



# Effect of Dietary Sea Buckthorn Pomace Supplementation on Growth Performance, Serum Biochemical, Immune, and Antioxidant Indexes in Weaned Piglets

Yibo Yan, Zhaohui Ding, Nanxin Liang, Kai Zhang, Lei Yue, Wengang Li\* and Xianyi Song\*

College of Animal Science, Shanxi Agricultural University, Taiyu 030801, P.R. China.

## ABSTRACT

Sea buckthorn pomace is an industrial by-product, rich in a variety of functional compounds, but it has not been effectively used yet. This experiment was conducted to evaluate the effects of sea buckthorn pomace (SBP) supplementary levels on production performance and serum metabolites in weaned piglets. A total of 40 28-day-old weaned piglets (Duroc × Landrace × Yorkshire; half female and half castrated male) with  $10 \pm 0.30$  kg of IBW were randomly assigned to 4 groups (1 female and 1 castrated male/pen and 5 replicates/group). Piglets were consumed a basal diet, or the basal diet supplemented with 0.5%, 1.0%, and 2.0% SBP for 30 d, respectively. The results showed that the ADG, ADFI, and FBW were greater ( $p < 0.05$ ), whereas the FCR was lesser ( $p < 0.05$ ) in 2.0% and 1.0% SBP group than in 0% SBP group. The concentration of C3 was greater ( $p < 0.05$ ) in 2.0% SBP group than in another group. The concentrations of IgM and IgG were greater ( $p < 0.05$ ) when SBP supplemented than 0% SBP group. The concentration of IgA was greater ( $p < 0.05$ ) in 2.0% SBP group than in 0% SBP and 0.5% SBP group. The concentration of T-AOC, activities of CAT and GSH-Px were greater ( $p < 0.05$ ) when SBP supplemented than 0% SBP group. The activity of SOD and concentration of MDA were lesser ( $p < 0.05$ ) in 2.0% and 1.0% SBP group than in 0% SBP group. It concluded that SBP could improve production, immune function, and antioxidant status on weaned piglets, and the appropriate level of SBP supplementation would be from 1.0% to 2.0%.

## Article Information

Received 06 May 2023

Revised 25 May 2023

Accepted 12 June 2023

Available online 24 October 2023

(early access)

Published 26 July 2024

## Authors' Contribution

Conceptualization, YY and WL. Methodology, YY, ZD. Investigation, ZD, NL, KZ and LY. Data curation, ZD. Writing original draft preparation, YY and XS. Writing review and editing, YY and WL. Project administration, WL. Funding acquisition, WL and XS. All authors have read and agreed to the published version of the manuscript.

## Key words

Sea buckthorn pomace, Piglets, Antioxidant status, Average daily gain, Immune

## INTRODUCTION

Weaning is the process of transitioning piglets from nursing from its mother to eating independently. In this period, the piglets were faced lots of stresses, which impaired intestinal structure and their barrier function, the mucosal immune system, and imbalanced intestinal flora, eventually resulting to a greater rate of diarrhea in weaned piglets and then result numerous economic losses (Ji *et al.*, 2019; Tang *et al.*, 2022). Hence, it needs to improve the immunity of piglets. With the prohibited that the use of antibiotics as feed additives, it was very urged to find an

alternative additive with lesser toxicity, side effects, and greater efficiency.

Sea buckthorn (*Hippophaë rhamnoides* L.), with high adaptability, rapid growth, and protect against erosion ability, has been widely cultivated in Western and Northwestern of China to protect ecosystems (Suryakumar and Gupta, 2011). In addition, sea buckthorn has numerous function compounds and was used in traditional medicine (Ren *et al.*, 2020; Żuchowski, 2022). Sea buckthorn pomace (SBP), as a by-product of sea buckthorn berry juice production, which was less investigated. Moreover, SBP could be used in animal diets due to it was enriched nutritional and functional (Yang *et al.*, 2021). This led us to hypothesize that the weaned piglets have a greater growth performance and antioxidant status when offered SBP. To test this hypothesis, we evaluated average daily gain (ADG), feed conversion ratio, and serum metabolites in piglets when offered diets differing in SBP levels.

## MATERIALS AND METHODS

### Experimental animals, design, diets and management

A total of 40 28-days old weaned piglets (Duroc ×

\* Corresponding author: liwg81508@163.com, sxnysxy@163.com  
0030-9923/2024/0005-2279 \$ 9.00/0



Copyright 2024 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Landrace × Yorkshire; half female and half castrated male) with similar initial body weight ( $10 \pm 0.30$  kg) were randomly assigned to 4 experimental diets supplemented with different levels of SBP (as air dry basis): (1) 0% SBP (basal diets group); (2) 0.5% SBP; (3) 1.0% SBP; and (4) 2.0% SBP. All piglets were divided into 20 pens, and each treatment had 5 replicate pens with 2 weaned piglets per pen (1 castrated male and 1 female). The experimental diets were formulated according to the nutrient requirements of weaned piglets followed by National Research Council (2012; Table I; 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 (length × width: 1.2 m × 0.8 m) with equipped a feeder and a nipple drinker, which had free access to feed (offered four times daily: 8:00, 12:00, 15:00, and 18:00) and water at all times. The experiment lasted for 30 days.

