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Silybum marianum seed extract supplementation positively affects the body weight of weaned piglets by improving voluntary feed intake

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Abstract

This study was conducted to evaluate the effects of dietary supplementation of *Silybum marianum* seed (SMS) extract on the growth performance, nutrient digestibility, fecal noxious gas emission, and hematology parameters in weaned piglets. A total of 120, 21-day-old weaned piglets ([Yorkshire × Landrace] × Duroc) were randomly assigned to 3 groups based on the average initial body weight (6.46 ± 0.45 kg). There were 8 replicate pens per treatment and 5 pigs (mixed sex) per pen. The experimental period was 42 days. Dietary groups included a basal diet, and a basal diet supplemented with 0.05% or 0.10% SMS extract. Feeding weaned piglets with SMS extract containing diet significantly increased average daily gain and average daily feed intake. Additionally, the supplementation of SMS extract had no significant effects on nutrient digestibility, serum hematology, and fecal noxious gas emission parameters. We considered that the supplementation of SMS extract had positive effects on the voluntary feed intake in weaned piglets, thus improving growth performance.

Keywords: Weaned piglet, Growth performance, Voluntary feed intake, *Silybum marianum*

INTRODUCTION

Silybum marianum, also known as milk thistle [1]. It has been employed as therapeutic agents for centuries in various pathologies due the unique flavonoid complex as its bioactive component [2]. The beneficial effects of *Silybum marianum* extract on human diseases are manifolds, including hepatocytes and lung tissue protection [3,4], anti-cancer [5], diabetes mellitus curation [6], asthmatic disorder alleviation [7], intestinal fibrosis avoidance [8], and cardiac lipotoxicity modulation [1]. In addition, it also has antioxidant [9], immunostimulatory [10], anti-inflammatory [11], and antibacterial [12] properties.

The bioactivity component in *Silybum marianum* extract is silymarin, an isomeric mixture of unique flavonoid complexes [13]. Dietary supplementation of *Silybum marianum* extract has been reported to increase milk production and milk nutrients in cows [14], ameliorate the toxic effects of toxic substances on poultry organs [9,15], and improve the development of mammary gland and reproductive

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Kim IH.
Data curation: Dang DX.
Formal analysis: Dang DX, Cho S.
Methodology: Kim IH.
Software: Dang DX. Cho S.
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Investigation: Kim IH.
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Ethics approval and consent to participate
This experiment was processed under the supervision of the Animal Care and Use Committee of Dankook University (Cheonan, Korea). The relevant protocol has been approved by the above committee (DK-1-2106).

performance in gilts [16], which was attributed to the antioxidant effects of flavonoid presented in the *Silybum marianum* extract [13]. Similarly, Cui et al. [17] reported that feeding weaned piglets with *Tartary Buckwheat* flavonoids containing diet positively increased nutrient digestibility, thus improving growth performance. You et al. [18] noted that dietary supplementation of flavonoid derived from the licorice improved the growth performance of weaned piglets by regulating intestinal health. However, to the best of our knowledge, no study has investigated the effects of *Silybum marianum* extract on the performance in weaned piglets.

We hypothesized that feeding weaned piglets with diets supplemented with *Silybum marianum* seed (SMS) extract may improve the growth performance and reduce the fecal noxious gas emission by increasing nutrient digestibility. The objective of this study was to evaluate the effects of dietary supplementation of SMS extract on growth performance, nutrient digestibility, fecal noxious gas emission, and hematology parameters in weaned piglets.

