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Dietary inclusion of mineral detoxified nano-sulfur dispersion on growth performance, fecal score, fecal microbial, gas emission, blood profile, nutrient digestibility and meat quality in finishing pigs

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Abstract

This study is to evaluate the effects of mineral Detoxified nano-Sulfur Dispersion (DSD) on growth performance, fecal score, fecal microbial, gas emissions, blood profile, nutrient digestibility and meat quality in finishing pigs. A total of 160 pigs with an initial body weight of 54.90 ± 5.10 kg were randomly assigned to 2 treatments include basal diet and basal diet with 10ppm DSD. During 10 week trial there were no differences in body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) between the control and DSD groups. Also, the fecal score, fecal microbiota, gas emission was not affected by DSD diet. Dietary inclusion of DSD tended to increase water holding capacity and decrease cooking loss and drip loss. At week 5, serum concentrations of glucose, calcium, total cholesterol, high-density level were increased, and triglyceride concentration was reduced in pigs fed with DSD than control diets. In summary, the inclusion of dietary DSD in the finishing pig diet has improved serum Ca, glucose concentrations and lipid profiles as well as it improves some meat quality traits.

Key words: DSD, finishing pigs, growth performance, blood profile, meat quality.

Introduction

Antibiotics, as growth promoters and therapeutic drug that reduces the risk of infectious diseases, have been widely used in animal production for many years (Barton 2000). Nevertheless, global antibiotics in animals have increased in the recent years with the advent of antibiotic-resistant bacteria that could pose a risk to human health (Huang et al. 2010). The use of antibiotics as growth promoters in animal feed has been prohibited in South Korea since 2011 (Global Agricultural Information Network, 2011). In this regard, it is mandatory to develop antibiotic-free diets for domestic animals including swine. An important effort has been made to identify the alternatives to antibiotics as growth promoters in the livestock industry (Hong et al. 2004; Cho et al. 2006).

Minerals play an essential role in the livestock industry. Minerals perform digestive and bio-synthesis process and growth of animals. Mineral detoxified nano-sulfur dispersion (DSD) (Patent No.: 10-1997773) is a useful product of organic dietary sulfur. The sources of organic sulphur is found in small amounts in grains, meat, eggs, fish and unpasteurized milk, medicinal plants and spices and vegetables. Nano minerals are the product of nano-biotechnology where particle sizes are reduced. Nano-minerals are mentioned to help better in growth, immunomodulation, bactericidal effects than regular products (Swain et al., 2015). Also, they are needed in a lower dose. Sulfur is an essential part of many enzymes and antioxidant molecules like glutathione and thioredoxin. Some sulfur containing compounds can efficiently form a line of defense against reactive oxygen and nitrogen species (Rezk et al., 2004). The transsulfuration pathway is well known as one of the most important metabolic pathways, related to both the synthesis of sulfur containing amino acids and the oxidative stress defense system (Mosharov et al., 2000; Persa et al., 2004). Therefore, sulfur containing antioxidants is essential in the maintenance of normal well-being of the cell and health (Mukwevho et al., 2014). In recent decades, Martin (1988) have stated that sulfur is widely

used as a dietary supplementation for its capability to enhance human health, reduce allergies and prevent asthma. Steely (1994) stated that phyto-genic sulfur concentration can be reduced by cooking and feed processing. Various roles of sulfur have been recognized, such as inclusion in amino acids, in addition to the activities of sulfur-related enzymes and biomolecule metabolism (Komarnisky et al., 2003). Though sulfur should be processed to remove its toxicity, In et al. (2012) developed and validated a method for detoxification through animal experiment. In the animal industry, the positive and negative effects of sulfur have gained much attention, with examination of dietary sulfur fed pigs, cattle and chicks for improvement of meat quality and growth performance (Kim et al., 2014a; Kim et al., 2014b; Park et al., 2003). A few reports have shown the effects of feeding diets containing plentiful sulfur content to cattle on the shelf life and tenderness of meat (Depenbusch et al., 2009; Koger et al., 2010). These comparing products are sulfur containing feed additive but not completely the same one.

