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Micelle silymarin supplementation to fattening diet augments daily gain, nutrient digestibility, decreases toxic gas emissions, and ameliorates meat quality of fattening pigs

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Abstract: To evaluate the impact of micelle silymarin (MS) on fattening pig growth, nutrient digestibility, toxic gas emissions and meat quality, 140 crossed fattening pigs were allocated to four treatments with seven repetition pens [(two barrows and three gilts)/pen] per treatment from the initial body weight of 51.0 kg ($_{SD}$ 2.86). The pigs were fed the basal diet containing 0%, 0.05%, 0.1%, and 0.2% MS for 10 weeks. The results showed a linear improvement in the average daily gain (ADG) of pigs during 5 to 10 weeks as the dietary MS dose increased (P = 0.041). The apparent total tract digestibility of nitrogen (N) was enhancive linearly (P = 0.017 and 0.031, respectively) in week 5 and week 10 as the dietary MS dose increased. The *Lactobacillus* populations in the faeces of pigs fed MS diets were linearly increased (P = 0.048) during week 5. The dietary supplement of MS decreased faecal H_2S concentrations in week 5 and NH_3 concentrations in week 10 (quadratic, P = 0.022 and 0.007, respectively). Moreover, dietary MS linearly diminished cooking loss (P = 0.010) and yellowness value at 45 min postmortem (P = 0.029), whereas the redness value linearly increased (P = 0.028 and 0.002, respectively) after 45 min and 24 h postmortem. Finally, the linear decrease (P < 0.001) of thiobarbituric acid reactive substance (TBARS) concentration and protein carbonyl in pigs fed MS diets was found, but a quadratic improvement (P < 0.031) of total antioxidant capacity (T-AOC) concentration was observed in the meat samples from MS-treated pigs. Taken together, supplementation of the graded level of MS to the basal diet exhibited dose-independent responses on ADG, N digestibility, toxic gas emissions and meat quality. Among the tested doses, 0.2% MS supplementation in the diet is found to be the most effective dose.

Keywords: apparent total tract digestibility; serum metabolites; faecal toxic gas; faecal microbiota

In high-density intensive farming, the growth rate of fattening pigs and their meat quality have been the principal issues especially for raisers, sellers, scholars, and consumers (Valaitiene et al. 2017). Since the high-quality pork with the higher nutritional value, excellent flavour, and ponceau has won high favour by consumers. It is beyond question that the nutrient intake by fattening pigs from the daily diet is the crucial factor for promoting the growth and ameliorating the meat quality. In feeding, the diet should contain functional additives for protecting the intestinal health from damage to better nutrient absorption in the case of ban on antibiotics, leading to elevated meat quality (Grela et al. 2020).

Silymarin, as a natural plant extract from milk thistle fruit, is worthy of attention. It contains polyphenols and flavonoids, and is mainly composed

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of silybin (the biologically active ingredient), silydianin, and silychristin, which are all natural flavonoid lignan compounds. So far the studies have shown that the function of normal silymarin is related not only to anti-oxidation (Surai 2015) and anti-inflammation (Nazemian et al. 2010) but also to the promotion of liver tissue regeneration and ease of liver poisoning and fatty liver (Gillessen and Schmidt 2020). In early researches, when 5% and 10% of *Silybum marianum* herb were fed to rabbits, the herbal fragrance and flavour of the meat were improved (Cullere et al. 2016). Meanwhile, 3 g/kg and 6 g/kg of Silybum seeds supplemented to the diet of growing-fattening pigs for 100 days promoted weight gain, improved carcass ratio and meat quality attributes such as redness and water-holding capacity (WHC) (Grela et al. 2020). Besides, muscles resisting oxidation and changes in lipid metabolism of broilers, rabbits, pigs and fish fed silymarin were reported (Schiavone et al. 2007; Cullere et al. 2016; Xiao et al. 2017; Grela et al. 2020). But normal silymarin has poor water solubility and low biological activity within mammalian bodies. Whereas, when the inner core of hydrophobic segment and outer shell of hydrophobic segment are coated, micelle silymarin (MS) is prevented from being decomposed in the oral cavity, and it is slowly released from the micellar solution. Evidence corroborates the fabulous absorbability of coated silymarin on cells *in vitro* and mouse intestines *in vivo* (Sui et al. 2010; Shangguan et al. 2015). Until now, there have been few studies about supplementing micelle silymarin in fattening diet.

Therefore, this study aimed to assess different levels of MS supplementation to fattening pig diet so as to explore their effects on performance, nutrient digestibility, gas emissions and meat quality.

MATERIAL AND METHODS

These pigs were raised at the swine experimental base of Dankook University in Cheonan (Republic of Korea). Procedures implemented on animals and sampling collection complied with the regulations set by the Animal Care and Use Committee of Dankook University. The micelle silymarin purchased from the Synergen Company (Gyeonggi-do, Republic of Korea) is composed of 10.8% silybin, 16.3% silydianin, and 7.0% silychristin, and coated with chitosan, with an effective content of 250 g/kg.

