Effect of simultaneous dietary supplementation of betaine, selenomethionine, and vitamins E and C under summer conditions in growing-finishing pigs

L. De Prekel, D. Maes, A. Van den Broeke, B. Ampe, M. Aluwé

Veterinary Sciences

Supplementary material and methods S1

Measurements in the slaughterhouse

Observations in the lairage area

All pigs were transported to a commercial slaughterhouse at 25 weeks of age. Upon arrival at the slaughterhouse, the same selected reference pigs were observed in the lairage area. Heat stress level was assessed upon entry to lairage and again one hour later. Heat stress was scored according to a tagged visual analogue scale of 0-150. Presence of panting, open mouth and drooling were also observed (yes/no) at the time of heat stress scoring. Skin colour (pink, red or red-spotted) and skin lesions (on a scale from 0 to 4) were also assessed at the same time as HS scoring.

Carcass traits

Pigs were slaughtered by exsanguination after carbon dioxide stunning. The 'AutoFOM III™' system (Frontmatec, Denmark) was used to define different carcass traits, as described by Kowalski et al. (2020). Warm carcass weight, cold carcass weight, carcass lean meat content, fat thickness, muscle thickness, dressing yield and lean tissue growth were determined.

Meat quality: instrumental

At 35 minutes post-mortem, the initial pH (Type HI98163 electrode FC2323, Hanna Instruments®, Temse Belgium) of all 57 animals was measured in the *M. longissimus thoracis et lumborum* next to the 7th rib (starting from the rostral side of the carcass). About 22h after slaughter, loin samples of the left side of all test animals were collected in the slaughterhouse. Twenty-four hours post-mortem, the ultimate pH was measured at the bottom, the middle and the top of the loin sample. Then the *M. longissimus thoracis et lumborum* was cut into meat slices and visible connective tissue and fat were removed. Water holding capacity was evaluated according to the gravimetric EZ-drip loss method of Christensen (2003), where two slices of 2.5cm were used. From each meat slice, a circular sample (Ø 25mm) between 9.5-10.5g was cut with a circular knife and weighed. Then, each sample was put into an EZ-drip loss container and stored for 48h at 4°C. The sample was weighed after 48h and the drip loss percentage calculated. Cooking loss and Warner-Bratzler (WB) shear force were measured according to the methods of Boccard et al. (1981) and Honikel (1998). Two slices of 2.5cm were put in a warm water bath of 75°C for 60 minutes and cooled down for a

minimum of 15 minutes by tap water. Then five cylindrical samples (Ø 1.27mm) per slice were cut parallel to the fibre direction and sheared perpendicular to the fibre direction using the Warner-Bratzler shear (Multitest 2.5-DV and AFG 250N, Mecmesin, UK). Afterwards, the average of all ten measurements per animal was calculated. Intramuscular fat content was measured according to the Bligh and Dyer method (Hanson J., 1963). First, two meat slices of 2.5cm were homogenised with a meat grinder and an ULTRA-TURRAX (ULTRA-TURRAX®, IKA®-Werke GmbH & Co. KG, Germany). Then, 100g of the homogenised sample was put in the NIRS device (NIRS DS2500 L™, FOSS, Denmark).The CIE-L*a*b* colour determinants were measured by three repeated measurements on 2.5cm meat slices with reflection spectroscopy (Miniscan® EZ 4500 L, Hunterlab, USA) after 30 minutes of blooming at 9 °C. Hue (colour vividness) and chroma (colour saturation index) were calculated according to the following formulas (AMSA, 2012):

$$Hue = \tan^{-1}\left(\frac{b*}{a*}\right)$$

$$Chroma = \sqrt[2]{a*^2 + b*^2}$$

With L*: lightness, a*:redness and b*:yellowness

When collecting the loin samples for meat quality, two carcasses were misplaced and were not included in the dataset.

Meat quality: sensory

Sensory evaluation of the loin samples was performed by a trained panel. Five sessions were organised in which 11 samples were served per session, with five or six samples per diet group. Each sample was scored by six trained panellists (according to Arildsen Jakobsen et al. (2014)) on different characteristics (fried odour, piggy odour, softness, juiciness, fried flavour, piggy flavour and acidic flavour) by a visual analogue scoring from 0-100. Between each sample, the panellists cleansed their palate by drinking water and eating an unsalted cracker. Each sample (slices of 2.0cm) was grilled on a clean pan (Tefal Ultra Compact Comfort GC3060, Tefal, Rumilly, France) to an inner temperature of 72°C. No herbs, salt or fat were added. Each slice was divided into six pieces and covered by a plastic cup to retain the aroma.

Statistical analysis

The effect of diet and observation time point during lairage (upon arrival and one hour after) on the heat stress score and skin lesions was evaluated using a linear mixed model with the pen as a random variable. The effect of diet on the different parameters of carcass traits, instrumental meat quality and sensory evaluations was also evaluated by a linear mixed model with pen as random variable for carcass traits and instrumental meat quality and panel time as random variable for the sensory meat quality. A post

hoc was performed when significant differences were found. The pen was considered as experimental unit.