MATERIAL AND METHODS

A total of 120, 21-day-old weaned piglets ([Yorkshire × Landrace] × Duroc) with the average initial body weight of 6.46 ± 0.45 kg were randomly assigned into 3 groups based on the initial body weight. There were 8 replicate pens per treatment and 5 pigs (mixed sex) per pen. The experimental period was 42 days. Dietary groups included a basal diet, and a basal diet supplemented with 0.05% or 0.10% SMS extract. Production processes of SMS extract were as follows: Dried SMS were pulverized and sieved through a 60-mesh size screen to produce a fine powder from which an ethyl alcohol extract (*Silymarin*) was made. In a nutshell, twenty grams of powder were defatted by soxhlation in three hundred milliliters of petroleum ether for sixteen hours. The defatted powder was then soaked in ethanol (300 mL) for ten hours before being evaporated in a vacuum drying oven (Binder, Tuttlingen, Germany) at 39℃. This product was composed of 10.8% silybin, 16.3% silydianin, and 7.0% silychristin, and coated by chitosan, with an effective content of 250 g/kg. Experimental diets were formulated to meet the nutrient requirements recommended by the National Research Council [19] (Table 1). All pigs were housed in an environmentally controlled room. The one-side stainless steel self-feeder and nipple drinker were installed in pens so as to give pigs free access to feed and water. The temperature during week 1 was maintained at 30℃ and then gradually reduced by 1℃ every week to maintain in 24℃. The humidity within the room was 60%. The protocol in this study (DK-1-2106) was reviewed and approved by the Dankook University Animal Care and Use Committee (Cheonan, Korea).

Experimental parameters measurement

Growth performance

Pigs were weighed individually on days 1, 8, 22, and 42 to measure their body weight. The value of average daily gain (ADG) was calculated based on the data of body weight. Daily feed intake was measured on a pen-basis to calculate the average daily feed intake (ADFI). The feed efficiency (gain to feed ratio) was calculated based on the values of ADG and ADFI.

Apparent nutrient digestibility

The chromium oxide at the dosage of 2 g/kg was supplemented to the diet of pigs during days 35–42 for determining the apparent total tract digestibility (ATTD) of dry matter (DM) and nitrogen, and the apparent energy retention. Representative feed samples in each dietary group were taken after mixing homogenously. On day 42, two pigs were randomly selected from each pen to take the fecal samples via the rectal massage method. All feed and fecal samples were stored at −20℃

Table 1. Ingredients and calculated chemical composition of diets (%, as fed)

Items	Feeding phases		
	Days 1–7	Days 8–21	Days 22–42
Ingredients (%)	100.00	100.00	100.00
Extruded corn	40.29	51.91	59.48
Soybean meal	9.74	17.05	22.46
Fermented soybean meal	10.00	5.50	3.10
Fish meal	7.45	2.00	-
Soybean oil	2.30	2.91	2.80
Monocalcium phosphate	0.66	1.10	1.23
Limestone	0.58	1.02	1.23
Sugar	3.00	2.00	2.00
Whey protein	11.00	7.00	3.00
Lactose	13.46	7.78	3.18
L-Lysine	0.56	0.71	0.63
DL-Methionine	0.15	0.13	0.09
L-Threonine	0.21	0.29	0.20
Choline Chl	0.10	0.10	0.10
Salt	0.10	0.10	0.10
Mineral mix[1)]	0.20	0.20	0.20
Vitamin mix[2)]	0.20	0.20	0.20
Calculated value (%)			
Metabolizable energy (MJ/kg)	14.40	14.20	14.00
Dry matter	91.00	88.00	99.00
Crude protein	20.00	18.00	18.00
Crude fat	4.67	5.25	5.26
Neutral detergent fiber	9.22	9.08	9.14
Acid detergent fiber	3.58	3.36	3.45
Ash	5.80	5.80	5.90
Lysine	1.60	1.50	1.40
Methionine	0.48	0.40	0.35
Calcium	0.80	0.80	0.80
Phosphorus	0.60	0.60	0.60
Lactose	20.00	12.00	5.00

[1)]Provided per kg of complete diet: 80 mg Fe (as $FeSO_4 \cdot 7H_2O$); 12mg Cu (as $CuSO_4 \cdot 5H_2O$); 85mg Zn (as $ZnSO_4$); 8mg Mn (as MnO_2); 0.28 mg I (as KI); 0.15mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

