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# Effects of sea buckthorn pomace on growth performance, serum metabolites and antioxidant indexes of growing pigs

Efeitos do bagaço de espinheiro marítimo no desempenho de crescimento, metabólitos séricos e índices antioxidantes de suínos em crescimento

Yibo Yan<sup>1</sup>; Nanxin Liang<sup>2</sup>; Zhaohui Ding<sup>2</sup>; Kai Zhang<sup>1</sup>; Lei Yue<sup>3</sup>; Wenjing Mei<sup>2</sup>; Wengang Li<sup>4\*</sup>; Xianyi Song<sup>4\*</sup>

# Highlights .

Adding sea buckthorn pomace improves the growth performance of growing pigs. Sea buckthorn pomace improves the immune and antioxidant functions of growing pigs. The optimal amount of sea buckthorn pomace for growing pigs is 1.5%.

# Abstract \_

Sea buckthorn pomace (SBP), an agro-industrial waste, containing useful nutrition compounds for animal production. This study aimed to evaluate the effect of growth performance and serum metabolisms in growing pig when consumed SBP. A total of 40 crossbred 70-day-old growing pig with initial body weight (IBW;  $30 \pm 1.5$  kg) were randomly assigned to 4 groups. The pigs were fed dietary supplementation of SBP (0.0%, 0.5, 1.5%, and 2.0%) for 30 d. It concluded that the appropriate level of 1.5% SBP supplementation could improve pig growth performance. Serum immune and antioxidant indexes performed best in 1.5% SBP supplementation group. We suggested that the appropriate level of SBP supplementation would be 1.5% for growing pig, which could improve their growth performance, serum immune and antioxidant indexes.

Key words: Sea buckthorn pomace. Growing pig. Growth performance. Serum antioxidants.

<sup>&</sup>lt;sup>1</sup> Profs. Drs., College of Animal Science, Shanxi Agricultural University, Taiyuan City, Shanxi Province, China. E-mail: 206434yyb@sxau.edu.cn; zhangkai2255@163.com

<sup>&</sup>lt;sup>2</sup> Graduate Student, College of Animal Science, Shanxi Agricultural University, Taiyuan City, Shanxi Province, China. E-mail: 841383525@qq.com; 1948708106@qq.com; 15896542294@163.com

<sup>&</sup>lt;sup>3</sup> Researchers, College of Animal Science, Shanxi Agricultural University, Taiyuan City, Shanxi Province, China. E-mail: leilei6136@163.com

<sup>&</sup>lt;sup>4</sup> Profs., College of Animal Science, Shanxi Agricultural University, Taiyuan City, Shanxi Province, China. E-mail: bobo10679888@163.com; sxnysxy@163.com

<sup>\*</sup> Author for correspondence

## Resumo

Bagaço de espinheiro marítimo (SBP), um resíduo agroindustrial, contendo compostos nutricionais úteis para a produção animal. Este estudo teve como objetivo avaliar o efeito do SBP sobre o desempenho e metabolismo séricos em suínos em crescimento. Um total de 40 suínos mestiços de 70 dias de idade com peso inicial (IBW) de 301,5 kg foram distribuídos aleatoriamente em 4 grupos. Os suínos foram alimentados com suplementação dietética de SBP (0,0%, 0,5, 1,5% e 2,0%) por 30 dias. Concluiu-se que o nível adequado de 1,5% de suplementação de SBP poderia melhorar o desempenho dos suínos. Os índices imunológicos e antioxidantes séricos apresentaram melhor desempenho no grupo de suplementação de SBP a 1,5%. Sugerimos que o nível adequado de suplementação de SBP seria de 1,5% para suínos em crescimento, o que poderia melhorar seu desempenho, índices séricos, imunológicos e antioxidantes. **Palavras-chave:** Bagaço de espinheiro-marítimo. Suínos em crescimento. Desempenho de crescimento. Antioxidantes séricos.

## Introduction \_

With an increasing feed cost in the animal production industry, it is necessary to operate for the alternative feed resources to conventional feeds, especially agricultural and industrial by-products (Yang et al., 2021). The use of agro-industrial waste, as functional feed materials, could be a promising strategy that would reduce feed costs while the nutritional qualities of the feed are still maintained. As a by-product of processing, pomace not only shows a high yield but also rich in nutrients such as organic acids, polyphenols, vitamins, minerals, and dietary fiber (Dannenberger et al., 2018; Tamkutė et al., 2019). Hence, in recent years, numerous reports focus on the use of pomace to develop new ingredient to alleviate the shortage of feed resources for pig production have continued to emerge (Correia et al., 2017; Alfaia et al., 2022; Zou et al., 2022).

