

MODIFICATION OF FATTY ACID COMPOSITION IN GOAT MEAT USING LINESEED OIL: EFFECTS ON LIPID PEROXIDATION

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Abstract – The objective of this study was to determine the antioxidant activity (AA) and the fatty acid composition of meat from goats supplemented with different n-6: n-3 fatty acid ratios. Isonitrogenous and isocaloric experimental diets contained either sunflower, palm kernel or linseed oil were used to adjust the dietary n-6: n-3 fatty acid ratios (FAR) to be 2.27:1 (LR), 5.01:1 (MR), and 10.38:1 (HR). Twenty-one five-month old male Boer goats weighing 13.66 ± 1.07 Kg were allocated randomly to the three dietary treatment groups and fed for 100 days. The *longissimus dorsi* (LD) muscle was sampled. The muscles were vacuum-packed and conditioned for 1, 3 and 6 days in a chiller at 4°C. The thiobarbituric acid reactive substance (TBARS) values for the LD muscle for all treatment groups after a 6-day post-mortem aging period were significantly ($P < 0.05$) higher than at day 1. The LR group had the highest TBARS value compared to the HR group treatment group after 6 days of post-mortem aging. Linseed oil increased the C18:3n-3 in the LR group and this is responsible for the lower ratio n-6: n-3 fatty acids ratio. Consequently, this is also associated with an increase in the TBARS value.

Key Words – Chevon, Fatty Acid, Goats, n-3 polyunsaturated fatty acids, TBARS

I. INTRODUCTION

High dietary cholesterol and fats intake are often implicated as the main cause of atherosclerosis and cardiovascular disease in human populations [1]. Current opinion in human nutrition advocates a reduction in the overall consumption of saturated fatty acids (SFAs), trans-fatty acids (TFAs) and cholesterol, while emphasizing the need to increase the intake of n-3 polyunsaturated fatty acids (PUFAs) [1,2]. Such nutritional recommendations with regard to fat consumption are largely due to several studies demonstrating

positive correlations between the intake of SFA and the incidence of heart diseases, which lead to rise in serum low-density-lipoprotein (LDL) cholesterol as SFA intake increases [3,4].

Alteration of the lipid content and fatty acid (FA) composition of foods can be an effective way to improve the consumer's health. Long chain (LC) n-3 PUFA are important in some tissues such as the brain and retina and may also be important in the maintenance of human health by preventing and protecting against metabolic diseases [2]. Simopoulos [5] reported that the n-6: n-3 FA ratio (FAR) should be between 1:1 and 2:1 to promote normal growth and development of human infants and adults. These justify the efforts to enhance the levels of the beneficial n-3 PUFA in meats and meat products. However, increasing the content of unsaturated fatty acids in foods would also lead increased peroxidativity. Peroxidative damages are typically associated with negative changes on the food products and would adversely affect the consumer health. Therefore, the purpose of this study was to determine if fatty acid composition in chevon could be changed by feeding different levels of n-6: n-3 fatty acid ratio in the goat diets with emphasis on lipid oxidation.

II. MATERIALS AND METHODS

Animals, Diets, and Management

Twenty-one five-month old male Boer goats weighing 13.66 ± 1.07 Kg (mean initial body weight $\pm$ standard error) were randomly assigned to different treatment groups. Goats were allocated randomly to three dietary treatment groups where the experimental diets were formulated to have n-6: n-3 FAR of 2.27:1, 5.01:1, and 10.38:1. The linseed oil was used as a source of α -linolenic acid (C18:3n-3) while sunflower oil was used as a source of linoleic acid (C18:2n-6). The

experimental diets were daily fed at 3.7% of BW (DM basis), with adjustments made weekly according to the changing BW. The diets were formulated to be isonitrogenous and isocaloric and to meet the energy and protein requirements of growing goats. All goats had free access to water and a mineral block. The feeding trial lasted for 100 days with a three week adaptation period. At the end of the 100 day trial, all the animals were slaughtered in accordance with the standard slaughter procedures outlined in the MS 1500:2004 (Department of Standards Malaysia, 2004). The *longissimus dorsi* (LD) muscle from 12th to 15th rib were removed from the carcass. All samples were stored at -80°C until analyzed for FA and antioxidant activity. The LD meat were vacuum-packed and conditioned for 1, 3 and 6 days in a chiller at 4°C.

