



UNIVERSITI PUTRA MALAYSIA

***RUMEN METABOLISM, CARCASS TRAITS AND MEAT QUALITY
IN GOATS FED BLEND OF CANOLA OIL AND PALM OIL***

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By

ADEYEMI KAZEEM DAUDA

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

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DEDICATION

This thesis is dedicated to Almighty Allah (S.W.T)



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

RUMEN METABOLISM, CARCASS TRAITS AND MEAT QUALITY IN GOATS FED BLEND OF CANOLA OIL AND PALM OIL

By

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December 2015

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Faculty : Agriculture

Consumption of ruminant meat has been implicated in the incidence of chronic diseases in human due to the imbalance in its fatty acid (FA) profile. This justifies the need to modify the FA composition of ruminant meat. Dietary supplementation of unsaturated fats is an effective strategy for modifying the FA composition of ruminant meat. However, unsaturated fats could have detrimental effects on rumen microbial metabolism and meat quality. The use of dietary fat blends has been accentuated as a cheaper and readily available alternative for modifying tissue lipids in ruminants compared with rumen inert fats. Nonetheless, the impact of fat blends on rumen metabolism has been highly variable and inconsistent and its effects on meat quality remain obscure. Thus, there is need for specific studies in different production systems to permit tailored decisions and informed choices in the utilization of fat blends in ruminant nutrition. Due to the FA composition and antioxidant contents of canola and palm oils, this study was conducted to examine the effects of blend of canola oil and palm oil on *in vitro* and *in vivo* rumen metabolism, nutrient intake and digestibility, growth performance, serum biochemistry, carcass attributes and meat quality in goats. The study was conducted in two phases.

The first phase consisted of two *in vitro* experiments. The first *in vitro* experiment evaluated the effects of blends of canola oil (CO) and palm oil (PO) and forage (F) to concentrate (C) ratios on rumen fermentation and apparent biohydrogenation (BH) of fatty acids. The treatments included three concentrate to forage (oil palm fronds, OPF) ratios (C:F; 75:25, 50:50 and 25:75) and six blends of canola oil and palm oil (CO:PO; 0:0, 100:0, 80:20, 50:50, 20:80 and 0:100) supplemented at 5% of the dry matter (DM) of the substrate and incubated at 39 °C for 48 h. The pH declined ($P < 0.05$) while the gas production and volatile fatty acids (VFA) increased as the C:F increased in the control (oil-free) substrates compared with the oil-based substrates. The acetate and methane concentrations were lower ($P < 0.05$) while the propionate was higher in oil-based substrates than the control substrates. Regardless of the C:F, oil supplementation decreased gas production, VFA, DM and organic matter (OM)

digestibilities, saturated fatty acids (SFA), and BH of C18:3n-3 and C18:2n-6, and enhanced the polyunsaturated fatty acids (PUFA) and BH intermediates. There were significant interactions between C:F and CO:PO for gas production, rumen fermentation, and BH of FA. The combination of 50:50, C:F and 80:20, CO:PO yielded higher concentration of unsaturated FA and had minimal adverse effects on rumen fermentation.

The second *in vitro* trial investigated the effects of graded levels of 80% canola oil and 20% palm oil (BCPO) on rumen fermentation and BH of FAs. The BCPO was supplemented to the basal substrate consisting of 50% concentrate and 50% OPF at the rate of 0, 2, 4, 6, and 8%. Supplementation of BCPO did not affect ($P > 0.05$) gas production and rumen fermentation. Nonetheless, increasing level of BCPO enhanced ($P < 0.05$) the BH of C18:1n-9 but decreased ($P < 0.05$) the BH of C18:2n-6 and C18:3n-3. After 24 h incubation, the concentration of SFA decreased ($P < 0.05$) while that of PUFA and BH intermediates increased ($P < 0.05$) with increasing level of BCPO.

The second phase of the study assessed the nutrient intake and digestibility, growth performance, rumen metabolism, serum biochemistry, carcass traits, tissue lipids and meat quality in goats fed diets supplemented with graded levels of BCPO. Thirty Boer crossbred bucks (4-5 months old and BW, 20.53 ± 0.6 kg) were randomly assigned to diets containing 0, 4 and 8% BCPO, fed daily for 100 d and slaughtered. Diet had no effect ($P > 0.05$) on growth performance and feed efficiency in goats. Dietary BCPO did not affect the intake and digestibility of nutrients except ether extract. The total VFA, acetate, butyrate and methane concentration decreased ($P < 0.05$) with increasing level of BCPO in diet. However, propionate; ammonia nitrogen and rumen pH did not differ ($P > 0.05$) among the treatments. The populations of total protozoa and methanogens were lower ($P < 0.05$) while the populations of total bacteria, *Ruminococcus albus*, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* were higher ($P < 0.05$) in the oil-fed goats than the control goats. The ruminal proportion of C18:3n-3 and total FA increased ($P < 0.05$) while the proportion of C18:2n-6 decreased ($P < 0.05$) with increasing level of BCPO in diet.

