Animal Review
2014 Vol. 1, No. 3, pp. 37-44
ISSN(e): 2409-6490
ISSN(p): 2412-3382
© 2014 Conscientia Beam. All Rights Reserved.

INFLUENCE OF DEHYDRATED *MEDICAGO SATIVA* ON QUALITY CHARACTERISTICS OF MARCHIGIANA BEEF

Giuseppe Martino[1]† --- Lisa Grotta[2] --- Valentina Ponzielli[3]

[1,2,3] *Faculty of BioSciences and Technologies for Agriculture Food and Environment, University of Teramo, Mosciano S.A., Italy*

ABSTRACT

The composition of cattle diets is one the most important parameters influencing meat quality .Effects of pasture alone and supplementation with dehaydrated Medicago sativa on carcass and meat quality were studied in Marchigiana beef cattle, in particular lipid and stability.

Meat quality measurements were made on Longissimus dorsi (LD) muscle in 20 animals slaughtered at 660-700 kg.

The amount of lipids in the control group was lower with higher percentage of polyunsaturated fatty acids (PUFA), especially linoleic (C18:2) and linolenic (C18:3) acid. There were significant differences ($P \leq 0.01$) of TBARS values that were higher in the group fed with supplementation of Medicago sativa than in the other group, the control one.

Keywords: Dehydrated *medicago sativa* (*Alfalfa*), Pasture, Meat quality, Fatty acids, Lipid oxidation, TBARS, Meat quality.

Contribution/ Originality

Today people try more and more to replace soybean with other GMO-free protein foods. This study documents how the use of dehydrated *Alfalfa* meal in calf nutrition instead of soybean enriches the meat in polyunsaturated acids but, at the same time, promotes the processes of lipid oxidation.

1. INTRODUCTION

Meat quality is influenced by several factors as breeding, slaughtering, dissection and commercial distribution [1, 2]. Several studies showed the relationship between pasture-feeding alone or supplemented and meat quality (refrences). Ruminant tissues fatty acid profiles can be influenced by animal production system (encompassing diet and physical environment/facility used to produce or finish animals) or nutritional background [3-9].

† Corresponding author
© 2014 Conscientia Beam. All Rights Reserved.

There are increasing interest in manipulating meat fatty acids, especially saturated fatty acids, which have been implicated in diseases associated with modern life, especially in developed countries.

Furthermore, lipids quality is particularly important considering their oxidation [3, 8] . Infact, lipid peroxidation not only is one of the major causes of quality deterioration in raw and cooked meat, but it is also considered to be important for the development of atherosclerosis, and it is thought to be involved in ageing and other clinical disorders, such as cancer or cardiovascular and liver diseases [9].

Oxidation of polyunsaturated fatty acids (PUFA) leads to the formation of hydro- and endo-peroxides, which undergo fragmentation in order to yield a wide range of reactive intermediates, including alkanals, alkenals, hydroxyalkenals and MDA [10]. Some of these compounds, as MDA, are seen to be mutagenic [13-12].

As a result of policies against the use of GMO flours/meals in animal and human feeding and prejudices of consumers towards GMO foods, in recent years the use of dehydrated alfalfa meal as a protein source instead of large-scale produced genetically modified soybean is increasingly spreading.

This study evaluated the effects of pasture-feeding alone and with the supplementation of dehydrated *Medicago sativa* on carcass and meat quality of Marchigiana beef cattle in particular lipid quality and stability.

2. MATERIALS AND METHODS

2.1. Animals and Diets

The experiment was conducted at a herd of Marchigiana beef cattle site in the province of Pescara (Abruzzo, Italy) during the spring of 2012

To evaluate meat quality, 20 Marchigiana beef cattle were studied: animals were slaughtered at 680-700 Kg live weight.

These animals were divided into two groups, a control group (CG) and an experimental group (EG) and assigned to a different nutritional treatment for 90 days before slaughter.

All animals received a food base consisting of corn silage and wheat straw and 7.5 kg / head / day of feed supplement.

As regards the experimental group, a pasture with the same protein and energy content as the control group was used, but with the supplementation of dehydrated *Medicago sativa*.

The formulation of the experimental feed supplement was: 35% of maize meal, 25% of *Medicago sativa* meal, 15% of barley meal, 15% of residue of flour, 6% of soybean extraction meal, 1.5% of saponified fats, vitamins and minerals.

