THE EFFECT OF *MORINGA OLEIFERA* LEAF MEAL (MOLM) ON FATTY ACID COMPOSITION AND OXIDATIVE STABILITY OF PORK

Xola P. Nduku[1], Thobela T. Nkukwana[2], Arnold Hugo[3], and Voster Muchenje[1]*

1Department of Livestock and Pasture Science, University of Fort Hare, Eastern Cape 5700, South Africa

2Agricultural Research Council - Animal Production Institute, Private Bag X2, Irene 0062, Pretoria, South Africa.

3Department of Microbial Biochemical and Food Biotechnology, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa

* vmuchenje@ufh.ac.za

Abstract – Effects of *Moringa oleifera* leaf meal (MOLM) as a feed additive on fatty acid (FA) composition, health lipid indices (atherogenicity and desaturase) and Thiobarbituric acid reactive substances (TBARS) of the *Longissimus thoracis et lumborum* (LTL) muscle was determined for 24 male pigs, i.e., 12 Large White (LW) and 12 Kolbroek (KB) aged six weeks. Four pigs of each breed were individually housed and randomly subjected to one of three dietary treatments formulated to contain 0% (T1), 2.5% (T2) and 5% (T3) MOLM, for eight weeks. Inclusion of up to 5% MOLM in pig diets resulted in a desirable increase in muscle *n*-3 content and reduced the *n*-6: *n*-3 fatty acid ratio. However, there were no significant differences (P>0.05) caused by the inclusion of MOLM in pig diets on muscle TBARS.

Key Words – Antioxidants, consumer health, *Moringa oleifera* leaves, fatty acids profiles, TBARS.

I. INTRODUCTION

The fatty acid composition has a major impact on consumer's perception on the health status of the meat [6]. Pork has a high *n*-6: *n*-3 ratio, which is associated with cancer, hypertension and cardiovascular diseases [13]. Unfavourable levels of polyunsaturated fatty acids (PUFAs) in pork negatively affect the oxidative stability and shelf life of the meat as a result of their susceptibility to lipid oxidation [8]. Lipid oxidation results in the development of rancidity, thereby negatively affecting sensory characteristics (colour, texture and flavour) and nutritional quality of the meat [9]. Nutritionists recommend pig diets to be balanced between *n*-3 PUFA and *n*-6 PUFA to produce good quality pork with no human health implications. The use of natural antioxidants especially from plants, to stabilize meat is increasing and considered to be safer than synthetic antioxidants [7]. These plants are essential with antioxidant properties which pass antioxidant compounds to meat [4]. One such plant with the potential to be used as an antioxidant and that could have a positive influence on the fatty acid composition in pig tissue is *Moringa oleifera* (M. oleifera).

The leaves of the M. oleifera plant contain phytochemicals such as, carotenoids, vitamins, minerals, amino acids, flavonoids and phenolics and favourably high levels of *n*-3 fatty acids [5]. Studies have reported dietary supplementation with MOLM to result in improved fatty acid profiles in breast meat from broiler chickens [8] and reduced *n*-6: *n*-3 ratios in goat meat [9]. There is, however, scarce information on the dietary use of MOLM on the fatty acid composition of pork from pigs fed in the early phase of production. The present study, therefore evaluated the potential of MOLM as a feed additive on the fatty acid profile and oxidative stability of pork.

II. MATERIALS AND METHODS

Ethical considerations were made in this study to conform to the national and international standards governing the usage of animals. Permission to use animals was obtained from the Ethical Clearance Committee of the University of Fort Hare (Certificate Reference Number: MUC011 SNDU01). Twenty four male pigs (12 Large White, 12 Kolbroek) at six weeks of age, weighing an average of 10 kg were obtained from a small scale commercial piggery in Fort Cox College of Agriculture and Forestry. All the pigs were housed individually and each pen was equipped with

feeding trough for feed and nipple water. Each pig served as an experimental unit. The pigs were randomly assigned to one of three maize-soyabean meal basal feeds in mash form, formulated to contain different levels of MOLM, and consisted of the control (T1, no MOLM); treatments 2 (T2) and treatment 3 (T3) which contained 2.5% and 5%, respectively for seven weeks. All the dietary treatments were formulated to be isonitrogenous and isoenergetic for the weaner (6 -8 weeks) and grower (9-13 weeks) phases. The pigs were allocated to the 3 treatment groups, at the rate of 4 pigs per treatment for each of the two breeds. Pigs had *ad libitum* access to feed and water and feeding was done twice daily, i.e., at 0800 hours and at 1500 hours, with half of the daily rations being given at each feeding time. At the end of the eight week feeding trial, the pigs were prepared for slaughter. Meat samples from the *Longissimus thoracic et. lumborum* (LTL) muscle were collected 24 hours post mortem from the right side of each carcass, cut into 25 mm thick loin chops, vacuum sealed (Gastrovac Pro, Henkovac, Netherlands) and stored at -20°C until the time of analysis. Total lipid from muscle samples were quantitatively extracted, according to the method of [2], using chloroform and methanol in a ratio of 2:1. Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. A 5 g sample of lean meat (removed from the middle of each cut) was used for Thiobarbituric acid-reacting substances (TBARS) analysis using the aqueous acid extraction method of [10] to determine lipid oxidation. A 2 g sample of back fat (BF) (inner + outer layer) was also removed for the lipid extraction, using the [2] method. Thiobarbituric acid-reacting substances were expressed as micrograms of malonaldehyde (MDA) per gram of meat.

