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Effects of Sweet Potato Vine Silage Supplementation on Meat Quality, Antioxidant Capacity and Immune Function in Finishing Pigs

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Abstract

Background

Sweet potato vine is the stem and leaf of sweet potato and is nutritious as feedstuff. Silage is an effective solution to retain nutritional value and is beneficial for the preservation. This article explored the effects of Sweet potato vine silage (SPVS) supplementation on meat quality, antioxidant capacity and immune function in finishing pigs. 180 finishing pigs (Berkshire×Licha Black) with body weight of 74.54 ± 3.32 kg were randomly divided into three groups with six pigs per pen and six replicate per treatment: control diet (CON), CON supplemented with 2.5% SPVS (LSPVS) and 5.0% SPVS (HSPVS).

Results

It showed that eye muscle area in the LSPVS group was significantly increased and carcass weight in the HSPVS was significantly reduced. Cooking loss in both HSPVS and LSPVS were significantly reduced. Hepatic level of glutathione peroxidase (GSH-PX) was significantly upregulated in LSPVS but downregulated in HSPVS. In serum, HSPVS decreased glutathione (GSH) level and increased GSH-PX level. HSPVS significantly reduced hepatic IL-1 level and LSPVS significantly reduced IL-12 level and increased IL-8 level. Moreover, HSPV promoted the secretion of IgM and IgG in serum.

Conclusions

SPVS supplementation improved animal performance, meat quality, antioxidant capacity and immune performance in finishing pigs, which provide a new alternative to improve animal health and substitute traditional feedstuff.

Background

Sweet potato is known as an important crop in tropical and subtropical regions. The sweet potato vine is the stem and leaf of the sweet potato. As a waste and by-product of sweet potato, sweet potato vine has a highly nutritional value, with a relatively high content of proteins, soluble fiber, and insoluble fiber. Moreover, the mineral [12], vitamins, lutein [2] and flavonoids[7] are rich in the leaves. Abundant polyphenols [1] and antioxidants such as vitamin C and flavonoids have high free radical scavenging activity[5] and could enhance the body's immune function [6]. Selenium, an essential trace element in animal [8], is rich in sweet potato vine and plays an important role in improving growth performance, meat quality and antioxidant capacity of broilers [9] [10] [11].

In addition to enhancing immunity, anti-oxidation, anti-aging, anti-tumor, and hypoglycemia [3–4], sweet potato vine regulated intestinal health [13] and improved the reproductive performance in sows [14]. Dietary supplementation with spinach or sweet potato leaves improved the growth performance of growing pigs

[15]. However, one problem is that sweet potato vines decay within a short term after harvesting due to the high water content. Hence, silage becomes an alternative way to solve this issue. The silage is not affected by external factors and is kept under anaerobic conditions. In the absence of air, the fermentation of soluble carbohydrates in forages results in a variety of end products, ultimately resulting in the preservation of a forage crop as silage [16]. Therefore, the nutrients in silage could be retained to the maximum extent. Sweet potato vine silage (SPVS) provides a stable feed with a high recovery of dry matter, energy, and highly digestible nutrients compared with the fresh crop.

As previously described, dietary SPVS supplementation have been proved to increase dry matter intake and milk yield[17] and reduce the ruminal nitrogen degradation[18] in ruminants. However, it remains unclear for the effects of SPVS supplementation on growth performance and health status in pigs. In this study, the effects of different dosage of SPVS supplementation on meat quality, antioxidant capacity and immune function were studied.

Materials And Methods

Animals and experimental design

The protocols used in this experiment were approved by the Northeast Agricultural University Institutional Animal Care and Use Committee, and the ethical treatment of animals used in this study complied with the Animal Welfare Committee protocol (#NEAU-[2013]-9) at Northeast Agricultural University (Harbin, China).