**Table I. Ingredients and chemical composition of experimental diets and Sea Buckthorn Pomace (as air basis).**

Ingredient	Basal diet	SBP
Corn grain, ground (%)	60.0	-
Wheat bran (%)	5.0	-
Soybean meal (%)	20.0	-
Premix (S1512) (%)	15.0	-
Total	100	-
<b>Chemical composition</b>		
Digestive energy (MJ/kg)	15.42	-
Dry matter (%)	93.89	95.85
Crude protein (%)	22.0	9.16
Ether extract (%)	5.42	9.25
Ash (%)	6.18	6.13
Crude fiber (%)	1.62	18.20
Calcium (%)	0.75	0.71
Phosphorus (%)	0.51	0.43
Lysine (%)	1.30	-

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 weaned piglets were weighed individually on d 1 and 30 before morning feeding (08:00) and their average daily gain (ADG) were calculated over the 30 d. The average daily feed intake (ADFI) was also recorded and calculated over the 30 d, feed conversion ratio (FCR) was

calculated according to ADFI to ADG. The feed, SBP, and orts (100 g) were collected before morning feeding on d 1, 16, 30. The precaval vein blood samples of all animals were collected individually in evacuated tubes (XY16, Hebei Xiangyuan medical devices Co., Ltd, Shijiazhuang City, China) on day 30 before morning feeding, settled at room temperature for 1 h, and then centrifuged at  $1500 \times g$  (4°C; TGL-16M, Cence Co., Ltd, Changsha, China) for 20 min, and the serum was stored at -20°C for further analyzed.

#### *Chemical analysis*

Feed, SBP, and orts were freeze-dried for 80 h (LGJ-18S, Songyuan Freeze dryer, Beijing City, China), ground, pass through a 1 mm screen (Leinuo Jixie, Xinxiang City, China), and then stored at room temperature for later analyzed. The DM of feed, SBP, and orts samples were tested by drying at 105°C for 36 h in a forced air oven (DHG-9053A, Shanghai Yiheng Instrument Co., Ltd; method 925.45; AOAC, 2006) and organic matter (OM) was calculated as loss in dry weight upon complete combustion of a sample at 650°C for 6 h in a muffle furnace (SX2-4-10N, Shanghai Yiheng Instrument Co., Ltd; method 942.05; AOAC, 2006). Total nitrogen content in feed, SBP, and orts were measured by the micro-Kjeldahl method (K1100, Hanon instruments, Jinan, China), and CP was calculated as N concentration multiply by 6.25. Ether extract in feed, SBP, and orts were measured by using a reflux system (Ankom XT 15, Fairport, NY, USA) with petroleum ether at 90°C for 1 h (method 920.29; AOAC, 2006). The crude fiber was determined by acid hydrolysis (0.128 mol/L H<sub>2</sub>SO<sub>4</sub>) and alkaline hydrolysis (0.313 mol/L NaOH) which analyzed by a Ankom A200 fiber analyzer for 30 min, respectively (Ankom Technology, Fairport, NY, USA; Van Soest *et al.*, 1991).

The piglets blood routine analysis was measured by an automatic hematology analyzer (Mindray BS-420 biochemical analyzer; Beijing Huaying Bioengineering Institute, Beijing, China). Serum total protein, albumin, globulin, high density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), glucose, Ca, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured 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 complements C3 (C3), complement C4 (C4), immunoglobulin M (IgM), immunoglobulin G (IgG), interleukin-2 (IL2p), interleukin-4 (IL4p), interleukin-6 (IL6p), immunoglobulin A, (IgA), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (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).

#### Statistical analysis

The data were subjected to one-way ANOVA with SAS 9.3 (SAS Inst. Inc, Cary, NC) with the model:  $Y = \mu + SBPL + e$ ,  $Y$  = dependent variable;  $\mu$  = treatment mean value; SBPL = effect of SBP supplemented level;  $e$  = residual error. Duncan's multiple-range test was used for multiple mean comparisons in the procedure. The  $p$  value  $< 0.05$  was considered statistically significant.

## RESULTS

#### Growth performance

As designed, the IBW was no differences ( $p > 0.05$ ) among 4 groups (Table II). The ADG, ADFI, and FBW were greater ( $p < 0.05$ ), whereas the FCR was lesser ( $p < 0.05$ ) in 2.0% and 1.0% SBP group than in 0% SBP group.

The ADG, ADFI, FBW, and FCR were no differences ( $p > 0.05$ ) between 0% and 0.5% SBP group.

