DIFFERENT DIETARY PROTEIN- AND PUFA LEVEL AFFECTS LIPOGENIC PROTEIN EXPRESSION AND FATTY ACID CONCENTRATIONS IN PORCINE MUSCLE

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Abstract – In total 40 male Landrace pigs were allocated into five feeding groups. The animals were fed with two different levels of protein and two different types of vegetable oil. The muscle fatty acids concentrations were significantly affected by the diet resulted in higher *n*-3 FA concentration of linseed oil containing high- and reduced protein diets compared to sunflower oil diets. However, the meat quality of the muscle was not diet affected. The protein expression of mature Sterol regulatory element binding protein 1c (mSREBP-1c) in the muscle of high protein fed pigs was increased; however not the precursor form of SREBP-1c (pSREBP-1c) and the protein expression of stearoyl-CoA-desaturase (SCD). The clarification of mechanisms regulating *de novo* synthesis of fatty acids and fat partitioning in different tissues in pigs can be a step in designing the strategies for producing pigs with desirable fatty acid profile and fat content.

Key Words – fatty acids, meat quality, pig muscle, SREBP-1, SCD

I. INTRODUCTION

Manipulation of the fatty acid composition of farm animal muscle and adipose tissues has been of great interest in recent years, which is related to an increasing demand on production of meat with desirable nutritional and technological quality [1]. At the present time the main strategies are genetic selection and/or dietary manipulations. Dietary strategies used to customise fatty acid composition of pig fat have been proven to be very effective because dietary fatty acids can be incorporated into pig muscle and adipose tissues with little modifications [2]. Selected MUFA and PUFA have a number of health benefits. The majority of the health benefits have been associated with *n*-3 PUFA [3]. There is increasing amount of evidence that the two fat depots, the subcutaneous fat and the intramuscular muscular fat (IMF), can be regulated by independent mechanisms. IMF is the last fat depot to develop, and it may respond to dietary manipulations in a different manner when compared to other adipose tissues depots [4]. However, the mechanisms regulating *de novo* fatty acid synthesis and fat partitioning in pigs remain unclear. The present study investigated the effect of reduced protein diet in combination with different vegetable oils on protein expression of lipogenic enzymes and fatty acid profile in porcine muscle.

II. MATERIALS AND METHODS

In total 40 male Landrace pigs (castrates) were used in the diet experiment. The animals were allocated into five feeding groups (each n=8) at a live weight of approximately 60 kg (Table 1). The pigs were fed *ad libitum* from 60 kg to 100 kg live weight and restricted to 2.8 kg/day until 120 kg. The animals in the experimental groups (1-4) were fed with two different levels of protein and two different types of vegetable oil. The animals in the control group were fed with a regular pig diet without plant oil supplementation. The chemical- and fatty acid composition of the diet of the five groups (HPD-SO, HPD-LO, RPD-SO, RPD-LO and CON) are presented in Table 1. The HPD was formulated to contain 19.6% crude protein, and the RPD was formulated to contain 15.5% crude protein. Sunflower oil was used in the diet group HPD-SO and RPD-SO and linseed oil in diet group HPD-LO and RPD-LO as fat sources. The diets contained the same level of metabolisable energy (ME) of approximately 13.6 MJ/kg. The acquisition of the diet data was performed for each single animal. The pigs were weighted

Table 1 Experimental design, chemical- and fatty acid composition of the diet groups (based on original matter)

	Group 1 (HPD-SO)	Group 2 (HPD-LO)	Group 3 (RPD-SO)	Group 4 (RPD-LO)	Group 5 (CON)
Number (n)	8	8	8	8	8
Feeding	High protein diet with sunflower oil	High protein diet with linseed oil	Reduced protein iet with sunflower oil	Reduced protein diet with linseed oil	Control
Start weight (kg)	$68.84_{1.60}$	$69.69_{1.60}$	$69.36_{1.60}$	$69.01_{1.60}$	$69.24_{1.60}$
Live weight at slaughter (kg)	$120.56_{0.75}^{a}$	$121.38_{0.75}^{a}$	$123.19_{0.75}^{a}$	$120.62_{0.75}^{a}$	$117.80_{0.75}^{b}$
Chemical compost.					
Dry matter	90.0	88.7	89.8	89.6	89.3
Crude protein	19.6	19.4	15.7	15.0	17.4
Crude fat	5.2	5.7	5.9	6.3	3.4
Crude fibre	4.7	4.6	5.3	5.5	4.2
Crude ash	4.9	4.9	4.8	5.0	4.8
Starch	36.9	36.2	40.0	40.7	40.1
ME (MJ/kg)	13.7	13.7	13.5	13.5	13.2
Amino acids (g/kg)					
Lysine	0.93	0.95	0.82	0.82	0.96
Methionine	0.26	0.27	0.22	0.22	0.24
Cysteine	0.57	0.58	0.49	0.48	0.54
Threonine	0.60	0.61	0.48	0.45	0.56
Fatty acids (%)					
16:0	10.30	10.03	9.89	9.52	12.44
18:0	3.38	3.84	3.43	3.75	1.84
18:1*cis*-9	22.81	17.29	22.96	18.01	32.46
18:2*n*-6	52.94	29.43	51.69	31.77	39.07
18:3*n*-3	6.66	36.11	8.10	33.80	6.74