Diet had no effect on serum antioxidant enzyme (AE) activities and lipid oxidation. Goats fed 4 and 8% BCPO had higher ($P < 0.05$) serum total cholesterol and HDL cholesterol, n-3 FA and α and γ -tocopherol than the control goats. Dietary BCPO had no effect ($P > 0.05$) on carcass and non-carcass components but induced significant changes in the FA composition of omental, perirenal and mesentery adipose tissues in goats. Dietary BCPO beneficially altered the FA composition of *longissimus lumborum*, *semimembranosus*, *infraspinatus* and *gluteus medius* muscles, kidney and liver in goats. Dietary BCPO had no effect on tissue AE activities. However, goats fed 4 and 8% had higher tissue carotenoids and tocopherols over a 7 d *postmortem* chill storage compared with the control goats. Diet had no effect on the physicochemical and sensory properties but enhanced the oxidative stability of lipid, myoglobin and myofibrillar proteins in chevon over chill

storage. *Postmortem* ageing had significant impact on the oxidative stability of myofibrillar proteins, lipid and myoglobin in goats.

Dietary supplementation of BCPO can be used to enhance the beneficial fatty acids in muscles and offal without compromising rumen microbial metabolism, growth performance, serum biochemistry, carcass traits and meat quality in goats.



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**METABOLISMA RUMEN, CIRI KARKAS DAN KUALITI DAGING
KAMBING YANG DIBERI MAKAN CAMPURAN MINYAK KANOLA DAN
KELAPA SAWIT**

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Penggunaan daging ruminan telah dikaitkan dengan kejadian penyakit kronik dalam manusia yang disebabkan oleh ketidakseimbangan komposisi asid lemak (FA). Ini mewajarkan keperluan untuk mengubah suai komposisi asid lemak daging ruminan. Suplemen pemakanan lemak tidak tepu adalah satu cara yang berkesan untuk mengubah suai komposisi asid lemak dalam daging haiwan ruminan. Walau bagaimanapun, lemak tidak tepu boleh mempunyai kesan mudarat ke atas metabolisme mikrob rumen dan kualiti daging. Kaedah penggunaan campuran lemak dalam makanan dikatakan lebih murah bagi mengubah suai tisu lemak dalam haiwan ruminan. Walau bagaimanapun, kesan pemakanan campuran lemak ke atas metabolisme rumen adalah berbeza dan tidak konsisten manakala kesannya terhadap kualiti daging masih lagi belum diketahui. Oleh yang demikian, kajian khusus ke atas sistem pengeluaran berbeza bagi menentukan keputusan dan pilihan yang tepat melibatkan penggunaan campuran lemak dalam pemakanan ruminan adalah diperlukan. Komposisi sedia ada asid lemak dan antioksidan di dalam minyak kanola dan minyak sawit telah mendorong kepada kajian bagi mengenal pasti kesan pemakanan campuran minyak kanola dan minyak sawit ke atas metabolisme rumen *in vitro* dan *in vivo*, kadar serapan nutrien dan kebolehcernaan, prestasi pertumbuhan, biokimia serum, ciri karkas dan kualiti daging kambing. Kajian ini telah dijalankan melalui dua fasa.

Fasa pertama terdiri daripada dua eksperimen *in vitro*. Eksperimen *in vitro* pertama telah dijalankan untuk menilai kesan campuran minyak kanola (CO) dan minyak kelapa sawit (PO) dan foraj (F) kepada konsentrat (C), ke atas penapaian rumen dan penghidrogenan bio (BH) asid lemak. Rawatan terdiri daripada tiga kepekatan nisbah foraj (pelepah kelapa sawit, OPF) (C:F; 75:25, 50: 50 dan 25:75) dan enam pencampuran minyak sawit dan minyak kanola (CO:PO; 0:0, 100:0, 80:20, 50: 50, 20:80 dan 0:100) ditambah kepada 5% bahan kering (DM) dalam substrat dan di inkubasi pada suhu 39 °C untuk selama 48 jam. Nilai pH menurun ($P < 0.05$) manakala pengeluaran gas dan pengeluaran asid lemak meruap (VFA) meningkat

dengan peningkatan C:F di dalam substrat kawalan (*oil-free*) jika dibandingkan dengan substrat berasaskan minyak. Kepekatan asetat dan metana didapati lebih rendah ($P < 0.05$) manakala propionat pula lebih tinggi dalam substrat berasaskan minyak berbanding dengan substrat kawalan. Tanpa mengira C:F, suplemen minyak telah mengurangkan pengeluaran gas, VFA, DM dan pencernaan bahan organik (OM), asid lemak tepu (SFA), dan BH C18:3n-3 dan C18:2n-6, dan asid lemak tidak tepu (PUFA) dan perantaraan BH. Terdapat interaksi yang signifikan antara C:F dan CO:PO bagi pengeluaran gas, penapaian rumen dan BH FA. Gabungan 50: 50, C:F dan 80:20, CO:PO telah menghasilkan kepekatan asid lemak tidak tepu yang lebih tinggi dan mempunyai kesan buruk yang minimum pada penapaian rumen.