Sea buckthorn (*Hippophae rhamnoides*), with a high adaptability, rapid

growth, and protect against erosion ability, has been widely cultivated in dry areas. It was not only used for a fruit, but also for an herb due to possesses numerous edible nutritional and medicine composition (Olas, 2016; Lee et al., 2021; Ran et al., 2021). Sea buckthorn pomace (SBP), accounting for 20% of the total fruit weight, is a by-product of sea buckthorn processing. It is usually discarded as waste which could impair the environment and cause the economic loss. Previous studies have been showed the SBP was abundance in vitamin, carotenoids, polyphenols, fatty acids, and sterols (Jones et al., 2019; K. Wang et al., 2022a; Z. Wang et al., 2022b). Hence, it was urged to find an environment-friendly way to utilize the SBP.

This study was conducted to evaluate the growth performance and antioxidant properties of SBP offered to growing pig which aimed to promote the use of this extremely valuable residual resource in the animal production.

## Materials and Methods \_\_\_\_\_

## Animals and treatments

A total of 40 70-day-old healthy growing pig (Duroc × Landrace × Yorkshire; 20 female and 20 castrated male) with similar initial body weight (IBW; 30  $\pm$ 1.5 kg) were randomly assigned to 4 experimental diets with supplemented SBP (as air dry basis): 1) 0% SBP (basal diets); 2) 0.5% SBP (basal diets containing 0.5% SBP); 3) 1.5% SBP (basal diets containing 1.5% SBP); 4) 2.0% SBP (basal diets containing 1.5% SBP). All animals were divided into 20 pens, and each treatment had 5 replicate pens with 1 castrated male and 1 female pig. The experimental diets were formulated to meet the nutrient requirements of growing pig (Table 1; National Research Council [NRC], 2012; Aonong group; Xiamen City, China). The SBP was obtained from Shanxi Xincheng Veterinary Drug Co., LTD (Taigu, China). The animals were housed in 20 cages with equipped a feeder and a nipple drinker and had free access to feed (offered four times daily: 7:00, 12:00, 17:00, and 21:30) and water at all times. The experiment lasted for 30 days. All experimental procedures were in accordance with the guideline established by the Regulation for the Administration of Affairs Concerning Experimental Animals, and approved by the Animal Welfare and Research Ethics Committee at Shanxi Agricultural University (SXAU-EAW-2021P. NL.003028001).

#### Table 1

#### Ingredient composition and nutritional levels in experiment diet

Items	content
Ingredient	
Corn, %	65
Soybean meal, %	15
Wheat bran, %	5
Premix (S1512) , %	15
Total, %	100
Nutritional level	
Digestible energy, MJ/kg	13.2
Dry matter, %	86.0
Crude protein, %	25.0
Ether extract, %	6.01
Crude fiber, %	10.00
Ash, %	30.0
Calcium, %	4.00
Phosphorus, %	1.00
Lysine, %	0.70
Methionine, %	0.20

The contents of vitamins and trace elements per kilogram of diets were as follows: VA 2260 IU; VD 388 IU; VE 18 IU; Cu 7 mg; Fe 98 mg; Mn 7 mg; I 0.25 mg; Except for DE, all the compositions of diet were measured values.

# Procedures and sample collection

The pigs were weighed individual on day 1 and 30 before morning feeding (07:00) and their ADGs were calculated over the 30 days. The average daily feed intake (ADFI) was calculated over the 30 days by daily feed intake, feed conversion ratio was calculated according to ADFI to ADG. Feed and SBP (100 g) sample were collected before morning feeding on d 1, 16, and 30.

The precaval vein blood samples of all animals were collected in evacuated tubes on the day 30 prior to morning feeding. The blood samples were centrifuged at  $3000 \times g$  (4 °C) for 20 mins, and the serum was stored at -20 °C for further analyzed.

# Chemical analysis

Feed and SBP samples were freezedried for 72 h, ground to pass through a 1 mm screen, and then stored at room temperature. The DM of feed samples were determined by drying at 105°C for 24 h in a forced air oven (method 925.45) and organic matter (OM) was measured as loss in dry weight upon complete combustion of a sample at 550°C (Association of Official Analytical Chemists [AOAC], 2006) for 6 h in a muffle furnace (method 942.05). Total nitrogen content in feed samples were determined by the micro-Kjeldahl method (K1100, Hanon instruments, Jinan, China), and CP was calculated as N × 6.25. Ether extract (method 920.29) was measured by using a reflux system (Ankom XT 15, Fairport, NY, USA) with petroleum ether at 90°C for 1 h. The crude fiber was analyzed using the Ankom A200 fiber analyzer (Ankom Technology, Fairport, NY, USA ; Van Soest et al., 1991).