Different small letter (a, b) devote significant effect of diet ($P \leq 0.05$)

during the diet experiment once a week. All animals will be slaughtered at an average live weight of 120 kg in the abattoir of the Leibniz Institute for Farm Animal Biology in Dummerstorf (Germany). The slaughter and dressing procedures are in accordance with EU specifications. For fatty acid analysis, samples of the *M. longissimus* d. were thawed at 4°C. After homogenization (Ultra-Turrax, IKA Staufen, Germany; T25, 3 x 15 sec, 12,000 rpm) and adding C19:0 as an internal standard, the total lipids were extracted in duplicates with chloroform/methanol (2:1, v/v) at room temperature. After trans esterification, the FAMEs were analysed by capillary GC using a Perkin Elmer gas chromatograph (Autosys XL) with a flame ionisation detector and split injection using a SIL 88 CB column (100m x 0.25 mm, (Perkin Elmer Instruments, Shelton, United States) [5]. For protein expression analysis, the proteins were extracted by homogenizing muscle tissues in ice-cold lysis buffer. The proteins were separated by SDS-PAGE on gels (7.5%) in a Criterion™ electrophoresis unit (Bio-Rad, Germany). After transfer, the proteins were incubated with primary antibodies for pSREBP-1 (sc-367), for mSREBP-1 (sc-366, Santa Cruz Biotechnology, USA) and for SCD (ab39969, United Kingdom). After incubating with a secondary antibody the blot were developed using the chemiluminescence. Signal intensity was normalized on tubulin as reference protein [6].

III. RESULTS AND DISCUSSION

The average daily gain (ADG) was not affected by different experimental diets, except the control group (CON) showed lower ADG compared with experimental groups. The back fat and muscle area was not influenced by different dietary protein combined with types of vegetable oil in male Landrace pigs (Table 2). A number of studies have demonstrated that IMF and subcutaneous fat content might be manipulated independently by dietary means. For example, feeding a low protein diet increases the level in IMF with much smaller effect or with no effect on subcutaneous fat

Table 2 Growth performance, carcass traits, meat quality and fatty acid concentrations (*longissimus* muscle) of Landrace pigs fed different diets