To our knowledge, there are no reported studies information on the effects of DSD supplementation in pig diets. Thus, the purpose of this current study was to investigate the inclusion of DSD on growth performance, fecal score, fecal microbial, gas emission, blood profile, nutrient digestibility, meat quality in finishing pig.

Materials and methods

The protocol for this experiment was reviewed and approved by the Animal Care and Use Committee of Dankook University (DK-1-1925), Cheonan, Republic of Korea, for animal experimentation. The mineral detoxified nano-sulphur dispersion (DSD) was supplied by Five N Signature co., (Incheon, Republic of Korea).

Preparation of DSD

The mineral detoxified sulphur dispersion (DSD) was supplied by Five N Signature co., (Incheon, Republic of Korea). The formulation process and product characteristics

supplied by the company are as follows: First mineral sulfur was detoxified by resin vapor produced by boiling pine in water. Plant juice like radish was mixed and stirred with the prepared sulfur and fermented. Then this purified sulfur water was mixed with purified nano-sulfur juice. It was heated to dissolve the vegetable sugar and chloride to get the detoxified nano-sulfur. The final product was a water stock solution of nano-sulfur. For this experiment, the sulfur content of the water stock was fixed to 2%. The final product contained only water and nano-sulfur molecule. The mineral detoxified nano-sulfur was diluted 1:1000 ratio in water and administered to pigs.

Animals and facilities

A total of 160 crossbred finisher pigs {(Landrace × Yorkshire × Duroc)} with initial average weight of 54.90 ± 5.10 kg (Mean $\pm$ SE) were used in 10-week experimental trial. Finisher pigs were divided into pens according to their initial body weight, 5 pigs per pen (3 gilts and 2 barrows). Thirty-two pens were randomly assigned to 2 treatments, where each pen was counted as one replication and each treatment had 16 replications. Pigs were fed either a basal diet (Control) or the basal diet supplemented with 10ppm DSD (Patent No.: 10-1997773). The corn-soybean meal diet was formulated as per recommendation of National research council (2012) (Table 1). Pigs were housed in a thermostatically controlled ambient environmental temperature (25°C) with the slatted plastic floor and equipped with one side self-feeder and nipple drinker. Each pig was individually identified by using tags and the feed and water were provided by ad libitum throughout the experimental period.

Sampling measurements

Initially, (body weight) BW was measured at week 5 and week 10. To calculate the average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F), the consumption of feed was recorded on a pen basis. To calculate apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and energy, 0.20% chromium oxide was added to

the diet as an indigestible marker for 7 days prior to fecal collection at week 5 and week 10. Fecal samples were collected randomly from at least 2 pigs (1 barrow and 1 gilt) per pen then pooled with representative sample and stored in a freezer at -20°C. All feed and fecal samples were freeze-dried and finely ground to pass through a 1 mm screen. DM and N amounts were determined using methods established by the Association of Official Analytical Chemists (AOAC, 2000). UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) was used to determine chromium in the diets and feces at the same time Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) was used to analyze energy by measuring the heat of combustion in the samples. The apparent total tract digestibility was calculated using the following formula: digestibility (%) = $\{1 - [(N_f \times C_d) / (N_d \times C_f)]\} \times 100$, where N_f = nutrient concentration in faeces (% DM), N_d = nutrient concentration in diet (% DM), C_d = chromium concentration in diet (% DM), and C_f = chromium concentration in faeces (% DM).

The fecal score was measured and recorded at week 5 and week 10 and it was determined by the following 1 = hard, dry pellets in a small and hard mass; 2 = hard, formed stool that remains firm and soft; 3 = soft, formed, and moist stool that retains its shape; 4 = soft, unformed stool that assumes the shape of the container; 5 = watery, liquid stool that can be poured. Fecal score and signs of stool consistency were recorded on a pen basis of an individual pig. The fecal microbial was measured at beginning of week 5 and at the end of week 10. The fecal sample were collected from each treatment and using an ice pack to maintain the temperature. One gram of composite fecal sample was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co.) and then homogenized. The fecal samples were placed on plating serial 10^3 to 10^7 dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI), and Lactobacillus medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *E. coli* and *Lactobacillus* respectively to determined colony counts of bacteria. *Lactobacillus* was incubated for 48 h at 39°C under

