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The effect of dietary fatty acid composition on adipose tissue quality and expression of genes related to lipid metabolism in porcine livers

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ABSTRACT

This study investigated different fatty acid profiles in dietary fat sources for pigs fed with corn DDGS (CDDGS) and determined their effect on adipose tissue quality and the expression of metabolism-related genes in the liver. The experiment was carried out on 32 fatteners (60–118 kg of body weight) divided into four groups. Group I (control): feed mixture with rapeseed oil; Group II: feed mixture with rapeseed oil and CDDGS; Group III: feed mixture with beef tallow and CDDGS; Group IV: feed mixture with coconut oil and CDDGS. The results confirmed the relationship between dietary fatty acid profile and acid content in fat tissue. CDDGS reduced SFA/UFA ratios and increased iodine values ($P < 0.001$) and TBA-RS in the subcutaneous adipose tissue of backfat. Replacing rapeseed oil in a diet including CDDGS with beef tallow or coconut oil enabled the pigs to accumulate more SFA in backfat and reduce iodine value. The genes selected in the present experiment, and which are believed to be associated with lipid metabolism, had been identified as DEGs (differentially expressed genes) in an RNA sequence experiment performed previously to explore the coexpression of these genes after different diets. Expression of the genes, evaluated by Real-Time q-PCR, was not affected in any case by the pig's sex. The expression levels in liver tissue for genes *APOA4, ACSL5, CYP2C49, CYP2B22,* and *GSTO1* were significantly different among groups, while for *CYP7A1* the differences identified by RNA sequencing were not confirmed. The expression levels of estimated genes were correlated with the fatty acid profile in a diet, but not with fattening performance results or carcass quality traits. To summarize, CDDGS included in feed mixtures can negatively influence subcutaneous adipose tissue traits important for processing and shelf-life. Dietary factors, such as fat sources characterized by a high degree of saturation, improved the hardness and oxidative stability of the backfat. The fatty acid profile of dietary fat sources affected the expression levels of genes associated with lipid metabolism in the liver. Coconut oil and beef tallow increased the expression levels of genes *ACSL5, APOA4, GSTO1, CYP2B22,* and *CYP2C49*. Moreover, most of the genes were co-expressed within the diet, which suggests the possibility of their co-regulation.

Abbreviations: CDDGS, corn dried distillers grains with solubles; SFA, sum of saturated fatty acids; UFA, sum of unsaturated fatty acids; MUFA, sum of monounsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; IV, iodine value of fat; TBA-RS, thiobarbituric acid reactive substances; DEG, differentially expressed genes.

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Further studies will be conducted on metabolic pathways in which the analyzed genes are involved to understand the molecular mechanisms of their activity and possible health effects of their overexpression.

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1. Introduction

Currently, pigs are characterized by satisfactory growth rate and lean meat accretion. However, thin subcutaneous adipose tissue and a low content of intramuscular fat reduce the meat's organoleptic value and limit its usefulness for processing (Morgan et al., 1994). It is known that fat content in tissue influences flavor and juiciness as well as tenderness and firmness. Disadvantageous features are often observed in pigs selected for lean tissue, because of the breeding selection towards increased meat content and reduction of backfat thickness, which is related to an increase in linoleic acid (C18:2) content in adipose tissue (Eggert et al., 2001). Linoleic acid content is inversely correlated with backfat thickness (Wood et al., 1989). In spite of the fact that oleic acid (C18:1) prevails in the fatty acid composition of pig tissue (about 40%), linoleic acid shows the highest correlation with fat firmness and its technological traits.

Moreover, a relationship was found between dietary fatty acid profiles and the adipose tissue fatty acid composition (Benz et al., 2011a, 2011b). In recent years, the use of corn dried distillers grains with solubles (CDDGS) has been gaining increasing attention. Corn grain, and consequently CDDGS, are characterized by a high content of unsaturated fatty acids (UFA), especially linoleic acid (C18:2), which are prone to oxidation, and feeding increased amounts of CDDGS to growing pigs increases the concentration of these fatty acids in pork adipose tissues (Xu et al., 2010a). The high content of UFA in adipose tissue has a negative effect on technological parameters, usefulness for processing, oxidative stability and quality during storage. Such animal products exhibit reduced firmness, causing problems with separation of layers during slicing and an oily appearance in the package (Carr et al., 2005). Moreover, the fat's organoleptic quality deteriorates since compounds formed during the fat oxidation process have a negative effect on the taste and flavor. It seems obvious that, for economic reasons, high amounts of CDDGS in the diet are desirable. However, the negative influence of dietary UFA on adipose tissue quality should be balanced by some feed additives or a saturated dietary fat source.

Since the type of fat added to the diet of animals significantly influences the composition of fatty acids in their tissues, and its metabolism depends on protein products of gene expression, studies attempting to determine the effect of animals' diet on the expression levels of genes are essential. Identification of molecular actions clarifying the mechanisms determining adipose tissue quality, liver protection from excess lipid accumulation and exhibiting other beneficial health actions are among the main topics of interest. Among genes related to lipid metabolism, we have selected those related to potential beneficial health effect. It has been indicated that apolipoprotein A IV (apoA-IV) is involved in a protective role in atherosclerosis and has antioxidative capacity (Qin et al., 1998; Stan et al., 2003), and it has been shown to promote cellular cholesterol efflux by activating reverse cholesterol transport and converting it at the liver into bile acids (Banach and Sinkiewicz, 2013). Acyl-CoAs enter multiple metabolic pathways as substrates for complex lipid synthesis or ß-oxidation (Bu and Mashek, 2010), but their role is tissue-dependent (Mashek et al., 2006). These observations indicate a role for ACSL in the channeling of intracellular lipids as a method to control energy metabolism. According to Oikawa et al. (1998), this synthetase enzyme utilizes a wide range of SFA, suggesting that *ACSL5* may provide the acyl-CoA used for the synthesis of cellular lipids in adipocytes. Cytochrome members of the P450 family (CYPs) act in the balance of the bile acids in the liver and in the steroid biosynthesis pathway (Moe et al., 2009). Porcine isoform *CYP2C49* is a member of the subfamily CYP2C, which metabolize drugs and steroids (Skaanild and Friis, 2005), one of the key enzymes in the metabolism of skatole. It is also involved in the synthesis of cholesterol from squalene and cholesterol conversion into steroid hormones as well as bile acids, which is beneficial for dietary fat digestion. However, the molecular mechanisms underlying the response of metabolism-related genes to dietary lipid intake or nutrient composition have not been completely defined and a species-specific mechanism of response may exist, so further studies are required. In recent years, pigs have become popular animal models for human nutrition and health studies. As pigs are the most similar to humans in terms of metabolism pathways, for example the protein apoA-IV shows high sequence identity (75.6%) with the human protein (Navarro et al., 2004b), this kind of experiment may yield useful nutritional information related to human nutrigenomics.

The aim of the study was to investigate different fatty acid profiles of dietary fat sources in pigs fed with mixtures including corn DDGS and to determine their effect on backfat subcutaneous adipose tissue quality and the expression level of six lipid metabolism-related genes (*APOA4, GSTO1, CYP7A1, ACSL5, CYP2B22*, and *CYP2C49*) in the liver, an organ crucial in lipid metabolism.

2. Material and methods

All procedures included in the experiment relating to the use of live animals were in agreement with the local Ethics Committee for Experiments with Animals.