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The effects of micelle silymarin on growth performance, nutrient utilisation, and blood profiles of weaning piglets

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ABSTRACT. Our previous study showed that micelle silymarin (MS) improved the antioxidant capacity of lactating sows and average daily gain of fattening pigs; however, its effect on weaned piglets has not yet been elucidated. The present work aimed to explore the influence of varying doses of MS on growth performance, blood profiles, nutrient digestibility, and antioxidant capacity of weaned piglets. One hundred and twenty weaned piglets (60 males and 60 females) at the age of 21 days (average body weight = 6.50 kg) were randomly divided into 3 dietary groups. Each treatment consisted of 8 replicate pens (5 pigs/pen). The following feeds were applied: a basal diet (without MS) and a basal diet with the addition of 0.05% MS or 0.1% MS. The feeding trial lasted 42 days. The results demonstrated that dietary MS linearly increased average daily gain over the entire period ($P = 0.011$). Average daily feed gain tended to be higher with increasing MS doses in the diet ($P = 0.058$). Faecal score and nutrient utilisation in terms of dry matter, nitrogen, and gross energy were not affected by MS doses ($P > 0.05$). On day 42, serum immunoglobulin M levels were elevated after the inclusion of both 0.05% and 0.1% MS in the diet ($P = 0.016$). Additionally, malondialdehyde levels showed a linear reduction on days 14 and 21 ($P = 0.005$), while glutathione levels were increased on day 21 ($P = 0.005$). In summary, supplementing the diet of weaning piglets with MS had no adverse effects on their health. Furthermore, including 0.1% MS in the diet of weaned piglets tended to improve growth performance and antioxidant capacity.

Introduction

In the swine industry, weaning piglets are forcibly transferred to a new environment and abruptly transitioned from breast milk to pelleted diets. This phenomenon is commonly referred to as ‘weaning stress’ (Zhang and Piao, 2022a). The consequence of this stress involve impairments in the intestinal barrier, diarrhoea, and growth arrest (Chen et al., 2021). Reports suggest that natural plant extracts have the potential to alleviate oxidative stress (Corino et al., 2021), help maintain a balanced intestinal microbiota and average daily gain in weaned piglets (Su et al., 2020).

Silymarin is extracted from the fruits and seeds of *Silybum marianum* L. (milk thistle) and contains natural flavonolignans, such as silibinin A and B (main active ingredient), silydianin, and silychristin (Gillessen and Schmidt, 2020). Due to its composition, silymarin has the capability to directly neutralise free radicals, activate antioxidant enzymes and non-enzymatic antioxidants (Surai, 2015). Silymarin has been utilised for more than 2 000 years to protect liver cells from toxicants and ameliorate conditions like alcoholic cirrhosis (Jaggi and Singh, 2016). However, oral administration of silymarin has shown limited bioavailability in the gastrointestinal tract (Wu et al., 2007; Gillessen and Schmidt, 2020) due to its rapid metabolism in the body and water-insoluble properties (Ladas et al., 2005). In contrast, when silymarin is coated with a micelle that consists of a hydrophilic shell and a lipophilic core, its bioavailability can be improved and stability in the blood (Javed et al., 2011; Wang et al., 2014). In our previous study, dietary 0.05%, 0.1%, and 0.2% micelle silymarin (MS) linearly improved average daily gain and decreased lipid oxidation products, such as malondialdehyde, in the meat of fattening pigs (Zhang and Kim, 2022). The addition of 0.05%, 0.1%, and 0.2% MS to lactating sows' diet also demonstrated its antioxidant capacity, as evidenced by higher levels of superoxide dismutase and lower levels of oxidised glutathione (Zhang et al., 2021). However, the effect of MS on weaned piglets have yet to be explored. We hypothesised that supplementing the diet of weaned piglets with MS could alleviate oxidative stress and promote their growth. Therefore, the primary objective of this study was to investigate the influence of various doses of MS on growth performance, nutrient utilisation and blood profiles of weaned piglets.

