cross weaner piglets of similar initial body weight randomly allocated to four groups (, , , and), each comprising six replicates of one pig. Group received a diet containing 22% SBM, group received the same diet supplemented with 0.02% -MN, group received a diet containing 37% SBM, and group received the 37% SBM diet supplemented with 0.02% -MN. The experimental period lasted 30 days. On day 30, 10 mL blood samples were collected from the carotid artery, mesenteric vein, and hepatic portal vein of all 24 piglets for serum -GM, -GM, and GM analysis. Additionally, 10 mL blood from the precaval vein was collected for serum biochemical analysis, and samples from the proximal jejunum and distal ileum were obtained for determination of SLC7A1, SLC7A11, and SLC38A2 gene expression.

The results demonstrated that compared with group , group exhibited significantly reduced average daily feed intake (ADFI) and ileal SLC7A1 gene expression ($P<0.05$), while showing significantly increased average daily -GM intake, carotid artery serum -GM content, mesenteric vein serum -GM and GM contents, and precaval vein serum alanine aminotransferase, aspartate aminotransferase activities, and urea nitrogen concentration ($P<0.05$). Group showed significantly decreased mesenteric vein serum -GM content and hepatic portal vein serum -GM and GM contents ($P<0.05$), along with significantly elevated precaval vein serum glucose, calcium, and high-density lipoprotein concentrations ($P<0.05$). Compared with group , group displayed significantly reduced hepatic portal vein serum GM content ($P<0.05$) and significantly upregulated jejunal expression of SLC7A1, SLC7A11, and SLC38A2 genes ($P<0.05$). These findings indicate that increased -GM intake reduces ADFI in piglets, and that serum -GM, -GM, and GM concentrations in the hepatic portal vein, carotid artery, and mesenteric vein increase proportionally with dietary -GM, -GM, and GM contents. When dietary -GM, -GM, and GM levels are elevated, -MN supplementation can reduce hepatic portal vein serum GM concentration and upregulate jejunal expression of SLC7A1, SLC7A11, and SLC38A2 genes.

Keywords: -mannanase; galactomannan; soybean meal; amino acid transporter; biochemical indices; weaner piglets

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1.1 Experimental Design

-Mannanase was purchased from Elanco (USA) with an activity of 360 MU/kg. Twenty-four healthy dual-cross castrated male weaner piglets at 21 days of age were selected and subjected to a 6-day adaptation period. At an initial body weight of (9.00±1.17) kg, the piglets were randomly assigned to four groups (, , , and) following a 2×2 factorial arrangement, with six replicates per group and one pig per replicate, housed individually. Each group received one of four experimental diets: group was fed a diet containing 22% soybean meal

(SBM), group received the 22% SBM diet supplemented with 0.02% -MN, group was fed a diet containing 37% SBM, and group received the 37% SBM diet supplemented with 0.02% -MN. The diets were formulated according to NRC (2012) nutrient requirements for piglets, with -GM, -GM, and GM contents adjusted based on SBM level. All diets were pelleted and maintained at consistent digestible energy levels. Diet composition and nutrient levels are presented in Table 1 .

1.2 Animal Management

The feeding trial was conducted at the Animal Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, located at Yong' an Branch of Hunan New Wufeng Co., Ltd. Experimental pigs were housed under identical environmental conditions in individual pens with ad libitum access to water and feed. Feed was provided four times daily (07:00, 11:00, 14:00, and 18:00), with feed intake recorded regularly. The facility utilized natural lighting and ventilation, and pigs followed standard immunization protocols. The adaptation period lasted 6 days, followed by a 30-day experimental period.

It should be noted that to ensure the continuity and integrity of this study investigating the effects of high-SBM diets and -MN on serum galactomannan content, biochemical indices, and intestinal amino acid transporter gene expression, piglets continued to receive diets supplemented with high zinc (zinc oxide) throughout the 30-day experimental period. This approach was specifically adopted for research purposes, and zinc usage should comply with national regulations in practical applications. At the conclusion of the trial, all 24 weaner piglets were anesthetized for blood collection from the carotid artery, mesenteric vein, and hepatic portal vein (10 mL each) for serum -GM, -GM, and GM analysis; precaval vein blood (10 mL) for serum biochemical analysis; and tissue samples from the proximal jejunum and distal ileum for amino acid transporter gene expression analysis. Following sampling, all experimental piglets were disposed of harmlessly according to national regulations.

