UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DIETARY LINSEED-BASED N-3 FATTY ACIDS ON GROWTH PERFORMANCE, FATTY ACID PROFILE, MEAT QUALITY AND RUMEN MUCOSAL MORPHOLOGY OF CROSSBRED BOER GOATS UNDER INTENSIVE CONDITIONS

KAMALELDIN ABUELFATH MOHAMED ABDELMAGED

FPV 2014 21

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By

KAMALELDIN ABUELFATH MOHAMED ABDELMAGED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

August 2014

DEDICATIONS

I am dedicating this thesis to the soul of my father, I am so grateful for your kindness, and I will never forget you

&

To my mother, my wife, and my whole family, with love and deep thanks

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF DIETARY LINSEED-BASED N-3 FATTY ACIDS ON GROWTH PERFORMANCE, FATTY ACID PROFILE, MEAT QUALITY AND RUMEN MUCOSAL MORPHOLOGY OF CROSSBRED BOER GOATS UNDER INTENSIVE CONDITIONS

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August 2014

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Faculty : Veterinary Medicine

Ruminant meats are characterized by a high ratio of saturated fatty acids (SFA), a low ratio of polyunsaturated fatty acids (PUFA), and a high ratio of PUFA n-6: n-3, which have been associated with modern life diseases. Different from beef and sheep, the information about the effect of the feeding dietary regime, in general, and n-3 PUFA, in particular, on fatty acid (FA) profiles of goat's edible tissues is scarce. This study was conducted to increase n-3 PUFA content and conjugated linoleic acids (CLA) in goat meat through feeding different levels of whole linseed, as a source of α-linolenic acid (ALA) n-3 PUFA, and investigate its effects on the growth performance, carcass characteristics, meat quality, and changes in rumen fermentation parameters and mucosal morphology. Twenty-four, 5-month old crossbred Boer bucks with a mean live weight of 14.23 ± 0.33 kg were assigned equally (n=8) into three isocaloric and isonitrogenous treatment diets that differed in the level of linseed. The diets L0, L10 or L20 contained 0%, 10% and 20% (w/w) whole linseed, respectively. The animals were slaughtered after a feeding period of 110 days. Upon slaughter, samples from liquor and tissues of the rumen were collected for analysis. The carcasses were dissected and samples from three muscles, *longissimus dorsi* (LD), *supraspinatus* (SS) and *semitendinosus* (ST), and internal and subcutaneous fats were taken for FA analyses. Subsamples from the muscles were aged for 1 or 7 days at 1- 4 °C before subjecting to the meat quality and lipid oxidation analyses.

The results revealed that the inclusion of linseed increased the proportion of ALA by 5.7, 6.75 and 7.0 fold, and 12.52, 10.25 and 11.67 fold in the LD, SS and ST muscles, for L10 and L20, respectively. The total n-3 PUFA was increased by 3.4, 3.2 and 3.6 fold, and 4.73, 4.14 and 5.5 fold in the LD, SS and ST muscles, for L10 and L20, respectively. In adipose tissues, the increment in ALA was 3.07 and 6.92 fold; and 3.00 and 7.54 folds in the subcutaneous and perirenal fats for L10 and L20, respectively, compared to L0. The proportions of beneficial long chain n-3 PUFA ecosapentaenoic,

docosapentaenoic, and docosahexaenoic in the muscles were significantly increased; nevertheless, these FA were not detected in the adipose tissues. The PUFA n-6:n-3 ratio decreased from 8.86, 7.55 and 6.65 in LD, SS and ST, respectively for L0 to 1.68, 1.87 and 2.0; and 1.16, 1.8 and 1.32 for L10 and L20, respectively. There was also a significant decrease in the SFA in all tissues except for the LD muscle and perirenal. The CLA increased in all tissues except for the LD muscle. At 20% inclusion (L20), the PUFA:SFA ratio was significantly higher in all studied tissues compared to the control group.