Animals, diets, and housing

One hundred and forty fattening pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of 51.0 kg (± 2.86) were raised in a 10-week trial and were assigned to four dietary treatments. Each treatment had 35 pigs (seven repetitions of five pens with two barrows and three gilts per pen) blocked on BW and fed either basal diet (control diet, 0% MS, Table 1) or basal diet supplemented with graded levels of MS (0.05%, 0.1%, and 0.2%). The basal diet composition was formulated to meet the nutritional recommendation of National Research Council (NRC 2012). Pigs had *ad libitum* access to water and feed through a semi-self-feeder and a nipple drinker when they were housed in pens (1.5 m × 1.5 m) with a controlled temperature of 20 °C and humidity of 55–70% set by the environmental control device during the entire period. Besides, light provision was manually controlled from 07:00 to 22:00 every day.

Sampling collection and measurement

Growth performance parameter. At the end of week 5 and 10, individual BWs of the pigs (after 8 h of starvation) were measured. Additionally, the total feed input and residual feed were calculated on a pen-to-pen basis to estimate the average daily gain (ADG), average daily feed intake, and conversion ratio (G/F) of pigs.

Backfat thickness and lean meat percentage. The measurements of backfat (BF) thickness and lean meat percentage (LMP) were executed at the end of the fifth week and tenth week. The ultrasonic instrument (Piglot 105; SFK Technology, Herlev, Denmark) was used to determine the BF thickness on the dorsal midline (65 mm, left side) of the last pair of ribs. The calculation of LMP was based on the measurement of BF thickness at two sites: at 7 cm to the midline (left side) between the 3rd and the 4th last lumbar vertebrae; at 7 cm to the midline (left side) between the 3rd and the 4th last ribs, and the depth of the muscle at the same point (Ao et al. 2019).

Apparent total tract digestibility. To evaluate the digestion and absorption of nutrients, a supplement of 2.5 g/kg chromic oxide (Duksan Techopia Co., Ltd, Cheonan, Republic of Korea) was mixed into all diets over the fifth week and tenth week.

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Table 1. Ingredients and composition of basal diet (as fed basis)

Ingredients	%
Maize	76.72
Soybean meal (48%)	15.32
Tallow	2.53
Molasses	2.00
DCP	1.35
Limestone	0.56
Salt	0.30
Methionine (99%)	0.07
Lysine (98.5%)	0.48
Threonine (99%)	0.14
Tryptophan (99%)	0.05
Mineral mix[1]	0.20
Vitamin mix[2]	0.20
Phytase	0.05
Choline (25%)	0.03
Total	100.00
Calculated composition	
Digestible energy (Mcal/kg)	14.39
Crude protein (%)	14.00
Calcium (%)	0.60
Total phosphorus (%)	0.55
Lys (%)	1.00
Met (%)	0.30
Thr (%)	0.65
Trp (%)	0.20
Crude fat (%)	5.39
Crude fibre (%)	2.34
Crude ash (%)	4.36

[1]Provided per kg of diet: Fe, 115 mg as ferrous sulphate; Cu, 70 mg as copper sulphate; Mn, 20 mg as manganese oxide; Zn, 60 mg as zinc oxide; I, 0.5 mg as potassium iodide; Se, 0.3 mg as sodium selenite

[2]Provided per kilogram of diet: vitamin A, 13 000 IU; vitamin D_3, 1 700 IU; vitamin E, 60 IU; vitamin K_3, 5 mg; vitamin B_1, 4.2 mg; vitamin B_2, 19 mg; vitamin B_6, 6.7 mg; vitamin B_{12}, 0.05 mg; biotin, 0.34 mg; folic acid, 2.1 mg; niacin, 55 mg; D-calcium pantothenate, 45 mg

Faecal matter was collected during the last three days of the fifth week and tenth week from randomly selected two pigs (one male and one female) from each pen per treatment by stimulating the pig anal sphincter to induce defecation, and 10% dilute sulfuric acid and toluene were used to fix the manure. Lastly, an equal proportion of three days' faeces were mixed, then stored in the fridge at –20 °C prior to analysis.