	Group 1 (HPD-SO) LSM_{SEM} (n = 8)	Group 2 (HPD-LO) LSM_{SEM} (n = 8)	Group 3 (RPD-SO) LSM_{SEM} (n = 8)	Group 4 (RPD-LO) LSM_{SEM} (n = 8)	Group 5 (CON) LSM_{SEM} (n = 8)	Significance
ADG (g)	$831.4_{18.38}^{a}$	$835.2_{18.30}^{a}$	$874.4_{18.38}^{a}$	$840.6_{18.38}^{a}$	$761.0_{18.38}^{b}$	0.002
ADFI (g)	$3135.6_{49.25}$	$3195.6_{49.25}$	$3191.4_{49.25}$	$3195.0_{49.25}$	$3214.2_{49.25}$	0.832
Back fat (mm)	$18.4_{1.03}$	$19.3_{1.03}$	$18.2_{1.03}$	$19.2_{1.03}$	$20.1_{1.03}$	0.710
Muscle area (cm^2)	$52.1_{2.01}$	$50.3_{2.01}$	$54.3_{2.01}$	$51.1_{2.01}$	$52.2_{2.01}$	0.692
Meat quality						
IMF (%)	$1.3_{0.17}$	$1.4_{0.17}$	$1.4_{0.17}$	$1.3_{0.17}$	$1.2_{0.17}$	0.789
pH_{45}	$6.3_{0.06}$	$6.4_{0.06}$	$6.4_{0.06}$	$6.4_{0.06}$	$6.2_{0.06}$	0.078
Color (L*)	$47.7_{0.56}$	$48.3_{0.56}$	$47.1_{0.56}$	$48.5_{0.56}$	$47.6_{0.56}$	0.380
a*	$7.6_{0.32}$	$7.4_{0.32}$	$7.4_{0.32}$	$7.3_{0.32}$	$7.7_{0.32}$	0.886
b*	$1.6_{0.23}$	$1.6_{0.23}$	$1.2_{0.23}$	$1.4_{0.23}$	$1.1_{0.23}$	0.440
Shear force (kg/cm^2)	$5.2_{0.27}$	$4.7_{0.27}$	$5.5_{0.27}$	$4.7_{0.27}$	$4.8_{0.27}$	0.189
Fatty acids (mg/100 g)						
Sum FA	$1404.1_{150.4}$	$1518.1_{150.4}$	$1487.1_{150.4}$	$1411.3_{150.4}$	$1293.5_{150.4}$	0.852
14:0	$16.8_{2.76}$	$20.5_{2.76}$	$19.2_{2.76}$	$16.9_{2.76}$	$15.1_{2.76}$	0.667
16:0	$3\ 31.8_{40.15}$	$368.2_{40.15}$	$354.2_{40.15}$	$321.0_{40.15}$	$302.9_{40.15}$	0.792
18:0	$162.9_{19.13}$	$183.5_{19.13}$	$183.3_{19.13}$	$164.3_{19.13}$	$154.5_{19.13}$	0.761
18:1*cis*-9	$475.3_{61.02}$	$508.4_{61.02}$	$500.6_{61.02}$	$469.4_{61.02}$	$467.4_{61.02}$	0.980
18:2*n*-6	$201.3_{10.70}^{a}$	$148.2_{10.70}^{b}$	$203.9_{10.70}^{a}$	$163.0_{10.70}^{a,b}$	$139.3_{10.70}^{b}$	0.0001
18:3*n*-3	$13.1_{4.05}^{a}$	$65.2_{4.05}^{b}$	$16.8_{4.05}^{a}$	$66.8_{4.05}^{b}$	$10.3_{4.05}^{a}$	3.92E-14
20:4*n*-6	$43.4_{1.01}^{a}$	$29.4_{1.01}^{b}$	$41.4_{1.01}^{a}$	$29.9_{1.01}^{b}$	$39.9_{1.01}^{a}$	4.17E-13
20:5*n*-3	$4.4_{0.54}^{a}$	$17.9_{0.54}^{b}$	$5.1_{0.54}^{a}$	$19.7_{0.54}^{b}$	$6.3_{0.54}^{a}$	2.04E-23
22:4*n*-6	$4.8_{0.14}^{a}$	$2.3_{0.14}^{b}$	$4.6_{0.14}^{a}$	$2.0_{0.14}^{b}$	$4.3_{0.14}^{a}$	3.97E-18
22:5*n*-3	$8.5_{0.29}^{a}$	$13.8_{0.29}^{b}$	$9.4_{0.29}^{a}$	$14.0_{0.29}^{b}$	$8.9_{0.29}^{a}$	3.95E-18
22:6*n*-3	$3.7_{0.23}$	$3.5_{0.23}$	$3.6_{0.23}$	$3.6_{0.23}$	$4.7_{0.23}$	0.005
Sum SFA	$524.3_{62.84}$	$587.9_{62.84}$	$570.2_{62.84}$	$515.9_{62.84}$	$486.8_{62.84}$	0.785
Sum MUFA	$584.2_{76.42}$	$629.8_{74.42}$	$614.2_{76.42}$	$575.7_{76.42}$	$578.5_{76.44}$	0.982
Sum PUFA	$294.9_{15.27}^{a}$	$299.6_{15.27}^{a}$	$301.9_{15.27}^{a}$	$319.0_{15.27}^{a}$	$227.6_{15.27}^{b}$	0.002
Sum n-3 FA	$32.4_{5.19}^{a}$	$108.9_{5.19}^{b}$	$38.4_{5.19}^{a}$	$113.0_{5.19}^{b}$	$32.2_{5.19}^{a}$	8.56E-16
Sum n-6 FA	$262.5_{11.30}^{a}$	$190.7_{11.30}^{b}$	$263.5_{11.30}^{a,b}$	$206.0_{11.30}^{b}$	$195.4_{11.30}^{a,b}$	1.21E-05
Ratio *n*-6 /*n*-3 FA	$8.2_{0.12}^{a}$	$1.8_{0.12}^{a,b}$	$6.9_{0.12}^{a,b}$	$1.8_{0.12}^{a,b}$	$6.1_{0.12}^{b}$	5.02E-32