Statistical analysis

Data for fatty acid composition and TBARS values were analysed using General Linear Model (GLM) procedures of [12] and pair wise comparisons of LSMeans were done. The least significant difference (LSD) method was used to separate the means. Interactions were not significant at (P>0.05). The statistical model used was: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijk}$

III. RESULTS AND DISCUSSION

Table 1. Proximate and total % fatty acid composition of *Longissimus thoracic et. lumborum* muscle as affected by breed and dietary treatment

		Dietary Treatments		
		T1	T2	T3
		(n = 8)	(n = 8)	(n = 8)
Nutrient composition				
IMF%	L	2.42±0.4	2.76±0.5	1.67±0.5
	W	64	36	36
	K	2.75±0.4	3.48±0.4	2.36±0.4
	B	64	64	64
FFDM%	L	18.95^{Aa}±	21.34^{Ab}±	22.35^{Ab}±
	W	0.605	0.699	0.699
	K	20.86^{Ba}±0	22.45^{Aa}±	22.13^{Aa}±
	B	.605	0.605	0.605
Moist%	L	77.68±0.	75.90±1.	76.00±1.
	W	872	007	007
	K	75.59±0.	73.49±0.	75.51±0.
	B	872	872	872
Fatty acids (%)				
Total	L	40.3^{b}±1.6	40.51^{b}±1.	34.01^{a}±1.
SFA	W	80	94	941
	K	40.09±1.	41.64±1.	39.23±1.
	B	680	680	680
Total	L	40.78±2.	43.98±2.	46.22±2.
MUFA	W	379	747	747
	K	45.93±2.	47.43±2.	44.94±2.
	B	379	379	379
Total	L	18.96±1.	15.51±2.	19.77±2.
PUFA	W	862	150	150
	K	13.98±1.	12.96±1.	15.83±1.
	B	862	862	862
Total *n*-6	L	18.18±1.	14.55±2.	18.31±2.
	W	873	162	162
	K	13.49±1.	10.72±1.	14.80±1.
	B	873	873	873
Total *n*-3	L	0.78^{a}±0.1	0.96^{Ba}±0.	1.46^{Bb}±0.
	W	06	123	123
	K	0.65^{a}±0.1	0.60^{Aa}±0.	1.03^{Ab}±0.
	B	23	106	106
PUFA:SFA	L	0.47^{ab}±0.	0.38^{a}±0.0	0.60^{Bb}±0.
	W	056	64	064
	K	0.35±0.0	0.28±0.0	0.41^{A}±0.0
	B	56	56	56
PUFA:MUFA	L	0.49±0.0	0.36±0.0	0.44±0.0
	W	65	75	74
	K	0.31±0.0	0.24±0.0	0.36±0.0
	B	65	65	65

n-6:*n*-3	L	23.37[b]±1.	15.10[a]±1.	12.61[a]±1.
	W	636	889	889
	K	20.82[b]±1.	18.46[ab]±1	14.25[a]±1.
	B	889	.636	636

[AB]Means in the same column with different superscripts are significantly different (P<0.05); [ab]Means in the same row with different superscripts are significantly different (P<0.05); *Moringa oleifera* leaf meal; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; *n*-6 – omega 6 fatty acids; *n*-3 – omega 3 fatty acids PUFA: SFA – polyunsaturated fatty acids and saturated fatty acids ratio; PUFA: MUFA – polyunsaturated fatty acids and monounsaturated fatty acids ratio; *n*-6: *n*-3 – omega 6 fatty acids and omega 3 fatty acids ratio.

The effects of pig breed and dietary treatment on the composition of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) fatty acids, omega-3 (*n*-3) fatty acids, and PUFA: SFA, PUFA: MUFA and *n*-6: *n*-3 ratios in pork muscle are also shown in Table 1. There were no significant (P>0.05) breed differences in the SFA across treatment groups. Treatment effects were observed in LW pigs, where inclusion of MOLM at 5% resulted in significantly (P<0.05) lower SFA level. Lower levels of saturated fatty acids are more desirable in meat. The values for the other fatty acids did not differ significantly (P>0.05) in both breeds, across the dietary treatments. There was however a general trend of increasing amounts of PUFAs and *n*-6 FAs as the MOLM inclusion level was increased. Although pork muscle samples from pigs fed on MOLM had slightly higher levels of PUFAs and *n*-6, they were not significantly (P>0.05) different from the 0% MOLM samples. Meat fatty acid composition is influenced by genetic factors, although to a lower extent than dietary factors [1]. The inclusion levels of MOLM at 2.5% and 5% levels in LW pigs, and 5% in KB pigs significantly increased (P<0.05) *n*-3 in pork muscle. The *n*-3 and *n*-6 FAs play an important role in human nutrition and are essential to human health [11], in the regulation of the cardiovascular system and immunological processes [3]. Inclusions of MOLM at 2.5% and/or 5% resulted in significantly (P<0.05) lower *n*-6 :*n*-3 fatty acids in pork from LW pigs and KB pigs. The recommended value of *n*-6: *n*-3 ratio is 4.0, even though it can be manipulated to be higher than this in some meats [6] with dietary inclusions that are likely to influence the long chain *n*-3 fatty acids. Although higher values of the *n*-6: *n*-3 ratio were obtained from the current study, they were reduced by the inclusion of MOLM in the pig diets.