Bali Black Pigs were provided by Shanxia Investment Company in Jiangxi Province, China. 180 finishing pigs (Berkshire×Licha Black) with an average initial body weight (BW) of 74.54 ± 3.32 kg were randomly assigned into following treatments: basal diet supplemented with 0 (CON), 2.5% SPVS (LSPVS), and 5% SPVS (HSPVS) on a dry matter basis. The pigs were preliminary fed with free access of basal diet for seven days (Table 1). Each treatment had six replicates with ten finishing pigs per replicate and the experiment lasted for 9 weeks. SPVS was made by Shanxia Investment Company and used freshly. The fresh sweet potato vines with a moisture content of 70%-75% were compacted and bundled, and then sealed with a plastic bag to create an anaerobic environment to make SPVS. The SPVS were unpacked every morning and a blender was used to proportionally mix the SPVS with the basal diet.

Table 1
Composition and nutrient levels of experimental diets (As fed-basis)

Item	Basal diet
Corn	719.7
Soybean meal	143.5
Wheat bran	80.0
Soybean oil	20.0
Dicalcium phosphate	9.0
Limestone	9.0
L-Lysine-HCL,78%	5.0
L-Threonine	1.0
DL-Methionine	0.5
L-Tryptophan	0.3
Salt	2.0
Premix*	10.0
Nutritional levels (Dry matter basis)	
Metabolic energy, MJ/kg	13.2
Crude protein, %	14.4
Ether extract, %	5.1
Crude fiber, %	2.5
Calcium, %	0.5
Total phosphorus, %	0.6
Lysine, %	1.0
Methionine, %	0.3
Threonine, %	0.6
Tryptophan, %	0.2

*The premix provided the following nutrients per kilogram of the complete diet: Vitamin A, 8 000 IU; Vitamin D3, 2000 IU; Vitamin E, 30 IU; Vitamin K3, 1.5 mg; Vitamin B1, 1.6 mg; Vitamin B6, 1.5 mg; Vitamin B12, 0.012mg; Niacin, 20 mg; Pantothenic acid, 15 mg; zinc (zinc oxide), 80 mg; iron (iron sulfate), 100 mg; copper (copper sulfate), 20 mg; manganese (manganese sulfate), 25 mg; iodine (potassium iodide), 0.3 mg; selenium (Sodium selenite), 0.2 mg.

Table 2
Ingredient proportions of basal feed and sweet potato vine silage

	Ctrl	Lspvs	Hspvs
Basal diet	100.0	90.9	81.8
SPVS[1]	0.0	9.1	18.2
Total	100.0	100.0	100.0
Nutritional levels (Dry matter basis)			
Metabolic energy, MJ/kg	13.2	12.1	10.9
Crude protein, %	14.4	13.3	12.2
Ether extract, %	5.1	4.8	4.4
Crude fiber, %	2.5	2.8	3.2
Calcium, %	0.5	0.5	0.4
Total phosphorus, %	0.6	0.5	0.5
Lysine, %	1.0	0.9	0.8
Methionine, %	0.3	0.3	0.2
Threonine, %	0.6	0.6	0.5
Tryptophan, %	0.2	0.2	0.2
1. SPVS: sweet potato vine silage			
2. Lspvs and Hspvs prepared by adding sweet potato vine silage to basal feed at ratio of 2.5% and 5.0% on dry matter basis, respectively.			

Diets and Feeding Management

The experimental diet was formulated according to the swine nutrient requirements of NRC (2012). The composition and nutritional value of the diets are presented in Table 1. Pigs of each duplicate were intensively raised in a pen with a feeder and a water-saving stainless steel drinker to allow the pigs to have free access to feed and water. All the groups were fed with a basal diet during the 7-day preliminary trial period. Subsequently, different treatment groups were fed with corresponding diets for 9 weeks. All pigs were housed in a temperature-controlled room. Over the whole trial period, the experimental animals were in good health.