Thawed feedstuff and faeces were weighed before being put into an electric oven (Daihan Scientific Co., Ltd, Seoul, Republic of Korea) and heated to 72 °C for 60 h until a constant weight of the product was obtained. Then all heated samples were ground and sieved with a 40-mesh screen. Parallel content determination was carried out for individual samples. Dry matter (DM) in diet and faecal samples was analyzed by Method 930.15 (AOAC 2000). Nitrogen levels were determined by using the principle of Method 920.40 (AOAC 2000), with Kjeltec^{TM} 8400 instrumentation (FOSS, Hillerød, Demark). For gross energy (GE) measurement, caloric content generated from the burning of feed and faeces was measured via a Parr 6400 calorimeter (Parr Instrument Co., Moline, IL, USA). The concentrations of Cr_2O_3 were determined using UV spectrophotometry (UV-1201; Shimadzu, Kyoto, Japan). Briefly, 2 g faecal and feed samples were mixed with a 2-ml mixture (KOH and K_2HPO_4), respectively, and incinerated in a muffle at 600 °C for 4 hours. After that, the samples were chilled for 15 min and filtered. Acetone (1 ml) and H_2SO_4 (2 ml) were blended with the filter, then the mixture was assayed via microcuvette. The Cr_2O_3 concentration was calculated as follows:

$$100 - [(NF/ND) \times (CrD/CrF) \times 100] \quad (1)$$

where:

NF, ND – nutrient concentration in the dry faeces and diet samples, respectively;

CrD, CrF – Cr concentration in the dry diet and faeces samples, respectively.

The calculation of apparent total tract digestibility (ATTD) of nutrients was done using the formula given below:

$$ATTD\ (\%) = [1 - \{(Nf \times Cr_2O_3d)/(Nd \times \times Cr_2O_3f)\}] \times 100 \quad (2)$$

where:

Nf, Nd – nutrient concentrations;

Cr_2O_3f, Cr_2O_3d – chromium concentrations, each in faeces and diet, respectively.

These values were all presented as percentages of the total dry matter.

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Microbial counts in excreta. At the end of week 5 and week 10, fresh faeces were received into sterile tubes, respectively, from the same two pigs for evaluating ATTD by stimulating the pig anal sphincter to induce defecation, and then put into an icebox and immediately transferred to the laboratory. The faecal samples were pooled in equal proportion on a pen basis on the operation table. One g of pooled faecal sample was diluted using 9 ml peptone broth of 10 g/l ranging from 10^3 to 10^7 (1% chroma, Becton, Dickinson, and Co., Franklin Lakes, NJ, USA). To authenticate the different bacteria, featured culture media were used to identify and culture specific microbes. The De Man, Rogosa, and Sharpe medium (CM0361B; Thermo Fisher Scientific, Waltham, MA, USA) was used to incubate *Lactobacillus* at 30 °C for 48 h, and Violet Red Bile Glucose Agar medium (Thermo Fisher Scientific, Waltham, MA, USA) was used to incubate *E. coli* at 37 °C for 24 h. The colony units (CFU/g) per gram of faeces were calculated, and the result was expressed by $\log_{10}$-transformed data. The identification of colonies depended on distinctive shape and colour according to the instructions of culture medium.

Faecal toxic gas. To analyze the influence of dietary MS on the faecal toxic gas production at week 5 and week 10, the fresh faeces (300 g) taken from the rectum of the pigs used for evaluating ATTD were pooled and collected into a 2.6-l airtight plastic crisper and fermented for 24 h at 25 °C. Before the measurement, the crisper was shaken manually to destroy the scabs on the surface and homogenize the samples. Then approximately 100 ml of upper air was sampled using a gas sampling pump by drilling a small hole in one side of the crisper. The measurement of H_2S, NH_3, and methyl mercaptan was conducted using Gastec detector tubes (No. 3La for NH_3, No. 4LK for H_2S, and No. 70 for mercaptans; Gastec Corp., Kanagawa, Japan).

Blood indices. At the termination of the fifth week and tenth week, randomly one pig from the known two pigs for sampling collection per pen (starvation for 8 h) was chosen for taking blood. Blood samples were collected using K_3EDTA evacuated tubes (3.0 ml; Greiner Bio-One, Kramsmenster, Austria) from the ear vein of pigs. Serum was obtained by centrifuging the blood sample immediately at 3 500 × *g*, 4 °C for 10 min, then frozen at a −20 °C freeze until analysis. The serum urea nitrogen, cholesterol, triglyceride, and total protein concentrations were analyzed using MGC240 Indiko Plus automatic biochemical analysis instrument (Thermo Fisher Scientific, Waltham, MA, USA), and the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was analyzed using matching kits (Product No.: C0010-2-1 and C009-2, respectively; Nanjing Jiancheng Institute of Bioengineering, Nanjing, China).

At the end of week 10, all pigs were executed at a local commercial slaughterhouse with the unified batch. Pigs underwent series of corona, bleeding, depilation, removing viscera (preserving kidney and belly fat), and being divided into two halves. Next, the carcass weights were weighed. Precisely, the left side longissimus thoracic (LT) muscle, in terms of meat quality, was cut between the tenth and the eleventh rib from randomly selected seven pigs of each treatment and stored at 4 °C of cold storage for 24 h.