1.3 Sample Collection

On day 30 of the experimental period, after a 12-hour fast, the 24 weaner piglets were slaughtered under anesthesia with Zoletil 50 (Virbac S.A., France). Blood samples (10 mL each) were first collected from the carotid artery and precaval vein. The abdominal cavity was then opened to collect 10 mL blood samples from the hepatic portal vein and mesenteric vein. All blood samples were allowed to clot for 3 hours before serum was harvested by centrifugation at 3,000 rpm for 10 minutes. The supernatant was transferred to centrifuge tubes and stored at -80°C. Tissue samples (2 cm) from the proximal jejunum and distal ileum were immediately collected, rinsed with physiological saline, wrapped in aluminum foil, snap-frozen in liquid nitrogen, and stored at -80°C.

1.4 Determination of -GM, -GM, and GM Contents in Feed Ingredients and Serum

The contents of -GM, -GM, and GM in feed ingredients and serum were determined using assay kits purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd., with detection performed by the same company.

1.5 Determination of Average Daily Feed Intake (ADFI) and Average Daily Intake of -GM, -GM, and GM

Daily feed intake was recorded throughout the experimental period to calculate ADFI from days 1 to 30. Based on feed intake and dietary -GM, -GM, and GM concentrations, the average daily intake of -GM, -GM, and GM was calculated for the same period.

1.6 Serum Biochemical Indices Analysis

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, and concentrations of urea nitrogen (UN), creatinine (CREA), glucose (GLU), calcium (Ca), phosphorus (P), high-density lipoprotein (HDL), and complement 4 (C4) were determined using Roche biochemical assay kits and a COBAS C311 automated biochemical analyzer (Roche, Germany).

1.7 Amino Acid Transporter Gene Expression Analysis

Frozen jejunal tissue was ground in liquid nitrogen, and total RNA was extracted using Trizol reagent (Invitrogen, USA). All RNA samples were diluted to 1 g/ L and treated with DNase I (Invitrogen, USA) according to the manufacturer' s instructions to eliminate genomic DNA contamination. The DNA-free RNA was reverse-transcribed using a reverse transcription kit from Shanghai Biological Engineering Co., Ltd. to generate cDNA in a 20 L reaction system, which was stored at -20°C for subsequent analysis. Primers were designed based on porcine gene sequences (http://www.ncbi.nlm.nih.gov/pubmed/) using Primer 5.0 software and synthesized by Shanghai Sangon Biotechnology Co., Ltd. Primer sequences and parameters are listed in Table 2 .

For quantitative PCR, 1 L of cDNA template was added to a reaction mixture containing 5 L SYBR Green Premix (2×, pre-mixed with MgCl , dNTPs, SYBR Green I dye, EX-Taq polymerase, and buffer), 0.5 L each of forward and reverse primers (0.4 mol/L), and deionized water to a final volume of 10 L. The PCR cycling conditions were: 1) initial denaturation at 95°C for 10 s; 2) amplification for 40 cycles of 95°C for 5 s and 60°C for 20 s; and 3) melting curve analysis from 60°C to 99°C at a heating rate of 0.1°C/s. After amplification, Ct values were obtained for -actin and target genes, and relative gene expression was calculated using the 2^(-ΔΔCt) method.

1.8 Statistical Analysis

Experimental data were initially processed using Excel 2007 and subsequently analyzed using the GLM procedure in SPSS 18.0 statistical software. The statistical model included the main effects of SBM level and -MN supplementation, as well as their interaction. Duncan' s multiple comparison test was used to evaluate differences among groups. Results are expressed as "mean ± standard error." Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

2.1 -GM, -GM, and GM Contents in Common Plant Feed Ingredients

As shown in Table 3 , which presents the measured -GM, -GM, and GM contents in common plant protein and energy feed ingredients, -GM content was markedly higher than -GM content in 19 of the 22 feed ingredients examined, with the exceptions being fermented soybean meal, broad beans, and black beans. Furthermore, total GM content exceeded the sum of -GM and -GM contents. These findings underscore the importance of considering -GM, -GM, and GM contents during diet formulation.