Sample Collection

When the body weight of finishing pigs reached about 110 kg, 6 finishing pigs with similar body weight were randomly selected from each treatment group (1 pig per pen) after a overnight fasting and slaughtered by electronic stunning. Blood samples were collected and centrifuged at 3500 r/min for 10

min. The serum and liver samples were collected and stored at -20°C for subsequent analysis. The longissimus dorsi muscle (the last thoracic vertebra of a left carcass to the 6th lumbar vertebra) samples were collected for meat quality determination.

Meat quality analysis

After slaughter, the blood, fur, viscera, head and hooves are removed and the remaining weight is the carcass weight. The eye muscle area was measured which is located between the first and second of the thoracic vertebrae of the longissimus dorsi. The width and thickness of eye muscle were measured with vernier caliper, and the eye muscle area was calculated. The backfat thickness of the shoulder, waist and hip on the left side of the carcass was measured with a vernier caliper and then averaged. The longissimus dorsi muscle was stored in a refrigerator at 4°C for following determination. Portable pH meter (DHS-2F) was used to determine pH values at 45 min and 24 h after slaughter. The electrode of the pH meter was inserted into the muscle at a depth of 1–3 cm, and make sure that the head of the electrode was completely embedded in the meat. The pH values were read after 5 seconds, and results from 3 determinations were averaged. L* (luminance), A * (redness) and B * (yellowness) values of longissimus dorsi muscle were measured by automatic Chroma Meters (CR-400, Japan) within 45–60 minutes after slaughter. The meat was trimmed to the size of 2 cm×3 cm×5 cm and weighed. The meat was suspended in the crisper with a fishing line (along the direction of muscle fiber without touching the inner wall), and stored at 4°C. After 24 hours, the weight was weighed and the drip loss was calculated. About 100 g longissimus dorsi muscle samples were taken and weighed. The samples were placed in a valve bag with a thermometer inserted and heated in 80°C water bath. The samples were take out when the thermometer reached 80°C. The cooking loss was calculated by averaging three repeats for each sample.

Blood biochemical index analysis

We tested the serum levels of albumin (ALB), low density lipoprotein cholesterol (LDL), blood urea nitrogen (BUN) and alkaline phosphatase (AKP) by using the commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The absorbance was measured by UV-2401PC ultraviolet spectrophotometer (Shimadzu Corporation, Japan). The sample processing, determination and calculation of the results were carried out in accordance with the operating steps of the kit instructions.

Antioxidant analysis

The levels of total superoxide dismutase (T-SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-PX), malonaldehyde (MDA) and total protein (TP) in liver or serum were determined. The absorbance was measured by UV-2401PC ultraviolet spectrophotometer (Shimadzu Corporation, Japan). The determination were operated according to the guidelines of commercial kits.

Immune function analysis

Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-12 (IL-12) and Tumor necrosis factor-α (TNF-α) were detected in the liver. In addition, the immunoglobulin A (IgA), immunoglobulin M

(IgM) and immunoglobulin G (IgG) in serum were detected. The absorbance was measured by UV-2401PC ultraviolet spectrophotometer (Shimadzu Corporation, Japan).

The assays were performed according to the instructions of commercial kits.

Statistical Analysis

The results were presented as the means and standard error of the means (SEM). All data were submitted to one-way ANOVA procedure of SPSS 25.0 software. The significance was declared at $p < 0.05$ and a statistical trend was considered for $0.05 \leq p < 0.10$.

Results

Effect of Sweet Potato Vine Silage on the meat quality of pigs

The meat quality of the three groups is shown in Table 3. Compared with the CON group, the eye muscle area of the LSPVS group was extremely significantly increased ($p < 0.01$) and the carcass weight of the HSPVS group was extremely significant decreased ($p < 0.01$). The cooking loss was significantly decreased ($p < 0.01$) in both LSPVS and HSPVS. For other meat quality indexes, there were no significant differences among three treatments.