Meat quality assay. The eye muscle area was measured by firstly painting the profile of the cross-section of LT at the tenth rib and then calculating the profile with a planimeter. The pH measurement was implemented after 45 min and 24 h postmortem resorting to a pH meter (Opto-Star, Matthaus, Germany) with the parallel in different positions of the meat sample. The probe of the pH meter was calibrated with the standard buffers of 4.86 and 7.0. The marbling and firmness scores were assessed by two professional judges referring to the National Pork Producer Council (NPPC 1999) at 24 h postmortem. The L* (lightness), a* (redness), and b* (yellowness) values for scoring the meat colour were taken into account and obtained from the average of three random readings using an Opto-Star meat colour tester (Matthaus, Germany) at posthumous 45 min and 24 h. The drip loss was evaluated by the formulation: the weight loss after suspension/initial diced meat weight. In brief, individually diced meat (40 ± 5 g) with a length and width of 3 cm was cut from LT and then put into a sealed sample bag in triplicate. The sample bags were suspended at 4 °C for 24 h, 72 h, and 120 h before reweighing. For the assay of cooking loss, approximately 100 g fresh muscle samples were steamed for 40 min in duplication, after which the samples were removed from the steamer, cooled, and reweighed. The ratio of water to meat area was then calculated, giving a measure of WHC (a smaller ratio indicates increased WHC). For assaying the intramuscular

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fat percentage, approximately 10 g muscle sample was first lyophilized in a vacuum freeze dryer (LG-J12S; Song yuan Freeze Dryer, Co., Ltd, Beijing, China) with the temperature of –45 °C and vacuum pressure of 4 Pa to get powder. Then the fat in LT was obtained by depurating the mixture of powder and petroleum ether using a Soxtec 2055 fat extraction system (Foss Tecator AB, Box 70, S-26321; Höganäs, Sweden).

To assess the effect of dietary MS on antioxidant properties, 20 g of LT were frozen at –20 °C for further analysis. Before the measurement of oxidative and antioxidant properties, the frozen muscle sample at 24 h postmortem was ground into powder in a sterile mortar together with liquid nitrogen, then 1 g powder abrasive was mixed with 9 ml 9% saline (at 4 °C), and then the mixture was centrifuged at 8 000 × *g*, 4 °C for 10 min to get a supernatant for assaying the thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (T-AOC) using commercial kits (Catalogue No.: abx257171, abx298877; Abbexa Ltd, Cambridge, UK) according to the instruction manual. The coefficient of variation (CV) of TBARS between inter- and intra-assay was both under 10%, with a sensitivity of 4.69 ng/ml. Also, the CVs of T-AOC were controlled within 10% (inter-assay) and 15% (intra-assay). TBARS values were quantified as malondialdehyde (MDA) equivalents (mg MDA/kg muscle). Considering the determination of oxidation products of protein, 2,4-dinitrophenylhydrazine (DNPH) carbonyl was assayed referring to the method of Zhang et al. (2019). Parallel determination was applied to each sample.

Data analysis

All data were checked for homogeneity of its variances and with the Shapiro-Wilk test to inspect its normality. Analysis was performed using the statistical model of the mixed procedure (SAS 9.4, Institute Inc., Cary, NC, USA):

$$Y_{ij} = \mu + T_i + e_{ij} \quad (3)$$

where:

Y – the analyzed variable;

μ – the overall mean;

T_i – the fixed effect of the i^{th} treatment;

e_{ij} – the error term specific to the sow identified assigned to the i^{th} treatment.

The linear and quadratic responses to the graded level of MS inclusion in the basal diet were analyzed using an orthogonal polynomial comparison. A probability of $P < 0.05$ was viewed as significant while instances of $0.05 < P < 0.1$ were not.

RESULTS

Growth performance, BF, LMP and carcass of fattening pigs. The effect of the graded level of MS on growth performance of fattening pigs is shown in Table 2, a linear effect ($P = 0.041$) of the dietary incremental MS dose on ADG of pigs was found during weeks 5 to 10, with higher ADG ($P = 0.030$) in pigs fed MS diets than in those receiving the control diet. An uptrend of the G/F ratio of MS-treated pigs from week 5 to week 10 was observed ($P = 0.089$) as the dietary MS dose increased. During the entire experiment, the ADG of pigs fed the graded level of MS showed trends in linear increment ($P = 0.081$), tended to be higher ($P = 0.098$) than in their control counterparts. However, the BF thickness, LMP, and carcass were not affected by the increase in MS levels added to the basal diet ($P > 0.05$) (Table 3).

Apparent total tract digestibility, faecal microbiota colonies and faecal toxicant content. The effect of MS on ATTD of fattening pigs is shown in Table 4. The ATTD of N was enhanced (linear effect, $P = 0.017$ and 0.031, respectively) in response to the increasing MS dose inclusion in the basal diet at week 5 and week 10, whereas the ATTD of DM and GE was not affected by dietary MS in the four groups ($P > 0.05$).

The faecal microbe counts as affected by the graded level of MS are presented in Table 5. The *Lactobacillus* counts were linearly increased ($P = 0.048$) in faecal samples of MS-treated pigs compared with control pigs during week 5.