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content in pigs [7]. However the present study using different dietary protein level did not resulted in differences in IMF. Comparable to other studies the meat quality parameter of *longissimus* muscle (Color L*, a*, b*, shear force) was not diet effected (Table 2), [8]. The fatty acid composition of *longissimus* muscle in Landrace pigs fed different diet is shown in Table 3. The reduced dietary protein level in *longissimus* muscle caused not variations of individual *n*-3 or *n*-6 fatty acid concentrations; however the significant changes in these FA concentrations are based on sunflower- or linseed oil diet supplementation. The concentrations of the *n*-6 FA (18:2*n*-6, 20:4*n*-6, 22:4*n*-6) in HPD-SO and RPD-SO group were elevated in porcine muscle fed sunflower oil containing diet compared with the CON group (Table 2). The concentrations of the *n*-3 FA (18:3*n*-3, 20:5*n*-3, 22:5*n*-3) in HPD-LO and RPD-LO group were increased up to 113 mg/100g (sum *n*-3 FA) in porcine muscle fed linseed oil containing diet compared with the CON group, except 22:6*n*-3 (Table 3). Also, other studies investigated effects of *n*-3 FA containing dietary fat sources on muscle FA profiles revealed increased concentrations for 18:3*n*-3 and 22:5*n*-3, however no diet effect on 22:6*n*-3 [1,8]. Diet did not affect the concentration of saturated fatty acids (14:0, 16:0, and 18:0). The mechanisms regulating *de novo* fatty acid synthesis and fat partitioning in pigs remain unclear until now. The key enzyme involved in MUFA biosynthesis is stearoyl-CoA-desaturase (SCD). SCD is under control of transcription factors like SREBP-1. The muscle

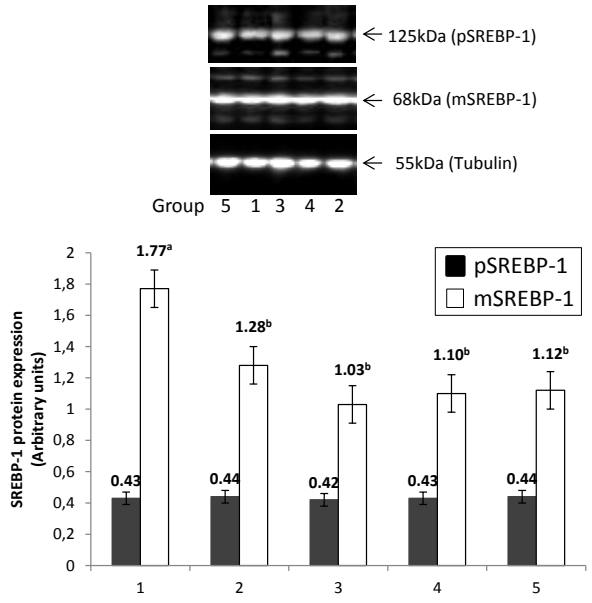

Fig.1 Protein expression of pSREBP-1 (125kDa) and mSREBP-1 (68kDa) in muscle of pigs fed different diets (normalized to tubulin, 55kDa) (a,b – significant effect of diet, <0.05)

protein expression of the SREBP-1 precursor (pSREBP-1) in the present study was not diet affected; however the active nuclear form of SREBP-1 (mSREBP-1) was significantly increased in muscle of HPD-SO-fed pigs (group 1) compared to all other diet groups (Fig. 1). For SCD, the protein expression in *longissimus* muscle pigs showed no influence of different dietary protein- or fatty acid level (data not shown). As a result the concentrations of reaction products of SCD, the MUFA, were not diet affected. In contrast, Doran et al. [7] detected increased SCD protein expression in pig muscle fed reduced protein diet but not in back fat tissue suggesting tissue-specific activation of the lipogenic enzyme expression.

IV. CONCLUSION

The clarification of mechanisms regulating *de novo* synthesis of fatty acids and fat partitioning in pigs is an important step in designing the strategies for producing pigs with desirable fatty acid composition and fat content.

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