Lipid Peroxidation

Lipid oxidation was measured using thiobarbituric acid-reactive substances (TBARS) according to the method of Lynch and Frei [6]. Meat samples (1 g) were homogenized in 4 mL 0.15 M KCl + 0.1mM BHT with UltraturraxTM homogenizer (1 min, medium speed). After homogenization, 200 µL of the sample were mixed with TBARS solution and then heated in a water bath at 95 °C for 60 min until the development of a pink color. After cooling, one ml of distilled water and three ml of n-butyl alcohol were added to the extracts and vortexed. The mixtures were centrifuged at 5000 rpm for 10 min. Absorbance of supernatant was read against an appropriate blank at 532 NM using a spectrophotometer (Secomam, Domont, France). The TBARS were calculated from a standard curve of 1, 1, 3, 3- tetraethoxypropane and expressed as mg malondialdehyde (MDA) /Kg sample.

Determination of fatty acid composition

The total fatty acids were extracted from meat based on the method of [7] modified by [8], using chloroform: methanol 2:1 (v/v) containing butylated hydroxytoluene to prevent oxidation during sample preparation. Transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAME) were carried out using KOH in methanol and 14 % methanolic boron trifluoride (BF₃) (Sigma Chemical Co. St.

Louis, Missouri, USA) according to methods by (10). The FAME were separated by gas chromatography (Agilent 7890A), using a Supelco SP 2560 capillary column of 100m x 0.25mm ID x 0.2 µm film thickness (Supelco, Inc., Bellefonte, PA, USA). The carrier gas was nitrogen at a flow rate of 1.2 ml/min. The split ratio was 1: 20 after injection of 1 µl of the FAME. The injector temperature was programmed at 250 °C and the detector temperature was 270 °C. The column temperature program started runs at 150 °C, for 2 min, warmed to 158 °C at 1 °C /min, held for 28 min, warmed to 220 °C at 1 °C /min, and then held for 20 min to achieve satisfactory separation. The peaks of samples were identified and concentrations calculated based on the retention time and peak area of known standards (Sigma Chemical Co., St. Louis, Missouri, USA). The fatty acid concentrations are expressed as g/100g of total identified fatty acids measured in each sample. A 3 × 3 factorial design (diets × postmortem aging periods) was employed for TBARS value. Fatty acid data was analyzed by one-way ANOVA, using the MIXED procedure of the SAS software package, version 9.1 (SAS Inst. Inc., Cary, NC). Differences of P < 0.05 were considered to be significant. The data were checked for normality using PROC UNIVARIATE of SAS software and the results in the tables are presented as means ± standard error of the mean.

III. RESULTS AND DISCUSSION

Lipid Oxidation in Chevon

The thiobarbituric acid reactive substance (TBARS) values for the LD muscles across all the dietary n-6: n-3 FAR after a 6 day postmortem aging period were significantly (P < 0.05) higher than the day 1 (Table 1). The highest TBARS value belonged to the LR group compared to the HR treatment group after 6 days of aging. In the HR treatment, the rate of lipid oxidation was not significantly (P > 0.05) lower compared to other treatments at different postmortem aging periods. The aging period significantly (P < 0.05) increased the TBARS value in all treatment groups (Table 1).

Table 1. Antioxidant activity (mg MDA/Kg meat) in the LD muscle of goats fed diet with different n-6: n-3 FAR at different post mortem aging periods.

Treatment	1day	3day	6day	SEM	P-value
LR	0.45 ^c	0.81 ^b	1.25 ^a	0.07	0.001
MR	0.39 ^b	0.78 ^{ab}	1.08 ^a	0.04	0.017
HR	0.30 ^b	0.72 ^{ab}	0.93 ^a	0.04	0.041

LR: low n-6: n-3 FAR; MR: medium n-6: n-3 FAR; HR: high n-6: n-3 FAR. ^{a,b,c} Means within rows with different superscripts are different among treatments (P < 0.05).