The blood routine analysis included white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoalobin (MCH), Mean corpusular hemoglobin concerntration (MCHC), platelet (PLT), red blood cell distribution widthstandarddeviation (RDW-SD), red blood cell volume distribution width (RDW-CV), platelet distribution width (PDW), mean platelet volume (MPV), platelet -larger cell ratio (P-LCR), plateletcrit (PCT), Neutrophil (NEUT#), LYMPH#, monocyte (MONO#), eosinophil(EO#), (BASO)#, Neutrophil Basophils (NEUT), LYMPH, MONO, EO, was performed using an automatic hematology analyzer (Mindray BS-420 biochemical analyzer; Beijing Huaying Bioengineering Institute, Beijing, China). Serum total protein, albumin, globulin, low-density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol (TC), atherosclerosis index (AI), creatinine, blood urea nitrogen (BUN), glucose, free fat acids (FAA), serum ammonia (SEA), aminotransferase(AST), aspartate aminotransferase alanine (ALT), free hemoglobin (FH), β-hydroxybutyric acid (BHBA), immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A were determined by using an automatic biochemistry analyzer (Hitachi 7160, Hitachi High-Technologies Corporation, Tokyo, Japan), following the protocols of commercial kits (Biobase biotech Co. LTD, Jinan, China). The serum superoxide dismutase (SOD), malondialdehyde (MDA), glutathioneperoxidase (GSH-px), catalase (CAT), and total antioxidant capacity (T-AOC) were measured by enzyme-linked immune sorbent assay (ELISA) kits (Lab systems Multiskan MS Type 352, Helsinki, Finland). The serum metabolites were determined at Beijing Huaying Bioengineering Institute (Beijing, China).



#### Statistical analyses

The data were presented as means ± SD. The data were subjected to one-way ANOVA with SPSS 20.0, significant differences amongst treatments were determined using Duncan's multiple range tests. The P values < 0.05 was considered statistically significant.

#### **Results and Discussion**

#### Growth performance

As designed, the IBW was no differ (P > 0.05) among the 4 groups (Table 2). The FBW, ADFI, and ADG were greatest (P < 0.05) in 1.5% SBP group and lowest (P < 0.05) in 2.0% SBP group. The F: G was lowest (P < 0.05) in 1.5% SBP group and greatest (P < 0.05) in 0% SBP group. The SBP contains bioactive substances which have been recognized to possess antioxidative and antimicrobial properties. Studies showing the use of pomace by-products have inconsistent results basically in terms of growth performance (Pop et al., 2015; Yang

et al., 2021). For example, supplementation of 5% or 10% grape pomace was reported to show no significant improvement on growth performance of broiler chickens (Chamorro et al., 2015), whereas inclusion of 7.5% grape pomace reduced the average daily feed intake (Kumanda et al., 2019) and supplemented from 1% to 2% grape pomace could improve body weight (Pop et al., 2015). This would be explained by the pomace by-products levels, bioactive substances, and animal species. In the present study, we found that both ADFI and ADG were greatest in 1.5% SBP group and lowest in 2.0% SBP group, which indicated that 0.5% to 1.5% SBP groups had a better response than in 2.0% SBP group. A previous study found that supplementing with flavones of sea buckthorn fruits quadratically increased ADG, ADFI, and final BW which could explained the 2.0% SBP group have a lesser ADG and ADFI than in 0.5% and 1.5% SBP groups (Ma et al., 2015). In addition, these results provided an insight that the pigs could adapt to 0.5 % to 2.0 % SBP groups as feeds ingredient and it was no toxicity for growing pigs.

#### Table 2

Growth performance of pig fed diets in which supplemented with SBP in a corn-soybear	n meal diet

Items	SBP levels				
items	0%	0.5%	1.5%	2.0%	
IBW, kg	31.80±1.79	31.67±1.92	30.60±1.14	31.01±1.48	
FBW, kg	54.24±2.04 <sup>b</sup>	55.45±1.54 <sup>b</sup>	58.35±1.42°	51.24±3.94ª	
ADFI, kg/d	1.87±0.29 <sup>b</sup>	1.88±0.26°	1.93±0.31 <sup>d</sup>	1.68±0.28ª	
ADG, kg/d	0.75±0.05ª	0.79±0.06 <sup>b</sup>	0.93±0.04°	0.71±0.04ª	
F: G	2.52±0.18°	2.38±0.19 <sup>b</sup>	2.09±0.09ª	2.38±0.13 <sup>b</sup>	

IBW = Initial body weight; FBW = Final body weight; ADFI = average daily feed intake; ADG = average daily gain; F:G = the ratio of feed to gain; SBP = seabuckthorn pomance.

