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Dietary vitamin A restriction affects adipocyte differentiation and fatty acid composition of intramuscular fat in Iberian pigs

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ABSTRACT

The aim of this study was to investigate whether dietary vitamin A level is associated with differences in adipocyte differentiation or lipid accumulation in Iberian pigs at early growing (35.8 kg live weight) and at finishing (158 kg live weight). Iberian pigs of 16.3 kg live weight were allocated to two feeding groups, one group received 10,000 IU of vitamin A/kg diet (control); the other group received a diet with 0 IU of vitamin A (var) for the whole experimental period. The dietary vitamin A level had no effect on growth performance and carcass traits. The early suppression of vitamin A increased the preadipocyte number in *Longissimus thoracis* (LT) muscle in the early growth period ($P < 0.001$) and the neutral lipid content and composition (higher MUFA and lower SFA content) at the end of the finishing period ($P < 0.05$). Vitamin A restriction in young pigs increases their lipogenic potential without affecting carcass traits.

1. Introduction

Adipocyte differentiation is an important factor for fat accumulation in the body. Adipocytes are derived from fibroblast-like preadipocytes and grow in size by accumulation of lipids in the cytoplasm in association with terminal differentiation (Hausman, Campion, & Martin, 1980). In the early stage of adipocyte differentiation, many adipocyte characteristic genes are sequentially activated and play established roles in promoting the differentiation process (Ntambi & Kim, 2000).

Adipocyte differentiation is regulated by various kinds of hormones (Boone, Gregoire, & Remacle, 2000; Gregoire, Smas, & Sul, 1998). Furthermore, it is well known that fat-soluble vitamins, especially metabolites of vitamin A and D, modulate adipocyte differentiation in cultured cells in mammals (Kawada et al., 1990). All-*trans* retinoic acid (RA, the active metabolite of vitamin A) and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) inhibit adipocyte differentiation in cultured cells at a supraphysiological concentration (Kawada et al., 1990; Sato & Hiragun, 1988; Suryawan & Hu, 1997). However, very low concentration (1 pM — 10 nM) of RA stimulates adipocyte differentiation (Safonova et al., 1994).

Due to its role in reproduction, growth, development and immune response, commercial pig diets in the European Union contain vitamin A concentrations approximately six- to ten-fold higher than NRC recommendation (1317 IU/kg diet) (Fraga and Villamide, 2000). However, studies in vivo showed that a dietary level of 1300 IU of vitamin A for 11 weeks was associated with a higher intramuscular fat (IMF) content in *Longissimus thoracis* (LT) muscle than a diet with 13,000 IU in pigs (Olivares, Reya, Daza, & Lopez-Bote, 2011). However, in another experiment, Olivares, Daza, Rey, and Lopez-Bote (2009a) found no effect of dietary vitamin A on IMF content in pigs. Thus, the effect of vitamin A on body fat accumulation in swine remains unclear. Also, previous studies have found that dietary vitamin A concentration alters fatty acid composition of adipose tissue in sheep (Daniel, Salter, & Buttery, 2004), beef (Siebert et al., 2006) and pigs (Olivares, Daza, Rey, & Lopez-Bote, 2009b; Olivares et al., 2009a, 2011) but no effect was found on the fatty acid composition of IMF in pigs (Olivares et al., 2011). These experiments have been performed with different animals (ruminant vs. non-ruminant), genotypes (Duroc vs. lean pigs) and different times of supplementation or restriction of vitamin A. Both, IMF content and fatty acid composition are determinant factors affecting meat quality (Wood et al., 2008) and they are of special interest in high quality meat products, such as those obtained from Iberian pigs. Moreover, the effects of dietary vitamin A level have never been

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assessed in this breed. On the other hand, Iberian pigs have a high adipogenic and lipogenic potential, which could modify the effects of vitamin A restriction on fatness and thus, the use of dietary vitamin A restriction as a strategy to increase IMF should be tested in Iberian pigs.

