Meat Science 145 (2018) 230–237

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Improving pork quality traits by a short-term dietary hydroxy methionine supplementation at levels above growth requirements in finisher pigs

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ARTICLE INFO

Keywords:
Amino acid
HMTBA
Muscle
Glutathione
Antioxidant capacity
Technological quality

ABSTRACT

The effects of dietary methionine (Met) supplies above growth requirements on tissue biology and pork quality were studied. At 70 kg, 45 crossbred pigs were fed a control (CONT) diet adequate in Met (0.22% Met) up to 105 kg. For the last 14 days before slaughter, pigs were fed with the CONT diet or with diets where the Met level was increased to Met3 (0.66% Met) or Met5 (1.10% Met). Growth performance and carcass composition did not change with the treatment. Pigs fed the Met5 treatment displayed lower TBARS and higher glutathione levels ($P \leq .05$), along with higher ultimate pH ($P < .01$) and lower drip, lightness and hue ($P \leq .10$) in the longissimus muscle, compared to the CONT and Met3 pigs. Extra-dietary Met improved ham's technological quality in the Met3 and Met5 groups ($P \leq .05$). Thus, dietary Met supplementation improves pork quality without impairing growth or carcass traits.

1. Introduction

Methionine (Met) is an essential amino acid (AA) sustaining growth and development. In young growing pigs, dietary Met deficiency decreased growth rate and protein accretion (Conde-Aguilera, Barea, Le Floc'h, Lefaucheur, & van Milgen, 2010; Conde-Aguilera, Lefaucheur et al., 2016) and this was associated with greater lipid deposition in carcass and muscle (Castellano et al., 2015). In finishing pigs, dietary Met deficiency also resulted in higher muscle glycolytic potential (Conde-Aguilera, Cobo-Ortega, Mercier, Tesseraud, & van Milgen, 2014). Altogether, changes in muscle energy storages (lipid and glycogen) with dietary Met supply may subsequently influence some meat quality traits, including the extent of *postmortem* (p.m.) pH decline. Importantly, Met also shows non-proteinogenic functions (Tesseraud, Métayer-Coustard, Collin, & Seiliez, 2009). Specifically, Met is the precursor of cysteine, which is essential for the synthesis of glutathione and taurine, two major cellular non-enzymatic antioxidants. Oxidative stresses occur all along the value chain of animal production including meat storage, with consequences on juiciness, tenderness, flavor and warmed-over flavor, odour and rancidity of products (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). However, oxidation processes may be delayed, retarded or even prevented by applying an antioxidant strategy such as vitamin E and(or) selenium (Shahidi & Wanasundara, 1992). In young pigs, dietary Met deficiency reduced the amount of glutathione in the liver and muscle, resulting in an elevation of antioxidant enzyme activity in the muscle and adipose tissues (Castellano et al., 2015; Conde-Aguilera, Lefaucheur et al., 2016). Conversely, Met supplementation, especially when provided in the form of 2-hydroxy-4 (methylthio) butanoic acid (HMTBA) that leads to a higher synthesis of cysteine, taurine and reduced glutathione than DL-Met with additional benefits in terms of antioxidant capacities (Martin-Venegas, Geraert, & Ferrer, 2006; Vazquez-Anon, Bertin, Mercier, Reznik, & Roberton, 2017; Zhang, Li, & Wang, 2015), may thus modify tissues' protective capacities through glutathione synthesis. Therefore, the question arises whether HMTBA might also help to prevent oxidative stress and delay p.m. oxidation processes in pork when supplied in pig diet beyond growth requirements, with possible consequences for meat quality traits such as water-holding capacity (Huff-Lonergan & Lonergan, 2005) or color stability during storage.

To date, very few studies have however considered the effects of dietary Met at levels above growth requirements and its consequences on muscle composition and meat quality traits. Recently, Li et al. (2017) reported that low-birth-weight piglets receiving L-Met-supplemented diets above the standard requirement from 40 kg up to slaughter at 100 kg of body weight (BW) exhibited increased muscular glutathione and lower lipid peroxidation, with positive effects on pork quality (higher ultimate pH and reduced drip loss during loin meat storage) when compared with pigs fed a control diet. Moreover,

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https://doi.org/10.1016/j.meatsci.2018.06.040
Received 29 May 2018; Received in revised form 18 June 2018; Accepted 28 June 2018
Available online 30 June 2018
0309-1740/

chickens fed HMTBA-supplemented diet had greater glutathione levels and reduced lipid peroxidation, compared to those receiving a DL-Met-supplemented diet (Swennen et al., 2011).

Considering this information altogether, we hypothesized that a short-term dietary Met supplementation (as HMTBA) in finishing pigs may influence muscle energy stores and antioxidant capacity and thereby potentially influence pork quality traits, without impairing growth performance or carcass composition. This study aimed at determining the effects of dietary HMTBA supply at levels largely above total sulfur amino acid requirements during the last two weeks before slaughter on growth performance, body composition and muscle biochemical properties at slaughter and meat quality in pigs.

2. Material and methods

2.1. Experimental design, diets and management

The experiment was performed in the INRA experimental facilities (UEPR, 35590 Saint-Gilles, France) in compliance with European Union (directive 86/609/CEE) and French legislation (Décret n°2001–464 29/05/01; agreement for animal housing number C-35–275–32). The technical and scientific staffs had individual accreditation from the French Minister to experiment on living animals. At approximately 70 kg of BW, 45 crossbred ((Large White × Landrace) × Piétrain) female pigs free of the halothane-sensitive (n) and RN$^-$ alleles were chosen from 15 litters and assigned in-litter to 3 dietary treatments (n = 15/group). They were all housed in individual pens (85 × 265 cm) placed in the same room on a concrete floor. They were first fed a control diet (CONT) formulated to meet nutritional requirements based on NRC (2012) guidelines from 70 kg to 105 kg of BW. For the last fourteen days before slaughter, pigs were then fed either the control diet (CONT group) or an HMTBA-supplemented diet with a calculated Met value 3 times (Met3 group, 0.66% Met) or 5 times (Met5 group, 1.10% Met) greater than that in the CONT diet (Table 1). Diets were isoenergetic and iso-nitrogenous. Their detailed composition are presented in Table 1. All pigs had free access to feed and water until the end of the experiment, i.e. 24 h before slaughter.