**Table II. Effects of dietary supplementation of different levels of SBP on performance in weaned piglets.**

Items	SBP levels			
	0%	0.5%	1.0 %	2.0 %
IBM (kg)	10.51±0.25	10.28±0.37	10.61±0.28	10.51±0.32
FBM (kg)	19.71±1.32 <sup>b</sup>	20.13±1.54 <sup>b</sup>	22.24±1.43 <sup>a</sup>	23.62±1.38 <sup>a</sup>
ADG(kg/d)	0.31±0.13 <sup>c</sup>	0.32±0.17 <sup>bc</sup>	0.38±0.07 <sup>b</sup>	0.47±0.19 <sup>a</sup>
ADFI(kg/d)	0.52±0.23 <sup>c</sup>	0.54±0.17 <sup>c</sup>	0.61±0.17 <sup>b</sup>	0.67±0.23 <sup>a</sup>
FCR	1.70±0.10 <sup>c</sup>	1.69±0.09 <sup>c</sup>	1.60±0.14 <sup>b</sup>	1.42±0.18 <sup>a</sup>

In the same row, values with no letter or the same small letter superscripts mean no significant difference ( $P > 0.05$ ), while with different small letter superscripts mean significant difference ( $P < 0.05$ ). The same as below. IBM, initial body mass; FBM, final body mass; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SBP, seabuckthorn pomace.

**Table III. Effects of sea buckthorn pomace on blood physiological indexes in weaned piglets.**

Items	SBP levels			
	0%	0.5%	1.0%	2.0%
WBC ( $\times 10^9/L$ )	24.97±5.42 <sup>a</sup>	21.08±4.31 <sup>b</sup>	15.43±0.61 <sup>b</sup>	13.15±1.78 <sup>b</sup>
RDW-CV (%)	22.44±1.18	21.34±0.9	21.12±2.28	22.1±2.56
PDW (fL)	18.14±4.11	15.28±1.57	15.08±1.6	15.38±0.50
MPV (fL)	11.94±1.18	11.6±0.58	11.44±0.45	11.44±0.85
P-LCR (%)	40.98±7.69	39.72±5.19	38.02±3.04	36.82±3.76
PCT (%)	0.10±0.10 <sup>c</sup>	0.23±0.07 <sup>b</sup>	0.37±0.23 <sup>a</sup>	0.48±0.28 <sup>a</sup>
NEUT ( $\times 10^9/L$ )	0.41±0.24	0.58±0.46	0.65±0.69	0.50±0.35
LYMPH ( $\times 10^9/L$ )	8.51±2.31	8.79±3.53	9.86±5.48	10.70±4.51
MONO ( $\times 10^9/L$ )	0.66±0.21	0.55±0.38	0.53±0.28	0.44±0.15
EO ( $10^9/L$ )	0.09±0.15 <sup>a</sup>	0.23±0.27 <sup>b</sup>	0.36±0.06 <sup>b</sup>	0.45±0.19 <sup>b</sup>
BASO ( $10^9/L$ )	5.65±1.19	4.37±0.37	6.47±1.30	4.99±2.59
NEUT (%)	2.64±1.35	3.54±2.02	3.18±1.75	2.88±1.02
LYMPH (%)	56.02±7.68	58.68±7.47	54.68±6.64	63.84±8.70
MONO (%)	2.72±2.92	2.88±1.34	1.48±0.44	2.58±1.41
EO (%)	0.60±1.04 <sup>c</sup>	2.82±1.04 <sup>b</sup>	1.92±0.40 <sup>ab</sup>	2.14±0.51 <sup>a</sup>
BASO (%)	38.02±7.95	32.08±9.72	39.24±8.16	28.56±7.62
RBC ( $\times 10^{12}/L$ )	6.22±0.32 <sup>c</sup>	6.53±0.61 <sup>c</sup>	7.45±0.42 <sup>b</sup>	7.52±0.45 <sup>a</sup>
HGB (g/L)	87.23±7.61	96.15±8.59	92.58±14.53	89.91±8.41
HCT (%)	43.71±6.71	44.53±4.65	42.28±5.69	46.78±8.31
MCV (fL)	78.32±4.76	79.28±5.41	81.13±7.25	77.78±5.53
MCH (pg)	14.15±0.61	14.24±0.67	14.83±0.57	14.39±0.54
MCHC (g/L)	156±6.31	147.6±4.21	164±5.53	153.8±4.72
PLT ( $\times 10^9/L$ )	415.2±54.53	414.6±63.42	458.4±63.87	434.4±79.02

SBP, seabuckthorn pomace; WBC, white blood cells; RDW-CV, red blood cell volume distribution width; PDW, platelet volume distribution width; MPV, mean platelet volume; P-LCR, large platelet ratio; PCT, thrombocytosis; NEUT, absolute neutrophil count; LYMPH, absolute lymphocytes count; MONO, absolute monocyte count; EO, absolute eosinophil count; BASO, absolute basophil count; RBC, red blood cell; HGB, hemoglobin concentration; HCT, Hematocrit; MCV, mean corpuscular volume; MCH, mean Corpuscular hemoglobin; MCHC, mean red blood cell hemoglobin concentration; PLT, platelets.

