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Dietary supplementation with monosodium L-glutamate modifies lipid composition and gene expression related to lipid metabolism in growing pigs fed a normal- or high-fat diet

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ABSTRACT

Monosodium glutamate (MSG) is used worldwide as a flavor enhancer. Its effects on body weight and fat content are debated. Using the pig model in which animals were fed normal- or high-fat isocaloric diets, the present study tested whether dietary MSG modified body weight, relative amount of carcass fat, expression of genes related to fatty acid metabolism, and carcass lipid composition. A total of 32 growing pigs were randomly assigned to four dietary treatment groups: normal-fat diet, normal-fat diet + 30 g/kg MSG, high-fat diet, or high-fat diet + 30 g/kg MSG. There were 8 animals per treatment group. After a 30-day feeding period, body weight and carcass composition were measured. The expression of genes related to lipid metabolism in *longissimus dorsi* muscle and white adipose tissue was also assessed. Our results showed that MSG supplementation given with either normal- or high-fat diet did not affect body weight and the relative amount of total carcass fat. However, MSG supplementation in pigs fed the normal- or high fat diet modified the lipid content and fatty acid profiles in skeletal muscles. MSG modified differently the expression of genes related to lipid metabolism in the muscles and white adipose tissue of animals depending on the normal- or high-fat diet used. In addition, the expression of myosin heavy chains in skeletal muscle fibers was modified by MSG supplementation in both normal- and high-fat groups of animals. Collectively, our results indicate that MSG supplementation is not obesogenic but differentially regulates gene expression related to lipid metabolism, lipid composition and muscle fiber composition in the skeletal muscle of pigs.

1. Introduction

Glutamate is one of the most abundant amino acids in alimentary proteins (Kong et al., 2012). This amino acid is present in its free form in food and is used as an additive in the form of monosodium glutamate (MSG). Glutamate, an acidic amino acid, has critical roles in metabolism and regulates key physiological processes (Brosnan and Brosnan, 2013; Fu et al., 2013). Because MSG is used worldwide as a flavor enhancer, it appears necessary to document the effects and consequences of MSG consumption in terms of beneficial or deleterious effects of this compound. Glutamate has been reported to participate in synthetic and oxidative pathways in tissues (Blachier et al., 2009; Wu, 1998), to enhance diet-induced thermogenesis in the brown adipose tissue of young adult rats (Smriga et al., 2000), to modify adiposity in adult rats (Collison et al., 2011; Kondoh and Torii, 2008), to activate taste receptors in the digestive tract (San Gabriel and Uneyama, 2013), and to regulate the release of several hormones (Iwatsuki and Torii, 2012). Regarding the effects of MSG on body weight and fat mass, conflicting reports have been published (Kondoh and Torii, 2008; Collison et al., 2011; Tordoff et al., 2012). In humans, controversial data suggest that MSG consumption, depending on the overall context, increases (He et al., 2011) or has no effect on the risk of becoming overweight (Shi et al., 2010). In post-weaning pigs, a recent study found that dietary supplementation with up to 4% MSG was safe and improved growth performance (Rezaei et al.,

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2013), pointing to a potential use of glutamate as a dietary supplement in animal feeding. In growing pigs, two studies have shown that the venous and arterial concentrations of glutamate, aspartate, and alanine increase rapidly after supplementation with high doses of MSG (Blachier et al., 1999; Zhang et al., 2012). However, there is limited information regarding the effects of dietary supplementation with MSG on the metabolism of growing pigs, especially those fed a high-fat diet.

A major goal of pig production is to increase skeletal muscle growth and reduce excess fat accretion. However, fat and fatty acids, in either adipose tissue or skeletal muscle, contribute importantly to various aspects of meat quality and are central to the nutritional value of meat (Wood et al., 2004). Especially, intramuscular fat has a role in the tenderness and juiciness of cooked meat (Wood et al., 2008). Feeding strategies could change several aspects of meat quality, through the effect on muscle/fat ratio and composition (Tan et al., 2011; Wang et al., 2011; Zhou et al., 2014), although the underlying mechanisms have not been elucidated yet. Thus, it appears interesting to evaluate the metabolic and phenotypic effects of the combination of dietary fat and MSG, taking into account that previous work from our group have shown that dietary supplementation with MSG and fat modifies tissue composition in growing pigs (Feng et al., 2014). In this context, the aim of the present study was to answer to the following questions: (i) Does a 30-day supplementation period with MSG increase the relative fat mass of pigs in two different dietary contexts (i.e. normal-fat diet and high-fat diet)? (ii) Does such supplementation modify the lipid content and fatty acid profile in muscles? (iii) Does MSG modify the expression of genes related to fatty acid metabolism in muscle and white adipose tissue? (iv) Given that changes in intramuscular lipid content have been shown to be associated with modified composition of muscle fiber type (Kim et al., 2008; Ryu and Kim, 2005), does MSG supplementation modify the expression of myosin heavy chains in muscles?