The effect of increasing levels of MS on gas emissions is shown in Table 6. The H_2S concentrations in week 5 and NH_3 in week 10 were markedly declined (quadratic, $P = 0.022$ and 0.007, respectively) in response to the ascending MS dose in the diet, but they were universally lower ($P = 0.022$ and 0.033, respectively) in the faeces of treated fattening pigs than in the control pigs during week 5.

Serum metabolites. As shown in Table 7, serum cholesterol concentrations in pigs fed MS had the tendency towards decline ($P = 0.079$) compared to those in the control diet during week 5, and they

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Table 2. Effect of the graded level of micelle silymarin on growth performance of fattening pigs (n = 35)

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
Body weight (kg)								
Initial	51.00	51.01	50.99	50.98	1.17	0.988	0.998	0.996
Week 5	79.03	79.38	79.68	80.05	1.54	0.637	0.939	0.996
Week 10	108.27	109.80	109.96	110.93	1.91	0.384	0.362	0.792
0–5 weeks								
ADG (g/day)	801	811	820	830	15	0.275	0.163	0.829
ADFI (g/day)	2 233	2 235	2 239	2 249	38	0.849	0.751	0.969
G/F	0.359	0.363	0.366	0.369	0.006	0.272	0.186	0.749
5–10 weeks								
ADG (g/day)	836	869	865	882	14	0.030	0.041	0.423
ADFI (g/day)	2 728	2 729	2 694	2 668	44	0.561	0.288	0.942
G/F	0.307	0.319	0.322	0.331	0.009	0.123	0.089	0.674
0–10 weeks								
ADG (g/day)	818	839	842	856	14	0.098	0.081	0.615
ADFI (g/day)	2 475	2 482	2 467	2 459	28	0.859	0.593	0.895
G/F	0.331	0.338	0.342	0.349	0.007	0.147	0.091	0.707

ADFI = average daily feed intake; ADG = average daily gain; G/F= average daily gain/average daily feed intake; SEM = pooled standard error of the mean

Table 3. Effect of the graded level of micelle silymarin on backfat thickness and lean meat percentage in fattening pigs (n = 35)

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
Initial								
BF thickness (mm)	13.23	13.22	13.23	13.25	0.09	0.993	0.864	0.892
LMP (%)	57.12	57.13	57.12	57.15	0.13	0.906	0.869	0.961
Week 5								
BF thickness (mm)	15.29	15.31	15.37	15.48	0.11	0.483	0.246	0.935
LMP (%)	54.44	54.52	54.58	54.66	0.12	0.314	0.209	0.837
Week 10								
BF thickness (mm)	17.20	17.42	17.06	17.40	0.20	0.224	0.783	0.222
LMP (%)	52.65	52.64	52.60	52.74	0.18	0.950	0.713	0.867
Carcass	89.40	93.09	89.34	91.46	1.29	0.206	0.803	0.629

BF = backfat; LMP = lean meat percentage; SEM = pooled standard error of the mean

sharply dropped (ANOVA, P = 0.079; quadratic, P = 0.026) during week 10 in treated pigs. In addition, a downward trend was observed in triglyceride concentrations (linear, P = 0.056) as MS addition increased.

Meat quality. Table 8 represents the effect of dietary MS supplementation on the meat quality of fattening pigs. The WHC of meat samples was documented as an upward linear trend (P = 0.080) as the dietary MS dose rose. Cooking loss of muscle samples was linearly reduced in MS-treated groups (P = 0.01) and markedly differed (P = 0.01) from that in the control. Moreover, drip loss at 72 h after slaughter was reduced (P = 0.035) in MS-treated groups

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Table 4. Effect of the graded level of micelle silymarin on apparent total tract digestibility in fattening pigs (%) (n = 7)

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
Week 5								
DM	72.04	72.25	72.59	74.72	2.08	0.637	0.338	0.755
N	65.81	66.04	72.51	80.26	4.54	0.185	0.017	0.755
GE	70.40	70.86	71.31	73.01	2.32	0.624	0.408	0.892
Week 10								
DM	76.29	76.87	76.98	77.20	1.01	0.539	0.554	0.797
N	67.12	67.16	67.90	67.98	0.98	0.289	0.031	0.521
GE	76.66	76.82	76.90	78.22	1.26	0.657	0.361	0.738

DM = dry matter; GE = gross energy; N = nitrogen; SEM = pooled standard error of the mean

Table 5. Effect of the graded level of micelle silymarin on faecal microbiota counts in fattening pigs ($\log_{10}$ CFU) (n = 7)

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
Week 5								
Lactobacillus	9.40	9.41	9.43	9.57	0.19	0.334	0.048	0.480
Escherichia coli	7.48	7.45	7.41	7.38	0.11	0.325	0.765	0.825
Week 10								
Lactobacillus	9.54	9.56	9.57	9.60	0.16	0.610	0.513	0.939
Escherichia coli	7.57	7.54	7.52	7.51	0.14	0.644	0.848	0.991

