COMBINED EFFECTS OF DIETARY ARGININE, LEUCINE AND PROTEIN LEVELS ON FATTY ACID COMPOSITION AND GENE EXPRESSION IN PIG MUSCLE

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Abstract – Intramuscular fat (IMF) content is one of the key meat quality traits. Some feeding strategies have been suggested to improve fat partitioning in pigs, mainly based on dietary amino acid supplementation and reduced protein diets. Therefore, the objective of this study was to evaluate the combined effects of arginine, leucine and protein levels on IMF, fatty acid composition and mRNA level of genes controlling lipid metabolism in pig *longissimus lumborum* muscle. Fifty four intact male pigs (Duroc × Pietrain × Large White × Landrace crossbred), from 59 to 92 kg of live weight were used. The reatments followed a 2 × 3 factorial arrangement, with two levels of arginine supplementation (0 vs. 1%) and three levels of basal diet (normal protein diet, NPD; reduced protein diet, RPD; and RPD with 2% of Leucine, RPDL). The results showed that dietary arginine supplementation did not affect IMF content, neither the percentage of main fatty acids. The increase in IMF content under the RPD, with or without leucine supplementation, was accompanied by increased *FASN* and *SCD* mRNA levels. RPD had a significant effect on fatty acid composition. Leucine supplementation of RPD did not change IMF, but increased 16:0 and 18:1*c*9 and decreased 18:2*n-6* in muscle.

Key Words – Amino acids, fatty acid composition, lipid metabolism

I. INTRODUCTION

Fat content and fatty acid composition in meat producing animals has received considerable attention in view of their implications for meat quality and human health. Furthermore, not only fat content but also fatty acid composition of IMF plays an important role in meat quality [1]. Some feeding strategies have been suggested to improve IMF content and fatty acid composition mainly based on dietary amino acid supplementation and reduced protein diets. Previous research on growing finishing pigs suggested that dietary supplementation with arginine increases IMF, thus improving fat partitioning [2]. Other study suggested that IMF of pork can be increased by feeding finishing pigs with high levels of leucine [3]. The mechanism associated to this effect can be explained by dietary-stimulated increase in steroyl-CoA desaturase (SCD) activity in pig muscle [4].

Recent results from our research group indicated that the increased IMF promoted by RPD is very likely due to lysine limitation and it is mediated via up-regulation of the adipogenic transcription factor PPARG and the lipogenic enzyme SCD [5]. In this study we investigated the potential cumulative effects of dietary arginine supplementation, reduced protein diet (RPD) and RPD with leucine supplementation on fatty acid composition in commercial crossbred pigs muscle and the effect on expression of genes controlling lipid metabolism.

II. MATERIALS AND METHODS

Fifty four commercial crossbred (25% Duroc, 25% Pietrain, 25% Large White and 25% Landrace) entire male pigs with an initial body weight of 58.9 (SD 1.59) kg were used. All the animals were randomly assigned to one of the six diets in a 2 × 3 factorial arrangement. The diets were isoenergetically formulated and differed in crude protein, arginine and leucine contents, as follows: 16.0% of crude protein (normal protein diet, NPD); 13.0% of crude protein (reduced protein diet, RPD); and 13.0% of crude protein plus leucine in the diet to achieve 2% (reduced protein diet with leucine, RPDL). The arginine treatment and the isonitrogenous control were obtained through supplementation of the basal diets with 1.0% of arginine and 2.05% of alanine, respectively. Pigs were slaughtered at an average

live body weight of 91.7 (SD 1.61) kg. After electrical stunning and exsanguination, samples of *longissimus lumborum* muscle were collected for IMF, fatty acid composition and gene expression analysis. IMF was extracted according to the Soxhlet method with previous acid hydrolysis [6]. The quantification of fatty acid methyl esters (FAME) were performed using a gas chromatograph as described by Madeira *et al.* [5]. The RNA was isolated and total RNA was reversed transcribed as described by Madeira *et al.* [5]. The gene expression was determined for real-time quantitative PCR. The results were analyzed using the MIXED procedure of SAS version 9.1, (SAS Institute, Inc., Cary, NC, USA). The contrasts between dietary protein level (NPD *v.* RPD, NPD *v.* RPDL and RPD *v.* RPDL) were performed. The level of significance was set at $P<0.05$.

III. RESULTS AND DISCUSSION

Results of IMF, fatty acid composition and partial sums of fatty acids in the *longissimus lumborum* muscle are presented in Table 1. IMF content was not affected neither by dietary arginine ($P=0.274$) nor by leucine supplementation of RPD ($P=0.801$). However, the reduction of protein level in the diet resulted in a significant ($P\leq0.001$) increase in IMF content by 45% and 48% for RPD and RPDL groups, respectively. In the present study, a 19% RPD fed during the growing-finishing phase of the commercial crossbred pigs resulted in 45-48% of increase in IMF. This is consistent with findings of previous studies, which indicated that dietary protein reduction increased IMF content in commercial crossbred pigs [4].