Table 3 Effect of Sweet Potato Vine Silage on the meat quality of pigs

Item	CON	LSPVS	HSPVS	p-Value
Eye muscle area,cm^2	52.68±2.55c	65.46±1.65a	56.16±4.68c	<0.01
Backfat thickness ,cm	3.34±0.31	3.57±0.14	3.11±0.20	0.15
Carcass rate,%	78.40±9.68	80.36±5.25	77.81±1.86	0.15
Carcass weight, kg	98.90±2.95a	98.95±2.13a	84.47±3.80b	<0.01
Drip loss,%	8.79±0.01	7.61±0.01	7.90±0.01	0.45
Cooking loss,%	35.89±0.018a	30.75±0.015b	28.27±0.012c	<0.01
pH_1	5.49±0.35	5.46±0.22	5.29±0.21	0.71
pH_{24}	4.65±0.09	4.06±0.32	4.31±0.29	0.18
L_1	41.37±1.90	38.34±0.03	40.93±1.78	0.13
A_1	5.80±0.78	7.80±0.50	6.70±0.83	0.14
B_1	3.46±0.37	2.81±0.11	3.79±0.95	0.46
L_{24}	44.41±1.48	40.69±5.46	47.16±3.55	0.31
A_{24}	6.86±1.28	8.39±0.25	5.73±1.16	0.26
B_{24}	5.68±1.09	8.67±0.82	6.65±1.41	0.65

CON: Basal diet; LSPVS: basal diet supplemented with 2.5% sweet potato vine silage; HSPVS: basal diet supplemented with 5% sweet potato vine silage.Values are mean ± SEM (n = 6).

Effect of Sweet Potato Vine Silage on the blood biochemical indexes of pigs

The blood biochemical indexes of the three groups is shown in Table 4. Compared with the CON group, LDL level in HSPVS group was extremely significantly increased ($p < 0.01$), but ALB and BUN had no significant alterations.

Table 4 Effect of Sweet Potato Vine Silage on the blood biochemical indexes of pigs

Item	CON	LSPVS	HSPVS	p-Value
ALB, g/L	39.42±8.68	50.14±3.15	45.42±4.71	0.14
LDL, mmol /L	11.06±0.34b	12.05±0.29b	14.30±1.69a	<0.01
BUN, mmol /L	1.95±0.91	1.90±0.59	1.56±0.32	0.73

CON: Basal diet; LSPVS: basal diet supplemented with 2.5% sweet potato vine silage; HSPVS: basal diet supplemented with 5% sweet potato vine silage. Values are mean ± SEM (n = 6).

Effect of Sweet Potato Vine Silage on the liver antioxidant capacity of pigs

The liver antioxidant capacity of the three groups is shown in Table 5. Compared with the CON group, CAT was extremely significantly reduced in the LSPVS group ($p < 0.01$), but extremely significantly increased in the HSPVS group ($p < 0.01$). GSH-PX was extremely significantly increased in the LSPVS group ($p < 0.01$), but significantly decreased in the HSPVS group ($p < 0.05$).

Table 5 Effect of Sweet Potato Vine Silage on the liver antioxidant capacity of pigs

Item	CON	LSPVS	HSPVS	p-Value
T-SOD,%	531.52±11.50	523.84±15.01	526.41±6.47	0.74
CAT,U/ml	25.33±0.31b	18.27±0.67 c	35.01±3.27a	<0.01
GSH, umol /L	41.26±4.61	43.46±0.56	42.38±7.35	0.30
GSH-PX,U/mg	185.82±20.72b	247.41±23.45a	139.±6.87c	<0.01
MDA ,nmol/mg	2.69±1.40	1.27±0.26	2.82±0.68	0.06
TP, ug/mL	14807.21±900.86	18413.47±493.61	13481.99±1321.49	<0.01

CON: Basal diet; LSPVS: basal diet supplemented with 2.5% sweet potato vine silage; HSPVS: basal diet supplemented with 5% sweet potato vine silage.Values are mean ± SEM (n =

6).