*Blood physiological indexes*

There were no differences on RDW-CV, PDW, MPV, P-LCR, NEUT ( $10^9/L$ ), LYMPH ( $10^9/L$ ), MONO ( $10^9/L$ ), BASO ( $10^9/L$ ), NEUT (%), LYMPH (%), MONO (%), BASO (%), HGB, HCT, MCV, MCHC, and PLT among 4 groups ( $p > 0.05$ ; Table III). The WBC, PCT, EO ( $10^9/L$ ), and EO (%) were greater ( $p < 0.05$ ) when SBP supplemented than in 0% SBP group. The RBC ( $10^{12}/L$ ) was greater ( $p < 0.05$ ) in 2.0% and 1.0% SBP group than in 0% SBP group.

*Serum biochemical indexes*

There were no differences ( $p > 0.05$ ) on total protein, albumin, globulin, A/G, HDL, LDL, and AST among 4 groups (Table IV). The serum ALT was lesser ( $p < 0.05$ ) in 2.0% and 1.0% SBP group than in 0% SBP group. In addition, the concentration of Ca was greater ( $p < 0.05$ ) in

SBP supplemented groups than in 0% SBP group.

*Serum immune indexes*

The C3 was greater ( $p < 0.05$ ) in 2.0% SBP group than in another 3 group (Table V). The IgM, IgG, were greater ( $p < 0.05$ ), whereas the IL2, IL4, and IL6 were lesser ( $p < 0.05$ ) when SBP supplemented compared to control group. The IgA was greater ( $p < 0.05$ ) in 2.0% SBP group than in 0% SBP and 0.5% SBP group. The C4 was no differences ( $p > 0.05$ ) among 4 group.

*Serum antioxidant factors*

The concentration of T-AOC, and activities of CAT, GSH-Px were greater ( $p < 0.05$ ) in SBP supplemented groups than in 0% SBP group (Table VI). The activities of SOD and MDA were lesser ( $p < 0.05$ ) in 2.0% and 1.0% SBP groups than in 0% SBP group.

**Table IV. Effects of dietary supplementation of different levels of SBP on serum biochemical indexes in weaned piglets.**

Items	SBP levels			
	0%	0.5%	1.0 %	2.0 %
Total protein (g/L)	62.46±6.34	61.07±4.88	66.5±5.14	59.85±3.68
Albumin (g/L)	23.75±3.74	23.21±2.75	22.49±1.12	20.74±0.34
Globulin (g/L)	38.72±3.44	37.86±3.77	44.02±4.27	39.11±3.69
Albumin/globulin	0.61±0.08	0.62±0.10	0.51±0.04	0.53±0.05
HDL (mmol/L)	0.82±0.15	0.82±0.17	0.72±0.10	0.69±0.09
LDL(mmol/L)	2.23±0.34	2.2±0.23	2.15±0.34	1.80±0.14
AST (U/L)	92.07±18.04	85.53±21.18	79.4±50.89	69.73±9.02
ALT (U/L)	106.23±13.13 <sup>a</sup>	103.32±18.03 <sup>a</sup>	86.67±6.84 <sup>b</sup>	85.47±5.83 <sup>b</sup>
Calcium (mol/L)	2.45±0.01 <sup>c</sup>	2.51±0.02 <sup>b</sup>	2.53±0.04 <sup>ab</sup>	2.56±0.05 <sup>a</sup>

HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, Aspartate transferase; ALT, alanine aminotransferase; SBP, seabuckthorn pomance.

**Table V. Effects of dietary supplementation of different levels of SBP on serum immune indexes in weaned piglets.**

Items	SBP levels			
	0%	0.5%	1.0%	2.0%
C3 (g/L)	0.29±0.05 <sup>b</sup>	0.44±0.05 <sup>b</sup>	0.46±0.03 <sup>b</sup>	0.48±0.11 <sup>a</sup>
C4 (g/L)	0.07±0.02	0.09±0.02	0.09±0.03	0.10±0.01
IgM (g/L)	0.61±0.03 <sup>c</sup>	0.7±0.07 <sup>b</sup>	0.71±0.03 <sup>b</sup>	0.94±0.05 <sup>a</sup>
IgA (g/L)	0.93±0.04 <sup>b</sup>	1.01±0.14 <sup>b</sup>	1.11±0.04 <sup>ab</sup>	1.20±0.14 <sup>a</sup>
IgG (g/L)	8.34±0.54 <sup>a</sup>	9.39±0.45 <sup>b</sup>	9.57±0.91 <sup>b</sup>	10.02±0.78 <sup>b</sup>
IL2 (pg/mL)	156.54±6.28 <sup>a</sup>	148.98±9.4 <sup>a</sup>	130.65±5.74 <sup>b</sup>	130.6±5.74 <sup>b</sup>
IL4 (pg/mL)	15.50±1.11 <sup>a</sup>	11.51±0.94 <sup>b</sup>	9.60±0.47 <sup>c</sup>	7.47±0.65 <sup>d</sup>
IL6 (pg/mL)	347.24±18.91 <sup>a</sup>	270.92±9.15 <sup>b</sup>	248.7±16.63 <sup>c</sup>	216.0±11.39 <sup>d</sup>

C3, complement 3; C4, complement 4; IgM, immunoglobulin M; IgA, immunoglobulin A; IgG, immunoglobulin G; IL2, interleukin 2; IL4, interleukin 4; IL6, interleukin 6; SBP, seabuckthorn pomance.