Kajian *in vitro* kedua telah menentukan kesan minyak kanola dan minyak sawit (BCPO), masing-masing pada tahap 80% dan 20% ,ke atas penapaian rumen dan BH FA. BCPO telah ditambah kepada substrat asas yang mengandungi 50% #kepekatan dan 50% OPF pada kadar 0, 2, 4, 6, dan 8%. Suplemen BCPO didapati tidak menjejaskan ($P > 0.05$) pengeluaran gas dan penapaian rumen. Walau bagaimanapun, pertambahan BCPO telah merangsang ($P < 0.05$) BH C18:1n-9 dan menurunkan ($P < 0.05$) BH C18:2n-6 dan C18:3n-3. Selepas 24 jam inkubasi, kepekatan SFA didapati telah menurun ($P < 0.05$) manakala PUFA dan perantaraan BH telah meningkat ($P < 0.05$) dengan peningkatan tahap BCPO.

Fasa kedua kajian ini telah menilai pengambilan nutrien dan kebolehcernaan, prestasi pertumbuhan, metabolisma rumen, serum biokimia, ciri kerangka, tisu lipid dan kualiti daging kambing yang diberi makan diet yang ditambah dengan BCPO yang mempunyai tahap bergred. Tiga puluh ekor kambing jantan Boer (4-5 bulan dengan berat, 20.53 ± 0.6 kg) telah dibahagikan secara rawak kepada beberapa rawatan diet yang mengandungi 0, 4 dan 8% BCPO, diberi makan setiap hari untuk 100 hari dan kemudiannya disembelih. Diet tidak memberi kesan ($P > 0.05$) pada prestasi pertumbuhan dan kecekapan pemakanan dalam kambing. Diet BCPO juga didapati tidak menjejaskan pengambilan dan pencernaan nutrien kecuali ekstrak eter. Kepekatan jumlah VFA, asetat, butirir dan metana menurun ($P < 0.05$) dengan pertambahan kadar BCPO di dalam diet. Walau bagaimanapun, propionat, nitrogen ammonia dan pH rumen didapati tidak berbeza ($P > 0.05$) di antara rawatan. Populasi jumlah protozoa dan *methanogen* didapati lebih rendah ($P < 0.05$) manakala populasi jumlah bakteria *Ruminococcus albus*, *Fibrobacter succinogenes* dan *Ruminococcus flavefaciens* adalah lebih tinggi ($P < 0.05$) pada kumpulan kambing yang diberi makan minyak berbanding kumpulan kambing kawalan. Perkadaran ruminal C18:3n-3 meningkat ($P < 0.05$) manakala perkadaran C18:2n-6 menurun ($P < 0.05$) dengan peningkatan tahap BCPO dalam diet. Diet tidak mempunyai kesan ke atas aktiviti enzim antioksidan (AE) dalam serum dan pengoksidaan lipid. Kambing yang diberi makan 4% dan 8% BCPO mempunyai jumlah kolesterol dan kolesterol HDL, n-3 FA dan α dan γ -Tokoferol lebih tinggi ($P < 0.05$) berbanding kumpulan kambing kawalan. Diet BCPO tidak memberi kesan ($P > 0.05$) keatas komponen kerangka dan bukan kerangka tetapi mempengaruhi komposisi FA omental, perirenal dan mesenteris tisu adipos pada kambing. Diet BCPO mengubah komposisi FA pada otot *longissimus lumborum*, *semimembranosus*, *infraspinatus* dan *gluteus medius*, buah pinggang dan hati kambing. Diet tidak mempunyai kesan ke atas aktiviti-aktiviti AE dalam tisu. Walau bagaimanapun, kambing yang diberi makan 4% dan 8%

mempunyai tisu karotenoid dan *tocopherols* yang lebih tinggi selepas 7 h *postmortem* dalam simpanan dingin berbanding kambing kawalan. Diet tidak mempunyai kesan ke atas sifat fizikokimia dan deria tetapi menambahbaik kestabilan oksidatif lipid, myoglobin dan protin myofibrillar dalam daging chevon semasa tempoh penyimpanan. Proses penuaan *postmortem* telah menunjukkan kesan ketara ke atas kestabilan oksidatif protin myofibrillar, lipid dan myoglobin dalam kambing.