Effect of Sweet Potato Vine Silage on the serum antioxidant capacity of pigs

The serum antioxidant capacity of the three groups is shown in Table 6. Compared with the CON group, GSH was extremely significantly decreased ($p < 0.01$) and GSH-PX was extremely significant increased ($p < 0.01$) in the HSPVS group.

Table 6 Effect of Sweet Potato Vine Silage on the serum antioxidant capacity of pigs

Item	CON	LSPVS	HSPVS	p-Value
GSH, umol /L	2580.56±221.73a	2128.7±7.90ab	1531.58±324.21b	<0.01
GSH-PX,U/mg	185.28±18.55b	184.56±11.96b	263.68±24.67a	<0.01
TP, ug/mL	692.82±119.77	521.31±93.39	688.03±8.97	0.08

CON: Basal diet; LSPVS: basal diet supplemented with 2.5% sweet potato vine silage; HSPVS: basal diet supplemented with 5% sweet potato vine silage.Values are mean ± SEM (n = 6).

Effect of Sweet Potato Vine Silage on the liver immune function of pigs

The liver immune function of the three groups is shown in Fig. 1. Compared with the CON group, IL-1β was extremely significantly decreased in the HSPVS group ($p < 0.01$), and both IL-6 ($p < 0.05$) and IL-8 ($p < 0.01$) were significantly increased and IL-12 was significantly decreased in the LSPVS group ($p < 0.05$).

Effect of Sweet Potato Vine Silage on the serum immune function of pigs

The liver immune function of the three groups is shown in Fig. 2. Compared with the CON group, IgG was extremely significantly increased in the HSPVS group ($p < 0.01$), while IGM was significantly increased in the HSPVS group ($p < 0.05$).

Discussion

This study demonstrated that dietary supplementation with 2.5% SPVS (in dry basis) produced positive effects on eye muscle area in finishing pigs. Eye muscle area is closely related to muscle development and carcass traits and is an important economic trait of pigs [19]. Fermented sweet potato vines optimize

amino acid composition and protein quality which have been found to increase eye muscle area [20]. Nogalski et al. found that cida silage and corn silage increased the composition and fat cover levels of cattle and carcass fat was positively correlated with slaughter percentage[21].

Cooking loss is the quality loss of raw meat due to cooking water loss in the process of processing mature meat. The cooking loss of meat is the result of protein denaturation caused by heat[22]. Pen et al. found that silage of potato by-products had no significant effect on cooking loss of Holstein cattle[23]. But this study found that cooking loss was significantly reduced in both groups fed sweet potato vines. Sweet potato vine also improved the tenderness of meat and enhance the flavor of the meat. Due to the high fiber content of sweet potato vine, pig digestibility is low[24], which may produce negative effects on pigs.

The antioxidant defense system includes small molecules of non-enzymatic antioxidants and enzymatic antioxidants. Enzyme systems include superoxide SOD, CAT and GSH-PX [25], which protect cells from oxidative damage caused by peroxides [26]. Vitamin E, widely found in sweet potato vines, could induce the Wnt10b/β-catenin signaling pathway, and thus modulate the activity of antioxidant enzymes in muscle [27]. Flavonoids from sweet potato vine have been found to have antioxidant capacity[28]. Flavonoids are a class of major plant secondary metabolites with many functions, such as pigmentation, antimicrobial activity and antioxidant activity[29]. SOD is the first line of anti-oxidation defense that catalyzes the conversion of superoxide radicals to hydrogen peroxide[30] while CAT is another antioxidant enzyme that breaks down hydrogen peroxide into water[31]. As previously described, the silage of purple corn straw rich in anthocyanins had good fermentation quality and could improve the antioxidant activity[32] because anthocyanins are a flavonoid-rich compound as well [33]. Hence, flavonoids could play a key role in improving antioxidant capacity in SPVS.