The final weight, total weight gain, and apparent digestibility were not affected by the treatments (P >0.05). However, the goats fed the L20 diet had lower (P < 0.05) feed intake (669.30 g/day) compared to L0 (705.21 g/day) or L10 (698.51 g/day). The gain: feed ratio (G:F) was higher (P< 0.05) in L20 compared to other treatment groups. The internal fat weight was heavier (P< 0.05) in L20 (550.57 g) compared to L10 (373.00 g), while in L0 was in between (469.40 g) with no difference (P>0.05). The percentage of lean was better (P< 0.05) in L10 (67.82%) compared to L0 (65.25%) or L20 (64.78%). There was no effect (P>0.05) on cooking loss, shear force or color for the different muscles at the 1-day postmortem aging time. However, at the 7-day aging time the color of all the muscles was significantly affected by the treatment diets. Subjectively, no differences were detected between the goat meat enriched in n-3 PUFA (L10 and L20) and the control (L0) in color, flavor or aroma. However, the tenderness, juiciness and overall acceptability of L20 meat were rated better than those of L0 and L10. The thiobarbituric acid reactive substances (TBARS) value in all muscles was increased with increasing the contents of n-3 PUFA and postmortem aging time. The lowest TBARS values were constantly shown by L0, while the highest values were indicated by the samples of L20 for the various muscles and aging times.

The rumen digesta of goat fed linseed (L10 and L20) had a significantly higher proportion of stearic acid, vaccenic, C18:2 trans-10, cis-12 CLA, and ALA compared to L0. The palmatic was significantly lower in L0 and L20 compared to L0. However, no significant effect was observed in the proportion of oleic, linoleic (LA), CLA isomer C18:2 cis-9, trans-11, and arachidonic. The pH of the rumen liquor and concentration of total volatile fatty acids (VFA) were not affected by the treatments (P<0.05). However, L10 and L20 had a higher (P<0.05) molar proportion of acetate, and lower (P<0.05) molar proportion of butyrate and valerate. compared to L0. Both inclusion levels of linseed in the diet (L10 and L20) significantly reduced the total bacteria, methanogens, and protozoa, in the rumen liquor compared to the L0. The effect of the dietary treatments on cellulolytic bacteria, varied between the individual species. Both inclusion levels of linseed resulted in a significant decrease in the population of the *Fibrobacter succinogenes, and Rumunococus flavefaciens* compared to L0, with

no significant difference between the groups fed linseed diets (L10 and L20). Nevertheless, the population of *Rumunococus albus* was not affected by the different dietary treatments. The inclusion of linseed in diets also induced some changes in rumen mucosal morphology. The animals fed linseed (L10 and L20) had longer papillae in the atrium ruminis, dorsal rumen wall, and caudoventral blind sac regions compared to the control (L0) and it was only significantly higher in the ventral rumen wall for L20. In addition, feeding linseed significantly increased the papillae width in the caudoventral blind sac and the ventral rumen wall regions. The papillae area was greater in the most studied regions of the rumen (dorsal rumen wall, caudoventral blind sac, and ventral rumen wall) of L10 and L20 compared to L0.

It is concluded that both inclusion levels (10% and 20%) of linseed as a source of n-3 PUFA in goats diets resulted in producing meat highly enriched with n-3 PUFA with desirable n-6:n-3 ratio without any adverse effect on the growth performance, carcass characteristics or meat quality. The inclusion of linseed at 10% (w/w) resulted in improving the goat carcass characteristics, while at 20% (w/w) resulted in improving G:F ratio and the eating quality of goat meat. Moreover, the inclusion of linseed decreased the rumen microorganism involved in methanogenesis, and increased the surface area of the rumen papillae.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PEMAKANAN ACID LEMAK N-3 BERASASKAN BIJI RAMI KE ATAS PRESTASI PERTUMBUHAN, PROFIL ASID LEMAK, KUALITI DAGING DAN MORFOLOGI MUKOSA RUMEN DALAM KAMBING BOER KACUKAN DITERNAK SECARA INTENSIF

Oleh

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Daging ruminan mempunyai nisbah asid lemak tepu yang tinggi, nisbah asid lemak politaktepu yang rendah, dan nisbah n-6:n-3 yang tinggi, yang terlibat dalam penyakit berkaitan dengan gaya hidup zaman moden. Berbeza dengan daging lembu dan biri-biri, maklumat tentang kesan makanan rejim secara umum dan n-3 politaktepu asid lemak (PUFA) khususnya, terhadap profil asid lemak tisu kambing yang boleh dimakan adalah terhad.