[2)]Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D_3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 μg vitamin B_{12}.

until analysis. Before chemical analysis, samples were dried and further grinding as powder, which was smaller than 1-mm, following the description of Dang et al. [20]. The contents of DM and nitrogen in feed and fecal samples were determined by following the procedure of Association of Official Analytical Chemists [21] by using the method 930.15 and 968.06, respectively. In addition, the energy in feed and feces were determined by a bomb calorimeter (Parr 6100, Parr Instrument, Moline, IL, USA). The chromium levels were analyzed via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The ATTD was calculated relative to chromium concentrations [20].

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Fecal gas emission

Fresh fecal samples were randomly collected from two pigs in each pen via the method of rectal massage on days 1, 7, 21, and 42 to measure fecal ammonia (NH_3), hydrogen sulfide (H_2S), total mercaptans (R-SH), carbon dioxide (CO_2), and acetic acid emission by the method provided by Dang et al. [20]. In brief, Samples from the same pen were mixed and stored into 2.6-L sealed plastic boxes, which had a small hole in the middle and were sealed by adhesive plaster. The fecal samples were then stored at room temperature (25℃) for fermented 24 h. Subsequently, air samples (100 mL) were taken from the head-space above the surface of excreta through the small hole by a gas-sampling pump (model GV-100S, Gastec, Kanagawa, Japan), the sampling height was about 2.0 cm.

Hematology parameters

On days 1, 7, 21, and 42, two pigs per pen were selected randomly and were bled for collecting blood samples via anterior vena cava puncture into K_3EDTA vacuum tubes or clot activator vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The blood samples were collected during 11:00 to 12:00 h in order to exclude the circadiurnal fluctuations in hormone concentrations and stored at −4℃. Blood samples from clot activator vacuum tubes were centrifuged (3,000×g) for 15 min at 4℃ to obtain serum samples and then stored at −20℃ until analysis. The concentrations of blood urea nitrogen (BUN) and creatinine in serum were analyzed by an automatic biochemical blood analyzer (HITACHI 747, Hitachi, Tokyo, Japan). The concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) in serum were determined using an enzyme-linked immunosorbent assay kit (ELISA Starter Accessory Package, Bethyl Laboratories, Montgomery, TX, USA). In addition, whole blood samples from the K_3EDTA vacuum tube were analyzed for white blood cell (WBC), red blood cell (RBC), and lymphocyte concentrations using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

Statistical analysis

All data were subjected to the MIXED procedures of SAS (SAS Inst, Cary, NC, USA), with the following statistical model: $Y_{ijk} = \mu + t_i + r_k + e_{ijk}$, where Y_{ijk} was an observation on the dependent variable ij, μ was the overall population mean, t_i was the fixed effect of SMS extract treatments, r_k was the pen as a random effect, and e_{ijk} was the random error associated with the observation ijk. Orthogonal polynomials were used to assess the linear and quadratic effects of increasing dietary concentrations of supplemental SMS extract. The replicate pen was used as the experimental unit. Variability in the data was expressed as the SEM, $p < 0.05$ was considered to be statistically significant.

RESULTS

Dietary supplementation of SMS extract linearly increased ADG during days 1–7 ($p = 0.029$), 8–21 ($p = 0.005$), 22–42 ($p = 0.023$), and 1-42 ($p = 0.012$), as well as ADFI during days 1–7 ($p = 0.021$), 8–21 ($p = 0.049$), 22–42 ($p = 0.019$), and 1-42 ($p = 0.029$), whereas had no significant effects on feed efficiency (Table 2).

Feeding weaned piglets on SMS extract containing diet had no significant effects on apparent DM digestibility, nitrogen digestibility, and energy retention (Table 3).

Weaned piglets fed the diet supplemented with SMS extract did not affect the fecal NH_3, H_2S, R-SH, acetic acid, and CO_2 emission during all experimental period (Table 4).