The TBARS values in LD muscle were not affected by the different n-6: n-3 FAR on the same day of aging. Vieira *et al.* [9] also showed that feeding dairy ewes with different vegetable oils had no effect on the TBARS value of LD muscle without aging. Lipid peroxidation is a complicated process in which unsaturated fatty acids are transformed into fatty acid hydroperoxides, and later, into secondary products. Malonaldehyde as measured by the TBARS analysis is formed primarily by the oxidation of unsaturated fatty acids, with the reaction being more intense as the level of unsaturation of the fat increases [10]. The non-significant difference from the TBARS value between treatment groups at the same aging time can be explained by the occurrence of similar amount of total PUFA in the LD muscle. The aging period significantly increased the TBARS value in all treatment groups, which are in agreement with other reports for example Luciano *et al.* [11] for lamb meat after feeding with concentrate or herbage. Vantansever *et al.* [12] reported the oxidative stability of steaks and ground beef from steers fed fish oil which contained high LC n-3 PUFA was lower, but feeding linseed produced no deleterious effects when compared with the oxidative stability of meat from steers fed a diet of 40% concentrates based on barley, sugar beet molasses and a palm oil supplement.

Fatty Acids Profile of LD Muscle

Fatty acid composition of the goat LD muscle fed different dietary n-6: n-3 FAR is presented in Table 2. The total SFA, monounsaturated FA (MUFA) and PUFA were not affected by dietary n-6: n-3 FAR. However total n-3 PUFA increased with decreasing n-6: n-3 FAR. In contrast, the content of CLA isomers decreased when the n-6:

n-3 FAR is lower in the diet. The major increase in this ratio was observed in the HR diet with the highest level of n-6: n-3 FAR (Table 2).

Decreasing the dietary n-6: n-3 FAR by about 4.5 folds (from 10.38 to 2.27; Table 2) via oil supplementation, reduced the n-6:n-3 FAR by about 3 folds in the LD muscle (from 12.09 to 3.84). When the C18:2n-6 in the diet was replaced with C18:3n-3 from linseed oil, the n-6: n-3 FAR in muscle was reduced by about half, making it a more attractive meat for hypercholesterolemic for individuals at risk to metabolic diseases such as diabetes.

Table 2. Fatty acid composition of the LD muscle in growing goats fed diets with different n-6: n-3 FAR.¹

Fatty Acids	Dietary n-6: n-3 FAR				P-Value
	LR	MR	HR	SEM	
SFA ²	41.96	42.04	40.43	0.30	0.542
UFA ³	58.04	57.96	59.57	0.30	0.311
MUFA ⁴	45.69	45.85	46.78	0.44	0.183
PUFA n-3 ⁵	2.33	1.40	0.88	0.12	0.001
PUFA n-6 ⁶	8.96	9.62	10.63	0.19	0.087
Total trans FA ⁷	1.11	1.38	2.27	0.05	0.012
Total CLA ⁸	1.05	1.09	1.28	0.05	0.001
n-6 : n-3 FAR	3.84	6.88	12.09	0.68	0.001

LR: low n-6: n-3 FAR; MR: medium n-6: n-3 FAR; HR: high n-6: n-3 FAR. ¹The data are expressed as the g/100g of identified fatty acids. ²SFA= sum of C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0.

³UFA= sum of C14:1+C16:1+C17:1+C18:1+C18:2+C18:3+C20:4+C22:6+C20:5n-3+C22:5-3+C22:6n-3. ⁴MUFA = sum of C14:1+C16:1+C17:1+C18:1. ⁵PUFA n-3 = sum of C18:3n-3+C20:5n-3+C22:5 n-3+C22:6n-3. ⁶PUFAn-6 = sum of 18:2n-6+20:4n-6. ⁷Total trans FA= C18:1trans

⁸Total CLA= sum of cis-9 trans-11CLA + cis-12 trans-10CLA.