# Blood physiological indexes

The MCH, PDW, P-LCR, PCT, NEUT#, LYMPH#, MONO#, EO#, BASO#, NEUT, MONO, EO, and BASO no differ among the 4 groups (P > 0.05; Table 3). The WBC was lesser, whereas the RDW-SD was greater in 0.5%, 1.5%, and 2.0% SBP groups than in 0.0% SBP group. The RDW-CV was greatest in 1.5% SBP group and lowest (P < 0.05) in 0.0% SBP group. The MCHC and LYMPH were greatest (P < 0.05) in 0.0% SBP group and lowest (P < 0.05) in 1.5% SBP group. The RBC, HGB, HCT, MCV, PLT, and MPV were greatest (P < 0.05) in 2.0% SBP group and lowest (P < 0.05) in 0.0% SBP group. As a part of the immune system, WBC could help the body to fight off infections and diseases. The RBC, as an oxygen carrier, located in every cell which need oxygen to grow, reproduce, and stay healthy. In addition, it was the largest part of a complete blood count. Our result showed a greater RBC in 1.5% and 2.0% SBP groups which indicated that the blood loss, hemolysis, or decreased their production may cause in 0% SBP group. The PLT was increased in SBP groups which indicated these groups have a better capability to prevent and stop bleeding.

## Serum metabolites indexes

The serum concentrations of total protein, albumin, globulin, LDL, and glucose were not influenced by SBP supplementation levels (P > 0.05; Table 4). The serum concentrations of TC, AI, FAA, FH, and BHBA were greatest (P < 0.05) in 0.0% SBP group

and lowest (P < 0.05) in 2.0% SBP group. The serum concentrations of HDL, IgM, IgG, and IgA were greatest (P < 0.05) in 0.2% SBP group and lowest (P < 0.05) in 0.0% SBP group. The serum concentrations of creatinine, BUN, SEA, AST, and ALT were lowest (P < 0.05) in 1.5% SBP group and greatest in 2.0% SBP group. The serum biochemical components could reflect the metabolic condition and then use to monitor the health status of animals (Liu et al., 2022, 2023). Commonly, a greater serum TC concentration means a higher risk of cardio-cerebrovascular disease in mammal. Our results showed that the TC was lesser in 1.5% and 2.0% SBP groups and HDL was greater in 2.0% SBP group, which in agreement with a previous study on pig offered Aronia melanocarpa Pomace (Ren et al., 2022). The AST and ALT, as the important indicators that reflect liver damage, were increased as the liver was damaged (Schomaker et al., 2020). In the present study, we found the serum AST and ALT were greatest in 2.0% SBP group, which influenced the nutrition metabolism. The TC represents the combined amount of LDL and HDL. The concentrations of serum IgA, IgG, and IgM were increased in SBP groups, which showed SBP could enhancing humoral immune response in growing pig. Serum IgG concentration were ranged between 8.32-10.06 g/L, which accounts for ~80% of the total serum immunoglobulin because it is the vital role to resist the invasion of viral, bacterial, and fungal infections (Larsson, 2008).

#### Table 3

Blood physiological indexes of pig fed diets in which supplemented with SBP in a corn-soybean meal diet