anaerobic condition. The MacConkey agar plates were incubated at 37°C for 24 h. *E. coli* and *Lactobacillus* colonies were counted soon after removal from the incubator. To analyze the content of NH₃ and H₂S in fecal, 300 g fresh fecal samples were collected from each cage and placed in plastic boxes (polyvinyl, 25 × 35 cm) At week 5 and end of the experiment the collected samples were fermented in an incubator at (28°C) according to the method described by Cho et al., (2008). Concentrations of NH₃ and H₂S were measured within the range of 5.0 to 100.0 ppm (No. 3La, detector tube; Gastec Corp., Kanagawa, Japan) and 2.0 to 20.0 ppm (4LK, detector tube; Gastec Corp.) on d 1, 3, and 5 after fermentation.

During 5th, and 10th week blood samples (10) were randomly collected from pigs of each treatment and poured in vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), and stored at 4°C. Approximately 3 mL blood samples were centrifuged at 4,000 × g for 15 min at 4°C, to separate the serum. An automatic biochemistry analyzer (Advia 120, Bayer, Tarrytown, NY) was used to estimate the serum enzyme activity SOD, glucose, TG, cholesterol and calcium following manual instructions. On day 70, 8 pigs per treatment were slaughtered at a local commercial slaughterhouse. Then meat samples were chilled at -4°C for 24 hours. Employing a Minolta CR410 (Minolta Co, Japan) chromameter, meat quality was evaluated by measuring the Longissimus muscle color parameters of lightness (L*), redness (a*), and yellowness (b*). Duplicate pH values for each sample were measured using a pH meter (Testo205, Testo, Germany). Approximately, 2 g of Longissimus muscle sample was used to measure drip loss according to the plastic bag method described by Honikel (1998). The water-holding capacity (WHC), procedure described by Kauffman et al. (1986) was followed in this experiment. In short, a 0.3-g sample was pressed with 3,000 g of weight for 3 min at 26°C on a piece of filter paper (125-mm-diameter). The areas of the pressed sample and the expressed moisture were delineated and then determined using a digitizing area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan). The ratio of water: meat area was calculated;

giving a measure of WHC (a smaller ratio indicates increased WHC).

Statistical analysis

All data were statically analyzed by t test using the SAS program as a randomized complete block design (SAS Inst. Inc., Cary, NC), with the pen serving as an experimental unit. The results are significant at $P < 0.05$ level and $P < 0.10$ was considered as trend.

Results and Discussion

The current research was aimed to evaluate the effect of DSD supplementation in finishing pig's growth performance, fecal score, fecal microbial, gas emission, blood profile, nutrient digestibility, meat quality. Detoxified nano-sulfur dispersion supplementation in finishing pig's growth performance was shown in Table 2. The inclusion of DSD did not have any significant effect ($P > 0.05$) on growth performance (BW) during week 5 and week 10. There was no significant effect ($P > 0.05$) of 10ppmDSD supplementation on ADG, ADFI, and G: F of during week 5, 10 and overall experimental period. According to Jacobson et al. (1967) and Kennedy and Siebert (1972) reported that feed intake was significantly improved by using sulfur supplementation in cattle and sheep. According to our knowledge there is limited research on the sulfur supplementation on growth performance, so we cannot justify this controversy. Thus, contradictory was resulted in various experiments due the variation in amount of DSD dosage, different animals and differences in dietary compositions. In addition, there was no significant effect in nutrient digestibility of DM, N, GE due to the dietary DSD supplementation ($P > 0.05$; Table 3) in our study. DSD might be has a more direct effect on growth efficiency than on the digestibility of nutrients in poultry and pigs. Previous study Roy et al. (2012) demonstrated that nano-sulfur component against the antibiotic resistant bacteria had positive result and proved its anti-microbial activity. Caridi (2002) reported different species of *Lactobacillus* having an inhibitory effect on *E. coli*. So, increase in *Lactobacillus*

count may have reduced *E. coli* count by nutrient use competition or by producing lactic acid in the digestive system. Yang et al. (2010) indicated that the species and populations of microorganisms in the digestive tract influence the digestibility of nutrients and affect gut health conditions. Lactic acid bacteria are considered as beneficial intestinal bacteria, whereas coliforms are considered as major bacteria that often cause gut health problems such as diarrhea in animals. But the present study revealed that dietary inclusion of DSD in finishing pig had no significant effect on fecal microbiota of *Lactobacillus* counts and *E. coli* count during week 5 and 10 (Table 5).

