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Conjugated linoleic acid supplementation during late gestation and lactation of sows affects myofiber type in their litters

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ABSTRACT

The purpose of this study is to determine the effect of dietary conjugated linoleic acid (CLA) supplementation of pregnant and lactating sows on muscle growth and myofiber type of their piglets. There are thirty healthy, pregnant Rongchang sows with similar body condition and closed expected day of parturition for the experiment *in vivo*. Three experiment groups carry out with/without CLA (1%, 2% and 0%). The experiment began on 85th day of gestation and ended at 28th day after piglet birth. The results show that (1) piglets from CLA-fed sows had heavier longissimus dorsi and higher percentages of slow myofibers than control piglets ($P < 0.05$); (2) moreover, real-time PCR showed that CLA supplementation induce increasing expression of MyHC 1, MyOG, and MyOD genes while decreasing expression of MyHC 2a, MyHC 2x, and MSTN genes in piglets. We also check the expression of genes which appear in 2 *in vitro* experiment. c9, t11-CLA acted to induce the differentiation of cultured pig skeletal muscle cells and up-regulates the expression levels of MyHC 1 and MyOG, but t10, c12-CLA strongly inhibited cellular differentiation and down-regulates expression of MyOG. These findings suggest that dietary CLA during early life may affect muscle development and myofiber type in pigs.

1. Introduction

The growth and development of muscle is a complex process including the formation of muscle fibers during the fetal period, transformation and development of muscle fibers after birth, and regeneration of muscle during adulthood. The number of muscle fibers in pigs is determined during the fetal period (Wigmore and Stickland, 1983). There are two major periods of muscle growth during the fetal development in pigs; the first occurs at 35–60 days gestation and mainly primary fibers are formed while the second occurs at 54–90 days of gestation and mainly secondary fibers are formed (Ashmore et al., 1973). After birth, there is only a slightly increase in total fiber number from day 0 until day 28; that increase is considered the third wave of myofiber formation (Berard et al., 2011). The muscle fiber changes that occur after birth in piglets mainly consist of fiber type transformation and myofiber enlargement and extension (Stickland and Handel, 1986; Wigmore and Stickland, 1983). Therefore, the formation of fibers during the fetal period and fiber type transformation during early postnatal life both have important effects on muscle growth in pigs.

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Muscle fiber types can be divided into two main categories: slow-twitch (type 1) and fast-twitch (type 2) muscle fibers. The fast-twitch fibers can be further categorized into types 2a, 2b, and 2x (Blaauw et al., 2013; Lexell, 1995; Starkey, 2014). Fiber type transformation is controlled by a combination of genes and affected by various factors, such as nutrition, disease, and stress (Lefaucheur, 2010; Lee et al., 2012; Ryu and Kim, 2005). It is important to note that maternal nutrition (maternal diet) also plays a crucial role in the growth, development, and transformation of muscle during the fetal period, and this effect extends into adulthood (Pond et al., 1992; Zhu et al., 2006).

Conjugated linoleic acid (CLA) is an 18-carbon fatty acid that has gained increasing attention because of its diverse physiological effects in humans and animals; it has been shown to have anti-cancer, anti-obesity, and immune enhancing activity (Anne et al., 2005; Hassan et al., 2013; Helen, 2000; Yanwen and Peter, 2004). In addition, CLA affects muscle fiber growth, myoblast differentiation, and energy metabolism *in vitro* (Donna and Erin, 2006; Hommelberg et al., 2010; Vaughan et al., 2012).

The effects of CLA on pigs have already been widely investigated. Corderoa et al. (2010) reported that feeding 1% CLA to finishing swine increased IMF in heavy pigs. Feeding 2% CLA to growing pigs significantly decreased back fat accumulation *via* the induction of adipocyte apoptosis (Qi et al., 2014), and dietary CLA supplementation significantly increased the dry matter content of the *longissimus dorsi* muscle (Han et al., 2011). These experiments demonstrate CLA has strong influences on the physiological status and growth of pigs, specifically on muscle and fat.