Besides, sweet potato vines contain a variety of immune-boosting substances. For example, sweet potato vines contain selenium which affects specific immunity and non-specific immunity. Specific immunity includes humoral immunity and cellular immunity [34] which mainly rely on T lymphocytes and B lymphocytes to play an immune role [35]. Peplowski et al. demonstrated that dietary selenium supplementation improved the immune activity of weaned pigs [36]. In addition, the addition of organic selenium could also improve the activity of GSH-Px and the antioxidant capacity of the body[37]. In this experiment, the addition of LSPVS in the feed increased the level of GSH-Px, which may be related to the presence of selenium.

Immunoglobulins are a group of proteins that have antibody activity and are important index reflecting the body's humoral immune function. Si et al. [38] found that the addition of papyriformis silage significantly increased serum IgA, IgG and IgM contents of dairy cows, which may be due to the fact that flavonoids in papyriformis could regulate animal immune function. Huang et al. [39] found that the number of white blood cells, phagocytosis of macrophage cells, proliferation of T, B lymphocyte, and

serum IgG content of the mice were significantly increased by dietary supplementation with total flavonoids.

Dietary supplementation with SPVS led to a decline of TNF-α in this experiment, which was in accordance with previous study[40]. In sweet potato vine, another abundant mineral is potassium[41], increased extracellular concentration of which could increase the number of T cells [42]. T cells mediate lymphocyte transport and immune function [43]. In addition to selenium and potassium, the immune regulator in sweet potato vine is vitamin C which is an antioxidant that protects against oxidative damage. Vitamin C scavenges intracellular oxidizing free radicals (O^-) [44] [45]. Meanwhile, vitamin C improves the memory function of $CD3^+$ and $CD8^+$ T cells [46].

Conclusions

In conclusion, dietary supplementation with SPVS improved the growth performance, antioxidant capacity and immune capacity in finishing pigs. Specifically, LPSVS treatment improved the production performance while HSPVS supplementation improved the antioxidant capacity and immune performance. Therefore, further studies are needed to investigate the influence of SPVS supplementation on animal performance in different stages of pigs. SPVS provides a new alternative to substitute part of feedstuff.

Abbreviations

SPVS:Sweet potato vine silage; BW:body weight;DHS-2F:Portable pH meter; L*:luminance; A *: redness;B *: yellowness;ALB:albumin;LDL:low density lipoprotein cholesterol;BUN:blood urea nitrogen;AKP:alkaline phosphatase;T-SOD:total superoxide dismutase;CAT:catalase;GSH:glutathione ;GSH-PX:glutathione peroxidase ; MDA:malonaldehyde;TP:total protein;IL-1β:Interleukin-1β;IL-6:Interleukin-6; IL-8:Interleukin-8;IL-12:Interleukin-12;TNF-α:Tumor necrosis factor-α; IgA:immunoglobulin A;IgM:immunoglobulin M;IgG:immunoglobulin G.

Declarations

Ethics approval and consent to participate

Experimental procedures and animal use were approved by the Northeast Agricultural University.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional file.

Competing interests

We declare that we have no competing interests.

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Authors' contributions

RBW, BS and QQM designed the study;RBW and BS mainly wrote and revised the manuscript; QQM, BMS, ASS reviewed the manuscript;ZYY,RBW,QQM,HZ,QLZ and CNB conducted the experiments and determination of parameters;ZYY,RBW and BS contributed to animal feeding and data analyses. All authors read and approved the final manuscript.