The dietary supplementation of DSD on noxious gas emission in finishing pigs shown in Table 6. There was no significant effect on fecal gas emission (NH_3 , H_2O , Methyl mercaptan, CO_2 , and Acetic acid) between control and DSD groups at week 5 and 10. Previously, Yan et al. (2011) stated that fecal noxious gas emission was associated with nutrient digestibility, as decreased digestibility may reduce the microbial fermentation substrate in the large intestine. thus, decrease fecal noxious gas emission. In our study, during week 5, and 10 no significant difference ($P > 0.05$) were observed on fecal score associated with the inclusion of DSD diet (Table 4). Until now, there is no investigation on the dietary inclusion of DSD on the fecal microbiota counts, gas emission, and fecal score of swine. Hence, more studies are needed with different levels of DSD to know the exact cause for the absence of a substantial change in the excreta microbiota, gas emission, and fecal score in our experiment.

In the present study, dietary supplementation of DSD tended to reduced cooking loss ($p = .057$) and drip loss ($p = .094$ and $.081$) from analyzed meat samples on d5 and d7, respectively. In addition, pH value was significantly increased in DSD group than control group. A trend in increment in WHC was observed in pig fed with DSD supplementation ($p = .06$) (Table 8). However, no significant ($P > 0.05$) effects were found in meat color, sensory evaluation, and Longissimus muscle area (LMA). Generally, meat pH is a direct expression of

the quality of content in muscle acid and influences the sheer intensity, drip loss and color of the meat (Hossain et al. 2015). Balasubramanian et al. (2018) stated that drip loss was an ordinary indicator and indicates in better meat quality. In the present study, however, no effect on meat color fed with DSD diet in finishing pig was observed. From this perspective, we believe that DSD has a beneficial effect on meat quality. The effect of DSD supplementation on blood profile in finishing pig is shown in Table 7. In the present study, the concentrations of glucose, calcium, and lipid profiles (high-density level (HDL), low-density level (LDL) and Total cholesterol) were significantly increased ($P < 0.05$) respectively, whereas triglyceride concentration was significantly decreased during week5. Puvaca et al., (2015) stated that bioactive sulfur components have anti-inflammatory and antioxidant effects. In this regard, the dietary inclusion of DSD may be provide efficient anti-oxidant or free radical activity in pigs. The DSD supplementation diet in finishing pigs had no effect on SOD (superoxide dismutase). At week10, there was no significant difference on SOD Glucose, Calcium, Total cholesterol, HDL, LDL and Triglyceride. White and Venkatesh (2011) informed that total cholesterol and triglycerides plays a major role in humans and animal cell membrane like formation, fat transfer, steroid hormones synthesis and blood sugar from the kidney. Hosoda et al. (2006) suggested that the concentration of serum triglyceride, Total cholesterol, HDL cholesterol, and LDL cholesterol can be used to assess glucose and lipid metabolism. This research was conducted by environmentally controlled facility and there was no scavenging facility provide to pigs, so they did not have variation of stress. However, comparable research is not available to explore the reason for this outcome.

Conclusion

The current study will be the base of future research. The inclusion of DSD in the finishing pig diet at week 5 has improved serum Ca, glucose concentrations and lipid profiles as well as it improves some meat quality traits. In addition, it failed to show significant effects

of growth performance, fecal score, nutrient digestibility, gas emission, and fecal microbial. However, further research with different levels of DSD is required to understand the effect of DSD in finishing pig.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Table 1. Composition of finishing pig diets (as fed-basis).