The feed supplement of the control group contained the same share of maize meal, barley, fats, vitamins and minerals (without residue of flour and dehydrated *Medicago sativa*) ,but with the addition of 8.5% of soybean extraction meal, 16% of bran, 10.5% of beetroot, 5% of corn gluten feed and 5% of sunflower extraction meal.

© 2014 Conscientia Beam. All Rights Reserved.

These two kind of fodders didn't differ for the content of fibre, proteins and moisture, while lipid and mineral values were higher in experimental fodder than in the control one (Tab.1).

Furthermore, the palmitic (C16:0) and oleic acid (C18:1) were higher in the control diet than in the experimental fodder that contained a significantly higher percentage of linoleic acid (C18:3) (Tab.2). All the animals were slaughtered at an average age of 20-22 months.

2.2. Sample Collection and Storage

L*ongissimus dorsi* muscle (LD) was sampled from the 7th to 12th rib 24 hours after slaughter and stored at -20°C for laboratory analysis.

Meat quality measurements (pH, Moisture, Ash and Protein) were made on LD with procedures quoted from the "Animal Science and Production Association" (ASPA).

Samples used for MDA determination were stored refrigerated from 2 to 4°C until 14 days from slaughtering and some shares were picked up to analysis at 6, 10 and 14 days and then stored frozen at -20°C until the use.

2.3. Reagents

All the chemicals used were reagent grade commercial products and were used without any further purification. TBA: 2-thiobarbituric acid (Sigma-Aldrich, Italy) in acetic acid 90% (Carlo Erba, Italy); TCA: trichloroacetic acid (Carlo Erba, Italy) in distilled water; HCl: hydrochloric acid (Carlo Erba, Italy) in distilled water; $HClO_4$: perchloric acid (Carlo Erba, Italy) in distilled water; BHT: butylated hydroxytoluene (Sigma-Aldrich, Italy) in methanol (Carlo Erba, Italy); standard solution (STD): 1,1,3,3-Tetramethoxypropan 99% (Malonaldhyde Bis(Diethylacetal) (Sigma-Aldrich, Italy) in methanol (Carlo Erba, Italy); sodio solfato anidro (Carlo Erba, Italy); chloroform and methanol (Sigma-Aldrich, Italy) 2:1; sodium chloride (Sigma Aldrich, Italy); esano (Sigma Aldrich, Italy); acido solforico concentrato 96% (Carlo Erba, Italy); catalyst (copper catalyst Foss); NaOH al 40% (Carlo Erba Italy); nitric acid at 65% (Sigma Aldrich, Italy); hydrogen peroxide at 30% (Sigma Aldrich, Italy).

2.4. Meat Colour Determination

Meat colour (CIE $L^*a^*b^*$) was measured using a Minolta CR-300, calibrated with standards, on *Longissimus dorsi* muscle picked out near the bone, a part without defects of colour, damages or excess of connective tissue, 14 days after slaughtering.The colour was expressed with CIELAB system where L^* is the brilliance, a^* is red-green index and b^* is yellow-blue index.

2.5. Mineral Determination

Minerals were calculated on ~1.9 g fresh meat, put in Teflon containers (TFM), where were added 4 ml of nitric acid at 65% and 1 ml of hydrogen peroxide at 30%. Samples were mineralized.

After dilution, the samples were analysed using spectrophotometer Perkin-Elmer A300. Mineral values were expressed in mg/kg.

© 2014 Conscientia Beam. All Rights Reserved.

2.6. Fatty Acid Analysis Determination

Total fat for fatty acid analysis extracted with the method of Folch was transmethylated into methyl esters (FAME) at room temperature by using sodium methylate (0.2M) in methanol. FAME composition was determined by gas chromatography using gas chromatograph Fisons HRGC MEGA 2 with flame ionisation detection (FID) equipped with a VARIAN column CP-SIL 88 of 100 m. the carrier gas was hydrogen. Oven temperature programming was as follows: 160°C held for 1 min; 175°C at 4°C/min, held for 28 min; 215°C at 3°C/min, held for 30 min; 160°C at 10°C/min.

Fatty acid identification was carried out with standard mixture and fatty acid values were expressed in percentage.

2.7. Lipid Oxidation Determination (TBARs Test)

The extent of lipid oxidation in the LD was assessed by the 2-thiobarbituric acid (TBA) distillation method of Tarladgis, et al. [13] modified.