References

AMSA. (2012). AMSA Meat Color Measurement Guidelines. *Meat and Muscle Biology*. https://doi.org/10.22175/mmb.12473

Arildsen Jakobsen, L. M., Vuholm, S., Aaslyng, M. D., Kristensen, M., Sørensen, K. V., Raben, A., & Kehlet, U. (2014). Sensory characteristics and consumer liking of sausages with 10% fat and added rye or wheat bran. *Food Science & Nutrition*, *2*(5), 534–546. https://doi.org/10.1002/FSN3.126

Boccard, R., Buchter, L., Casteels, E., Cosentino, E., Dransfield, E., Hood, D. E., Joseph, R. L., MacDougall, D. B., Rhodes, D. N., Schön, I., Tinbergen, B. J., & Touraille, C. (1981). Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the commission of the European communities' (CEC) beef production research programme. *Livestock Production Science*, *8*(5), 385–397. https://doi.org/10.1016/0301-6226(81)90061-0

Christensen, L. B. (2003). Drip loss sampling in porcine m. longissimus dorsi. *Meat Science*, *63*(4), 469–477. https://doi.org/10.1016/S0309-1740(02)00106-7

Hanson J., S. W. F. & O. (1963). Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochem. J., 89 : 101-1O2*.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, *49*(4), 447–457. https://doi.org/10.1016/S0309-1740(98)00034-5

Kowalski, E., Vossen, E., Millet, S., Ampe, B., Callens, B., van Royen, G., de Smet, S., & Aluwé, M. (2020). Performance and carcass, loin and ham quality in crossbreds from three terminal sire lines. *Meat Science*, *167*. https://doi.org/10.1016/j.meatsci.2020.108158

Supplementary Tables

Table S1: Effect of diet (control- (CF) and summer feed (SF)) per phase (starter- (10-15 weeks of age) and grower phase (15-25 weeks of age)) on average daily feed intake, average daily gain and feed conversion ratio of growing-finishing pigs housed during the summer season

Parameter	Phase	Diet		SEM	P-value
		CF	SF		Diet
Average daily feed intake [g/day]	Starter	1645	1562	32.1	0.328
	Grower	2518	2472	48.6	0.690
Average daily gain [g/day]	Starter	856	787	16.8	0.165
	Grower	905	882	13.3	0.478
Feed conversion ratio	Starter	1.92	1.99	0.01	0.310
	Grower	2.78	2.81	0.03	0.446

CF = Control feed; SF = Summer Feed

Table S2: Effect of diet (control- (CF) and summer feed (SF)), observation time point in the lairage area (upon arrival and one hour after) and its interactions on heat stress score and skin lesions of growing-finishing pigs housed during the summer season

Parameter	Observation time point	Diet		SEM	P-value		
		CF	SF		Diet	Observation time point	Diet x observation time point
Heat stress score	upon arrival	27.7[a]	26.2[a]	1.63	0.587	<0.001	n.s.
	one hour after arrival	19.0[b]	17.5[b]	1.58			
Skin lesions	upon arrival	0.4	0.4	0.11	0.913	0.255	n.s.
	one hour after arrival	0.6	0.6	0.12			

n.s. = non-significant interaction; CF = Control feed; SF = Summer Feed

[a- b] Values within a row with different superscripts differ significantly at $P < 0.05$

Table S3: Effect of diet (control (CF) and summer feed (SF)) on carcass traits and meat quality (instrumental and sensory) of growing-finishing pigs housed during the summer season

Parameters	Diet		SEM	P-value
	CF	SF		Diet
Carcass traits				
Fasted weight [kg]	119.0	116.0	1.49	0.476
Warm carcass [kg]	97.2	94.6	1.24	0.409
Cold carcass [kg]	95.3	92.8	1.21	0.409
Lean meat content [%]	63.8	63.4	0.38	0.637
Fat thickness [mm]	8.4	8.6	0.37	0.787
Muscle thickness [mm]	67.6	65.5	0.66	0.243
Dressing yield [%]	80.6	79.8	0.20	0.503
Lean tissue gain [g/day]	449.0	429.0	4.90	0.172
Meat quality: instrumental				
pH_i	6.5	6.5	0.04	0.545
pH_{24}	5.6	5.6	0.01	0.346
Drip loss [%]	7.3	6.3	0.26	0.439
Cooking loss [%]	30.6	28.3	0.29	0.217
Shear force [N]	30.4	32.4	0.91	0.553
Intramuscular fat [%]	1.9	2.1	0.09	0.363
Lightness L*	57.1	57.4	0.27	0.603
Redness a*	6.5	6.2	0.08	0.332
Yellowness b*	14.9	15.1	0.07	0.480
Hue	66.6	67.6	0.25	0.172
Chroma	16.3	16.3	0.08	0.975
Meat quality: sensory				
Fried odour	30	28	2.50	0.681
‘Piggy’ odour	18	16	0.94	0.298
Tenderness	49	44	2.13	0.218
Juiciness	45	48	1.25	0.253
Fried flavour	22	20	2.01	0.550
‘Piggy’ flavour	25	26	0.84	0.541
Acidic flavour	11	12	0.78	0.611

CF = Control feed; SF = Summer Feed