THE COMBINATION OF ARGININE AND LEUCINE SUPPLEMENTATION OF REDUCED CRUDE PROTEIN DIETS IN PIGS IMPROVES MEAT EATING QUALITY

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Abstract – Intramuscular fat (IMF) has been shown to be positively related to quality of meat and plays an important role in consumers' perceptions of cooked pork palatability. This study was designed in order to investigate the effect of dietary arginine supplementation, protein reduction (PR) and leucine supplementation on meat quality, IMF and sensory analysis in pork *longissimus dorsi* muscle. Fifty four entire male pigs from a commercial crossbred (Duroc × Pietrain × Large White × Landrace) with an initial and final average live weight of 59 kg and 92 kg, respectively, were used in this experiment. The IMF was extracted according to the Soxhlet method with previous acid hydrolysis. pH and temperature were measured at 45 min and 24 h *postmortem* and color measurements at 24 h *postmortem*. For sensory analysis were used a trained sensory panel analysis. Dietary arginine supplementation had no effect (*P*>0.05) on IMF content but produced meat off-flavor and, under normal protein diet, increased meat tenderness and overall acceptability. The PR increased (*P*<0.001) IMF. Leucine addition did not affect (*P*>0.05) IMF. There was an increase of juiciness in PR and leucine addition, which accompanied the increase of IMF content. Dietary PR enhances pork eating quality but negatively affected carcass characteristics of pigs.

Key Words – amino acids, intramuscular fat, sensory analysis

I. INTRODUCTION

Pork is one of the most consumed meats in the world, and thus, an important source of dietary fat [1]. Pigs reared in intensive systems have become leaner to satisfy consumers and faster growing to improve feed efficiency [2]. However, it is well known that pork eating quality was negatively affected by IMF reduction [3]. IMF content of pork plays an important role in consumers' perceptions of cooked pork palatability [4], and it has been suggested that an IMF content between 2.5 and 3.0% is necessary for consumer acceptability of cooked pork [5]. Earlier studies have shown that the IMF levels can be increased without increasing subcutaneous fat with dietary protein restriction [6,7] and Leu supplementation [8,9] during the growing-finishing period of pigs. However, dietary Arg supplementation has been reported as being beneficial for promoting IMF content, skeletal muscle gain, carcass lean content and improved meat quality [10,11].

Our group reported recently that the increased IMF promoted by dietary crude protein reduction in pigs is due to lysine limitation, which improves pork sensory attributes [12]. Thus, the objective of this study was to investigate the additive/interaction effects with Arg and/or Leu supplementation on reduced protein diets as a strategy for increasing IMF and improving meat quality characteristics and sensory attributes on *longissimus dorsi* muscle of pork.

II. MATERIALS AND METHODS

Fifty four crossbred entire male pigs with four genetic lines (50% Large White × 50% Landrace gilts mated to 50% Duroc × 50% Pietrain boars) with a mean live body weight of 58.9 ± 1.6 kg were selected. Before the beginning of the experiment, all animals were housed and fed the same conventional feed management (based on starter and growth concentrates). Afterward, groups of pigs (3 pens with individual control of feed intake, containing 3 pigs each), were randomly assigned to 1 of the 6 isoenergetic (14 MJ ME/kg) dietary treatments. There were six dietary treatments, normal crude protein (CP)