3.5 g of fresh meat was added 50 ml of aqueous trichloroacetic acid (TCA) at 8% and 500 μl of methanolic butylated hydroxytoluene (BHT) at 0.01%. So, samples were Ultra-Turraxed with Ultra Turrax T25 for 10 min and then distilled. 2 ml of distilled were mixed with 2 ml of TBA in acetic acid (90%) at 0.02 M. Following incubation for 1 h at 80°C, reaction mixtures were cooled to room temperature and submitted to spectrophotometry (Perkin Elmer Lambda 20 UV/vis spectrophotometer) against blank reaction mixture at 534 nm.TBARS values were obtained by multiplying the absorbance readings by a factor and expressed as mg malonaldehyde per kg sample. TBARS were measured at 6, 10 and 14 days.

3. STATISTICAL ANALYSIS

All the data were statistically analysed by one-way ANOVA using SPSS 9.0 for windows. The significant differences were determined using t-Test at the level of $P < 0.05$.

4. RESULTS AND DISCUSSION

4.1. Animal and Carcass Characteristics

Carcass incidence on live weight (yield) and pH24 values did not differ between feeding treatments. Furthermore, all samples didn't show significant differences of colour between the control and experimental groups (Tab. 3).

4.2. Composition Characteristics

The results of the proximate compositional analysis are shown in Table 4.

No significant differences in moisture, lipid, protein and mineral levels were determined between control and experimental samples. It has been shown a similar composition of meat for protein (~22%), moisture (~76%), and fat content (~1.3%).Control and experimental meats have

© 2014 Conscientia Beam. All Rights Reserved.

shown a good quota of iron, zinc and calcium. These meat quality characteristics weren't influenced by different pastures used in this study.

4.3. Fatty Acid Profile

The fatty acid profiles, extracted from LD muscle by chloroform/methanol according to the method of Folch, et al. [14] are shown in Table 5.

Experimental samples had higher percentage of polyunsaturated fatty acids (PUFA), as docosapentaenoic (C22:5) and linoleic acid (C18:2) and, especially, linolenic (C18:3) ($P<0.001$) acid than control ones. This is possible probably because *Medicago sativa* is particularly rich of polyunsaturated fatty acids.

Instead, the monounsaturated fatty acid (MUFA) levels of experimental samples are lower than the PUFA.

There was no significant difference in the percentage of oleic acid (C18:1) that was higher in the control group. The meat of this group had also a major level of total monounsaturated fatty acids and palmitic acid (C16:0). The control meat had shown higher values of saturated fatty acids (SFA), cause of several diseases, but less subject to oxidation than unsaturated fatty acids.

4.4. Lipid Oxidation (TBARS) of LD of Animals Studied

The extent of lipid oxidation was determined by monitoring malonaldehyde (MDA) formation by means of the TBA assay. Results have shown an equal increase of MDA values between the control and the experimental samples.

TBARS were measured at 6, 10 and 14 days.

The values significantly stood out($P \leq 0.01$), however, after 10 days of refrigerated storage between the two types of samples (Tab.6). MDA levels of experimental group were higher than the control ones, probably because in this kind of meat there was a major percentage of polyunsaturated fatty acids (PUFA). In fact, in literature, it has been shown that the diet influences the MDA development and extent [15].

5. CONCLUSIONS

In the described work, we have evaluated the effects of pasture-feeding alone and with the supplementation of dehydrated *Medicago sativa* on carcass and meat quality of Marchigiana beef cattle in particular with reference to animal breeding parameters and lipid quality and stability.

The present study indicates that there were lower lipid values and higher polyunsaturated fatty acids (PUFA) percentages, especially docosapentaenoic (C22:5),linoleic (C18:2) and linolenic (C18:3) acid, in the experimental meat.This aspect is in conformity with levels of polyunsaturated fatty acids of *Medicago sativa* which were higher than those in a diet without this supplementation.

Furthermore, there has been a significant difference ($P \leq 0.01$) between the two types of studied meat. The experimental meat has showed MDA levels which were higher than those in

© 2014 Conscientia Beam. All Rights Reserved.

the control meat, probably because in this kind of meat there was a major percentage of polyunsaturated fatty acids (PUFA), particularly subject to oxidation.