**Table VI. Effects of dietary supplementation of different levels of SBP on serum antioxidant factors in weaned piglets.**

Items	SBP levels			
	0%	0.5%	1.0%	2.0%
T-AOC (U/mL)	7.1±0.66 <sup>d</sup>	8.01±0.74 <sup>c</sup>	9.11±0.64 <sup>b</sup>	10.54±0.63 <sup>a</sup>
SOD (U/mL)	5.04±1.11 <sup>a</sup>	4.55±0.42 <sup>ab</sup>	3.94±0.68 <sup>b</sup>	3.07±0.19 <sup>b</sup>
GSH-Px (U/mL)	252.78±9.89 <sup>d</sup>	275.89±16.07 <sup>c</sup>	297.56±34.5 <sup>b</sup>	335.83±20.1 <sup>a</sup>
CAT (U/mL)	40.6±4.1 <sup>d</sup>	43.71±1.62 <sup>c</sup>	46.91±1.73 <sup>b</sup>	52.03±3.06 <sup>a</sup>
MDA (nmol/mL)	45.62±1.55 <sup>a</sup>	40.42±6.79 <sup>a</sup>	35.2±2.58 <sup>b</sup>	27.31±2.58 <sup>c</sup>

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

## DISCUSSION

### *Effect of SBP on growth performance*

The piglets faced numerous stresses when weaned from the sow (Campbell *et al.*, 2013). It could impact their intestinal health and then causes lesser production efficiencies, and greater morbidity and mortality. The sea buckthorn, as a traditional medicine, possesses numerous health benefits and has been used all over the world. As the by-products, SBP was used in pig and lambs to improve their performance and meat quality (Dannenberger *et al.*, 2018; Qin *et al.*, 2020). In the present study, SBP improved the ADG of piglets and showed a lower FCR compared to 0% SBP piglets group, which may partially be explained by the improved serum immune indexes and antioxidant factors. These observations are in agreement with Dufourny *et al.* (2021) who reported that apple pomace improve growth performance in piglets. In addition, ADFI was increased in 1.0% and 2.0% SBP groups, which in agreement with previous studies in broiler offered phenolic compounds (Mountzouris *et al.*, 2011; Viveros *et al.*, 2011). This could explain by intestinal surface area and digestive enzyme activities could enhanced, which lead to a greater nutrient absorption in small intestine (Mountzouris *et al.*, 2011; Viveros *et al.*, 2011).

### *Effect of SBP on blood physiological indexes*

The WBC help fight infection and inflammation and a high WBC count relected one of the following has increased the making of white blood cells, which means an infection or immune system issue. In the present study, the blood WBC counts decreased when supplemented SBP and it explained by SBP could improve immune. The RBC, as one of the major components of blood, could help carry oxygen throughout body. In the present study, the number of RBC ranged between 6.22 and  $7.52 \times 10^{12}/L$ , which was similar in a previous study (Lei *et al.*, 2023). In addition, The RBC increased when supplementary SBP,

which means blood loss, hemolysis, or decreased their production may cause in 0% and 0.5% SBP group.

### *Effect of SBP serum biochemical indexes*

The serum ALT concentration could reflect the production and muscle within reference ranges in animal. A previous study showed that a decreased ALT concentration suggesting a positive effect on piglets (Yang *et al.*, 2022). In the present study, we found that ALT concentration decreased in 1.0% and 2.0% SBP group, which showed that SBP have a positive effect on piglets. Total proteins, which includes albumin and globulin, was the main and most abundant constituents in serum. It has numerous essential physiological functions, and are regards as the indicators to evaluate health status of piglets (Che *et al.*, 2017; Figueroa *et al.*, 2003; Gómez *et al.*, 2002). Albumin, as mainly a protein, which is grows in the liver and associated with metabolism, and globulin is mainly as an immunoglobulin. In the present study, there were no differ on total proteins, albumin, and globulin. A previous study showed that the serum total proteins, albumin, and globulin were increased when supplemented with sea buckthorn leaves in Altay sheep (Liu, 2019). However, Yao *et al.* (2023) has demonstrated that serum total protein and album concentration were no differ in sea buckthorn flavonoids groups. The reason could explain the supplemented sea buckthorn levels and animal is different, and more research need in the future study. Calcium, as a macromineral, is necessary for bone matrix formation and maintenance, and take part in the blood coagulation process and enzymes activity. Our results showed that the concentrations of serum calcium were greater in SBP groups, which related with a greater calcium concentration in dried sea buckthorn pomace (724 mg/kg; Nour *et al.*, 2021).