Results of our study on effects of arginine supplementation are in line with that of Go *et al.* [9] who found that dietary arginine supplementation does not increase IMF in pigs. However, Tan *et al.* [2] reported an increase in IMF content in experiments that used 1% of dietary arginine supplementation. This discrepancy might be explained by the use of pigs with distinct genetic background, mainly different predisposition for fat deposition. Regarding the dietary leucine supplementation of RPD, our results are in disagreement with those of Hyun *et al.* [10], who described an increase of IMF with leucine supplementation of diets restricted in lysine. In sum, our results do not indicate any additional effect of dietary arginine and/or leucine supplementation on increased IMF promoted by the RPD alone. The predominant fatty acids in IMF were 18:1*c*9 (32-37% of total FAME), 16:0 (21-22%), 18:2*n-6* (12-16%), 18:0 (11-12%), 18:1*c*11 (4%) and 20:4*n-6* (3-4%) for all experimental groups.

Expression analysis of key genes associated with lipid metabolism has presented in Figure 1. In terms of dietary effects on fatty acid composition, the animals fed the diet supplemented with arginine had a lower percentage of *n-3* long-chain PUFA, which was mainly due to a decrease in DPA level. The DPA decrease was accompanied by the lower expression of *FADS1* gene and the higher expression of *SREBP1* gene. In contrast to *FADS1* gene, our study did not find significant effects of arginine on *SCD* gene expression. This is not in agreement with findings of Tan *et al.* [11], who reported that arginine supplementation increased 18:1*c*9 and decreased 18:0 and 18:2*n-6*, which was explained by the arginine-dependent activation of SCD, a key enzyme in the formation of oleic acid. Our study indicated that supplementation of RPD with leucine changed the fatty acid profile in pig muscle. The up-regulation of *SCD* ($P=0.09$) and *FASN* in muscle were consistently reflected by higher proportions of 18:1*c*9 and 16:0 fatty acids. Moreover, most of the muscle PUFA decreased under the dietary leucine supplementation. Thus, the increase in 18:1*c*9 and 16:0 should result in a direct replacement of the majority of PUFA in muscle lipids.

IV. CONCLUSION

Arginine supplementation of pig diets, either alone or in combination with RPD and/or leucine, does not seem to be useful to increase IMF content or to change fatty acid composition. The supplementation of RPD with leucine seems to increase MUFA content. Our results also indicate that lipogenesis might be differently regulated in pig muscle. These data contribute to understand the mechanisms of dietary regulation of fat partitioning and could help to improve pig feeding strategies to address industry needs.

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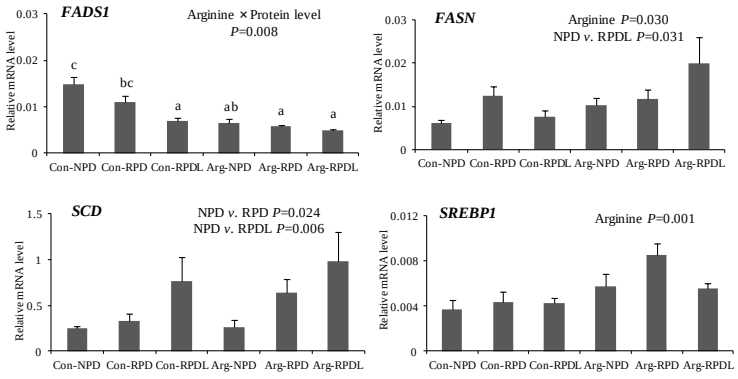
Figure 1. Effect of dietary arginine, leucine and protein levels on gene expression in *longissimus lumborum* muscle of pigs.



FADS1, Fatty acid desaturase 1, FASN, fatty acid synthase, SCD, stearoyl-CoA desaturase, SREBP1, sterol regulatory element binding protein 1. Con, control diet; NPD, normal protein diet; RPD, reduced protein diet; RPDL, reduced protein diet with leucine addition. Values are means, with their standard errors represented by vertical bars. [a,b] Mean values within a row with unlike letters were significantly different ($P<0.05$). "Arginine" and arginine × protein level mean significant effect of arginine or interaction between arginine and protein level, respectively.