Material and methods

Weaned piglets were raised at Dankook University's swine facility (Gongzu City, South Korea). All feeding procedures adhered to the Guiding Principles for the Care and Use of the Animal Committee of the Dankook University (Protocol number: DK-1-2116). The animal experiment received ethical approval on August 4, 2022.

Micelle silymarin processing

Milk thistle seeds were pressed and infiltrated with acetone lye. Subsequently, the mixture was concentrated and extracted with a non-polar solvent. The dried extract contained ≥ 80% silymarin. Then the extracts were micellised using various emulsifiers to obtain a minimum concentration of 20% micellar silymarin. The product was provided by Synergen (Gyeonggi-do, South Korea).

Animals and diets

A total of 120 weaned piglets at 21 days of age were randomly assigned to 3 dietary groups (Table 1). They had an average body weight of 6.5 ± 1.0 kg (means ± SD), and there were 60 male and 60 female piglets. Each treatment consisted of 8 replicates (5 piglets/pen), with 2 males or 3 females in each pen. The three dietary groups received different diets. One group was provided with a basal diet, while the other two groups received the basal diet with the addition of either 0.05% or 0.1% micelle silymarin. The basal diets were divided into 3 phases (days 1–7, 8–21, and 22–42) and were formulated to meet or exceed NRC (2012) recommendations for pigs weighing 5–20 kg.

Table 1. Formula and composition of basal diet

Items	Phase 1 (days 1–7)	Phase 2 (days 8–21)	Phase 3 (days 22–42)
Expanded maize	17.02	16.22	-
Expanded oat	15	-	-
Maize	14.3	29.56	44.78
Wheat	-	10.0	20.0
Fermented soy protein	2.3	-	-
Potato protein	1.5	-	-
Dehulled soybean meal 43%	7.0	17.0	23.34
Extruded full fat soybean	-	5.0	-
Plasma protein 70%	5.0	-	-
Dried milk powder	10.0	-	-
Krill powder	2.0	2.0	2
Cheese powder	3.5	4.0	-
Wheat bran	2.5	3.0	2.5
Soybean oil	1.9	3.2	-
Animal fat	-	-	3.69
Lactose	14.0	6.0	-
Monocalcium phosphate	0.78	1.06	0.74
Limestone	0.67	0.65	1.1
Salt	0.1	0.2	0.3
ZnO	0.25	0.25	-
Choline chloride 50%	0.31	0.15	0.09
Lysine 78%	0.48	0.5	0.38
Methionine 98%	0.4	0.32	0.32
Threonine 98%	0.37	0.29	0.21
Tryptophane 98%	0.12	0.1	0.05
Vitamin[1]/Mineral mixture[2]	0.50	0.50	0.50
Total	100.00	100.00	100.00
Chemical composition			
digestible energy, kcal/kg	3730	3570	3490
metabolizable energy, kcal/kg	3530	3420	3330
crude protein, %	19.00	18.00	18.00
crude fat, %	7.00	7.00	6.00
crude ash, %	4.00	4.50	4.80
crude fibre, %	2.40	3.30	3.60
total lysine, %	1.47	1.30	1.20
calcium, %	0.80	0.80	0.80
phosphorus, %	0.55	0.55	0.50

[1] provided per kg of basal diet: mg: Cu ($CuSO_4 \cdot 5H_2O$) 12, Mn (MnO_2) 8, Fe ($FeSO_4 \cdot 7H_2O$) 100, Zn (as $ZnSO_4$) 80; I (KI), 0.28, Se ($Na_2SeO_3 \cdot 5H_2O$) 0.15; [2] provided per kg of basal diet: IU: vit. A 11 000, vit. D_3 1 100; vit. E 44; mg: vit. K 4.4, riboflavin 8.3, niacin 50, thiamine 4, pantothenic acid 27; μg: vit. B_{12} 33

Feeding trial

Piglets were housed together in a single facility for a period of 42 days. The pens measured 150 cm in length, 76 cm in width, and 140 cm in height. Initially, during the first week, the temperature was maintained at 29–30 °C using additional heating lamps. Subsequently, the temperature was gradually decreased by approximately 2 °C each week until it reached a stable level of 24 °C. Relative humidity was maintained at 50–62% throughout the study period. All piglets had free access to tap water and received feed daily.