### *Effect of SBP serum immune indexes*

In general, effective functioning in the immune system

was necessary for protection against lots of diseases, which can undesirably affect the performance and welfare of farm animals. The serum C3 and C4 proteins are very commonly tested individual complement proteins. As we all know, SBP was abundance in phenolic compounds, vitamin C, unsaturated fatty acids, and phytosterols (Olas *et al.*, 2018). Serum immunoglobulin, included IgA, IgG, and IgM, can reflect the immune status of the piglet (Liu *et al.*, 2022). In the present study, we found that the concentration of serum IgA, IgG, and IgM were increased in SBP groups, which means SBP could enhancing humoral immune response and reducing the occurrence of diseases in weaning piglets. In addition, serum IgG concentrations were ranged between 8.34-10.12 g/L, which in agreement with a previous study demonstrated IgG take up~80% of the total serum immunoglobulin and is the major role in resisting the invasion of microorganism infections (Larsson, 2008).

Generally, the IL-2 is related with cellular immunity and plays a vital role in animal immune function. The IL-4, as a cytokine, associated with pleiotropic functions, which could plays an important role to develop Th2 responses. The IL-6, as a vital mediator of the inflammatory response, was used for the markers to evaluate systemic pro-inflammatory cytokine activation and vital inducers for acute-phase proteins. The concentrations of IL-2, IL-4, and IL-6 decreased in SBP groups, which could explain the piglets were health when offered SBP.

#### *Effect of SBP serum antioxidant factors*

Redox imbalance could impact animal health and growth. Numerous nutritional strategies were widely accepted to improve antioxidant capacity, for example, fruit pomace (de Souza *et al.*, 2019; Schneider *et al.*, 2020), mineral (Liu *et al.*, 2020; Yu *et al.*, 2020; Wang *et al.*, 2022), and then maintain animal growth and production. The T-AOC concentration, CAT, SOD, and GSH-Px activities, and the content of MDA could reflect the antioxidant and lipid peroxidation status of organism tissues, respectively. Antioxidant enzymes, included SOD, GSH-Px, and CAT, are the important antioxidant components in defense systems involved in scavenging reactive oxygen species and maintaining the redox equilibrium. The MDA, as a metabolite produced end products by lipid peroxidation, was commonly regards as oxidative stress indicator. In the present study, we founded that the concentration of T-AOC, activities of CAT, and GSH-Px were improved and concentration of MDA was lesser when supplemented SBP, which could explain by numerous bioactive compounds, such as polyphenols, flavonoids in SBP (Luntraruru *et al.*, 2022). Previous studies demonstrated that dietary supplementary sea buckthorn

flavonoids could improve serum antioxidant capability in meat rabbit and Guangxi Small Hemp ducks (Du *et al.*, 2023; Yao *et al.*, 2023). Interestingly, the SOD was lesser in 1.0% and 2.0% SBP groups than in 0% SBP group. The inconsistency in serum SOD could be due to differences in the dosage of SBP and the breed and physiological status of the animals. The antioxidant activity in SBP group could explained by their chemical components, such as flavonoids and polysaccharides etc. (Olas, 2016; Liu *et al.*, 2021), which have lots of phenolic hydroxyl groups and benzene ring structures related to antioxidant. In addition, at present, there is no reference standard for the optimal amount of sea buckthorn pomace in different animals, breeds and physiological stages. The level of additive content in this study is based on the characteristics of the physiological stage of weaning piglets and the more conservative level adopted after literature research. This also shows the limitations of the conclusions of this study. Further research should be carried out on the basis of increasing the dose, and at the same time, the optimal amount of additives for pigs in other physiological stages should be studied.

## CONCLUSION

The piglets have a greater ADG and lesser feed conversion ratio in SBP groups. In addition, the serum immune and antioxidant capability were improved in SBP groups. It concluded that SBP could improve growth performance, immune and antioxidant status of weaned piglets, and the appropriate level of SBP supplementation would be from 1.0% to 2.0%.

#### *Funding*

This research was funded by the Central Guidance on Local Science and Technology Development Fund of Shanxi Province (grant number 2017GA630002) and Biological Breeding Project of Shanxi Academy of Agricultural Sciences (17YZGC110).

#### *Ethics statement*

All experimental procedures were in accordance with the guideline established by the Regulation for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, 2004), and approved by the Animal Welfare and Research Ethics Committee at Shanxi Agricultural University.

#### *Statement of conflict of interest*

The authors have declared no conflict of interest.