In a previous study, we demonstrated that dietary supplementation with 0.5–1.0% CLA accelerated muscle fiber growth and increased MyHC1 gene expression in 30–90 kg growing-finishing pigs (Huang et al., 2014), which indicates that CLA promotes muscle growth in pigs. The total number and type of muscle fibers in pigs is, to a large extent, determined during late gestation; however, the effects of CLA on muscle fibers during the fetal period and early postnatal development are still unclear. In this study, we investigated whether CLA had a direct influence on the growth and type transformation of muscle fibers in fetal and early postnatal pigs *in vivo* and *in vitro*.

2. Materials and methods

2.1. Animal experiment

The animals used in this study were RongChang pigs, a local Chinese pig breed with outstanding meat quality. Thirty healthy, pregnant, multiparous sows with similar body condition and closed expected day of parturition were randomly assigned to one of three groups ($n=10$ per group): control group (0 CLA), 1% CLA group, and 2% CLA group. There were 10 replicates in each group with 1 sow per replicate. Their feed was supplemented with different levels of a CLA mixture with a c9, t11-CLA: t10, c12-CLA ratio of 1:1 (Aohai Biotechnology Co, Ltd., Qingdao, China). The experiment began on day 85 of gestation and ended at piglet weaning (day 28 after birth). During the experimental period, feed and water were offered to the animals *ad libitum*, and the dietary formulation met the Chinese meat-fat type pig-feeding standard (NY/T 95-2004).

Seven healthy piglets (from differential sows) were selected by depending on their average weight and slaughtered for each experimental group at each time point (1st, 7th, 14th, 28th day after birth), respectively. Their longissimus dorsi muscles were separated and weighed, and a portion of the muscle was snap frozen in liquid nitrogen and preserved at −80 °C for immunohistochemistry and RNA extraction. Another portion of muscle was fixed in 4% neutral formalin for paraffin sections and hematoxylin and eosin (HE) staining.

2.2. Gas chromatography

Milk samples were collected from the sows on 1st and 28th day after parturition, and venous blood samples were collected from the piglets on day 28, for determination of CLA content. Gas chromatography was used to determine the CLA content as per the agricultural industry standard of the People's Republic of China (NY/T 1671-2008).

2.3. Density and diameter of myofibers

The muscle samples were embedded in OCT freezing medium at −20 °C for 2 h and then a cryostat (CM1950, Leica, Germany) was used to section the tissue at −20 °C for histologic analysis. The HE-stained muscle sections (8 μm) were used to observe the morphology of the muscle tissue; the images of the slides were captured by a digital microscope camera on a biological microscope (YS100, Nikon, Japan). An image analysis program (Image Pro Plus 5.0, Media Cybernetics, USA) was used to evaluate all parameters in the section. At least 50 fibers in each picture were measured to obtain the mean fiber diameter, and the number of fibers in 1 mm^2 was used to determine the fiber density.

Table 1
Primer sequences of PCR.

Gene	Primer sequences (5′→3′)
MYHC 1	F: AAGGGCTTGAACGAGGAGTAGA
	R: TTATTCTGCTTCCTCCAAAGGG
MYHC 2A	F: GCTGAGCGAGCTGAAATCC
	R: ACTGAGACACCAGAGCTTCT
MYHC 2B	F: ATGAAGAGGAACCACATTA
	R: TTATTGCCTCAGTAGCTTG
MYHC 2X	F: AGAAGATCAACTGAGTGAACT
	R: AGAGCTGAGAAACTAACGTG
MyoG	F: TCTACCAGGAACCCCACTTCTA
	R: GTCCCCAGCCCCTTATCTT
MyoD	F: CGATTGAGGGCATTATCAGAACA
	R: CCCAAGTGTCAGCCACAAGAG
MSTN	F: GGACCTGACCGACTACCTCA
	R: AATGAGAACAGCGAGCAA
β-actin	F: GCGGCATCCACGAAACTAC
	R: TGATCTCCTTCTGCATCCTGTC
18s RNA	F: CCCACGGAATCGAGAAAGAG
	R: TTGACGGAAGGGCACCA