Sampling and analysis

Body weight (BW) of all animals was measured in the morning on days 7, 21, and 42 after overnight fasting, followed by the calculation of the corresponding feed consumption. Average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio were calculated on days 1–7, 8–22, and 23–42. Additionally, all piglet mortalities that occurred during the study were recorded to ensure accurate adjustment of growth performance calculations.

After checking BW on days 7, 21, and 42, 1 piglet in each pen was selected with a body weight close to the pen average, and blood from anterior vena cava was collected into 2 heparin tubes.

Whole blood (3 ml) was used to analyse the *leukocyte,* red blood cell, and lymphocyte counts using an ADVIA 120 automatic blood analyser (Bayer Global, Leverkusen, Germany). The remaining tubes (5 ml of blood) were centrifuged at 3500 *g*, 4 °C for 15 min. The obtained serum was refrigerated at −20 °C. Serum urine nitrogen was assayed using an urea nitrogen colorimetric detection kit (Thermo Fisher Scientific, Seoul, South Korea). Creatinine was analysed using a fluorometric method, specifically checking the red complex absorbance at the wavelengths of 538/587 nm (Catalogue numbers, ab65340; Abcam, Cambridge, England). For the determination of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), specific kits from Abcam were employed (Catalogue numbers: ab291065, ab190537, ab190536), with the coefficient of variation <10%. The determined ranges for IgG were 15.625–1000 ng/ml, and 12.5–400 ng/ml for IgM and IgA.

To analyse the effects of MS in alleviating oxidative stress in weaned piglets, serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione reductase (GR) were determined. Serum MDA reacts with thiobarbituric acid to form a reddish-brown product under acidic conditions at 95 °C, and this product can be detected at 532 nm. Consistent with the principle that SOD catalyses $O_2^-$to a stable compound, thus avoiding the reduction of nitro blue tetrazole to blue formazan by O_2^-. Formazan can be detected at 562 nm. Glutathione reductase reduces oxidised glutathione in the presence of reduced coenzyme II, chromogen reacts with the thiol group of the reduced glutathione to produce a yellow compound that can be detected at 405 nm. All kits were purchased from Cell Biolabs, Inc. (San Diego, CA; Catalogue numbers: SAT-330, SAT-340, SAT-812, respectively).

Faecal score

Faeces consistency was assessed at weeks 1, 2, 3, and 6. The stool consistency criteria were adopted from Lan et al. (2016) as follows: 1 – hard and dry pellets; 2 – hard, formed stool that remains firm and soft; 3 – soft, formed and moist stool that retains its shape; 4 – unformed stool; 5 – watery stool. Scores were assigned after thorough observations of the stool consistency of individual pigs, and the average stool score per pen was recorded twice daily.

Nutrient utilisation

To evaluate the effects of silymarin on nutrient digestibility of weaned piglets, the endogenous marker Cr_2O_3 (0.25%) was added to the diet during days 35–42. After a 4 day transition period, faeces were collected by massaging the straight long sphincter from the same 2 pigs in each pen twice daily for 3 consecutive days. Subsequently, the 3-day faeces from each pen were proportionally mixed. To obtain dry matter, both feed and faeces were dried overnight at 105 °C (Nørgaard et al., 2014). Nitrogen was measured using a Kjelec ™ 8100 distillation unit, applying method 976.05 (AOAC, 2002). Gross energy was calculated based on the calories of combustion of the feed and faeces; the gross energy was obtained using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Cr_2O_3 was determined using a spectrophotometer (Lei and Kim, 2014). The formula for the calculation of nutrient utilisation was as follows: apparent nutrient digestibility (ATTD) = $1 - (Nf \times Cr_2O_3d) / (Nd \times Cr_2O_3f)\}] \times 100$, where Nf and Nd represented nutrient concentration, and Cr_2O_3f and Cr_2O_3d represented chromium concentration, each in faeces and diet, respectively. These values were all presented as percentages of the total dry matter.