10ms0%0.5%1.5%2.0%WBC, × 109/L27.97±6.49°18.08±4.77°17.44±0.98°14.07±1.78°RBC, ×1012/L6.38±0.34°6.49±0.61*b7.12±0.5°7.47±0.45°HGB, g/L98.00±5.2°100.20±11.03°106.20±7.63*b117.60±9.84*HCT, %53.36±2.08°54.50±5.96°59.92±5.87°66.76±4.67*MCV, fL83.7±3.14°83.96±4.53°84.08±3.38°90.26±4.04*MCH, pg15.38±0.8615.44±0.7614.94±0.2715.90±0.84MCHC, g/L176.00±5.15°177.60±5.22°184.00±3.54*183.80±3.96*PLT, × 109/L394.20±64.23°404.60±60.02°438.60±67.77°564.40±89.07*RDW-SD, fL57.36±1.49*61.6±4.48*63.56±2.94*63.36±2.96*PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98**12.66±0.41*PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.64±0.86LYMPH, %58.48±1.31*57.98±1.33*52.4±3.99* </th <th>ltomo</th> <th colspan="4">SBP levels</th>	ltomo	SBP levels			
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HGB, g/L98.00±5.2°100.20±11.03°106.20±7.63°b117.60±9.84bHCT, %53.36±2.08°54.50±5.96°59.92±5.87°66.76±4.67°MCV, fL83.7±3.14°83.96±4.53°84.08±3.38°90.26±4.04°MCH, pg15.38±0.8615.44±0.7614.94±0.2715.90±0.84MCHC, g/L176.00±5.15°177.60±5.22°184.00±3.54°183.80±3.96°PLT, × 109/L394.20±64.23°404.60±60.02°438.60±67.77°564.40±89.07°RDW-SD, fL57.36±1.49°61.6±4.48°63.56±2.94°63.36±2.96°RDW-CV, %20.7±1.08°21.84±1.3°22.32±0.95°21.18±0.59°PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°12.66±0.41°P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°MONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	WBC, × 109/L	27.97±6.49 <sup>b</sup>	18.08±4.77ª	17.44±0.98ª	14.07±1.78ª
HCT. MCV, FL53.36±2.08°54.50±5.96°59.92±5.87°66.76±4.67°MCV, FL83.7±3.14°83.96±4.53°84.08±3.38°90.26±4.04°MCH, pg15.38±0.8615.44±0.7614.94±0.2715.90±0.84MCHC, g/L176.00±5.15°177.60±5.22°184.00±3.54°183.80±3.96°PLT, × 109/L394.20±64.23°404.60±60.02°438.60±67.77°564.40±89.07°RDW-SD, fL57.36±1.49°61.6±4.48°63.56±2.94°63.36±2.96°RDW-CV, %20.7±1.08°21.84±1.3°22.32±0.95°21.18±0.59°°PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°°12.66±0.41°P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°MONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	RBC, ×1012/L	6.38±0.34ª	6.49±0.61 <sup>ab</sup>	7.12±0.5 <sup>bc</sup>	7.47±0.45°
MCV, fL83.7±3.14°83.96±4.53°84.08±3.38°90.26±4.04°MCH, pg15.38±0.8615.44±0.7614.94±0.2715.90±0.84MCHC, g/L176.00±5.15°177.60±5.22°184.00±3.54°183.80±3.96°PLT, × 109/L394.20±64.23°404.60±60.02°438.60±67.77°564.40±89.07°RDW-SD, fL57.36±1.49°61.6±4.48°63.56±2.94°63.36±2.96°RDW-CV, %20.7±1.08°21.84±1.3°°22.32±0.95°21.18±0.59°PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°°12.66±0.41°P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°MONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	HGB, g/L	98.00±5.2ª	100.20±11.03ª	106.20±7.63ab	117.60±9.84 <sup>b</sup>
MCH, pg15.38±0.8615.44±0.7614.94±0.2715.90±0.84MCHC, g/L176.00±5.15°177.60±5.22°184.00±3.54°183.80±3.96°PLT, × 109/L394.20±64.23°404.60±60.02°438.60±67.77°564.40±89.07°RDW-SD, fL57.36±1.49°61.6±4.48°63.56±2.94°63.36±2.96°RDW-CV, %20.7±1.08°21.84±1.3°°22.32±0.95°21.18±0.59°°PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°°12.66±0.41°P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°MONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	HCT, %	53.36±2.08ª	54.50±5.96ª	59.92±5.87ª	66.76±4.67 <sup>b</sup>
MCHC, g/L176.00±5.15°177.60±5.22°184.00±3.54°183.80±3.96°PLT, × 109/L394.20±64.23°404.60±60.02°438.60±67.77°564.40±89.07°RDW-SD, fL57.36±1.49°61.6±4.48°63.56±2.94°63.36±2.96°RDW-CV, %20.7±1.08°21.84±1.3°22.32±0.95°21.18±0.59°PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°12.66±0.41°P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°MONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	MCV, fL	83.7±3.14ª	83.96±4.53ª	84.08±3.38ª	90.26±4.04 <sup>b</sup>