Kajian ini dijalankan untuk meningkatkan kandungan n-3 asid lemak PUFA dan asid linoleik berkonjugat (CLA) di dalam daging kambing melalui pemakanan biji rami pada tahap berbeza sebagai sumber α - linolenik (ALA) n-3 asid lemak, dan menyelidik kesan ke atas prestasi pertumbuhan kambing , ciri-ciri karkas, kualiti daging, dan perubahan parameter dalam penapaian rumen dan morfologi mukosa.

Dua puluh empat ekor Boer jantan kacukan berusia 5 bulan dengan berat hidup min 14.23 ± 0.33 kg telah dibahagi (n=8) kepada tiga diet rawatan isocaloric dan isonitrogenous yang berbeza dalam tahap biji rami. Diet L0, L10 dan L20 masing-masing mengandungi 0%, 10% dan 20% (w/w) biji rami. Haiwan disembelih selepas 110 hari tempoh pemakanan. Karkas telah dibedah dan sampel dari tiga otot, longissimus dorsi (LD), supraspinatus (SS) dan semitendinosus (ST) , dan lemak dalaman dan lemak subkutaneus telah diambil untuk analisis asid lemak. Otot berusia 1 atau 7 hari pada 1-4°C telah disubsample untuk analisis kualiti daging dan lipid pengoksidaan.

Keputusan menunjukkan bahawa biji rami meningkatkan ALA kepada gandaan 5.7, 6.75 dan 7.0, dan gandaan 12.52, 10.25 dan 11.67 pada otot LD, SS dan ST, masing-masing untuk L10 dan L20. Jumlah n-3 PUFA telah meningkat sebanyak gandaan 3.4, 3.2 dan 3.6; dan gandaan 4.73, 4.14 dan

5.5 pada otot LD, SS dan ST, masing-masing untuk L10 dan L20. Dalam tisu adipos, kenaikan ALA adalah pada gandaan 3.07 dan 6.92; dan gandaan 3.00 dan 7.54 untuk lemak subkutaneus dan perirenal, masing-masing untuk L10 dan L20, apabila dibandingkan dengan L0.

Nisbah rantaian panjang n-3PUFA ecosapentaenoic, docosapentaenoic, dan docosahexaenoic di dalam otot telah meningkat dengan ketara; bagaimanapun, asid lemak ini tidak dapat dikesan dalam tisu adipos. PUFA n-6/n-3 telah berkurangan daripada 8.86, 7.55 dan 6.65 masing-masing dalam LD, SS dan ST, untuk L0 kepada 1.68, 1.87 dan 2.0; dan 1.16, 1.8 dan 1.32, masing-masing untuk L10 dan L20. Terdapat juga penurunan ketara dalam SFA dalam semua tisu kecuali otot LD dan perirenal. PTK yang meningkat dalam semua tisu kecuali LD otot. Pada tahap 20% (L20), PUFA/SFA adalah jauh lebih tinggi dalam semua tisu berbanding dengan kumpulan kawalan.

Berat terakhir, jumlah berat badan, dan penghadaman jelas tidak terjejas oleh rawatan ($P>0.05$). Kambing L20 mempunyai pengambilan makanan (669.30 g/hari) yang lebih rendah ($P<0.05$) berbanding L0 (705.21 g/hari) atau L10 (698.51 g/hari). Nisbah kenaikan:makanan adalah lebih tinggi ($P<0.05$) pada L20 berbanding rawatan lain. Berat lemak dalaman adalah lebih berat ($P<0.05$) untuk L20 (550.57 g) berbanding L10 (373.00 g), manakala bagi L0 adalah (469.40 g), tanpa perbezaan yang signifikan dari kedua-duanya. Peratusan tanpa lemak adalah lebih baik ($P<0.05$) dalam L10 (67.82) berbanding L0 (65.25) atau L20 (64.78). Tidak ada kesan yang ketara kepada kehilangan memasak, daya ricih dan warna pada otot yang berbeza pada hari pertama penuaan. Pada hari ke-7, penuaan warna semua otot terjejas dengan ketara. Tiada perbezaan ketara antara daging kambing diperkaya dengan n-3 PUFA (L10 dan L20) dan kawalan (L0) dalam sifat-sifat warna, rasa, dan aroma. Walau bagaimanapun, kelembutan, kelembapan dan penerimaan keseluruhan daging L20 telah diberi nilai yang lebih baik berbanding dengan L0 dan L10. Nilai TBARS telah meningkat dalam semua otot pada masa penuaan yang berbeza dengan peningkatan isi kandungan n-3 PUFA. Nilai-nilai lebih tinggi adalah didapati adalam kumpulan L20. Pemanjangan masa penuaan mempunyai pengaruh penting dalam pengoksidaan lipid otot dengan meningkatkan nilai-nilai TBARS semua kumpulan .