Statistical analysis

All data were checked for homogeneity of variances and normality using the Shapiro-Wilk test.

The GLM orthogonal polynomial comparison was used to analyse linear and quadratic responses to graded dietary level of micelle silymarin. The difference was significant at $P < 0.05$, while $P < 0.1$ indicated a tendency. Data are presented as mean ± standard error of the mean (SEM).

Results

Growth performance and nutrient utilisation

As displayed in Table 2, ADG showed a significant increase throughout the study period ($P < 0.1$) and ADFI tended to increase during days 1–7, 22–42, and 1–42 ($P < 0.1$) with increasing MS dose. There was no significant effect observed on BW or G/F ratio. Table 3 shows no differences in the digestibility of dry matter, nitrogen or gross energy of weaned piglets during days 35–42 between the 3 groups.

Table 2. Effect of micelle silymarin (MS) on growth performance of weaned piglets

Items	MS level			SEM	*P*-value	
	0	0.05%	0.1%		linear	quadratic
Days 1–7						
ADG, g/day	260.19^{b}	268.73^{ab}	276.11^{a}	3.99	0.010	0.907
ADFI, g/day	290.73	297.77	302.97	4.81	0.087	0.878
G/F ratio	0.893	0.899	0.899	0.010	0.290	0.935
Days 8–21						
ADG, g/day	450.88^{b}	458.30^{ab}	463.82^{a}	4.18	0.043	0.854
ADFI, g/day	588.75	593.00	599.48	5.99	0.219	0.881
G/F ratio	0.766	0.773	0.774	0.009	0.559	0.796
Days 22–42						
ADG, g/day	490.26^{b}	504.43^{ab}	511.00^{a}	5.99	0.023	0.610
ADFI, g/day	813.00	834.58	843.29	12.01	0.089	0.667
G/F ratio	0.603	0.605	0.606	0.009	0.852	0.950
Overall						
ADG, g/day	438.79^{b}	449.77^{ab}	456.13^{a}	4.42	0.011	0.673
ADFI, g/day	651.21	664.58	671.96	7.32	0.058	0.742
G/F ratio	0.674	0.677	0.679	0.007	0.644	0.955

0 – basal diet (without MS); 0.05% – 0.05% MS added to basal diet, 0.01% – 0.01% MS added to basal diet; ADG – average daily gain, ADFI – average daily feed intake, G/F – gain/feed ratio, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$. Each treatment contains 8 replicate pens

Table 3. Effect of micelle silymarin (MS) on nutrient digestibility of weaned piglets on days 35-42

Items	MS level			SEM	*P*-value	
	0	0.05%	0.1%		linear	quadratic
Dry matter, %	80.69	81.24	81.43	1.72	0.765	0.932
Nitrogen, %	80.15	79.73	80.21	1.77	0.982	0.837
Gross energy, %	80.17	80.36	80.75	1.71	0.814	0.962

0 – basal diet (without MS); 0.05% – 0.05% MS added to basal diet; 0.01% – 0.01% MS added to basal diet, SEM – standard error of the mean. Each treatment collected faeces from 16 piglets

Faecal score

Dietary MS supplementation exerted no effect on faecal scores of weaned piglets in the entire study period ($P > 0.05$; Table 4).