PLT, × 109/L394.20±64.23°404.60±60.02°438.60±67.77°564.40±89.07bRDW-SD, fL57.36±1.49°61.6±4.48b63.56±2.94b63.36±2.96bRDW-CV, %20.7±1.08°21.84±1.3°b22.32±0.95b21.18±0.59°bPDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°b12.66±0.41°bP-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°bMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	MCH, pg	15.38±0.86	15.44±0.76	14.94±0.27	15.90±0.84
RDW-SD, fL57.36±1.49°61.6±4.48°63.56±2.94°63.36±2.96°RDW-CV, %20.7±1.08°21.84±1.3°°22.32±0.95°21.18±0.59°°PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°°12.66±0.41°P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°MONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	MCHC, g/L	176.00±5.15ª	177.60±5.22ª	184.00±3.54 <sup>b</sup>	183.80±3.96 <sup>b</sup>
RDW-CV, %20.7±1.08a21.84±1.3ab22.32±0.95b21.18±0.59abPDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66a11.72±0.45a12.12±0.98ab12.66±0.41bP-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.07BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	PLT, × 109/L	394.20±64.23ª	404.60±60.02°	438.60±67.77ª	564.40±89.07 <sup>b</sup>
PDW, fL17.16±1.7815.88±1.2316.26±2.4116.98±2.01MPV, fL11.22±0.66ª11.72±0.45ª12.12±0.98ªb12.66±0.41bP-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99ª54.32±6.22ªbMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	RDW-SD, fL	57.36±1.49ª	61.6±4.48 <sup>b</sup>	63.56±2.94 <sup>b</sup>	63.36±2.96 <sup>b</sup>
MPV, fL11.22±0.66°11.72±0.45°12.12±0.98°b12.66±0.41°P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31°57.98±1.33°52.4±3.99°54.32±6.22°°MONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	RDW-CV, %	20.7±1.08ª	21.84±1.3 <sup>ab</sup>	22.32±0.95 <sup>b</sup>	21.18±0.59 <sup>ab</sup>
P-LCR, %37.62±1.6737.16±5.3241.74±6.8242.18±4.91PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	PDW, fL	17.16±1.78	15.88±1.23	16.26±2.41	16.98±2.01
PCT, %0.10±0.180.26±0.220.29±0.330.24±0.29NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	MPV, fL	11.22±0.66ª	11.72±0.45 <sup>a</sup>	12.12±0.98 <sup>ab</sup>	12.66±0.41 <sup>b</sup>
NEUT#, ×109/L8.57±3.016.70±0.986.61±2.536.13±2.63LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	P-LCR, %	37.62±1.67	37.16±5.32	41.74±6.82	42.18±4.91
LYMPH#, ×109/L14.18±5.0110.86±4.178.88±2.488.63±3.81MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	PCT, %	0.10±0.18	0.26±0.22	0.29±0.33	0.24±0.29
MONO#, ×109/L0.66±0.210.55±0.380.53±0.280.44±0.15EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	NEUT#, ×109/L	8.57±3.01	6.70±0.98	6.61±2.53	6.13±2.63
EO#, ×109/L0.39±0.320.33±0.160.34±0.120.23±0.05BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	LYMPH#, ×109/L	14.18±5.01	10.86±4.17	8.88±2.48	8.63±3.81
BASO#, ×109/L0.42±0.160.41±0.150.37±0.220.32±0.17NEUT, %40.40±4.7638.72±5.635.40±1.735.36±0.86LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	MONO#, ×109/L	0.66±0.21	0.55±0.38	0.53±0.28	0.44±0.15
NEUT, %         40.40±4.76         38.72±5.6         35.40±1.7         35.36±0.86           LYMPH, %         58.48±1.31 <sup>b</sup> 57.98±1.33 <sup>b</sup> 52.4±3.99 <sup>a</sup> 54.32±6.22 <sup>ab</sup> MONO, %         3.10±1.25         3.08±1.29         2.76±0.55         2.90±1.05	EO#, ×109/L	0.39±0.32	0.33±0.16	0.34±0.12	0.23±0.05
LYMPH, %58.48±1.31b57.98±1.33b52.4±3.99a54.32±6.22abMONO, %3.10±1.253.08±1.292.76±0.552.90±1.05	BASO#, ×109/L	0.42±0.16	0.41±0.15	0.37±0.22	0.32±0.17
MONO, % 3.10±1.25 3.08±1.29 2.76±0.55 2.90±1.05	NEUT, %	40.40±4.76	38.72±5.6	35.40±1.7	35.36±0.86
	LYMPH, %	58.48±1.31 <sup>b</sup>	57.98±1.33 <sup>b</sup>	52.4±3.99ª	54.32±6.22 <sup>ab</sup>
EO, % 2.22±1.62 1.68±0.33 1.58±0.45 1.50±0.63	MONO, %	3.10±1.25	3.08±1.29	2.76±0.55	2.90±1.05
	EO, %	2.22±1.62	1.68±0.33	1.58±0.45	1.50±0.63
BASO, % 2.28±0.61 2.18±0.26 1.90±0.89 1.76±0.30	BASO, %	2.28±0.61	2.18±0.26	1.90±0.89	1.76±0.30