When soybean oil (0 or 8% of dietary DM) was fed to lambs consuming a basal diet of conditioned lucerne hay or pelleted lucerne hay, the n-6: n-3 FAR in *longissimus thorax* increased from 2.1 to 5.0 [13]. The n-6: n-3 FAR found in LR treatment (3.84) was within this range. In the LR treatment, only 53.15% of n-3 PUFA are long chain n-3 PUFA. This is important considering that health benefits of n-3 FA are mostly associated with n-3 LC-PUFA, and that humans cannot derive adequate amounts of n-3 LC-PUFA from the metabolism of 18:3n-3 in vivo. Consistently, the decrease in the dietary n-6: n-3 FAR ratio, caused

by replacement of sunflower oil with linseed oil in the diets, decreased the n-6: n-3 FAR of intramuscular lipids. The LR treatment group resulted in n-6: n-3 FAR below 4, which is the maximum recommended value for human diets by Simopoulos [5].

IV. CONCLUSION

Reducing the dietary n-6: n-3 FAR reduced the n-6: n-3 FAR in the goat meat. The increase in total n-3 PUFA of the meat was mainly attributed to the higher C18:3n-3 levels from linseed oil. It should be noted that the increase in meat unsaturated fatty acid content occurred with minimal effects on lipid peroxidation status of the meat. In summary, increasing the n-3 PUFA content of animal products can be a sustainable way to improve the nutritional value of meat.

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REFERENCES

- Griel AE, & Kris-Etherton PM (2006) Beyond saturated fat: The importance of the dietary fatty acid profile on cardiovascular disease. *Nut Rev* 64(5), 257-62.
- Kris-Etherton PM, & Innis S (2007) Dietary Fatty Acids – Position of the American Dietetic Association and Dietitians of Canada. American Dietetic Association Position Report. *J Am Diet Assoc* 107(9), 1599-1611.
- Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH & Willett WC (1997) Dietary fat intake and the risk of coronary heart disease in women. *New Eng J of Med* 337, 1491-9.
- Posner BM, Cobb JL, Belanger AJ & Cupples LA, D'Agostino R B, Stokes J (1991) Dietary lipid predictors of coronary heart disease in men. The Framingham Study. *Arc of Intern Med* 151, 1181-7.
- Simopoulos AP (2004) Omega-6/omega-3 essential fatty acid ratio and chronic diseases. *Food Rev Int* 20(1), 77-90.
- Lynch SM & Frei B (1993) Mechanisms of copper- and iron dependent oxidative modification of human low-density lipoprotein. *J Lipid Res* 34(10), 1745-1751.
- Folch J, Lees M & Sloane Stanely GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226(1), 497-509.
- Rajion MA, McLean JG, Cahill RN & NP Cahill R (1985) Essential Fatty Acids in the Fetal and Newborn Lamb. *Australian J Biol Sci* 38(1), 33-40.
- Vieira C, Fernández-Diez A, Mateo J, Bodas R, & Soto S, Manso T (2012) Effects of addition of different vegetable oils to lactating dairy ewes' diet on meat quality characteristics of suckling lambs reared on the ewes' milk. *Meat Sci* 91(3), 277-283.
- Janero DR (1990) Malonaldehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Bio Med*, 9, 515-540.
- Luciano G, Monahan F J, Vasta V, Pennisi P, Bella M & Priolo A (2009) Lipid and colour stability of meat from lambs fed fresh herbage or concentrate. *Meat Sci* 82(2), 193-199.
- Vatansever L, Kurt E, Enser M, Scollan ND, Richardson RI, Nute GR & Wood JD (1998) The quality of beef from steers fed supplements of omega-3 fatty acids. *Proceedings 44th International Congress of Meat Science and Technology*, 30 August-4 September, 1998, pp. 656-657.
- Santos-Silva J, Mendes I A, Portugal PV & Bessa, RJB (2004) Effect of particle size and soybean oil supplementation on growth performance, carcass and meat quality and fatty acid composition of intramuscular lipids of lambs. *Livestock Prod Sci* 90, 79-88.