Table 2. Effect of dietary supplementation of *Silybum marianum* seed (SMS) extract on the growth performance in weaned piglets[1)]

Items	SMS extract (%)			SEM	*p*-value	
	0	0.05	0.10		Linear	Quadratic
ADG (g)						
Days 1–7	261.32	269.36	274.86	4.078	0.029	0.802
Days 8–21	442.50	461.68	468.82	5.992	0.005	0.421
Days 22–42	487.76	508.18	516.00	8.145	0.023	0.535
Days 1–42	521.92	543.45	552.10	7.764	0.012	0.506
ADFI (g)						
Days 1–7	292.86	299.64	305.72	4.701	0.021	0.938
Days 8–21	597.50	612.50	619.11	9.392	0.049	0.647
Days 22–42	892.14	914.28	921.79	10.679	0.019	0.484
Days 1–42	697.86	706.01	718.21	7.925	0.029	0.794
Feed efficiency						
Days 1–7	0.89	0.90	0.90	0.006	0.470	0.681
Days 8–21	0.74	0.75	0.76	0.008	0.181	0.697
Days 22–42	0.55	0.56	0.56	0.009	0.338	0.868
Days 1–42	0.75	0.77	0.77	0.009	0.112	0.326

1)Values represent the means of eight pens with 5 pigs per replicate pen (n = 40) per treatment for body weight and eight pens (n = 8) per treatment for ADG, ADFI, and feed efficiency.
ADG, average daily gain; ADFI, average daily feed intake.

Table 3. Effect of dietary supplementation of *Silybum marianum* seed (SMS) extract on the nutrient digestibility in weaned piglets[1)]

Items (%)	SMS extract (%)			SEM	*p*-value	
	0	0.05	0.10		Linear	Quadratic
Dry matter	80.26	80.89	81.19	1.716	0.707	0.938
Nitrogen	78.60	79.31	79.61	1.766	0.691	0.925
Energy	79.28	80.15	80.50	1.714	0.618	0.902

1)Values represent the means of eight pens with 2 pigs per replicate pen (n = 16) per treatment.

The concentrations of WBC, RBC, lymphocyte, IgG, IgM, IgA, BUN, creatinine in blood were not affected by SMS extract supplementation (Table 5).

DISCUSSION

In this study, dietary supplementation of SMS extract increased ADG and ADFI of weaned piglets in a dose-dependent manner. Excellent voluntary feed intake promotion and body weight improvement properties of SMS extract have been demonstrated in experimentally induced toxic responses in poultry [22,23]. In addition, Jiang et al. [24] noted that feeding sows with an SMS extract containing diet significantly increased the feed intake, and subsequently improved the reproductive performance. The increase in ADFI means animals intake more nutrients, which is beneficial to the improvement of growth performance [25]. Aromatic components in plants as natural supplements in nutrition have been shown to improve the growth of animals by promoting feed intake [26]. Indeed, the supplementation of SMS has been reported to regulate the digestive process, it could stimulate the production of bile acids in the liver, increase the secretion of digestive juice, thus improving the appetite [13]. Hashem et al. [27] noted that dietary supplementation of silymarin improved appetite and feed intake, thus ameliorate the decrease of body weight induced by the chemical liver injury. Study in β-thalassemia intermedia treated with silymarin indicated that

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Table 4. Effect of dietary supplementation of *Silybum marianum* seed (SMS) extract on the fecal gas emission in weaned piglets[1)]