2.2 Effects of -MN and SBM on ADFI and Average Daily Intake of -GM, -GM, and GM

Table 4 summarizes the effects of -MN and SBM on ADFI and average daily intake of -GM, -GM, and GM. No significant differences were observed in ADFI or average daily intake of -GM, -GM, and GM between groups and ($P>0.05$). However, compared with group , group showed significantly higher ADFI ($P<0.05$). Additionally, average daily -GM intake was significantly greater in group than in group ($P<0.05$). Group exhibited significantly lower ADFI ($P<0.05$) and significantly higher average daily -GM intake ($P<0.05$) compared with group , indicating that high-level SBM diets without -MN supplementation substantially increase -GM intake in piglets ($P<0.01$).

2.3 Effects of -MN and SBM on Serum -GM, -GM, and GM Contents in Different Vascular Sites

The effects of -MN and SBM on serum galactomannan concentrations are presented in Table 5 . In carotid artery serum, -GM content was 37.70% lower in group than in group ($P>0.05$), and 16.90% lower in group than in group ($P>0.05$), suggesting that -MN reduced carotid artery -GM content ($P=0.02$). Serum -GM concentration was significantly elevated in group compared with group ($P<0.05$) and in group compared with group ($P<0.05$), demonstrating that excessive SBM supplementation markedly increased carotid artery -GM content ($P<0.01$).

In mesenteric vein serum, -GM content was significantly reduced in group compared with group ($P<0.05$), indicating that -MN decreased mesenteric

vein -GM content (P=0.01). Group showed significantly higher -GM and GM contents than group (P<0.05), while group exhibited significantly increased -GM and -GM contents compared with group (P<0.05). These results demonstrate that excessive SBM supplementation significantly elevated mesenteric vein serum concentrations of -GM (P=0.04), -GM (P<0.001), and GM (P<0.01).

In hepatic portal vein serum, -GM and GM contents were significantly lower in group than in group (P<0.05), and GM content was significantly reduced in group compared with group (P<0.05), indicating that -MN decreased hepatic portal vein serum -GM (P=0.02) and GM (P<0.01) contents. Serum -GM content was 40.24% higher in group than in group (P>0.05), while -GM content was significantly elevated in group compared with group (P<0.05), suggesting that excessive SBM supplementation increased hepatic portal vein serum -GM (P=0.03) and -GM (P=0.02) contents.

2.4 Effects of -MN and SBM on Serum Biochemical Indices in Precaval Vein

As shown in Table 6 , serum GLU, Ca, and HDL concentrations were significantly higher in group than in group (P<0.05), with P content also increased by 32.47% (P>0.05). These findings indicate that -MN supplementation significantly elevated precaval vein serum GLU (P<0.01), Ca (P<0.01), P (P=0.02), and HDL (P<0.01) concentrations.

Compared with group , group exhibited significantly increased serum ALT and AST activities and UN content (P<0.05). Group showed significantly higher AST activity and lower GLU content than group (P<0.05). These results demonstrate that excessive SBM supplementation significantly increased precaval vein serum ALT (P<0.01) and AST (P=0.01) activities and UN content (P<0.01), while significantly decreasing serum GLU concentration (P<0.01).

2.5 Effects of -MN and SBM on Intestinal Amino Acid Transporter Gene Expression

Table 7 presents the effects on intestinal amino acid transporter gene expression. Jejunal SLC7A11 expression was significantly higher in group than in group (P<0.05). Compared with group , group showed significantly elevated expression of jejunal SLC7A1, SLC7A11, and SLC38A2 genes (P<0.05). These findings suggest that -MN supplementation increased the relative expression of jejunal amino acid transporter genes SLC7A1 (P=0.03), SLC7A11 (P<0.01), and SLC38A2 (P=0.01).

Ileal SLC7A1 expression was significantly lower in group than in group (P<0.05), indicating that excessive SBM supplementation significantly reduced ileal SLC7A1 gene expression (P=0.01).

3 Discussion

Excessive galactomannan content in plant-based feed ingredients impairs gastrointestinal function, increases intestinal chyme viscosity, reduces feed utilization efficiency, and exerts detrimental effects on animal physiology. This study determined -GM, -GM, and GM contents in common feed ingredients and found that -GM content was markedly higher than -GM in most ingredients, with total GM content exceeding the sum of -GM and -GM. This suggests the presence of additional polysaccharide branches within the GM subfamily.