So, it is possible to affirm that pasture feeding with or without supplementation of *Medicago sativa* influences lipid content and quality of meats.

REFERENCES

[1] J. F. Young, K. Rosenvold, J. Stagsted, C. L. Steffensen, J. H. Nielsen, and H. J. Andersen, "Significance of preslaughter stress and different tissue PUFA levels on the oxidative status and stability of porcine muscle and meat," *J. Agric. Chem.*, vol. 51, pp. 6877-6881, 2003.

[2] G. Preziuso and C. Russo, "Meat quality traits of longissimus thoracis, semitendinosus and triceps brachii muscles from Chianina beef cattle slaughtered at two different ages," *Ital. J. Anim. Sci.*, vol. 3, pp. 267-273, 2004.

[3] J. D. Wood, R. I. Richardson, G. R. Nute, A. V. Fishe, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser, "Effect of fatty acids on meat quality: A review," *Meat Science*, vol. 63, 2003.

[4] K. S. Rhee, *Fatty acids in food and their health implications*, 2nd-Revised and Expanded ed. New York Marcel: Dekker Inc, 2000.

[5] K. S. Rhee, Y. A. Ziprin, C. E. Bishop, and D. F. Waldron, "Composition and stability of goat meat patties as affected by breed type and feeding regimen," *Journal of Food Science*, vol. 62, pp. 949-953, 962, 1997.

[6] K. S. Rhee, D. F. Waldron, Y. A. Ziprin, and K. C. Rhee, "Fatty acid composition of goat diets vs intramuscular fat," *Meat Science*, vol. 54, pp. 313-318, 2000.

[7] A. Rowe, F. A. F. Macedo, J. V. Visentainer, N. E. Souza, and M. Matsushita, "Muscle composition and fatty acid profile in lambs fattened in drylot or pasture," *Meat Science*, vol. 51, pp. 283- 288, 1999.

[8] D. K. Larick and B. E. Turner, "Influence of finishing diet on the phospholipids composition and fatty acid profile of individual phospholipids in lean muscle of beef cattle," *Journal of Animal Science*, vol. 67, pp. 2282-2293, 1989.

[9] W. N. Marmer, R. J. Maxwell, and J. E. Williams, "Effect of dietary regimen and tissue site on bovine fatty acid profiles," *Journal of Animal Science*, vol. 59, pp. 109-121, 1984.

[7] A. Yang, M. C. Lanari, M. Brewster, and R. K. Tume, "Lipid stability and meat colour of beef from pasture and grain-fed cattle with or without vitamin E supplement," *Meat Science*, vol. 60, pp. 41-50, 2002.

[8] B. Halliwell, "Establishing the significance and optimal intake of dietary antioxidants: The biomarker concept," *Nut. Rev.*, vol. 57, pp. 104 - 113, 1999.

[9] H. Esterbauer, J. Gebicki, H. Puhl, and G. Jürgens, *Free Radicals Biol. Med.*, vol. 13, pp. 341-390, 1992.

[10] A. K. Basu and L. J. Marnett, "Carcinogenesis," vol. 4, pp. 331-333, 1983.

[11] R. J. Shamberger, T. L. Andreone, and C. E. Willis, *J. Natl. Cancer Inst.*, vol. 53, pp. 1771-1773, 1974.

[12] J. Suttnar, L. Masova, and E. Dry, *J. Chromatogr. B.*, vol. 751, p. 193, 2001.

© 2014 Conscientia Beam. All Rights Reserved.

[13] B. G. Tarladgis, B. M. Watts, M. T. Younathan, and L. Dugan, "A distillation method for the quantitative determination of malonaldehyde in foods," *Journal of the American Oil Chemists Society*, vol. 37, pp. 44-50, 1960.

[14] J. Folch, M. Lees, and Stanley, "A simple method for the isolation and purification of lipids from animal tissues," *Journal of Biological Chemistry*, vol. 226, pp. 497-509, 1957.

[15] P. Gatellier, C. Hamelin, Y. Durand, and M. Renerre, "Effect of a dietary vitamin E supplementation colour stability and lipid oxidation of air and modified atmosphere-packaged beef," *Meat Science*, vol. 59, pp. 133-140, 2001.