diet (16% CP, NPD), reduced CP diet (13% CP, RPD), reduced CP diet with Leu addition to 2.0% (RPDL), normal CP diet supplemented with 1% Arg (16% CP, Arg-NPD), reduced CP diet supplemented with 1% Arg (13% CP, Arg-RPD) and reduced CP diet with Leu addition to 2.0% and supplemented with 1% Arg (13% CP, Arg-RPDL). The diets not supplemented with Arg were supplemented with an equivalent amount of Ala (2.05%) in order to be an isonitrogenous control. During the experimental period, animals were allowed to feed twice a day and water *ad libitum*. Throughout the experiment, pigs were weighted weekly just before feeding and slaughtered at an average live body weight of 91.7 ± 1.6 kg. Feed was removed 17 h before slaughter. After electrical stunning and exsanguinations, samples from *longissimus dorsi* muscle were collected from the right carcass for IMF and 24 hours after for sensory analysis. All samples were vacuum-packed immediately and stored at -20 ºC. The pH and temperature were measured in the *longissimus dorsi* muscle at 45 minutes and 24 hours *post-mortem* at half the length of each loin, using a pH meter equipped with a penetrating electrode. The meat color was measured at 24 h *post-mortem*, using a Minolta CR-300 chromometer and applying the CIE L*, a* and b* system 1 hour after air exposure to allow blooming. IMF was extracted according to the Soxhlet method with previous acid hydrolysis [13]. For sensory analysis, the samples were thawed and grilled to an internal temperature of 70 ºC. All samples were trimmed of external connective tissue and cut into cores with approximately ~2 × 2 × 2 cm^3, maintained at 60ºC and tasted as soon as possible. Twelve trained panelists for pork performed the sensory analysis in 6 sessions (9 samples for each session). The results were analyzed using the MIXED procedure of SAS version 9.1, (SAS Institute, Inc., Cary, NC, USA) with three orthogonal contrasts were constructed in order to test the dietary effects of Arg supplementation (Arg = (NPD + RPD + RPDL) *vs.* (Arg-NPD + Arg-RPD + Arg-RPDL)), protein reduction (PR = (NPD + Arg-NPD) *vs.* (RPD + RPDL + Arg-RPD + Arg-PRDL)), and Leu supplementation (Leu = (RPD + Arg-RPD) *vs.* (RPDL + Arg-PRDL)).

III. RESULTS AND DISCUSSION

The results for meat quality traits are shown in Table 1. Any pork quality traits were affected by Arg supplementation, PR and Leu supplementation (P>0.05). Dietary Arg and Leu supplementation had no effect on IMF content (P>0.05). However, RPD increased IMF content (P<0.001). However, some studies with dietary Arg supplementation [10,11] found an increased IMF content without changing pork quality. In contrast, Go *et al.* [14] did not find an increased IMF content in pigs fed diets with Arg, which is in agreement with our results. It is well known that Arg regulates the partitioning of dietary energy in favor of muscle protein accretion [15]. In contrast, the reduction of dietary CP from 16 % in NPD and Arg-NPD to 13% in RPD, RPDL, Arg-RPD and Arg-RPDL diets increased the IMF content by 45-48%. These results are in agreement with studies, in which low CP diets also increased IMF content in commercial crossbred pigs [6]. Wood *et al.* [16] reported that a diet with 16% less protein and lower levels of essential amino acids produced fatter pigs with greater IMF contents in the longissimus muscle. Also, it was recently shown by our research group that the increased IMF promoted by RPD in pigs is due to lysine limitation [12]. The consumer's purchase decision depends on meat color, which is an important factor of freshness and meat quality [17]. The pigs fed the diet with Leu addition tended to show a lower a* (P=0.057), the meat was less red, which can compromise the consumer choice. The Arg supplementation did not affect the color parameters, in contrast to a previous study, where Arg tended to increase the lightness of muscle at 24 h postmortem, which is consistent with a more rapid pH decline [14].

The trained sensory panel scores for *longissimus lumborum* muscle of pigs are presented in Table 2. Arg supplementation had higher tenderness (P<0.01) and overall acceptability (P<0.05). Also, the Arg supplementation had a significant (P<0.05) role on pork off-flavor. The meat from Arg supplemented groups had higher off-flavor values (0.96 vs. 0.71). PR increased juiciness (P<0.001) and flavor (P<0.05) scores and also Leu supplementation increased juiciness (P<0.05). Fernandez *et al.* [18] reported that

when IMF levels increase above approximately 2.5%, the tenderness, juiciness and flavor are significantly improved. In our study, the IMF levels are below 2.5% and, therefore, the scores of tenderness, juiciness and flavor were low. As a consequence, the overall acceptability was also low. Our results showed an increase of tenderness with Arg supplementation, and therefore in overall acceptability, but IMF did not increase. Moreover, the PR and Leu addition increased juiciness, which is in accordance with the increased IMF content in reduced protein treatment.