Rumen digesta daripada kambing yang diberi makan biji rami (L10 dan L20) mempunyai kadar asid stearik (C18:0), vaccenic (C18:1 trans- 11), CLA C18:2 trans -10, cis- 12, dan ALA (C18:3 n-3) yang lebih tinggi berbanding dengan L0. Palmatic asid (16:0) adalah jauh lebih rendah dalam kedua-dua kumpulan diberi makan biji rami berbanding dengan L0. Namun, perkadaran oleik (C18:1 n-9), linoleik (C18:2 n-6), CLA isomer C18:2 cis-9, trans-11, dan arakidonik (C20:4 n-6) adalah tidak dipengaruhi dengan ketara. pH cecair rumen dan kepekatan jumlah asid lemak

meruap (VFA) tidak terjejas dengan ketara oleh rawatan. Walau bagaimanapun, L10 dan L20 mempunyai perkadaran molar butyrate dan valerate yang lebih tinggi berbanding dengan L0. Kedua-dua tahap biji rami dalam pemakanan (L10 dan L20) mengurangkan jumlah bakteria methanogens, protozoa, dan kulat anaerobik dalam rumen kambing dengan ketara berbanding dengan L0. Kesan rawatan pemakanan pada populasi bakteria sellulolitik , termasuk Fibrobacter succinogenes , Rumunococus Albus , dan flavefaciens Rumunococus , adelah berbeza antara individu. Kedua-dua tahap biji rami (L10 dan L20) menyebabkan penurunan ketara dalam populasi Fibrobacter succinogenes , dan Rumunococus flavefaciens berbanding L0 , tanpa perbezaan yang siknifikan dengan kumpulan yang diberi makan diet biji rami (L0 dan L20). Walau bagaimanapun, populasi Rumunococus albus tidak terjejas oleh rawatan pemakanan yang berbeza.

Kambing yang di beri makan biji rami (L10 dan L20) mempunyai papillae yang lebih panjang dalam ruminis atrium, dorsal dinding rumen, dan kantung caudoventral berbanding kawalan (L0), manakala di ventral dinding rumen, hanya di L20 mempunyai nilai yang tinggi dengan ketara. Pemakanan biji rami juga telah meningkatkan lebar papillae dengan ketara dalam caudoventral dan ventral dinding rumen, tetapi tiada perbezaan yang ketara didapati di kawasan lain. Kawasan papillae adalah lebih tinggi di kawasan rumen yang dikaji (dorsal dinding rumen, kantung caudoventral, dan ventral dinding rumen) daripada L10 dan L20 berbanding dengan kawalan. Skor warna epitelium rumen adalah lebih tinggi dalam L0 berbanding dengan haiwan yang diberi makan biji rami (L10 dan L20).

Kesimpulannya, kedua-dua tahap (10% dan 20%) biji rami sebagai sumber n-3 PUFA untuk diet kambing menghasilkan daging yang diperkaya dengan n-3 asid lemak dengan nisbah n-6:n-3 yang wajar tanpa kesan buruk ke atas prestasi pertumbuhan, ciri-ciri karkas dan kualiti daging. Kemasukan biji rami pada 10% (w/w) memperbaiki ciri-ciri karkas kambing, manakala 20% (w/w) meningkatkan nisbah kenaikan makanan dan kualiti daging kambing. Selain itu, tahap kemasukan biji rami mempertingkatkan kawasan permukaan penyerapan rumen papila dan mengurangkan mikroorganisma rumen yang terlibat dalam methanogenesis.

ACKNOWLEDGEMENTS

All thanks and praise be to the Almighty Allah for giving me the health, patience, and subjected to me very helpful people to complete this work. I'm indebted to my wonderful supervisor, Prof. Dr. Md Zuki Bin Abu Bakar @ Zakaria. It is difficult to find suitable words to show my deepest thanks and gratitude, because he gave me a chance to work with him, and supporting me with his knowledge, guidance, time and money.

I am also thankful to the members of my supervisory committee, Assoc. Prof. Dr. Goh Yong Meng, and Dr. Awis Qurni Bin Sazili, for their help, suggestions and support during my study.