Items	Finisher1	Finisher2
Ingredients (%)		
Corn	79.21	83.13
Soybean meal (48%)	16.2	12.48
Tallow	1.72	1.65
Tri-calcium phosphate	1.18	1.04
Lysine	0.44	0.41
Limestone	0.41	0.38
Salt	0.2	0.2
Tryptophan (10%)	0.2	0.3
Threonine (99%)	0.13	0.12
Vitamin mix ²	0.12	0.12
Mineral mix ¹	0.1	0.1
Methionine (99%)	0.06	0.04
Choline (25%)	0.03	0.03
Total	100	100
Calculated value		
Crude protein, %	14.5	13
Fat, %	4.69	4.7
Ash, %	3.85	3.49
Fiber, %	2.43	2.37
Lys, %	1	0.87
Thr, %	0.66	0.59
Ca, %	0.61	0.54
P, %	0.54	0.5
Met, %	0.29	0.26
Trp, %	0.18	0.17
ME, kcal/kg	3300	3300

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

² Provided per kilograms of diet: vitamin A, 13,000 IU; vitamin D₃, 1,700 IU; vitamin E, 60 IU; vitamin K₃, 5 mg; vitamin B₁, 4.2 mg; vitamin B₂, 19 mg; vitamin B₆, 6.7 mg; vitamin B₁₂, 0.05 mg; biotin, 0.34 mg; folic acid, 2.1 mg; niacin, 55 mg; D-calcium pantothenate, 45 mg.

Table 2. The effect of mineral detoxified nano-sulfur dispersion on growth performance in finishing pigs¹

Items	CON	TRT1	SEM ²	<i>P</i> -value
Body weight, kg				
Initial	54.89	54.9	0.01	0.58
Week 5	80.71	81.17	0.31	0.32
Week 10	109.82	111.15	0.71	0.20
Week 5				
ADG, g	738	744	10	0.65
ADFI, g	2224	2218	11	0.69
G/F	0.332	0.335	0.003	0.39
Week 10				
ADG, g	832	842	15	0.62
ADFI, g	2797	2805	20	0.79
G/F	0.297	0.3	0.003	0.52
Overall				
ADG, g	785	793	11	0.17
ADFI, g	2511	2511	15	0.97
G/F	0.312	0.315	0.003	0.41

¹Abbreviation: CON, Basal diet; TRT1, CON + 10ppm mineral detoxified nano-sulfur dispersion.

²Standard error of means.

Table 3. The effect of mineral detoxified nano-sulfur dispersion supplementation on nutrient digestibility in finishing pigs¹

Items, %	CON	TRT1	SEM ²	<i>P-value</i>
Week 5				
Dry matter	70.38	71.22	1.34	0.67
Nitrogen	68.64	68.93	1.12	0.86
Energy	69.3	69.18	1.19	0.95
Week 10				
Dry matter	67.01	68.19	1.07	0.46
Nitrogen	65.13	66.17	1.72	0.68
Energy	66.79	67.04	1.46	0.91

¹Abbreviation: CON, Basal diet; TRT1, CON + 10ppm mineral detoxified nano-sulfur dispersion.

²Standard error of means.

Table 4. The effect of mineral detoxified nano-sulfur dispersion supplementation on fecal score in finishing pigs¹

Items	CON	TRT1	SEM ²	<i>P-value</i>
Fecal score ³				
Initial	3.2	3.21	0.03	0.87
Week 5	3.14	3.13	0.05	0.85
Week 10	3.11	3.1	0.06	0.91

¹Abbreviation: CON, Basal diet; TRT1, CON + 10ppm mineral detoxified nano-sulfur dispersion.

²Standard error of means.

³Fecal score = 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes shape of container; 5 watery liquid that can be poured.

Table 5. The effect of mineral detoxified nano sulfur dispersion supplementation on fecal microbial in finishing pigs¹

Items, log ₁₀ cfu/g	CON	TRT1	SEM ²	<i>P-value</i>
Week 5				
<i>Lactobacillus</i>	7.64	7.67	0.06	0.73
<i>E. coli</i>	5.46	5.43	0.09	0.82
Week 10				
<i>Lactobacillus</i>	7.69	7.76	0.07	0.45
<i>E. coli</i>	5.60	5.66	0.05	0.40

¹Abbreviation: CON, Basal diet; TRT1, CON + 10ppm mineral detoxified nano-sulfur dispersion.