## REFERENCES

- Association of Official Analytical Chemists (AOAC), 2006. *Official methods of analysis*, 18<sup>th</sup> edition. AOAC, Arlington, VA, USA.
- Campbell, J.M., Crenshaw, J.D. and Polo, J., 2013. The biological stress of early weaned piglets. *J. Anim. Sci. Biotechnol.*, **4**: 19. <https://doi.org/10.1186/2049-1891-4-19>
- Che, L.Q., Peng, X., Hu, L., Wu, C., Xu, Q., Fang, Z.F., Lin, Y., Xu, S.Y., Li, J., Feng, B., Tian, G., Zhang, R.N., Sun, H., Wu, D. and Chen, D.W., 2017. The addition of protein-bound amino acids in low-protein diets improves the metabolic and immunological characteristics in fifteen to thirty-five kg pigs. *J. Anim. Sci.*, **95**: 1277-1287. <https://doi.org/10.2527/jas.2016.0990>
- Dannenberger, D., Tuchscherer, M., Nürnberg, G., Schmicke M. and Kanitz, E., 2018. Sea Buckthorn pomace supplementation in the diet of growing pigs-effects on fatty acid metabolism, HPA activity and immune status. *Int. J. mol. Sci.*, **19**: 596. <https://doi.org/10.3390/ijms19020596>
- de Souza, D.R., Willems, J.L. and Low, N.H., 2019. Phenolic composition and antioxidant activities of saskatoon berry fruit and pomace. *Fd. Chem.*, **290**: 168-177. <https://doi.org/10.1016/j.foodchem.2019.03.077>
- Du, X.Y., Liu, M., Li, J.T., Yang, G.Q., Guo, D.X., Guo, D.X., Zhu Y.J. and Li, F.F., 2023. Effect of seabuckthorn flavonoids on growth performance, slaughtering performance, serum biochemical indexes, antioxidant capacity and immune function of meat rabbit. *Chin. J. Anim. Nutr.*, **35**: (in Chinese).
- Dufourny, S., Antoine, N., Pitchugina, E., Delcenserie, V., Godbout, S., Douny, C., Scippo, M.L., Froidmont, E., Rondia, P., Wavreille, J. and Everaert, N., 2021. Apple pomace and performance, intestinal morphology and microbiota of weaned piglets-a weaning strategy for gut health? *Microorganisms*, **9**: 572. <https://doi.org/10.3390/microorganisms9030572>
- Figuroa, J.L., Lewis, A.J., Miller, P.S., Fischer R.L. and Diedrichsen, R.M., 2003. Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine. *J. Anim. Sci.*, **81**: 1529-1537. <https://doi.org/10.2527/2003.8161529x>
- Gómez, R.S., Lewis, A.J., Miller, P.S. and Chen, H.Y., 2002. Growth performance, diet apparent digestibility, and plasma metabolite concentrations of barrows fed corn-soybean meal diets or low-protein, amino acid-supplemented diets at different feeding level. *J. Anim. Sci.*, **80**: 644-653. <https://doi.org/10.2527/2002.803644x>
- Ji, F.J., Wang, L.X., Yang, H.S., Hu A. and Yin, Y.L., 2019. Review: The roles and functions of glutamine on intestinal health and performance of weaning pigs. *Animal*, **13**: 2727-2735. <https://doi.org/10.1017/S1751731119001800>
- Larsson, P.H., 2008. Purification of antibodies. *Methods mol. Med.*, **138**: 197-207. [https://doi.org/10.1007/978-1-59745-366-0\\_16](https://doi.org/10.1007/978-1-59745-366-0_16)
- Lei, L.L., Zhang, S.F., Zhang, Y.J., Lu, L.Z., Li, C., Liao, Y., Liu, W., Li Y.Y. and Shen, S.B., 2023. Effect of total mixed protein grass fermented diet on growth performance, blood indexes and carcass traits of finishing pigs. *Chin. J. Anim. Nutr.*, **35**: 813-822. (In Chinese).
- Liu, H., Yu, Q.F., Fang, C.K., Chen, S.J., Tang, X.P., Kolapo A.M. and Fang, R.J., 2020. Effect of selenium source and level on performance, egg quality, egg selenium content, and serum biochemical parameters in laying hens. *Foods*, **9**: 68. <https://doi.org/10.3390/foods9010068>
- Liu, N., Ma, X. and Jiang, X., 2022. Effects of immobilized antimicrobial peptides on growth performance, serum biochemical index, inflammatory factors, intestinal morphology, and microbial community in weaning pigs. *Front. Immunol.*, **13**: 872990. <https://doi.org/10.3389/fimmu.2022.872990>
- Liu, S., Xiao, P., Kuang, Y., Hao, J., Huang, T. and Liu, E., 2021. Flavonoids from sea buckthorn: A review on phytochemistry, pharmacokinetics and role in metabolic diseases. *J. Fd. Biochem.*, **45**: e13724. <https://doi.org/10.1111/jfbc.13724>
- Liu, Y.F., 2019. *Effects of Seabuckthorn leaves and flavonoids of seabuckthorn leaves on production performance, blood indexes, and lipid metabolism of Altay sheep*. Doctor thesis, Shihezi University. (In Chinese).
- Luntrarur, C.M., Apostol, L., Oprea, O.B., Neagu, M., Popescu, A.F., Tomescu, J.A., Muțescu, M., Susman, I.E. and Gaceu, L., 2022. Reclaim and valorization of sea buckthorn (*Hippophae rhamnoides*) by-product: Antioxidant activity and chemical characterization. *Foods*, **11**(3): 462. <https://doi.org/10.3390/foods11030462>
- Mountzouris, K.C., Paraskevas, V., Tsirotsikos, P., Palamidia, I., Steiner, T., Schatzmayr G. and Fegeros, K., 2011. Assessment of a phytogenic feed additive