SBP = seabuckthorn pomance; WBC = white blood cell; RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpusular hemoglobin concerntration; PLT = platelet; RDW-SD = red blood cell distribution width-standarddeviation; RDW-CV = red blood cell volume distribution width; PDW = platelet distribution width; MPV = mean platelet volume; P-LCR = platelet -larger cell ratio; PCT = plateletcrit; NEUT = Neutrophil; MONO = monocyte; EO = eosinophil; BASO = basophils; NEUT = Neutrophil.

## Table 4

Serum metabolites indexes of pig fed diets in which supplemented with SBP in a corn-soybean meal diet

SBP levels			
0%	0.5%	1.5%	2.0%
74.85±1.98	73.83±2.73	74.68±2.92	75.51±4.68
30.9±2.36	28.66±2.01	28.49±3.85	31.81±1.6
43.96±3.00	45.17±2.65	46.19±2.45	43.7±3.49
2.40±0.28°	2.28±0.24°	1.93±0.26 <sup>b</sup>	1.46±0.19ª
0.61±0.09ª	0.71±0.13ª	0.74±0.09ª	$0.91 \pm 0.06^{b}$
2.49±0.35	2.70±0.16	2.53±0.19	2.32±0.38
2.96±0.26 <sup>d</sup>	2.27±0.47°	1.62±0.36 <sup>b</sup>	0.88±0.22ª
131.72±5.66 <sup>b</sup>	130.07±7.07 <sup>b</sup>	113.42±8.74ª	153.05±5.48°
7.52±0.20°	6.23±0.83 <sup>b</sup>	5.14±0.66ª	8.33±0.48 <sup>d</sup>
4.61±0.05	4.95±0.40	5.28±0.64	4.79±0.59
0.74±0.29 <sup>b</sup>	0.73±0.41 <sup>b</sup>	$0.66 \pm 0.37^{b}$	0.58±0.24ª
26.08±0.61°	24.13±0.95 <sup>b</sup>	22.06±1.37ª	28.94±1.08 <sup>d</sup>
76.96±14.4ª	72.37±14.5ª	71.02±7.25ª	110.38±7.94 <sup>b</sup>
93.07±11.25ª	88.42±8.93ª	84.99±3.03ª	113.19±7.56 <sup>b</sup>
27.55±1.20°	26.13±0.80 <sup>b</sup>	25.10±0.79 <sup>b</sup>	23.54±0.82ª
0.19±0.07 <sup>b</sup>	$0.16 \pm 0.02^{b}$	0.15±0.03 <sup>b</sup>	0.09±0.03ª
0.61±0.04ª	$0.68 \pm 0.04^{b}$	0.72±0.05 <sup>b</sup>	0.92±0.03°
8.32±0.52ª	9.31±0.43 <sup>b</sup>	$9.54 \pm 0.89^{b}$	10.06±0.78 <sup>b</sup>
0.93±0.04ª	0.99±0.11ª	1.11±0.05 <sup>b</sup>	1.24±0.1°
	$74.85\pm1.98$ $30.9\pm2.36$ $43.96\pm3.00$ $2.40\pm0.28^{\circ}$ $0.61\pm0.09^{\circ}$ $2.49\pm0.35$ $2.96\pm0.26^{\circ}$ $131.72\pm5.66^{\circ}$ $7.52\pm0.20^{\circ}$ $4.61\pm0.05$ $0.74\pm0.29^{\circ}$ $26.08\pm0.61^{\circ}$ $76.96\pm14.4^{\circ}$ $93.07\pm11.25^{\circ}$ $27.55\pm1.20^{\circ}$ $0.19\pm0.07^{\circ}$ $0.61\pm0.04^{\circ}$ $8.32\pm0.52^{\circ}$	$0\%$ $0.5\%$ $74.85\pm1.98$ $73.83\pm2.73$ $30.9\pm2.36$ $28.66\pm2.01$ $43.96\pm3.00$ $45.17\pm2.65$ $2.40\pm0.28^{\circ}$ $2.28\pm0.24^{\circ}$ $0.61\pm0.09^{\circ}$ $0.71\pm0.13^{\circ}$ $2.49\pm0.35$ $2.70\pm0.16$ $2.96\pm0.26^{\circ}$ $2.27\pm0.47^{\circ}$ $131.72\pm5.66^{\circ}$ $130.07\pm7.07^{\circ}$ $7.52\pm0.20^{\circ}$ $6.23\pm0.83^{\circ}$ $4.61\pm0.05$ $4.95\pm0.40$ $0.74\pm0.29^{\circ}$ $0.73\pm0.41^{\circ}$ $26.08\pm0.61^{\circ}$ $24.13\pm0.95^{\circ}$ $76.96\pm14.4^{\circ}$ $72.37\pm14.5^{\circ}$ $93.07\pm11.25^{\circ}$ $88.42\pm8.93^{\circ}$ $27.55\pm1.20^{\circ}$ $26.13\pm0.80^{\circ}$ $0.19\pm0.07^{\circ}$ $0.16\pm0.02^{\circ}$ $0.61\pm0.04^{\circ}$ $0.68\pm0.04^{\circ}$ $8.32\pm0.52^{\circ}$ $9.31\pm0.43^{\circ}$	$0\%$ $0.5\%$ $1.5\%$ $74.85\pm 1.98$ $73.83\pm 2.73$ $74.68\pm 2.92$ $30.9\pm 2.36$ $28.66\pm 2.01$ $28.49\pm 3.85$ $43.96\pm 3.00$ $45.17\pm 2.65$ $46.19\pm 2.45$ $2.40\pm 0.28^{\circ}$ $2.28\pm 0.24^{\circ}$ $1.93\pm 0.26^{\circ}$ $0.61\pm 0.09^{\circ}$ $0.71\pm 0.13^{\circ}$ $0.74\pm 0.09^{\circ}$ $2.49\pm 0.35$ $2.70\pm 0.16$ $2.53\pm 0.19$ $2.96\pm 0.26^{\circ}$ $2.27\pm 0.47^{\circ}$ $1.62\pm 0.36^{\circ}$ $131.72\pm 5.66^{\circ}$ $130.07\pm 7.07^{\circ}$ $113.42\pm 8.74^{\circ}$ $7.52\pm 0.20^{\circ}$ $6.23\pm 0.83^{\circ}$ $5.14\pm 0.66^{\circ}$ $4.61\pm 0.05$ $4.95\pm 0.40$ $5.28\pm 0.64$ $0.74\pm 0.29^{\circ}$ $0.73\pm 0.41^{\circ}$ $0.66\pm 0.37^{\circ}$ $26.08\pm 0.61^{\circ}$ $24.13\pm 0.95^{\circ}$ $22.06\pm 1.37^{\circ}$ $76.96\pm 14.4^{\circ}$ $72.37\pm 14.5^{\circ}$ $71.02\pm 7.25^{\circ}$ $93.07\pm 11.25^{\circ}$ $88.42\pm 8.93^{\circ}$ $84.99\pm 3.03^{\circ}$ $27.55\pm 1.20^{\circ}$ $26.13\pm 0.80^{\circ}$ $25.10\pm 0.79^{\circ}$ $0.19\pm 0.07^{\circ}$ $0.16\pm 0.02^{\circ}$ $0.15\pm 0.03^{\circ}$ $0.61\pm 0.04^{\circ}$ $0.68\pm 0.04^{\circ}$ $0.72\pm 0.05^{\circ}$