Table 4. Effect of micelle silymarin (MS) on faecal score of weaned piglets

Items	MS level			SEM	*P*-value	
	0	0.05%	0.1%		linear	quadratic
Days						
1–7	3.14	3.11	3.18	0.10	0.775	0.660
8–14	3.46	3.39	3.20	0.04	0.568	0.315
15–21	3.02	2.92	2.84	0.09	0.161	0.899
36–42	2.60	2.46	2.41	0.11	0.223	0.783

0 – basal diet (without MS); 0.05% – 0.05% MS added to basal diet; 0.01% – 0.01% MS added to basal diet; SEM – standard error of the mean. Each treatment contains 8 replicate pens

Blood profiles

Table 5 shows that the supplementation of different percentages of MS did not affect the levels of serum metabolites. IgM concentrations increased linearly with increasing dietary MS dose only on day 42 ($P = 0.016$; Table 6). There was no discernible impact of MS supplementation on serum immunoglobulin levels in weaned piglets during the remaining days. Increasing doses of dietary MS linearly reduced ($P < 0.05$) MDA concentrations on days 7 and 21 (Table 7).

Table 5. Effect of micelle silymarin (MS) on blood profiles of weaned piglets

Items	MS level			SEM	*P*-value	
	0	0.05%	0.1%		linear	quadratic
Day 7						
leukocyte, 10^{3}	15.00	14.49	15.11	0.60	0.904	0.442
red blood cells, 10^{6}	5.54	5.78	5.67	0.19	0.632	0.471
lymphocyte, %	31.58	31.68	32.63	0.95	0.448	0.719
BUN, mg/dl	5.80	5.91	5.88	0.19	0.766	0.776
creatinine, mg/dl	1.09	0.98	1.10	0.08	0.883	0.251
Day 21						
leukocyte, 10^{3}	15.34	15.45	15.40	0.07	0.513	0.338
red blood cells, 10^{6}	6.02	6.04	5.92	0.25	0.774	0.814
lymphocyte, %	33.31	32.83	33.53	0.92	0.869	0.606
BUN, mg/dl	6.08	5.93	6.12	0.20	0.873	0.504
creatinine, mg/dl	1.25	1.23	1.29	0.02	0.169	0.148
Day 42						
leukocyte, 10^{3}	15.43	15.53	15.44	0.07	0.916	0.294
red blood cells, 10^{6}	6.20	6.29	6.17	0.19	0.928	0.681
lymphocyte, %	34.09	33.95	33.84	0.49	0.718	0.982
BUN, mg/dl	6.54^{ab}	6.46^{b}	6.82^{a}	0.11	0.087	0.125
creatinine, mg/dl	138	1.40	1.36	0.02	0.462	0.189

0 – basal diet (without MS); 0.05% – 0.05% MS added to basal diet; 0.01% – 0.01% MS added to basal diet; BUN – blood urine nitrogen, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$. Each treatment contains 8 replicate pens

Table 6. Effect of micelle silymarin (MS) on serum immunoglobulin levels in weaned piglets

Items	MS level			SEM	*P*-value	
	0	0.05%	0.1%		linear	quadratic
Day 7						
IgG, mg/ml	0.48	0.50	0.47	0.03	0.840	0.488
IgM, mg/ml	0.19	0.17	0.19	0.02	0.929	0.425
IgA, mg/ml	0.22	0.18	0.19	0.02	0.264	0.273
Day 21						
IgG, mg/ml	0.47	0.43	0.52	0.04	0.363	0.209
IgM, mg/ml	0.20	0.29	0.29	0.02	0.537	0.804
IgA, mg/ml	0.20	0.25	0.21	0.02	0.876	0.138
Day 42						
IgG, mg/ml	0.57	0.58	0.61	0.02	0.246	0.696
IgM, mg/ml	0.23^{b}	0.24^{a}	0.24^{a}	0.00	0.016	0.015
IgA, mg/ml	0.23	0.24	0.23	0.01	0.623	0.095

0 – basal diet (without MS); 0.05% – 0.05% MS added to basal diet; 0.01% – 0.01% MS added to basal diet; IgG – immunoglobulin G, IgM – immunoglobulin M, IgA – immunoglobulin A, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$. Each treatment contains 8 replicate pens