IV. CONCLUSION

Dietary Arg supplementation during the growing-finishing phase of commercial crossbred pigs does not affect IMF and pork quality traits, but slightly affect some sensory attributes. In addition, our data confirm that low CP diets increase IMF content in lean pig genotypes. Dietary Leu supplementation of low CP diets does not seem to have any additional effect on IMF or meat quality traits. However, the small increment in pork eating traits obtained with these feeding strategies indicates that this dietary approach might have limited use for the meat industry and consumers.

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Table 1. Meat quality traits of *longissimus lumborum* muscle from pigs[1]

Item	NPD	RPD	RPDL	Arg-NPD	Arg-RPD	Arg-RPDL	Significance levels		
							Arg[2]	PR[3]	Leu[4]
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	0.276	<0.001	0.781
Temperature, ºC									
45 min	21.6±0.94	22.2±0.94	21.8±0.94	20.7±0.94	22.5±0.94	20.9±0.94	0.534	0.405	0.289
24 h	7.88±0.808	7.81±0.808	7.40±0.808	7.50±0.808	8.07±0.808	7.24±0.808	0.889	0.934	0.449
pH									
45 min	6.10±0.074	5.87±0.074	6.03±0.074	5.93±0.074	6.12±0.074	6.09±0.074	0.417	0.823	0.398
24 h	5.77±0.063	5.60±0.063	5.65±0.063	5.62±0.063	5.65±0.063	5.64±0.063	0.494	0.267	0.759
Color measurements									
L*	55.5±1.43	54.2±1.43	55.8±1.43	52.9±1.43	53.5±1.43	54.7±1.43	0.213	0.793	0.330
a*	6.99±0.559	7.55±0.559	5.54±0.559	6.29±0.559	6.12±0.559	5.95±0.559	0.218	0.476	0.057
b*	4.10±0.584	4.39±0.584	3.46±0.584	3.27±0.584	3.25±0.584	3.17±0.584	0.121	0.816	0.392

[1] NPD = normal CP diet; RPD = reduced CP diet; RPDL = reduced CP diet with Leu supplementation.
[2] Contrast "Arg" = (NPD + RPD + RPDL) *vs.* (Arg-NPD + Arg-RPD + Arg-RPDL)
[3] Contrast "PR" = (NPD + Arg-NPD) *vs.* (RPD + RPDL + Arg-RPD + Arg-RPDL)
[4] Contrast "Leu" = (RPD + Arg-RPD) *vs.* (RPDL + Arg-RPDL)

Table 2. Sensory panel scores of *longissimus lumborum* muscle from pigs[1]

Item	NPD	RPD	RPDL	Arg-NPD	Arg-RPD	Arg-RPDL	SEM	Significance levels		
								Arg[2]	PR[3]	Leu[4]
Tenderness	5.08	5.31	4.74	5.29	5.16	5.75	0.158	0.005	0.680	0.959
Juiciness	3.10	3.61	4.12	3.65	3.81	3.87	0.128	0.111	<0.001	0.025
Flavor	3.99	4.10	4.39	4.11	4.36	4.23	0.130	0.480	0.047	0.508
Off-flavor	0.64	0.61	0.87	0.87	1.16	0.84	0.151	0.037	0.393	0.856
Overall acceptability	4.25	4.44	4.22	4.51	4.35	4.83	0.146	0.026	0.526	0.362

[1] NPD = normal CP diet; RPD = reduced CP diet; RPDL = reduced CP diet with Leu supplementation.
[2] Contrast "Arg" = (NPD + RPD + RPDL) *vs.* (Arg-NPD + Arg-RPD + Arg-RPDL)
[3] Contrast "PR" = (NPD + Arg-NPD) *vs.* (RPD + RPDL + Arg-RPD + Arg-RPDL)
[4] Contrast "Leu" = (RPD + Arg-RPD) *vs.* (RPDL + Arg-RPDL)
Sensory scores: scale with 8 points, with 1 = extremely tough, dry, weak, or negative for tenderness, juiciness, flavor, and overall acceptability, respectively, and with 8 = extremely tender, juicy, strong, or positive for tenderness, juiciness, flavor, and overall acceptability, respectively.