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Figures

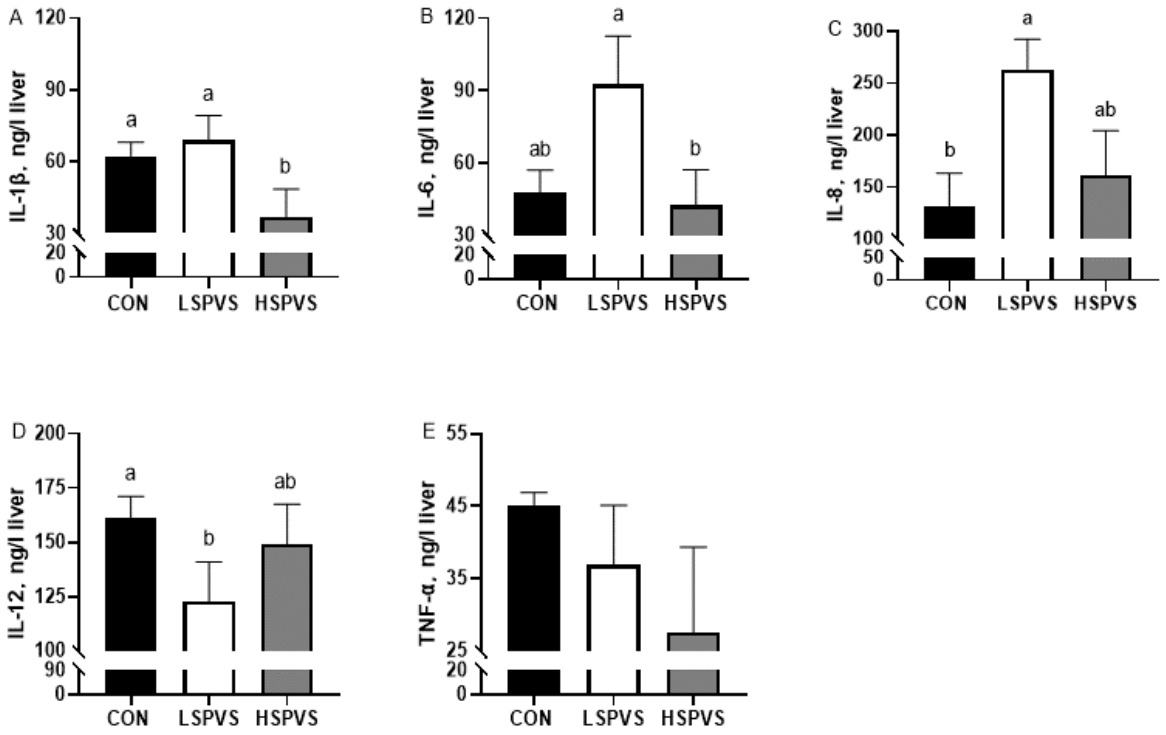

Figure 1

Effect of Sweet Potato Vine Silage on IL-1β (A), IL-6 (B), IL-8 (C), IL-12 (D), TNF-α (E) in liver immune function of pigs. CON: Basal diet; LSPVS: basal diet supplemented with 2.5% sweet potato vine silage; HSPVS: basal diet supplemented with 5% sweet potato vine silage.Values are mean ± SEM (n = 6).

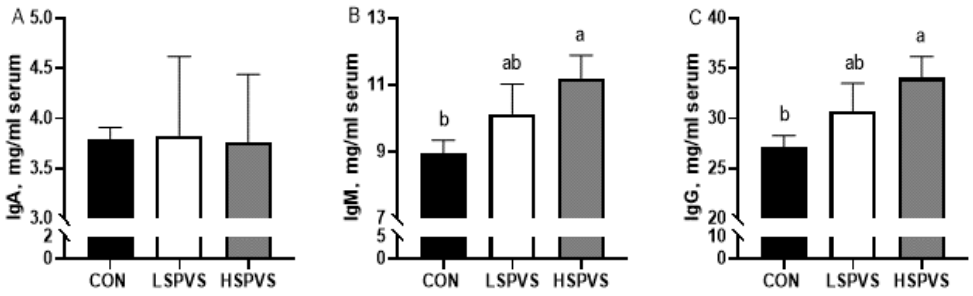

Figure 2

Effect of Sweet Potato Vine Silage on IgA (A), IgM (B), IgG (C) in serum immune function of pigs. CON: Basal diet; LSPVS: basal diet supplemented with 2.5% sweet potato vine silage; HSPVS: basal diet supplemented with 5% sweet potato vine silage. Values are mean ± SEM (n = 6).