SEM = pooled standard error of the mean

Table 6. Effect of the graded level of micelle silymarin on noxious gas emission in the faeces of fattening pigs (ppm) (n = 7)

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
Week 5								
Methyl mercaptans	4.83	4.52	4.92	4.77	0.19	0.649	0.847	0.910
NH_3	3.47	3.58	3.02	3.28	0.18	0.622	0.767	0.171
H_2S	6.83	5.86	6.10	6.63	0.25	0.022	0.904	0.022
Week 10								
Methyl mercaptans	5.00	4.77	4.75	4.98	0.25	0.919	0.360	0.883
NH_3	2.72	3.00	3.58	2.98	0.18	0.033	0.267	0.007
H_2S	6.35	6.87	6.30	6.20	0.19	0.616	0.180	0.291

SEM = pooled standard error mean

compared to control pigs. Whereas the supplementation of MS failed to affect the eye muscle area, firmness, intramuscular fat, and marbling in the four groups ($P > 0.05$). The a* value at 45 min postmortem linearly dropped ($P = 0.037$) as MS content increased in diets, and tended to be lower ($P = 0.077$) when compared with control pigs. Dietary MS tended to decline ($P = 0.052$) the L* value at 24 h postmortem compared with the control diet. There was a higher (linear, $P = 0.007$; quadratic, $P = 0.029$) a* value at 24 h postmortem corresponding to the increase in dietary MS, and a* value was significantly higher ($P = 0.005$) in meat samples of treated pigs compared to that in their counterparts. Finally,

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Table 7. Effect of the graded level of micelle silymarin on serum metabolites in fattening pigs (n = 7)

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
Week 5								
Total protein (g/l)	63.49	60.93	61.93	64.88	2.00	0.704	0.455	0.267
Urea nitrogen (mmol/l)	6.10	5.80	6.34	6.03	0.21	0.870	0.837	0.683
Cholesterol (mmol/l)	2.75	2.58	2.68	2.67	0.05	0.079	0.717	0.168
Triglycerides (mmol/l)	0.57	0.54	0.54	0.51	0.03	0.163	0.131	0.837
ALT (IU/l)	56.46	58.06	55.37	57.96	2.30	0.805	0.783	0.733
AST (IU/l)	32.87	33.12	31.36	30.42	1.22	0.393	0.109	0.938
Week 10								
Total protein (g/l)	71.85	70.95	69.13	71.10	1.37	0.369	0.680	0.220
Urea nitrogen (mmol/l)	5.96	6.03	6.40	6.33	0.21	0.246	0.175	0.463
Cholesterol (mmol/l)	2.99	3.02	2.47	2.55	0.12	0.004	0.212	0.026
Triglycerides (mmol/l)	0.603	0.607	0.555	0.535	0.03	0.257	0.056	0.928
AST (IU/l)	60.11	59.70	62.12	60.18	3.51	0.891	0.926	0.749
ALT (IU/l)	43.57	41.70	40.87	44.92	2.05	0.657	0.540	0.174

ALT = alanine aminotransferase; AST = aspartate aminotransferase; SEM = pooled standard error of the mean

Table 8. Effect of the graded level of micelle silymarin on meat quality attributes in fattening pigs (n = 7)

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
Eye muscle area (mm^2)	5 611	5 635	5 586	5 533	119	0.850	0.578	0.833
$pH_{45\ min}$	6.23	6.25	6.25	6.22	0.02	0.700	0.643	0.293
$pH_{24\ h}$	5.15	5.19	5.14	5.12	0.02	0.949	0.199	0.462
WHC (%)	51.09	50.81	56.37	53.64	1.33	0.117	0.080	0.101
Cooking loss (%)	34.88	31.73	31.64	29.94	1.14	0.010	0.010	0.341
Drip loss (%)								
24 h	1.93	1.58	1.84	2.17	0.18	0.747	0.144	0.140
72 h	4.41	4.20	3.96	3.85	0.18	0.035	0.475	0.809
120 h	6.28	6.05	6.58	6.90	0.34	0.139	0.183	0.248
Intramuscular fat (%)	1.57	1.61	1.51	1.58	0.03	0.907	0.759	0.440
Sensory evaluation								
Marbling	2.19	2.22	2.16	2.16	0.05	0.802	0.998	0.465
Firmness	2.84	2.63	2.77	2.71	0.09	0.183	0.547	0.471
Meat colour								
$L^*_{45\ min}$	44.22	43.99	44.32	43.69	0.37	0.624	0.380	0.580
$a^*_{45\ min}$	15.87	15.65	16.22	16.50	0.31	0.371	0.028	0.671
$b^*_{45\ min}$	7.83	7.98	7.36	7.48	0.25	0.449	0.184	0.653
$L^*_{24\ h}$	52.68	52.81	52.61	52.73	0.41	0.052	0.158	0.162
$a^*_{24\ h}$	14.20	14.05	14.47	14.71	0.13	0.178	0.002	0.522
$b^*_{24\ h}$	6.38	6.40	6.29	6.20	0.07	0.271	0.029	0.740

L*a*b* = luminosity index, yellowness index, and redness index; SEM = pooled standard error of the mean; WHC = water-holding capacity

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the b* value at 24 h postmortem in treated groups was reduced (linear, $P = 0.003$) with the increase in dietary MS dose.