Several authors have established that vitamin A exerts its effects on adipose tissue via regulation of expression of genes involved in adipogenesis and lipid metabolism (Bonet, Ribot, Felipe, & Palou, 2003; Daniel et al., 2004; Fernandez et al., 2011; Schwarz, Reginato, Shao, Krakow, & Lazar, 1997). Indeed, vitamin A, and specifically its metabolite, RA, is well known as a potent transcriptional regulator. Balmer and Blomhoff (2002) established that more than 500 genes are regulated by RA. Many genes are involved in the adipogenic process, from the very beginning, when *C/EBP* and *PPAR* (mainly *PPARG*) families are induced, to the final differentiation process, when mature adipocytes express genes involved in lipid metabolism such as ATP citrate lyase, malic enzyme, acetyl-CoA carboxylase, fatty acid synthase and others (Gregoire et al., 1998; Rosen, Walkey, Puigserver, & Spiegelman, 2000). The expression of these genes is considered a signal of the mature adipocyte phenotype. However, most of the available data in the literature are obtained from adipocyte culture studies. Results coming from studies in vivo are scarce and to our knowledge, there is no information about the effect of a restriction of dietary vitamin A on gene expression in pigs.

The objective of this study was to investigate how dietary vitamin A restriction affects gene expression in young pigs (35.8 kg) and impacts adipocyte differentiation and lipid accumulation in Iberian pigs at early growth (35.8 kg) and finishing (158 kg) periods.

2. Materials and methods

2.1. Animals and diets

Animal manipulations were done in compliance with the regulations of the Spanish policy for animal protection RD1201/05, which meets the European Union directive 86/609 about the protection of animals used in experimentation. The experiment was specifically assessed and approved (report CEEA 2010/003) by the Spanish National Institute for Agricultural and Food Research and Technology (INIA) Committee of Ethics in Animal Research. The trial was conducted at CIA Dehesón del Encinar (Oropesa, Toledo, Spain).

Thirty-eight castrated male (Torbiscal Pure Iberian) were randomly selected from a population. They were weaned at four weeks of age at a live weight (LW) of 11.7 ± 2.2 kg and were housed in pens until 2 months of age (average weight of 16.3 ± 2.5 kg) when piglets were randomly assigned to the two treatment groups, housed individually and given the experimental diets. One group was fed a vitamin A-enriched starter diet (10,000 IU vitamin A/kg diet) (control) and the other group received a starter diet formulated with no vitamin A (var, the same content in all periods) added in the premix (Table 1) from 16.3 ± 2.5 kg LW to 32.2 ± 4.5 kg LW. Diets were adjusted to meet requirements depending on the growing period. The pigs were changed to the corresponding control (10,000 IU vitamin A/kg diet) and vitamin A-restricted growing (from 32.2 ± 4.5 kg LW to 101 ± 4.1 kg LW) and finishing diets (from 101 ± 4.1 kg LW to 158 ± 7 kg LW). The pigs were fed 3.5% LW restriction until four months of age, 3% LW restriction until eight months and 2.5% LW restriction from this age until slaughter. The pigs had ad libitum access to water.

Ingredients, chemical composition and main fatty acids of experimental diets are shown in Table 1. Diets were formulated according to general guidelines proposed by De Blas, Gasa, and Mateos (2013) for Iberian pigs.

Table 1
Ingredient composition, calculated analysis (g/kg, as-fed basis unless stated otherwise) and fatty acid composition of the experimental diets.