Table 7. Effect of micelle silymarin (MS) on oxidant-antioxidant indices in serum of weaned piglets

Items	MS level			SEM	*P*-value	
	0	0.05%	0.1%		linear	quadratic
Day 7						
MDA, nmol/ml	6.21	5.93	5.87	0.09	0.012	0.296
SOD, U/ml	33.61	33.63	34.78	0.93	0.395	0.636
GR, U/ml	1.60	1.56	1.54	0.03	0.152	0.666
Day 21						
MDA, nmol/ml	5.95^{a}	5.84^{a}	5.58^{b}	0.08	0.005	0.466
SOD, U/ml	34.65	35.17	34.83	0.82	0.878	0.675
GR, U/ml	1.58^{b}	1.62^{ab}	1.66^{a}	0.02	0.005	0.582
Day 42						
MDA, nmol/ml	5.89	5.81	5.79	0.05	0.129	0.624
SOD, U/ml	34.90	35.08	35.77	0.77	0.444	0.797
GR, U/ml	1.55	1.59	1.63	0.03	0.055	0.925

0 – basal diet (without MS); 0.05% – 0.05% MS added to basal diet; 0.01% – 0.01% MS added to basal diet. MDA – malondialdehyde, SOD – superoxide dismutase, GR – glutathione reductase, SEM – standard error of the mean. ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$. Each treatment contains 8 replicate pens

In addition, GR concentration exhibited a significant increase ($P = 0.005$) on day 21 in response to the rising MS levels in the diet and displayed an increasing tendency ($P = 0.055$) on day 42. No effect of MS supplementation on serum oxidant-antioxidant markers in weaned piglets was observed on the other days.

Discussion

The present results indicate that the inclusion of 0.1% MS in the diet contributed to an increased ADG, corresponding to higher feed consumption. These results are consistent with the findings of Dang et al. (2022), who reported that *Silybum marianum* seed extracts at concentrations of 0%, 0.05%, and 0.1% had a linear positive effect on both ADG and ADFI in weaning piglets. Similarly, Grela et al. (2020) observed that adding 6% milk thistle seeds to the diet increased BW, ADG, and ADFI of pigs weighing between 25 and 115 kg. It should be emphasized that feed consumption is a crucial factor affecting growth, as emphasized by Dang et al. (2022). In our previous experiment, 0.05% and 0.1% dietary MS improved feed intake of lactating sows (Zhang et al., 2021). Traditionally, silymarin has been used in Chinese medicine to treat liver diseases. As reported by Chand et al. (2011), 10 g/kg milk thistle supplemented in broilers' diet containing 3 g/kg aflatoxin helped alleviate the damage caused by aflatoxin and resulted in similar ADG compared to chickens administered a control diet. It has been speculated that MS protects hepatocytes and maintains normal health status. Hashem et al. (2016) demonstrated that silymarin supplements promoted the appetite of rats with liver injury. A study on olive flounder showed that MS improved feed intake and ADG, while *Silybum marianum* contributed to higher ADFI and BW in Japanese quail (Khazaei et al., 2022). Silymarin contains more than 300 compounds, most of which are polyphenols and flavonoids (Hashem et al., 2016). Bioactive compounds in plants have been reported to induce higher activity of digestive enzymes, such as lipase, amylase or proteases (Srinivasan, 2005). This phenomenon was further observed in a study on yellow croaker larvae by Yao et al. (2020), where dietary silymarin supplements were found to improve trypsin and leucine-aminopeptidase activity. These results suggest that the higher feed intake observed in the present study may be linked to the enhancement of intestinal digestive enzyme activities.