2.2. Growth performance

Pigs were weighed individually at the beginning of the dietary treatment period, one week after its start and the day before slaughter. Individual feed consumption was measured weekly (feed offered minus refusals) during the test period. Average daily gain, average feed intake and feed conversion ratio (FCR) were calculated for each pig during the test period. Backfat thickness was measured at the last rib level on the left and right sides of the body by an ultrasound apparatus (VETKOP-LUS, Noveko Inc., Boucherville, QC, Canada) on all pigs at the beginning and end of the test period.

2.3. Slaughter and carcass measurements

Pigs were conducted to the slaughterhouse (INRA, 35590 Saint-Gilles, France) in two successive batches at 1-week intervals (n = 21 and n = 24 pigs for batches 1 and 2, respectively), with a balanced representation of the 3 dietary groups in each batch, with the heaviest pigs of each dietary group slaughtered in the first batch. All pigs fasted for 24 h before slaughter. The day before slaughter, pigs from the same dietary group (n = 7 for batch 1 or n = 8 for batch 2) were loaded onto a lorry, transported together to the slaughterhouse (5 min) and kept in lairage in the same pen without mixing with pigs from the other groups. All pigs had free access to water. The next day, pigs were taken alternately from each group and slaughtered by electrical stunning (350 V – 4 A) followed by jugular exsanguination in compliance with the current national regulations applied in slaughterhouses. Just after slaughter, the hot carcass (trimmed of digestive, reproductive, and respiratory tracts), the entire perirenal fat (around kidneys) and the liver were weighed. Carcass dressing was calculated as the ratio of hot carcass weight to final BW.

Table 1
Composition of experimental diets.

Diet	CONT	Met3	Met5
Ingredients[a] (g/kg as fed basis)			
Maize	661	661	661
Wheat	100	100	100
Soybean meal 48	160	160	160
Wheat middlings	10.0	5.0	0
Palm oil	30	30	30
Calcium carbonate	12.8	12.8	12.8
Dicalcium phosphate	9.8	9.8	9.8
Vitamin/mineral premix[b]	10	10	10
Salt	4.0	4.0	4.0
L-Lys HCl	2.2	2.2	2.2
L-Thr	0.1	0.1	0.1
L-Trp	0.4	0.4	0.4
DL-HMTBA	0	5.0	10.0
Calculated nutrient composition[c] (%)			
Crude protein	13.7	13.6	13.6
Crude Fat	5.82	5.81	5.79
Ash	4.80	4.78	4.76
Cellulose	2.59	2.57	2.54
Net energy (MJ/kg)	10.38	10.41	10.44
Lys[d]	0.73	0.73	0.73
Met			
TSAA	0.45	0.83	1.27
Thr[d]	0.46	0.46	0.46
Trp[d]	0.13	0.13	0.13
Arg[d]	0.80	0.79	0.79
His[d]	0.35	0.35	0.35
Ile[d]	0.54	0.53	0.53
Leu[d]	1.24	1.23	1.23
Phe[d]	0.64	0.64	0.64
Phe + Tyr[d]	1.11	1.11	1.10
Val[d]	0.61	0.61	0.61
Analyzed (%)			
Met[e]	0.20	0.59	1.02
Cys[e]	0.25	0.24	0.25

Abbreviation used: TSAA, total sulfur amino acid.

[a] CONT, a control diet, Met3 and Met5, Methionine-supplemented diets.

[b] Vitamins (/kg diet) A (7500 UI), D3 (1500 UI), E (20 UI), B1 (0.5 mg), K3 (1.0 mg), B2 (3.0 mg), B5 (10.0 mg), B6 (1.0 mg), B12 (0.015 mg), PP (1500 mg) and minerals iron (80 mg), iodine (0.50 mg), copper (15 mg), manganese (40 g), zinc oxide (80 mg) and sodium selenite (0.25 mg) together with phytase (500 FTU).

[c] Calculated, adjusted for 86.3% of dry matter.

[d] Calculated from the calculated ileal digestibility values of the ingredients, and adjusted for 86.3% of dry matter.

[e] Analysed methionine equivalent (Total Met + HMTBA) and cystine levels (Agostini, Dalibard, Mercier, Van der Aar, & Van der Klis, 2015).

After 24 h of chilling at 4 °C, the weight of the cold carcass and of wholesale cuts from the right carcass side (ham, loin, backfat, shoulder and belly) were recorded. Carcass drip loss (using hot and cold carcass weights) and composition (percentage proportion of wholesale cuts to the right side) were calculated and used to determine lean meat content (LMC) according to the eq. LMC (%) = 25.08 + 0.73% ham +0.87% loin - 1.23% belly (Daumas, 2008).

2.4. Tissue sampling and measurements of meat quality traits

Just after evisceration (i.e. 12 min after exsanguination), liver samples were taken, immediately frozen in liquid nitrogen and stored at −76 °C until the determination of glutathione contents.

Thirty minutes after slaughter, samples of *longissimus lumborum* muscle (LM) were taken from the carcass's right side at the last rib level, cut into small pieces and immediately frozen in liquid nitrogen; samples