Dietary GM supplementation has been shown to reduce intestinal villus height and Lactobacillus populations in piglets, thereby decreasing nutrient utilization efficiency. In the current study, increasing dietary SBM level from 22% to 37% reduced feed intake while significantly increasing -GM intake, suggesting that -GM may be a factor contributing to reduced feed consumption. Galactomannan is a thermostable polysaccharide component released from cell walls during hyphal tissue growth and is essential for Aspergillus survival. Consequently, excessive GM consumption by piglets may increase the risk of diseases such as aspergillus pneumonia, posing serious health threats. By measuring serum GM concentrations at different vascular sites, this study clarified GM absorption patterns in various body compartments. Increasing dietary SBM level from 22% to 37% significantly elevated -GM concentrations in both carotid artery and mesenteric vein serum, indicating that -GM may be the primary factor affecting physiological changes in piglets. Previous research has reported that GM impairs gastrointestinal function and increases intestinal chyme viscosity. The current finding that elevated dietary SBM level increased mesenteric vein serum concentrations of -GM, -GM, and GM suggests that altered GM balance in the intestine may underlie changes in intestinal physiological function, though the specific mechanisms require further investigation.

The liver is a component of the innate immune system. In hepatic portal vein serum, supplementation with -MN at 22% dietary SBM level significantly reduced -GM and GM concentrations, suggesting that -MN may enhance immune function by decreasing hepatic portal vein GM content. Serum biochemical indices serve as indicators of pathological processes and responses to therapeutic interventions. In experimental animal models, liver injury can be assessed through serum ALT and AST activities, with elevated levels indicating severe hepatocellular damage or necrosis, while increased serum UN concentration typically reflects reduced glomerular filtration rate due to renal injury. The present study demonstrated that increasing dietary SBM level from 22% to 37% significantly increased serum ALT, AST activities, and UN content, suggesting that SBM disrupts physiological and biochemical homeostasis and increases disease risk in piglets. Serum GLU and Ca concentrations reflect energy status and bone calcium absorption, respectively, while HDL promotes health by metabolizing excess cholesterol from extrahepatic tissues and preventing its accumulation. The observation that -MN supplementation at 22% SBM level significantly increased serum GLU, Ca, and HDL concentrations suggests that -MN enhances

energy utilization efficiency, promotes calcium absorption, and maintains cholesterol balance in piglets.

-Mannanase degrades GM in soybean meal into galactomannan oligosaccharides, which eliminate antinutritional factors, improve diet digestibility, and enhance nutrient absorption including amino acids. Amino acid transporters regulate intracellular amino acid concentrations and downstream signaling of amino acid receptors through coupled transport processes, delivering nutritional information and nutrients to cellular sites of need. SLC7A1 mediates the uptake and transport of basic amino acids (arginine and lysine) in mammalian cells, with upregulated SLC7A1 expression increasing intracellular concentrations of these basic amino acids. SLC7A11 encodes the cystine/glutamate transporter xCT, and its upregulation promotes glutamate absorption. SLC38A2 maintains intracellular L-glutamine levels and facilitates neutral amino acid transport into cells, with higher expression correlating with increased tissue neutral amino acid concentrations. The current results showed that -MN supplementation in the 37% SBM diet significantly increased jejunal expression of SLC7A1, SLC7A11, and SLC38A2. This effect may occur through -MN-mediated disruption of excessive GM -1,4-glycosidic bonds in the mesenteric vein, thereby promoting increased expression of these transporter genes. Conversely, increasing dietary SBM level from 22% to 37% reduced ileal SLC7A1 expression, suggesting that SBM may decrease intestinal absorption of basic amino acids (arginine and lysine) by downregulating SLC7A1 expression.

4 Conclusions

1. -GM content is higher than -GM content in most common feed ingredients.

2. Increased average daily -GM intake in weaner piglets is associated with reduced ADFI.

3. Elevated dietary -GM, -GM, and GM contents increase serum concentrations of -GM, -GM, and GM in the hepatic portal vein, carotid artery, and mesenteric vein of weaner piglets.