I would like to extend my thanks and appreciations to all lecturers and staff members of the Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, and Department of Animal Science, Faculty of Agriculture. Special thanks to Dr. Mahdi Ebrahimi for the technical assistance in fatty acid analysis, and Dr. Intan Shameha Abdul Razak for her help in morphology analysis. Also, I would like to acknowledge all staff of the ruminant farm and slaughter house, Department of Animal Science, UPM for their kind cooperation during the field study. My thanks also extend to Department of Veterinary Services Malaysia (DVS) and the field staff the of the goats farm in Kampung Kuala Pah, Jelebu, Negeri Sembilan for providing the animals for this research.

Special thanks are due to all friends, Malaysian and international for their fine co-operation and moral support during the hard times.

I am most grateful to the University of Khartoum in providing me the opportunity to pursue this candidature, and for granting me the study leave and scholarships during my study.

Unlimited thanks to all my family members with special thanks to my mother for her successive support and to my beloved wife Samah Awad Ali for her patience, encouragement and support, and kind caring of me and my treasured children.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ADF	acid detergent fiber
ALA	α-linolenic acid
ANOVA	analysis of variance
°C	degrees Celsius
°C/min	degrees Celsius per minute
cal	Calorie
CLA	conjugated linoleic acids
cm	Centimeter
cm^2	square centimeter
CP	crude protein
C_T	threshold cycle
d	Day
DM	dry matter
DMI	dry matter intake
DHA	docosahexaenoic acid
EDTA	ethylenediamine tetra-acetic acid
EFA	essential fatty acids
EE	ether extracts
EPA	eicosapentaenoic acid
FA	fatty acids
FCR	feed conversion ratio
g	Gram
G: F	g of gain/kg of feed
GLM	general linear model
h	hour
Kg	kilogram
L	Liter
LA	linoleic acid
L0	control diet; containing 0% whole linseed, ,
L10	diet containing 10% whole linseed
L20	diet containing 20% whole linseed
LD	*longissimus dordi* muscle
m	Meter
M	Molar
MDA	malondialdehyde
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimolar
mmol/L	millimoles per liter
MUFA	Monounsaturated fatty acids / Monoenoic fatty acids
n-3:n-6 ratio	Total n-3 PUFA to Total n-6 PUFA ratio
NDF	neutral detergent fiber
nm	Nanometer

P:S ratio	Total PUFA to Total SFA ratio
PUFA	Polyunsaturated fatty acids
SEM	standard error of means
sec	Second
SFA	Saturated fatty acids
SS	*Supraspinatus* muscle
ST	*Semitendinosus* muscle
TBARS	thiobarbituric acid reactive substances
U:S ratio	Total UFA to Total SFA ratio
UFA	Unsaturated fatty acids
VLDL	Very Low Density Lipoprotein
w/v	weight per volume
w/w	weight per weight
WHC	Water Holding Capacity
wk	Week
µl	microliter
µM	micromolar
µmol/h	micromoles per hour

CHAPTER 1

GENERAL INTRODUCTION

With increasing consumer awareness about the health problems associated with unhealthy food, much attention has been paid to the physiological functions of food, which involves the role of food in improving the humans' healthiness by modulating physiological systems (Dentali, 2002). Red meat is long well-known as a main nutritional source of protein and important nutrients, such as minerals and vitamins. Some of these nutrients, such as, vitamin B12, folic acid, and iron, either do not exist or have a lower bioavailability in other foods. However, recent studies indicated that meat intake may raise the risk of cardiovascular diseases (CVD), some cancers (Wood *et al*., 2004) and inflammatory diseases (McAfee *et al*., 2010) due to its high saturated fatty acids (SFA) content, low ratio of polyunsaturated fatty acids (PUFA) to SFA and a high PUFA n-6:n-3 ratio (Enser *et al.,* 1998; Simopoulos, 2002b; Cabiddu *et al.,* 2010). Thus, to overcome this challenging issue and to launch a new market to fulfil consumer demand by the possible alternative foods, research in meat production has focused on altering the fatty acids (FA) content of meat towards the desirable. The alteration of meat FA composition is generally based on increasing the level of n-3 PUFA and conjugated linoleic acids (CLA) (Hocquette *et al.,* 2010).