²Standard error of means.

Table 6. The effect of mineral detoxified nano sulfur dispersion supplementation on gas emission in finishing pigs¹

Items, ppm	CON	TRT1	SEM ²	<i>P-value</i>
Week 5				
NH ₃	1.5	1.6	0.7	0.87
H ₂ S	4.5	3.3	1.1	0.49
Methyl mercaptan	11	10.8	0.9	0.87
CO ₂	2125	2150	260	0.95
Acetic acid	3.4	3.6	1.5	0.93
Week 10				
NH ₃	2.2	1.9	0.7	0.77
H ₂ S	5.3	5.8	0.8	0.70
Methyl mercaptan	12.8	12.4	0.5	0.60
CO ₂	2175	2275	504	0.90
Acetic acid	3.2	3.3	1	0.95

¹Abbreviation: CON, Basal diet; TRT1, CON + 10ppm mineral detoxified nano-sulfur dispersion.

²Standard error of means.

³NH₃, ammonia; H₂S, hydrogen sulphide; CO₂, carbon dioxide.

Table 7. The effect of mineral detoxified nano sulfur dispersion supplementation on blood profile in finishing pigs¹

Items	CON	TRT1	SEM ²	<i>P-value</i>
Week 5				
SOD, %	63.8	61.8	2	0.50
Glucose, mg/dL	71.6 ^b	95.6 ^a	2.84	0.00
Calcium, mg/dL	8.9 ^b	9.9 ^a	0.28	0.03
Total cholesterol, mg/dL	71.6 ^b	89.7 ^a	4.21	0.01
HDL/C, mg/dL	28.4 ^b	39.4 ^a	1.21	0.00
LDL/C, mg/dL	38.6	46.2	2.62	0.07
Triglyceride, mg/dL	55.2 ^a	25.7 ^b	7.43	0.02
Week 10				
SOD, %	58	59	2.72	0.80
Glucose, mg/dL	91.3	84.8	2.57	0.11
Calcium, mg/dL	9.4	9.5	0.22	0.78
Total cholesterol, mg/dL	89.5	80.9	4.1	0.17
HDL/C, mg/dL	35.6	32.1	1.67	0.17
LDL/C, mg/dL	45.7	40.2	2.47	0.15
Triglyceride, mg/dL	68.5	64.9	7.65	0.74

¹Abbreviation: CON, Basal diet; TRT1, CON + 10ppm mineral detoxified nano-sulfur dispersion.

²Standard error of means.

³SOD, superoxide dismutase; HDL, high density lipoprotein; LDL, low density lipoprotein.

^{a,b} Means in the same row with different superscripts differ ($P < 0.05$).

Table 8. The effect of mineral detoxified nano sulfur dispersion supplementation on meat quality in finishing pigs¹

Items	CON	TRT1	SEM ²	<i>P</i> -value
Meat color				
L*	57.01	55.85	1.14	0.49
a*	14.5	15.23	0.29	0.12
b*	7.77	7.83	0.3	0.90
Sensory evaluation				
Color	3.69	3.64	0.06	0.59
Marbling	2.8	2.77	0.04	0.57
Firmness	2.91	2.97	0.07	0.53
Cooking loss, %	24.50	21.99	0.78	0.06
Drip loss, %				
d1	3.38	2.75	0.52	0.42
d3	5.44	5.06	0.63	0.69
d5	10.9	10	0.33	0.09
d7	21.07	20.12	0.33	0.08
pH	7.15 ^b	7.44 ^a	0.74	0.03
Longissimus muscle area, cm ²	64.66	66.66	1.25	0.30
Water holding capacity, %	50.47	54.72	1.35	0.06

¹Abbreviation: CON, Basal diet; TRT1, CON + 10ppm mineral detoxified nano-sulfur dispersion.

²Standard error of means.