4. Dietary -MN supplementation reduces serum -GM, -GM, and GM concentrations while increasing precaval vein serum GLU, Ca, P, and UN contents, and upregulating jejunal expression of SLC7A1, SLC7A11, and SLC38A2 genes.

References

[1] JOËT T, LAFFARGUE A, SALMONA J, et al. Regulation of galactomannan biosynthesis in coffee seeds[J]. Journal of Experimental Botany, 2014, 65(1): 323–337.

[2] PEREIRA-NETTO A B, MENEGUIN R G, BIZ A, et al. A galactomannan-driven enhancement of the in vitro multiplication rate for the Marubakaido apple rootstock (Malus prunifolia (Willd) Borkh) is not related to the degradation of exogenous galactomannan[J]. Applied Biochemistry and Biotechnology, 2012, 166(1): 197–207.

[3] VAN ZYL W H, ROSE S H, TROLLOPE K, et al. Fungal -mannanases: mannan hydrolysis, heterologous production and biotechnological applications[J]. Process Biochemistry, 2010, 45(8): 1203–1213.

[4] MALGAS S, VAN DYK J S, PLETSCHKE B I. A review of the enzymatic hydrolysis of mannans and synergistic interactions between -mannanase, -mannosidase and -galactosidase[J]. World Journal of Microbiology and Biotechnology, 2015, 31(8): 1167–1175.

[5] LV J N, CHEN Y Q, GUO X J, et al. Effects of supplementation of beta-mannanase in corn-soybean meal diets on performance and nutrient digestibility in growing pigs[J]. Asian-Australasian Journal of Animal Science, 2013, 26(4): 579–587.

[6] SONG Y S, PÉREZ V G, PETTIGREW J E, et al. Fermentation of soybean meal and its inclusion in diets for newly weaned pigs reduced diarrhea and measures of immunoreactivity in plasma[J]. Animal Feed Science and Technology, 2010, 159(1): 41–49.

[7] PETRAITIENE R, PETRAITIS V, BACHER J D, et al. Effects of host response and antifungal therapy on serum and BAL levels of galactomannan and (1→3)- -D-glucan in experimental invasive pulmonary aspergillosis[J]. Medical Mycology, 2015, 53(6): 558–568.

[8] ZHOU P, LIU Y, YAN Q J, et al. Structural insights into the substrate specificity and transglycosylation activity of a fungal glycoside hydrolase family 5 -mannosidase[J]. Acta Crystallographica Section D, 2014, 70(11): 2970–2982.

[9] METZLER-ZEBELI B U, EBERSPÄCHER E, GRÜLL D, et al. Enzymatically modified starch ameliorates postprandial serum triglycerides and lipid metabolome in growing pigs[J]. PLoS One, 2015, 10(6): e0130553.

[10] KALAI K, NEHETE R S, GANGULY S, et al. Investigation of parasitic and bacterial diseases in pigs with analysis of hematological and serum biochemical profile[J]. Journal of Parasitic Diseases, 2012, 36(1): 129–134.

[11] LI Xuejian. Effects of -mannanase on performance and its mechanism in weaner piglets[D]. PhD Thesis. Shenyang: Shenyang Agricultural University, 2008.

[12] ZHANG Qin, MAO Shengyong, ZHU Weiyun. Application of -mannanase in animal production[J]. Feed Research, 2008, 40(9): 42–44.

[13] FERREIRA H C Jr, HANNAS M I, ALBINO L F T, et al. Effect of the addition of -mannanase on performance, metabolizable energy, amino acid di-

gestibility coefficients, and immune functions of broilers fed different nutritional levels[J]. Poultry Science, 2016, 95(8): 1848–1857.

[14] FOTIADIS D, KANAI Y, PALACÍN M. The SLC3 and SLC7 families of amino acid transporters[J]. Molecular Aspects of Medicine, 2013, 34(2/3): 139–158.

[15] MUSSINI F J, COTO C A, GOODGAME S D, et al. Effect of -mannanase on broiler performance and dry matter output using corn-soybean meal based diets[J]. International Journal of Poultry Science, 2011, 10(10): 778–781.

[16] VAN NEVEL C J, DECUYPERE J A, DIERICK N A, et al. Incorporation of galactomannans in the diet of newly weaned piglets: effect on bacteriological and some morphological characteristics of the small intestine[J]. Archives of Animal Nutrition, 2005, 59(2): 123–138.