Tab-1. Composition of the two kind of feed supplement

Moisture %	10.1	9.48
Dry Matter %	89.9	90.52
Crude protein %	13.97	13.87
Crude fat %	2.63	3.74
Crude cellulose %	7.73	7.33
Ca %	1.05	0.9
P %	0.64	0.53
Mg %	0.33	0.33
K %	0.63	0.83
Fe mg/Kg	256	613
Cu mg/Kg	4.07	3.63
Zn mg/Kg	47.62	42.22
Mn mg/Kg	44.6	44.91

Tab-2. Fatty acid composition of the fat extracted from the two kind of fodders (%).

Fatty acids	**Soybean**	**Medicago sativa**
Miristic (C16:0)	27.9	24.4
Stearic (C18:0)	2.96	3.11
Oleic (C18:1)	25.74	24.88
Linoleic (C18:2)	36.44	35.5
Linolenic (C18:3)	1.99	6.95

Tab-3. Animal and carcass characteristics.

	DIET	
Parameters	Soybean	Medicago sativa(alfalfa)
Yield (%)	65.90±1.45	65.38±1.56
pH	5.45±0.10	5.49±0.14
Colour		
L*	39.35±3.21	38.21±1.47
a*	21.00±2.43	21.58±2.36
b*	9.54±2.96	10.35±2.43

© 2014 Conscientia Beam. All Rights Reserved.

Tab-4. Analytical composition of meat of the two kind of animal groups (µ± DS).

	DIET	
Parameters	Soybean	Medicago sativa(alfalfa)
Moisture	76.31 ± 2.86	76.00 ± 0.72
Ash	1.13 ± 0.14	1.11 ± 3.98
Protein	21.75 ± 1.00	21.94 ± 0.87
Total fat. % t.q.	1.32 ± 0.21	1.27 ± 0.20
Fe mg/Kg t.q.	18.62 ± 3.99	18.79 ± 3.23
Cu mg/Kg t.q.	0.12 ± 4.51	0.17 ± 6.55
Zn mg/Kg t.q.	41.21 ± 6.27	40.74 ± 3.71
Ca mg/Kg	33.63 ± 4.66	34.87 ± 4.01

Tab-5. Fatty acid composition of beef (LD muscle) of control and experimental animals (µ±DS).

	DIET	
Fatty acids	**Soybean**	**Medicago sativa(alfalfa)**
C14:0	2.36 ± 0.50	2.30 ± 0.38
C15:0	0.34 ± 0.08	0.36 ± 0.05
C16:0	25.59 ± 2.47	25.39 ± 1.43
C17:0	0.85 ± 0.10	0.79 ± 0.13
C18:0	16.79 ± 2.29	15.79 ± 1.51
C20:0	0.10 ± 0.04	0.08 ± 0.05
SFA	**46.03**	**44.71**
C14:1	0.40 ± 0.17	0.35 ± 0.16
C16:1	2.30 ± 0.55	2.31 ± 0.62
C18:1T	2.15 ± 0.90	2.16 ± 0.85
C18:1ω9	29.70 ± 3.52	27.91 ± 4.19
C18:1 ω7	1.45 ± 0.25	1.53 ± 0.15
C20:1	0.12 ± 0.01	0.10 ± 0.06
MUFA	**36.12**	**34.36**
C18:2	12.73 ± 3.70	14.44 ± 3.03
C18:3	^{a} 0.42 ± 0.07	^{b} 0.59 ± 0.06
CLA	0.35 ± 0.05	0.36 ± 0.12
C20:3	0.62 ± 0.24	0.82 ± 0.20
C20:4	3.23 ± 1.44	4.18 ± 0.90
C20:5	0.11 ± 0.07	0.13 ± 0.06
C22:5	0.40 ± 0.14	0.40 ± 0.25
PUFA	**17.86**	**20.92**

a, b = $P \leq 0.05$

Tab-6. MDA values (mg/Kg) at 6, 10 and 14 days from slaughtering.

Days from the slaughtering	Soybean	Medicago sativa(alfalfa)
6	0.24±0.03	0.29±0.04
10	^{A} 0.29 ± 0.03	^{B} 0.38 ± 0.06
14	0.56 ± 0.07	0.60± 0.05

A,B = $P \leq 0.01$

Views and opinions expressed in this article are the views and opinions of the author(s), Animal Review shall not be responsible or answerable for any loss, damage or liability etc. caused in relation to/arising out of the use of the content.

© 2014 Conscientia Beam. All Rights Reserved.