Oxidative and antioxidant properties of muscle sample. As shown in Table 9, the concentrations of TBARS and protein carbonyl in meat samples from MS-treated pigs were both linearly decreased ($P < 0.001$, both) as the dietary MS dose increased, and also declined markedly ($P = 0.058$ and 0.019, respectively) in treated groups compared with the control. In addition, the concentrations of T-AOC were increased (quadratic effect, $P = 0.031$) with the dietary increasing level of MS.

DISCUSSION

The MS did not affect final BW, but improved ADG from week 5 to week 10, which was somewhat similar to the observation of Grela et al. (2020), who noted that 3% and 6% of silymarin in diets had a positive impact on BW, G/F ratio, and growth rate of pigs (from 25 kg to 115 kg) by improved feed intake. Williams (2007) demonstrated that dietary supplementation of 20 mg silymarin/kg BW led to a temporary improvement in G/F in the later period of weaning piglets, but in this study the improvement in G/F by dietary MS was not significant. We guessed that the variance in dose and processing technology of additives, and growth phases of pigs were all the reasons causing the difference in results as the relatively mature gastrointestinal tract was less affected by promoters when pigs grew up. Xiao et al. (2017) showed that dietary silymarin boosted the feed utilization of common carp and enhanced protein retention, which was consistent with the findings of the present study, showing higher ATTD of N in MS groups. Given that silymarin was responsible for stimulating secretion and excretion of bile acids, and bile acids expedited a secretagogue in digestive enzymes (Grela et al. 2020), explaining the enhancement in ATTD of N. In addition, high-expression of intestinal tight junction proteins and preferable intestinal morphology resulting from silymarin treatment (Wei et al. 2020) were conducive to the higher ATTD of N as the brush border and microvilli in robust intestinal morphology were available for absorbing nutrients (Wang et al. 2019). To the best of our best knowledge, superior growth is inseparable from gut microbes. The improved N digestibility was also probably facilitated by the flora balance which is reflected via the improved *Lactobacillus* population in response to increasing MS, leading to better metabolism and transformation of feed into body mass (Pot and Tsakalidou 2009).

The ATTD of N was enhanced under the effect of increasing MS during the fifth week while the NH_3 concentrations in the faeces during the tenth week and H_2S concentrations during the fifth week linearly declined in treated groups. The emission of noxious gases from animal excrements was partially influenced by nutrient digestibility and the intestinal decomposing bacteria (Yan et al. 2011) in addition to pH and interaction of chemical substances. Because it was the first time that silymarin was used for exploring the emission of toxic gases from pigs, explaining the cause is our attempt. At the normal level of CP in the study herein, the functional MS may promote N retention, resulting in the low concentrations of NH_3–N in the slurry. Another possibility is that the diminished deaminating bacterial population leads to lower decomposition of NH_3–N because silymarin may regulate the intestinal flora. Likewise, the same principle could also explain H_2S production as H_2S is derived from the decomposition of sulphur compounds by sulphur bacteria in the hindgut (Walker and Schmitt-Kopplin 2021). In short, a reduction in the emission of toxic gases not only reduces the pollutants to the

Table 9. Effect of the graded level of micelle silymarin on muscle oxidation and anti-oxidation properties in fattening pigs at 24 h postmortem

Items	Micelle silymarin (% of diet)				SEM	*P*-value		
	0	0.05	0.1	0.2		ANOVA	linear	quadratic
TBARS (mg MDA/kg)	4.83	4.89	4.43	4.14	0.14	0.058	< 0.001	0.822
Protein carbonyl (mmol/mg)	3.89	3.76	3.31	2.93	0.19	0.019	< 0.001	0.876
T-AOC (IU/mg)	8.57	8.16	8.23	8.89	0.21	0.552	0.144	0.031

SEM = pooled standard error of the mean; T-AOC = total antioxidant capacity; TBARS = thiobarbituric acid reactive substances

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environment and improves the feeding environment (Chu et al. 2011) but also it is conducive to protecting the hindgut mucosa from harm by toxic gas (Pimentel et al. 2013) although the specific mechanism of silymarin is unclear.