	Starter		Growth		Finishing	
	Control[a]	Var[b]	Control	Var	Control	Var
Ingredient						
Barley	280.0	280.0	500.0	500.0	453.2	453.2
Soybean meal (440 g CP/kg)	155.1	155.1	169.4	169.4	75.9	75.9
Wheat	250.0	250.0	290.3	290.3	300.0	300.0
Soybean protein concentrate (650 g CP/kg)	25.0	25.0				
Corn	194.9	194.9				
Whey powder, sweet (cattle)	25.0	25.0				
Full fat soybean toasted	20.0	20.0				
High oleic sunflower seed					120.0	120.0
Lard	17.0	17.0	10.0	10.0	20.0	20.0
Calcium carbonate	5.4	5.4	8.2	8.2	8.2	8.2
Dicalcium phosphate	13.6	13.6	12.0	12.0	12.0	12.0
Control—mineral and vitamin premix[c]	4.0	0	4.0	0	4.0	0
Var—mineral and vitamin premix[d]	0	4.0	0	4.0	0	4.0
Salt	4.0	4.0	4.5	4.5	4.0	4.0
L-Lysine (500 g/kg)	4.0	4.0	1.6	1.6	2.2	2.2
Methionine-OH	1.4	1.4				
L-Threonine	0.6	0.6				
Calculated analysis[e]						
Net energy (MJ/kg)	10.0	10.0	9.5	9.5	10.4	10.4
Crude protein	178.2	178.2	171.9	171.9	147.0	147.0
Crude fat	41.9	41.9	29.0	29.0	85.0	85.0
Crude fiber	35.7	35.7	40.9	40.9	55.2	55.2
Crude Ash	48.0	48.0	49.2	49.2	49.2	49.2
Fatty acid composition (g/100 g total fatty acids)						
C12:0	1.8	1.6	7.2	8.5	1.0	1.0
C14:0	2.0	2.0	3.6	3.5	1.1	1.2
C16:0	18.8	19.2	18.0	17.8	11.2	11.8
C16:1 n−9	0.1	0.8	0.8	0.9	0.6	0.7
C16:1 n−7	1.0	0.4	0.3	0.3	0.2	0.2
C17:0	0.5	0.2	0.0	0.0	0.1	0.1
C17:1	0.2	0.6	0.0	0.0	0.2	0.2
C18:0	6.4	5.8	3.9	4.2	3.9	4.2
C18:1 n−9	26.6	26.3	21.1	20.5	57.0	57.5
C18:1 n−7	1.7	1.4	1.3	1.3	0.2	0.2
C18:2 n−6	36.2	36.8	39.0	37.8	21.4	20.1
C18:3 n−3	3.1	3.3	3.6	3.9	1.5	1.5
C20:0	0.3	0.3	0.3	0.3	0.4	0.3
C20:1 n−9	0.6	0.6	0.6	0.6	0.6	0.6
C20:3 n−6	0.2	0.2	0.2	0.2	0.3	0.2
C20:4 n−6	0.5	0.4	0.2	0.2	0.4	0.2
SFA[f]	29.8	29.1	32.9	34.3	17.6	18.7
MUFA[g]	30.2	30.1	24.1	23.6	58.8	59.4
PUFA[h]	40.0	40.7	43.0	42.1	23.6	22.0

[a] Control = control diet. 10,000 IU vitamin A/kg feed supplementation.
[b] Var = vitamin A restricted diet. 0 IU vitamin A/kg feed supplementation.
[c] Control—mineral and vitamin premix provided per kg of feed: vitamin A, 10,000 IU; vitamin D3, 2000 IU; vitamin E, 26.7 mg; vitamin B1, 1.3 mg; vitamin B2, 4.0 mg; vitamin B12, 0.020 mg; vitamin B6, 1.3 mg; calcium pantothenate, 13.3 mg; nicotinic acid, 20 mg; biotin, 0.1 mg; folic acid, 0.1 mg; vitamin K3, 2 mg; Fe, 133.3 mg; Cu, 26.7 mg; Co, 0.30 mg; Zn, 133.3 mg; Mn, 76.7 mg; I, 1.3 mg; Se, 0.30 mg; ethoxyquin, 150 mg.
[d] Var—mineral and vitamin premix provided per kg of feed: vitamin A, 0 IU; vitamin D3, 2000 IU; vitamin E, 26.7 mg; vitamin B1, 1.3 mg; vitamin B2, 4.0 mg; vitamin B12, 0.020 mg; vitamin B6, 1.3 mg; Calcium pantothenate, 13.3 mg; Nicotinic acid, 20 mg; Biotin, 0.1 mg; Folic acid, 0.1 mg; vitamin K3, 2 mg; Fe, 133.3 mg; Cu, 26.7 mg; Co, 0.30 mg; Zn, 133.3 mg; Mn, 76.7 mg; I, 1.3 mg; Se, 0.30 mg; Ethoxyquin, 150 mg.
[e] According to Fundación Española Desarrollo Nutrición Animal (2010) (supplied per kg of diet).
[f] SFA (C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0); sum of saturated fatty acids.
[g] MUFA (C16:1n−9 + C16:1n−7 + C17:1 + C18:1n−9 + C18:1n−7 + C20:1n−9); sum of monounsaturated fatty acids.
[h] PUFA (C18:2n−6 + C18:3n−3 + C20:3n−9 + C20:4n−6); sum of polyunsaturated fatty acids.

2.2. Sample collection

Nine pigs per treatment were slaughtered at 4 months of age (early growing) and the remaining (n = 10) at 11 months of age (finishing) (Industrias Cárnicas Alonso, S.L., Toledo, Spain) when pigs reached the averaged weights of 35.8 ± 3.1 and 158 ± 7 kg LW, respectively. In the slaughterhouse, carcass length from the posterior edge of the