- effect on broiler growth performance, nutrient digestibility and caecal microflora composition. *Anim. Feed Sci. Technol.*, **168**: 223-231. <https://doi.org/10.1016/j.anifeedsci.2011.03.020>
- Nour, V., Panaite, T.D., Corbu, A.R., Ropota M. and Turcu, R.P., 2021. Nutritional and bioactive compounds in dried sea-buckthorn pomace. *Erwerbs-Obstbau*, **63**: 91-98. <https://doi.org/10.1007/s10341-020-00539-1>
- Olas, B., 2016. Sea buckthorn as a source of important bioactive compounds in cardiovascular diseases. *Fd. Chem. Toxicol.*, **97**: 199-204. <https://doi.org/10.1016/j.fct.2016.09.008>
- Olas, B., Skalski, B. and Ulanowski, K., 2018. The anticancer activity of sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson). *Front. Pharmacol.*, **9**: 232. <https://doi.org/10.3389/fphar.2018.00232>
- Qin, X, Zhang, T., Cao, Y., Deng, B., Zhang J. and Zhao, J., 2020. Effects of dietary sea buckthorn pomace supplementation on skeletal muscle mass and meat quality in lambs. *Meat Sci.*, **166**: 108141. <https://doi.org/10.1016/j.meatsci.2020.108141>
- Ren, R., Li, N., Su, C., Wang, Y., Zhao, X., Yang, L., Li, Y., Zhang, B., Chen J. and Ma, X., 2020. The bioactive components as well as the nutritional and health effects of sea buckthorn. *RSC Adv.*, **10**: 44654-44671. <https://doi.org/10.1039/D0RA06488B>
- Schneider, V.S., Bark, J.M., Winnischofer, S.M.B., Dos Santos, E.F., Iacomini M. and Cordeiro, L.M.C., 2020. Dietary fibres from guavira pomace, a co-product from fruit pulp industry: Characterization and cellular antioxidant activity. *Fd. Res. Int.*, **132**: 109065. <https://doi.org/10.1016/j.foodres.2020.109065>
- Suryakumar, G. and Gupta, A., 2011. Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). *J. Ethnopharmacol.*, **138**: 268-278. <https://doi.org/10.1016/j.jep.2011.09.024>
- Tang, X., Xiong, K., Fang, R. and Li, M., 2022. Weaning stress and intestinal health of piglets: A review. *Front. Immunol.*, **13**: 1042778. <https://doi.org/10.3389/fimmu.2022.1042778>
- Van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, **74**: 3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C. and Brenes, A., 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poult. Sci.*, **90**: 566-578. <https://doi.org/10.3382/ps.2010-00889>
- Wang, D., Zhang, Y., Chen, Q., Kuang, Y., Fan, J., Xu, X., Zhu, H., Gao, Q., Cheng, S., Cong, X. and Liu, Y., 2022. Selenium-enriched *Cardamine violifolia* improves growth performance with potential regulation of intestinal health and antioxidant function in weaned pigs. *Front. Vet. Sci.*, **9**: 964766. <https://doi.org/10.3389/fvets.2022.964766>
- Yang, A., Wang, K., Peng, X., Lv, F., Wang, Y., Cui, Y., Wang, Y., Qu, D., Zhou, J. and Si, H., 2022. Effects of different sources of calcium in the diet on growth performance, blood metabolic parameters, and intestinal bacterial community and function of weaned piglets. *Front. Nutr.*, **9**: 885497. <https://doi.org/10.3389/fnut.2022.885497>
- Yang, K., Qing, Y., Yu, Q., Tang, X., Chen, G., Fang R. and Liu, H., 2021. By-product feeds: Current understanding and future perspectives. *Agriculture*, **11**: 207. <https://doi.org/10.3390/agriculture11030207>
- Yao, B.N., Liao, F.Y., Yang, J.Y., Wang, J., Zhu, B.G. and Yang, S.L., 2023. Effects of dietary supplementation of sea buckthorn flavonoids on growth performance, meat quality, serum biochemical indexes and immune organ indexes of Guangxi small hemp ducks. *Chin. J. Anim. Nutr.*, **35**: 1638-1648 (in Chinese).
- Yu, Q.F., Liu, H., Yang, K. L., Tang, X.P., Chen S.J., Kolapo M. A., Degen A. and Fang, R.J., 2022. Effect of level and source of supplementary dietary zinc on egg production, quality, and zinc content and on serum antioxidant parameters and zinc concentration in laying hens. *Poult. Sci.*, **99**: 6233-6238. <https://doi.org/10.1016/j.psj.2020.06.029>
- Żuchowski, J., 2022. Phytochemistry and pharmacology of sea buckthorn (*Elaeagnus rhamnoides*; *syn. Hippophae rhamnoides*): Progress from 2010 to 2021. *Phytochem. Rev.*, **22**: 3-33. <https://doi.org/10.1007/s11101-022-09832-1>