Historically, for more than 2000 years silymarin has been known for protecting the liver and curing cap mushroom poisoning. The elevated serum AST and ALT activity is considered to damage liver cells (Onmaz et al. 2017), and AST activity acts as a representative sign for fat liver (Schiavone et al. 2007). In the present study, the levels of serum AST and ALT were similar among groups, which agreed with the findings of Schiavone et al. (2007) about the influence of silymarin on broiler blood. It seemed to reveal no impaired liver function. The result of this study showed that a downward trend of cholesterol in week 5 and a significant decline in week 10, and a linear downward trend of triglycerides were found in the serum of MS pigs. Since the majority of cholesterol levels in human blood originated from the meats we eat (Berger et al. 2015), mounting attention was given to lessen the content of fat and cholesterol in pork to weaken their effect on inducing human Alzheimer's disease, liver dysfunction, and atherosclerotic vascular disease (Maxfield and Tabas 2005). Because the decline of serum cholesterol precedes that in liver (Krecman et al. 1998), we may judge the MS-treated pigs had a low risk of fatty liver. The fat reduction effect of silymarin was reported in the breast of broiler chickens (Schiavone et al. 2007), and the cholesterol reduction effect in hypercholesterolaemia of rats and broilers (Krecman et al. 1998; Kralik et al. 2015; Morovat et al. 2016). We may explain the cause from silibinin, one of the effective constituents, which had the function of increasing lipolysis and impeding gluconeogenesis (Yao et al. 2013). Besides, polyphenols extracted from silymarin could restrain cholesterol absorption in the gut (Schiavone et al. 2007).

The oxidation of protein and lipid in meat diminishes juiciness and shortens the shelf life when pigs suffer heat stress and slaughter stress. As was reported, protecting lipids and proteins from being oxidized was essential for improving the meat quality (Cheng et al. 2020). It was demonstrated in the present study that TBARS and protein carbonyl concentrations in the meat samples of MS-treated pigs were both lower than in their control counterparts, and the T-AOC concentrations showed a quadratic response. Low TBARS concentrations of LT in MS-treated pigs were consistent with the reports of Schiavone et al. (2007) and Grela et al. (2020). Given that the structure of silybin, silydianin, and silychristin formed by hydroxyl groups on phenol and benzene that could combine with oxygen-free radicals, such as O_2^-, and silymarin could accelerate the glutathione synthesis to enhance the antioxidant property (Gillessen and Schmidt 2020). It is noteworthy to mention that the protein oxidation decreased the binding ability of protein and water due to a conformational change, resulting in poor juiciness after cooking.

Pork quality attributes are evaluated based on pH, colour, and WHC and determined by the *MyHC* family genes (Zeng et al. 2019). The muscle pH as a crucial parameter of meat quality reflects the speed of muscle glycolysis and muscle acidity. The sharp fall of muscle pH after slaughter would result in low meat quality due to more denatured protein (Hammelman et al. 2003) and short shelf life. Contrary to the findings of Grela et al. (2020), Cullere et al. (2016), and Kralik et al. (2015) on delaying the muscle pH decrease, no influence of muscle pH was observed in the MS groups in the present study. However, the pH is affected not only by diet but the body temperature before and after slaughter also affects the pH (Jacob and Hopkins 2014), and the process of glycolysis differed in different species (Grela et al. 2020), as well as there was a difference in the type and dosage of additives.

As mentioned in the literature, taste evaluation mainly depends on flavour and tenderness, including other parameters such as WHC and intramuscular fat. The linear trend of an increase in WHC, reduction in cooking loss, as well as reduced drip loss of muscle samples at posthumous 72 h from MS pigs in the present study agreed with the report by Grela et al. (2020). The high WHC and low drip loss were sought by consumers and retailers for freshness in sensory evaluation and retail display (Traore et al. 2012). Since the silymarin took the responsibility for improving the muscle antioxidant capacity to maintain the structure and function of the integral cell membrane, the preferable WHC was obtained (Cheng et al. 2020).

The vividness of pork colour directly affects the preference of consumers to purchase while the low intense L*, high red saturation (a*), and low yellow saturation (b*) are expected for bright red flesh. Previous reports had inconsistent results by silymarin products with improved a* value and decreased

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b* value in broiler meat, and colour parameters from pigs all towards processing advantage, but no chroma effect on rabbit meat (Kralik et al. 2015; Cullere et al. 2016; Grela et al. 2020). Our results demonstrated that treated pigs had the linearly increased a* value and linearly decreased b* value but no significance in the L* value of LT in MS-treated pigs. Lipid oxidation is also accompanied by the oxidation of pigments (Lynch and Faustman 2000). The high a* value primarily depends on the lower oxidation degree of Fe^{2+} in myoglobin (Boles and Pegg 2010) for raw meat and myoglobin denaturation when cooking. The antioxidant properties of micelle silymarin were formed in the given structure of silybin, silydianin, and silychristin by hydroxyl groups on phenol and benzene that could combine with oxygen-free radicals (Gillessen and Schmidt 2020).

CONCLUSION

Micelle silymarin addition to diets of fattening pigs augmented ADG and N digestibility, reduced NH_3 and H_2S emissions, and improved meat quality. Moreover, 0.2% micelle silymarin supplementation in this study achieved an optimal effect.

Conflict of interest

The authors declare no conflict of interest.

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