Items (ppm)	SMS extract (%)			SEM	*p*-value	
	0	0.05	0.10		Linear	Quadratic
NH_3						
Day 1	1.50	1.50	1.00	0.312	0.286	0.529
Day 7	1.13	1.25	1.13	0.395	1.000	0.802
Day 21	1.88	1.63	1.38	0.410	0.411	1.000
Day 42	2.88	2.25	2.13	0.460	0.279	0.668
H_2S						
Day 1	1.30	1.20	1.35	0.183	0.851	0.591
Day 7	1.65	1.48	1.63	0.134	0.898	0.348
Day 21	2.00	1.73	1.78	0.231	0.508	0.579
Day 42	2.28	2.30	2.10	0.171	0.487	0.603
R-SH						
Day 1	2.50	3.25	3.00	0.301	0.270	0.207
Day 7	3.13	3.25	3.50	0.232	0.283	0.831
Day 21	3.25	3.00	3.13	0.232	0.712	0.526
Day 42	3.75	3.00	3.38	0.331	0.443	0.198
Acetic acid						
Day 1	6.25	6.88	6.50	1.195	0.886	0.741
Day 7	7.63	7.13	7.25	1.249	0.837	0.843
Day 21	7.75	7.38	7.13	1.203	0.722	0.967
Day 42	7.75	7.63	7.50	1.142	0.880	1.000
CO_2						
Day 1	9,625.00	9,675.00	9,100.00	658.18	0.587	0.707
Day 7	9,850.00	9,250.00	9,850.00	487.34	1.000	0.341
Day 21	10,225.00	9,800.00	9,625.00	625.72	0.515	0.874
Day 42	9,775.00	10,100.00	10,050.00	516.33	0.715	0.774

[1)]Values represent the means of eight pens with 2 pigs per replicate pen (n = 16) per treatment.

NH_3, ammonia; H_2S, hydrogen sulfide; R-SH, methyl mercaptans; CO_2, carbon dioxide.

treated patients with silymarin increased the appetite [28]. Therefore, we considered that increased ADG as the result of dietary SMS extract supplementation was related to the greater ADFI.

In addition, the mechanism in growth performance improvement in pigs by dietary manipulation was also related to the nutrient digestibility enhancement and subsequently increase in feed efficiency [20]. However, in this study, dietary supplementation of SMS extract had no significant effects on nutrient digestibility and feed efficiency. Similarly, some researchers reported that dietary supplementation of SMS extract did not affect the dry matter and nitrogen digestibility in dogs [29], buffalos [30], and broiler chicks [23]. However, studies that evaluated the effects of dietary supplementation of SMS extract on the nutrient digestibility in pigs were still limited, no study can be used for comparison with our study. In this study, the supplementation of SMS extract neither had beneficial effect nor adverse effects in nutrient digestibility.

Fecal noxious gas is generated by the unabsorbed nutrients fermented by the microbiota in the intestine [31]. The improvement in nutrient digestibility is considered to be one of the strategies for decreasing the contents of unabsorbed nutrients, thus reducing the noxious gas emission [32]. Liu et al. [33] reported that feeding growing pigs with herbal extract containing diet limited the emission of fecal noxious gases by improving nutrient digestibility. Zhao et al. [34] found the fecal noxious

Table 5. Effect of dietary supplementation of *Silybum marianum* seed (SMS) extract on the hematology parameters in weaned piglets[1)]