Table 2 presents the TBARS as a measure of oxidative stability in pork muscle. The means (±SE) for average TBARS (mg MDA/kg meat) of pork muscle as affected by breed and dietary treatment were analysed. Significant (P<0.05) breed differences were observed at 0% MOLM (T1) inclusion level, where pork from LW pigs had significantly (P<0.05) higher TBARS values (0.26) than pork from KB pigs (0.15). There are limited studies that report the effect of pig breed on the oxidative stability of pork. The current study showed no significant differences (P>0.05) caused by the inclusion of MOLM in pig diets, in TBARS (measures of oxidative stability) of pork in both breeds. However, for all treatments, pork had lower TBARS values ranged between 0.15 to 0.26 in LW pigs, and 0.09 to 0.15 in KB pigs.

Table 2. Means (±SE) for average TBARS (mg MDA/kg meat) of *Longissimus thoracic et. lumborum* muscle as affected by breed and dietary treatment

	TBARS (mg MDA/kg meat)		
	T1	T2	T3
	n=8	n=8	n=8
LW	0.26[B]±0.035	0.15±0.041	0.18±0.041
KB	0.15[A]±0.035	0.14±0.035	0.09±0.035

[AB]Means in the same column with different superscripts are significantly different (P<0.05); MOLM (*Moringa oleifera* leaf meal); T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM); TBARS (thiobarbituric acid reactive substances); LW (Large White); KB (Kolbroek).

IV. CONCLUSION

Dietary *Moringa oleifera* leaf meal inclusion resulted in a positive increase in levels of *n*-3 fatty acids and reduced *n*-6: *n*-3 fatty acid ratio of pork. There was no significant effect of MOLM inclusion on the TBARS of pork muscle, however they were generally improved. Hence, MOLM may be a proficient source of natural antioxidants for pork.

ACKNOWLEDGEMENTS

The authors are grateful and the DST/NRF SA–Argentina Research Collaboration Fund for funding this study, and to the Govan Mbeki Research and Development Centre of the University of Fort Hare and NRF Innovation Bursary for financial assistance. Appreciation also goes to Fort Cox College of Agriculture and Forestry for use of their facilities.

REFERENCES

1. De Smet, S., Raes, K. & Demeyer, D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: A review. Animal Research 53: 81-98.
2. Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry 226: 497-509.
3. Grashorn, M. A. (2007). Functionality of poultry meat. Journal of Applied Poultry Research 16: 99-106.
4. Lahucky, R., Nuernberg, K., Kovac, L., Bucko, O. & Nuernberg, G. (2010). Assessment of the antioxidant potential of selected plant extracts — in vitro and in vivo experiments on pork. Meat Science 85: 779–784.
5. Moyo, B., Masika, P. J., Hugo, A. & Muchenje, V. (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African Journal of Biotechnology 10: 12925-12933.
6. Muchenje, V., Dzama K., Chimonyo, M., Strydom, P. E., Hugo, A. & Raats, J.G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chemistry 112: 279-289.
7. Mukumbo, F. E., Maphosa, V., Hugo, A., Nkukwana, T. T., Mabusela, T. P. & Muchenje, V. (2014). Effect of *Moringa oleifera* leaf meal on finisher on growth performance, meat quality, shelf life and fatty acid composition of pork. South African Journal of Animal Science 44 (No. 4).
8. Nkukwana, T. T., Muchenje, V., Masika, P. J., Hoffman, L. C., Dzama, K. & Descalzo, A. M. (2014). Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration. Food Chemistry 142: 255-261.
9. Qwele, K., Hugo, A., Oyedemi, S. O., Moyo, B., Masika, P. J. & Muchenje, V. (2013). Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. Meat Science 93: 455-462.
10. Raharjo, S., Sofos, J. N. S., & Schmidt, G. R. (1992). Improved speed specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C18 method for measuring lipid peroxidation in beef. Journal of Agricultural and Food Chemistry 40: 2182-2185.
11. Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine Pharmacotherapy 56: 365–379.
12. Statistical Analysis system (SAS), 2003. User's Guide: Statistics (Version 6 Ed.). Statistical Analysis System Institute Inc., Cary, NC, USA.
13. Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R. & Enser, M. (2003). Effects of fatty acids on meat quality: a review. Meat Science 66: 21-32.