Table 1. Effect of dietary arginine, leucine and protein levels on intramuscular fat (IMF; % muscle), fatty acid composition (% total fatty acids) and partial sums of fatty acids *longissimus lumborum* muscle of pigs

	Control						Arginine						Significance level				
	NPD		RPD		RPDL		NPD		RPD		RPDL		Arg	Dietary protein level			Arg×Prot
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		NPD *v.* RPD	NPD *v.* RPDL	RPD *v.* RPDL	
IMF	1.34	0.181	1.85	0.181	2.20	0.181	1.53	0.181	2.30	0.181	2.05	0.181	0.274	0.001	<0.001	0.801	0.780
Fatty acid composition																	
12:0	0.25	0.019	0.19	0.017	0.17	0.013	0.20	0.012	0.19	0.008	0.16	0.004	0.027	0.039	<0.001	0.004	0.185
14:0	0.99	0.033	0.92	0.031	1.04	0.041	1.01	0.041	1.01	0.036	1.04	0.022	0.201	0.409	0.254	0.025	0.518
15:0	0.21	0.022	0.20	0.011	0.16	0.012	0.17	0.020	0.19	0.010	0.14	0.006	0.088	0.094	0.076	0.103	0.223
16:0	21.6	0.38	20.6	0.28	21.6	0.32	21.3	0.28	21.1	0.29	21.5	0.22	0.952	0.051	0.858	0.011	0.372
16:1*c*7	0.32	0.011	0.29	0.011	0.26	0.012	0.34	0.015	0.28	0.004	0.30	0.012	0.060	<0.001	<0.001	0.566	0.068
16:1*c*9	2.36	0.121	2.35	0.093	2.66	0.109	2.41	0.080	2.61	0.129	2.67	0.125	0.237	0.427	0.025	0.109	0.509
17:0	0.40	0.046	0.39	0.026	0.35	0.033	0.43	0.048	0.44	0.028	0.33	0.025	0.573	0.932	0.097	0.010	0.471
17:1*c*9	0.26	0.025	0.21	0.017	0.21	0.025	0.21	0.016	0.21	0.016	0.22	0.015	0.418	0.161	0.316	0.758	0.397
18:0	11.8	0.25	10.6	0.26	10.7	0.19	11.4	0.20	10.7	0.27	10.8	0.20	0.636	0.047	0.093	0.739	0.254
18:1*c*9	32.3	1.35	33.7	0.81	36.1	1.15	33.9	0.71	34.2	0.88	37.1	0.53	0.190	0.389	0.002	0.004	0.848
18:1*c*11	3.79	0.102	3.87	0.072	3.98	0.060	3.59	0.076	4.01	0.084	4.02	0.094	0.028	0.007	0.001	0.406	0.162
18:2*n-6*	15.7	1.00	15.0	0.63	12.7	0.82	15.5	0.62	14.5	0.61	12.5	0.301	0.594	0.307	<0.001	<0.001	0.961
18:3*n-3*	0.41	0.022	0.35	0.015	0.29	0.023	0.44	0.023	0.34	0.018	0.30	0.006	0.780	<0.001	<0.001	0.006	0.977
20:0	0.16	0.012	0.13	0.008	0.15	0.009	0.13	0.007	0.13	0.009	0.14	0.008	0.089	0.166	0.687	0.258	0.268
20:1*c*11	0.57	0.024	0.56	0.023	0.64	0.024	0.56	0.031	0.51	0.018	0.62	0.022	0.211	0.221	0.032	<0.001	0.647
20:2*n-6*	0.42	0.016	0.44	0.022	0.37	0.027	0.44	0.030	0.38	0.019	0.39	0.013	0.671	0.410	0.048	0.147	0.085
20:3*n-3*	0.17	0.020	0.13	0.008	0.12	0.012	0.13	0.010	0.15	0.007	0.12	0.006	0.508	0.130	0.012	0.071	0.050
20:3*n-6*	0.41	0.035	0.43	0.025	0.36	0.034	0.38	0.029	0.41	0.026	0.33	0.018	0.280	0.526	0.117	0.008	0.975
20:4*n-6*	3.23	0.420	3.69	0.191	2.99	0.327	3.00	0.224	3.30	0.226	2.68	0.157	0.163	0.204	0.374	0.006	0.952
Fatty acid partial sums																	
SFA	35.2	0.467	33.1	0.581	34.1	0.457	34.3	0.436	33.5	0.464	34.1	0.312	0.636	0.006	0.130	0.087	0.338
MUFA	39.7	1.56	41.1	0.92	44.0	1.27	41.2	0.82	41.9	1.04	45.0	0.61	0.220	0.364	0.001	0.003	0.943
PUFA	21.6	1.52	21.2	0.87	17.8	1.27	20.9	0.89	20.0	0.92	17.1	0.49	0.311	0.597	0.002	0.001	0.964
n-6 PUFA	20.3	1.43	20.1	0.86	16.9	1.21	19.7	0.86	19.1	0.88	16.3	0.47	0.388	0.690	0.003	0.001	0.965
n-3 PUFA	1.28	0.097	1.12	0.047	0.91	0.066	1.13	0.046	0.93	0.051	0.74	0.029	0.001	0.009	<0.001	<0.001	0.934

NPD, normal protein diet; RPD, reduced protein diet; RPDL, reduced protein diet with leucine addition.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).