TC = total cholesterol; HDL = high-density lipoprotein; LDL = low density lipoprotein; AI = atherosclerosis index; BUN = blood urea nitrogen; FAA = free fatty acids; SEA = serum ammonia; AST = aspartate transaminase; ALT = Alanine transaminase; FH = free hemoglobin; BHBA =  $\beta$ -hydroxybutyric acid; IgM = immunoglobulins M; Ig G = immunoglobulins G; IgA = immunoglobulins A; SBP = seabuckthorn pomance.

## Serum antioxidant indexes

The serum T-AOC, SOD, GST-px, and CAT were greatest (P < 0.05) in 2.0 % SBP group and lowest (P < 0.05) in 0.0% SBP group and the MDA was greatest (P < 0.05) in 0.0% SBP group and lowest (P < 0.05) in 2.0% SBP group (Table 5). Numerous studies demonstrated that seabuckthorn and its by-product possesses a strong antioxidant activity (Górnaś et al., 2014; Lyu et al., 2022; Saracila et al., 2022). The antioxidant activity of the sea buckthorn and its by-product is one of the main functional activities which have strong correlation to the flavonoid monomers (Lyu et al., 2022). Oxidative stress may impair numerous biomolecules, for example, nucleic acids, proteins, and lipids and so on (Lyu et al., 2022). The MDA, as a biomarker of oxidative stress, which reflecting the body healthy condition and a greater MDA concentration was considered harmfully. In the present study, a reduction in MDA concentration in SBP group, showing SBP supplementation was effective against oxidative lipid damage in growing pig. The T-AOC, as a common parameter, which reflected the capability of antioxidant. The SOD is an enzyme which existing in all living cells. The GSH-px is a vital antioxidant substance which contributes to defending oxidative stress. The CAT could participate in the biodefence system to protect cell from H2O2 toxicities. Our results showed that the serum T-AOC, SOD, GSH-px, and CAT increased in 1.5% and 2.0 % SBP group, which could explain by flavones in SBP to enhance antioxidant capability at a certain extent.

#### Table 5

Serum antioxidant indexes of pig fed diets in which supplemented with SBP in a corn-soybean meal diet

ltomo	SBP levels			
Items	0%	0.5%	1.5%	2.0%
T-AOC, U/mL	6.88±0.31ª	7.83±0.27 <sup>ab</sup>	8.72±0.28 <sup>b</sup>	13.9±2.43°
SOD, U/mL	27.59±4.31ª	38.57±1.66 <sup>b</sup>	42.76±1.76°	54.81±3.45 <sup>d</sup>
GSH-Px, U/mL	273.36±15.14ª	306.94±21.4 <sup>ab</sup>	327.17±35.67 <sup>bc</sup>	375.19±61.98°
CAT, U/mL	42.31±1.34ª	44.21±1.33ª	48.18±2.94 <sup>b</sup>	50.56±4.32 <sup>b</sup>
MDA, nmol/mL	4.03±0.97 <sup>b</sup>	3.42±0.68 <sup>ab</sup>	3.29±0.33ab	2.63±0.63ª

T-AOC = total antioxidant capacity; CAT = catalase; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malondialdehyde; SBP = seabuckthorn pomance.

## Conclusion \_\_\_\_\_

It concluded that the appropriate level of 1.5% SBP supplementation could improve growth performance, serum immune and antioxidant indexes in growing pig.

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## Conflicts of interest \_

The authors declare no conflict of interest.

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