However, nutrient utilisation was not affected, which was in line with the findings of Dang et al. (2022). However, it is worth noting that Shanmugam et al. (2022) found that dietary MS supplementation (0.04% and 0.06%) improved nutrient utilisation in broilers. In our previous study, 0.1% and 0.2% MS addition to the diet led to a higher nitrogen utilisation in fattening pigs but not in lactating sows (Zhang et al., 2021; 2022b). This variation in nutrient utilisation could be attributed to factors such as intestinal villus morphology, barrier function, and microbiota composition, as indicated by

Shanmugam et al. (2022) and Zhang et al. (2022b). Further investigations are required to elucidate the specific mechanisms underlying these differences. Additionally, faecal scores in this study, which indirectly reflect the balance or dysbacteriosis of the intestinal microbiota, were similar in the 0.05% and 0.1% MS groups compared to the control group in a study of Lan et al. (2016), also showing a similar degree of diarrhoea in piglets from different groups. However, one drawback of the latter study was that the incidence of diarrhoea was not assessed.

Antioxidant capacity

Numerous studies have focused on the antioxidant capacity of silymarin extract *in vivo* and *in vitro* due to its high content of polyphenols and flavonoids. The antioxidant properties of MS depend on scavenging radicals or maintaining the optimal redox state of cells through the activation of a range of antioxidant enzymes and non-enzymatic antioxidants (Surai, 2015). In a rat model, silymarin extracts were shown to upregulate the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) in the liver and kidney (Milton and Muthumani, 2012). Nrf2 is responsible for the synthesis of antioxidant molecules that reduce oxidative stress (Viktorova et al., 2019). In an experiment involving fish, 100 and 200 mg/kg silymarin doses induced higher superoxide dismutase (SOD) and catalase activities, and increased the expression of SOD and glutathione peroxidase (Wang et al., 2014). In the current study, 0.1% MS in the diet reduced MDA concentrations and increased GR activity, which was consistent with most findings concerning silymarin extracts or MS. These studies have consistently reported improvements in GR, reduced glutathione (GSH), and SOD activity in various species, including lactating sows, rats, and fish (Milton and Muthumani, 2012; Viktorova et al., 2019; Zhang et al., 2021; Abdel-Latif et al., 2023). MDA is a by-product of fat oxidation and its cross-linking with lipoproteins is known to induce toxic effects. GR is one of the key antioxidant enzymes that catalyses the conversion of oxidized glutathione (GSSG) into its reduced and active form (GSH). This indicates that supplementary MS can alleviate oxidative stress in weaning piglets.

Immunity indices and serum metabolites

Leukocytes, red blood cells, and IgG, IgA, and IgM immunoglobulins are important components of the immunity system (Leonard et al., 2010). Silymarin has been shown to upregulate the expression of immunoglobulin M-2 in the liver and increase serum immunoglobulin levels (Wang et al., 2019; Abdel-Latif et al., 2023). We found higher serum IgM levels in the 0.1% MS group on day 42, which was consistent with the findings of Attia et al. (2017). On the other hand, red blood cells and leukocytes of rabbits supplemented with milk thistle seeds at doses of 5 g/kg and 10 g/kg were not affected, but higher IgG levels were recorded. IgM is known for its role in neutralizing harmful agents during the initial stages of diseases, and it plays a vital role in sterilization and complement activation (Su et al., 2018). Serum BUN levels reflects the waste products of protein metabolism. Elevated serum BUN and creatinine concentrations can be considered indicators of damaged hepatocytes and kidneys, as these organs are involved in nutrient metabolism, urine synthesis, and export (Holanda and Kim, 2020). The similar BUN and creatinine concentrations found in this study could indicate appropriately selected feed and control of the feeding environment, as well as a sign of undamaged liver.

Conclusions

In summary, the supplementation of silymarin (MS) to the diet of weaning piglets had no adverse effects on their health. The dose of 0.1% MS significantly increased average daily gain, which could be attributed to increased feed consumption. Additionally, it enhanced the body's antioxidant capacity, as indicated by higher reduced glutathione levels and lower malondialdehyde concentrations, and also led to elevated immunoglobulin M levels.

Conflict of interest

The Authors declare that there is no conflict of interest.

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