Items	SMS extract (%)			SEM	*p*-value	
	0	0.05	0.10		Linear	Quadratic
WBC (10^3/μL)						
Day 1	14.32	14.05	14.62	1.896	0.911	0.862
Day 7	13.76	14.20	15.40	2.353	0.635	0.896
Day 21	14.53	15.15	15.61	1.886	0.693	0.972
Day 42	17.44	16.40	18.16	2.202	0.822	0.616
RBC (10^6/μL)						
Day 1	5.47	5.75	5.90	0.540	0.585	0.925
Day 7	5.12	6.52	5.86	0.432	0.256	0.084
Day 21	6.12	5.31	6.40	0.479	0.694	0.141
Day 42	7.18	7.16	6.97	0.474	0.767	0.890
Lymphocyte (%)						
Day 1	29.48	30.33	29.93	2.814	0.913	0.860
Day 7	32.03	35.85	31.08	1.657	0.695	0.063
Day 21	32.68	30.65	30.45	2.343	0.519	0.758
Day 42	35.98	32.63	33.25	1.648	0.272	0.351
IgG (mg/dL)						
Day 1	0.44	0.55	0.48	0.036	0.395	0.076
Day 7	0.47	0.55	0.48	0.042	0.901	0.209
Day 21	0.42	0.55	0.48	0.056	0.489	0.207
Day 42	0.45	0.55	0.48	0.053	0.672	0.238
IgM (mg/dL)						
Day 1	0.16	0.17	0.19	0.034	0.653	0.885
Day 7	0.18	0.18	0.18	0.034	1.000	1.000
Day 21	0.18	0.18	0.18	0.034	1.000	1.000
Day 42	0.18	0.18	0.18	0.034	1.000	1.000
IgA (mg/dL)						
Day 1	0.16	0.16	0.17	0.026	0.895	0.761
Day 7	0.20	0.12	0.14	0.028	0.143	0.182
Day 21	0.13	0.20	0.14	0.030	0.955	0.118
Day 42	0.20	0.12	0.14	0.028	0.143	0.182
BUN (mg/dL)						
Day 1	5.75	6.50	6.25	0.677	0.614	0.561
Day 7	6.00	6.75	6.75	0.656	0.440	0.652
Day 21	6.25	6.75	6.50	0.635	0.787	0.641
Day 42	7.00	6.00	7.00	0.408	1.000	0.077
Creatinine (mg/dL)						
Day 1	1.04	1.03	1.03	0.026	1.000	0.705
Day 7	1.13	1.15	1.09	0.018	0.170	0.130
Day 21	1.20	1.21	1.20	0.017	0.918	0.679
Day 42	1.40	1.44	1.44	0.029	0.357	0.684

[1)]Values represent the means of eight pens with 2 pigs per replicate pen (n = 16) per treatment.

WBC, white blood cell; RBC, red blood cell; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; BUN, blood urea nitrogen.

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gas emission of weaned piglets was decreased by fermented medicinal plants supplementation, which was related to the increase of nutrient digestibility. However, in the present study, feeding weaned piglets with SMS extract containing diet had no positive effects on the nutrient digestibility, which was considered the reason for SMS extract supplementation did not affect the fecal noxious gas emission.

BUN and creatinine as metabolic waste products are discharged by the kidneys [35], thus the concentration of BUN and creatinine in the blood was used as an indicator of nephrotoxicity [20]. The renal histological damage protection effect of SMS extract has been demonstrated by Jaggi et al. [5]. In addition, plenty of studies have demonstrated that dietary supplementation of SMS extract had no significant effects on the serum creatinine levels in ducks [36], horses [37], and dogs [29]. Therefore, the results of this study indicated that dietary supplementation of SMS extract had no adverse effects on the renal function in weaned piglets.

The WBC, lymphocytes, IgG, IgA, and IgM in the blood play important roles in the immune system of animals [20,38]. High levels of the above parameters mean an improvement of immunity [38,39]. RBC are important cell types that transport oxygen [20]. The immunostimulatory properties of SMS extract have been reported in human [5,40]. However, the levels of RBC, WBC, IgA, and IgM in blood were not affected by SMS extract supplementation in the diet of dogs [29], broiler chicks [41], and rabbit bucks [42]. At present study, feeding weaned piglets with SMS extract containing diet had no significant effects on the blood immune parameters.

CONCLUSION

This study demonstrated that dietary supplementation of SMS extract improved the ADG of weaned piglets by promoting voluntary feed intake. In addition, the supplementation of SMS extract had no adverse effects on nutrient digestibility, renal function, and immune status. However, further experiments were needed to evaluate the mechanisms of SMS extract supplementation on the improvement of voluntary feed intake, which will be an interesting point for improving the growth of pigs.

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