The α-linolenic acid (ALA) n-3 PUFA is distinct by a double-bond starting at the third carbon from the methyl end. Since mammals lack the enzyme fatty acyl-CoA desaturase (Δ12 and Δ15) it cannot be synthesized de novo (Barceló-Coblijn and Murphy, 2009). Therefore, this FA must be present in the diet. In fact, n-3 PUFA, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been related with significant physiological and health benefits in human populations. These benefits include reduced incidence of cardiovascular diseases (CVD), atherosclerosis, hypertension (Adkins and Kelley, 2010), some cancers, inflammatory diseases and some mental and emotional disorders (Laviano *et al.,* 2013); in addition, to improve eye and brain development (Hooper *et al*., 2006), autoimmune (Engelhart *et al*., 2002) and learning ability (Hajjar *et al.,* 2012). Similarly, CLA is now well known as an anticarcinogenic, anti-atherosclerotic, antimutagenic, antioxidant (Waghmare, 2013), antibacteriogenic, anti-diabetogenic, and anti-obesity (Syvertsen *et al*., 2006), and has osteosynthetic effects (Benjamin and Spener, 2009).

Despite ruminal biohydrogenation, studies in beef (Albertí *et al*., 2013; He, *et al.,* 2012; Juárez *et al.,* 2012; Mapiye *et al.,* 2013; Nassu *et al*., 2011) and in sheep (Bas, *et al.,* 2007; Berthelot *et al.,* 2010; Berthelot, *et al.,* 2012; Delmote *et al*., 2008; Noci *et al.,* 2011) have shown that the inclusion of sources of ALA as linseed in the diet increase the concentration of n-3

PUFA in their meat (beef-sheep) up to 3-fold (Palmquist, 2009). Moreover, the incomplete biohydrogenation of ALA results in developing CLA isomers cis-10 cis-12 and cis-9 cis-11 (Lee and Jenkins, 2011).

Although goat meat is considered inferior to mutton and beef in tenderness and juiciness attributes, it is widely consumed in the tropics and sub-tropics. In fact, in recent decades, goat meat has gained a growing interest due to its preferable nutritional features, as it has low levels of fat and cholesterol (Madruga and Bressan, 2011), and a higher level of PUFA compared to beef or lamb (Banskalieva *et al.,* 2000). The naturally high level of PUFA may indicate that goats have the potential to deposit a high level of n-3 PUFA in their tissues. Enriching goat meat with n-3 PUFA and CLA together with its natural favorable nutritional characteristics enables goat meat to play an important role in human health as a functional food, especially for health-conscious consumers. However, information about the effect of the feeding dietary regime, generally, on the fatty acid profiles of the edible tissues of goat meat is relatively scarce (Lee and Kannan, 2012). Worse still, there is no report about the effects of feeding whole linseed, particularly, on the fatty acid profiles of the edible tissues of goats. However, in attempting to enrich meat with a high level of PUFA, the possible effects on growth performance, quality and oxidative stability of produced meat must be considered, as well as the effects on rumen physiology and morphology resulting from the feeding high level of PUFA.

We hypothesize that feeding goats with whole linseed as a source of ALA highly increase the n-3 PUFA, enhance CLA in the goat tissues, without adverse effects on the growth performance, and meat quality, and with some effects on physiology parameters and mucosal morphology of the rumen. Hence, this study was conducted in an attempt to achieve the following objectives:

Main objective

The main objective of this study was to increase the amount of n-3 PUFA and CLA in goat meat by feeding diets containing different levels of linseed as a source of ALA and investigate the impact on the growth performance, carcass characteristics, meat quality, and rumen fermentation, microbial population and mucosal morphology.

Specific objectives

1. To investigate the changes in the fatty acid composition of plasma, muscles and adipose tissues of goats fed diets containing different inclusion levels of whole linseed, as a source of n-3 PUFA, with emphasis on n-3 PUFA and CLA.

2. To investigate the effects of feeding diets containing different inclusion levels of whole linseed, as a source of n-3 PUFA on growth performance, apparent digestibility, and the carcass characteristics of goats.

3. To investigate the effects of enriching goat meat with n-3 PUFA on meat quality, sensory evaluation, and lipid oxidation stability at different postmortem aging times.

4. To investigate the effects of feeding diets containing different inclusion levels of whole linseed, as a source of n-3 PUFA on the rumen fermentation parameters, microbial populations, and rumen mucosa morphology of goats.

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