[17] HORIE M, TAMIYA H, GOTO Y, et al. Nonspecific elevation of serum Aspergillus galactomannan antigen levels in patients with rheumatoid arthritis[J]. Respiratory Investigation, 2016, 54(1): 44–49.

[18] WICK M J, LEITHÄUSER F, REIMANN J. The hepatic immune system[J]. Critical Reviews in Immunology, 2002, 22(1): 47–103.

[19] VAN SPIL W E, DE GROOT J, LEMS W F, et al. Serum and urinary biochemical markers for knee and hip osteoarthritis: a systematic review applying the consensus BIPED criteria[J]. Osteoarthritis & Cartilage, 2010, 18(5): 605–612.

[20] VATSALYA V, AVILA D, FRIMODIG J C, et al. Liver injury assessment by VetScan VS2 analyzer and most frequently used ALT/GTP reagent[J]. Gastroenterology & Hepatology, 2016, 4(4): 107.

[21] GUO Yanmei, GUO Chuanyong. Clinical study of extremely elevated serum aspartate aminotransferase[J]. Health Vocational Education, 2012, 30(11): 154–155.

[22] ERÇIN C N, DOĞRU T, ÇELEBI G, et al. The relationship between blood urea nitrogen levels and metabolic, biochemical, and histopathologic findings in nondiabetic, nonhypertensive patients with nonalcoholic fatty liver disease[J]. Turkish Journal of Medical Sciences, 2016, 46(4): 985–991.

[23] KANENGONI A T, CHIMONYO M, ERLWANGER K H, et al. Growth performance, blood metabolic responses, and carcass characteristics of grower and finisher South African Windsnyer-type indigenous and Large White×Landrace crossbred pigs fed diets containing ensiled corncobs[J]. Journal of Animal Science, 2014, 92(12): 5739–5748.

[24] LIEBEN L, VERLINDEN L, MASUYAMA R, et al. Extra-intestinal calcium handling contributes to normal serum calcium levels when intestinal calcium absorption is suboptimal[J]. Bone, 2015, 81: 502–512.

[25] WANG Baihui, YANG Lei, LUO Yulong, et al. Effects of different feeding regimes on intestinal microbiota and fatty acid metabolism in Sunit sheep[EB/OL]. (2018-10-20)[2017-08-01]. http://kns.cnki.net/kcms/detail/11.2206.TS.20170802.1547.022.html

[26] HUNDAL H S, TAYLOR P M. Amino acid transceptors: gate keepers of nutrient exchange and regulators of nutrient signaling[J]. American Journal of Physiology-Endocrinology and Metabolism, 2009, 296(4): E603–E613.

[27] MÄÄTTÄ K, KUNNAS T, NIKKARI S T. Contribution of SLC7A1 genetic variant to hypertension: the TAMRISK study[J]. BMC Medical Genetics, 2013, 14: 69.

[28] YIN J, REN W K, DUAN J L, et al. Dietary arginine supplementation enhances intestinal expression of SLC7A7 and SLC7A1 and ameliorates growth depression in mycotoxin-challenged pigs[J]. Amino Acids, 2014, 46(4): 883–892.

[29] LI H T, HE X, ZHOU Z Y, et al. Expression levels of SLC7A11 in the skin of Kazakh sheep with different coat colors[J]. Hereditas, 2012, 34(10): 1314–1319.

[30] PLOESSL K, WANG L, LIEBERMAN B P, et al. Comparative evaluation of 18F-labeled glutamic acid and glutamine as tumor metabolic imaging agents[J]. Journal of Nuclear Medicine, 2012, 53(10): 1616–1624.

[31] TANAKA K, YAMAMOTO A, FUJITA T. Functional expression and adaptive regulation of Na+-dependent neutral amino acid transporter SNAT2/ATA2 in normal human astrocytes under amino acid-starved condition[J]. Neuroscience Letters, 2005, 378(2): 70–75.

[32] PALII S S, THIAVILLE M M, PAN Y X, et al. Characterization of the amino acid response element within the human sodium-coupled neutral amino acid transporter 2 (SNAT2) system A transporter gene[J]. Biochemical Journal, 2006, 395(3): 517–527.

Note: Figure translations are in progress. See original paper for figures.