2. Material and methods

2.1. Animal housing and treatment

Thirty-two pigs (Duroc × Large White × Landrace, half of males and half of females) from 4 litters with an initial average body weight (BW) of 25.0 ± 1.3 kg were randomly assigned to four dietary treatment groups: normal-fat diet, normal-fat diet + 30 g/kg MSG, high-fat diet, and high-fat diet + 30 g/kg MSG (Feng et al., 2014). There were 8 animals per treatment group. The dietary supplementation with 30 g/kg MSG is considered as safe dose with reported measurable metabolic and physiological effects in pigs (Rezaei et al., 2013; Blachier et al., 1999; Zhang et al., 2012). The normal-fat diet was a corn- and soybean meal-based diet formulated to meet National Research Council (2012)- recommendations for growing pigs. The composition and nutrient level of the four experimental diets are shown in Table 1. Throughout the experimental period, the pigs were raised in individual cages and had free access to drinking water and their respective diet. The experimental procedures for this study were approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

2.2. Sample collection and preparation

At the end of the 30-day experimental period, the pigs were euthanized by intravenous injection of 4% sodium pentobarbital solution (40 mg/kg BW) at 12 h after the last feeding by jugular puncture (Kong et al., 2007). Samples of the *longissimus dorsi* (LD) muscle and the overlying subcutaneous adipose (white adipose) tissues were collected separately and immediately snap-frozen in liquid nitrogen, and stored at −80 °C until analysis.

Table 1
The composition and nutrient level of four experimental diets.

Items	Normal-fat diet	Normal-fat diet + 30 g/kg MSG	High-fat diet	High-fat diet + 30 g/kg MSG
Ingredient composition (%)				
Corn	71.37	70.30	59.80	59.58
Soybean meal	19.20	16.80	21.27	21.50
Corn starch	0.00	0.00	7.00	5.00
Corn gluten meal	5.00	7.00	2.50	3.10
MSG	0.00	3.00	0.00	3.00
Alanine	1.58	0.00	1.58	0.00
L-lysine · HCl	0.15	0.20	0.15	0.12
Soybean oil	0.00	0.00	5.00	5.00
Premix*	2.70	2.70	2.70	2.70
Calculated nutrient level				
Digestible energy (MJ/kg)	13.98	13.87	13.92	13.98
Crude protein (%)	17.93	17.95	17.88	17.91
Ether extract (%)	4.35	4.51	9.39	9.45
Ca (%)	0.60	0.58	0.59	0.59
P (%)	0.45	0.44	0.48	0.46
Lys (%)	0.83	0.83	0.85	0.83
Met (%)	0.26	0.28	0.25	0.25
Thr (%)	0.56	0.56	0.55	0.55

* Composition (%): $CaHPO_4$, 27.78; Mountain flour, 24.07; NaCl, 11.11; Medical stone, 12.33; Powdered rice hulls, 18.81; $FeSO_4$, 0.74; $ZnSO_4$, 0.74; Selenium powder (1%), 0.15; Iodine powder (1%), 0.15; $CuSO_4$, 0.37; $MnSO_4$, 0.30; Choline chloride, 2.22; Growth pig multidimensional, 1.11; Antioxidants (Ethoxyquin 66%), 0.11.

2.3. Determination of carcass composition

Carcass weight and composition were measured immediately post mortem. After removal of the head, legs, tail, and viscera, the carcass was split longitudinally. Each left-side carcass was weighed and then physically dissected into skin, skeletal muscle, fat, and bone for evaluation of carcass characteristics. These components were weighed, and the weights were multiplied by 2 in order to calculate the percentage of the whole carcass that was made up of each component.

2.4. Chemical analysis of skeletal muscle

The chemical composition of the LD muscle was analyzed in duplicate according to Association of Official Analytical Chemists (AOAC, 1996) methods. The dry matter content of the LD muscle was determined gravimetrically with oven drying at 110 °C for 24 h. The protein and lipid contents were measured using the Kjeldahl and Soxhlet extraction methods, respectively. The fatty acid composition of intramuscular fat was determined with gas chromatography as detailed in our previous report (Zhou et al., 2014). Briefly, lipids were extracted from the LD muscle using petroleum ether/anhydrous diethyl ether (1:1, v/v). Methyl esters of the lipids were obtained via saponification with a solution of KOH:methanol (4 mol:1 L), and the organic layer was aspirated for the analysis of fatty acids with gas chromatography using a capillary column (HP-INOWAX, 30 m × 2.5 mm × 2.5 μm). The gas chromatograph temperature program was as follows: initial temperature of 140 °C for 5 min, increase of 3 °C per min to 220 °C, 1 min at 220 °C, and then holding 40 min. The injector and detector temperatures were 240 °C and 260 °C, respectively.