Suplemen BCPO boleh digunakan untuk meningkatkan asid lemak baik di dalam otot dan organ dalaman tanpa menjejaskan metabolisma mikroba rumen, prestasi pertumbuhan, biokimia serum, ciri-ciri kerangka dan kualiti daging kambing.



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I certify that a Thesis Examination Committee has met on 28 December 2015 to conduct the final examination of Adeyemi Kazeem Dauda on his thesis entitled "Rumen Metabolism, Carcass Traits and Meat Quality in Goats Fed Blend of Canola Oil and Palm Oil" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

ADF	acid detergent fiber
ANOVA	analysis of variance
BCPO	blend of 80% canola oil and 20% palm oil
BH	Biohydrogenation
BI	band intensity
°C	degrees centigrade
°C/min	degrees centigrade per minute
cal	Calorie
CAT	Catalase
CLA	conjugated linoleic acid
cm	Centimetre
cm ²	square centimetre
d	Day
DM	dry matter
FA	fatty acids
FE	feed efficiency
g	Gram
GLM	generalized linear model
GM	<i>gluteus medius</i>
GPX	glutathione peroxidase
h	Hour
IS	<i>Infraspinatus</i>
Kcal	Kilocalories
L	Liter
LL	<i>longissimus lumborum</i>
m	Meter
MDA	Malondialdehyde
MHC	myosin heavy chain
MHCf	myosin heavy chain fast
MHCs	myosin heavy chain slow

min	Minute
mm	Milimeter
mmol/L	milimole per liter
MRA	metmyoglobin reducing activity
μL	Microliter
μmol/L	micromole per liter
mg	Milligram
mg/L	milligram per liter
mL	millilitre
mL min	millilitre per minute
MUFA	monounsaturated fatty acids
n-6/n-3	total n-6 PUFA to total n-3 PUFA ratio
NDF	neutral detergent fibre
PUFA	polyunsaturated fatty acids
RD	reflective density
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	standard error of means
SFA	saturated fatty acids
SM	<i>semimembranosus</i>
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
UFA	unsaturated fatty acids
VFA	volatile fatty acids
WHC	water holding capacity

CHAPTRE ONE

GENERAL INTRODUCTION

In recent time, consumers are cautioned against the consumption of ruminant meat because the fat it contains is more highly saturated and this was believed to be a factor predisposing to chronic diseases (Ashaye *et al.*, 2011; Blank *et al.*, 2012; World Health Organization, 2015). Nevertheless, reducing meat consumption could pose severe nutritional inadequacies for some important nutrients (McAfee *et al.*, 2010; Jiménez-Colmenero *et al.*, 2012). Thus, modifying the fatty acid (FA) composition of ruminant meat is essential (Scollan *et al.*, 2014; Mapiye *et al.*, 2015).

Dietary supplementation of unsaturated fats in ruminant's diet is an effective strategy for modifying the FA composition of ruminant meat (Shingfield *et al.*, 2012; Scollan *et al.*, 2014). However, altering muscle lipids in ruminants is an intricate task considering the detrimental effects of unsaturated fats on rumen microbial metabolism and the extensive biohydrogenation (BH) of unsaturated fatty acids (UFA) to saturated fatty acids (SFA) (Shingfield *et al.*, 2012).

Rumen inert fats are commonly used in ruminant nutrition to protect dietary UFA from rumen biohydrogenation and to forestall their adverse effects on rumen fermentation (Bauman *et al.*, 2003; Putnam *et al.*, 2003). However, rumen inert fats are expensive (Dewhurst *et al.*, 2003; Doreau *et al.*, 2011), and may not be readily accessible for peasant farmers especially in the developing countries. Since feed accounts for the major cost of ruminant production, supplementing ruminant ration with low cost non-inert fats like blends of animal and vegetable fats may be a viable option to address these problems (Jenkins, 1993; Dewhurst *et al.*, 2003).

The efficacies of blended fat in ruminant nutrition have been espoused. Blended fats bear resemblance to ruminally inert fats and may enhance ruminal fermentation compared with single fats (Jenkins, 1993). Blends of vegetable oils and/or animal fats have less impact on rumen fermentation in steers (Zinn, 1989a; Brandt and Anderson, 1990) and dairy cows (Palmquist and Conrad, 1980; Palmquist, 1991) and modified tissue lipids in lambs (Jerónimo *et al.*, 2009; Ferreira *et al.*, 2014). Nonetheless, the impact of dietary oil blends on rumen metabolism and fat accretion in ruminants has been highly variable and inconsistent in the published literature. Thus, there is need for additional studies in different production systems to permit tailored decisions and informed choices in the utilization of oil blends in ruminant nutrition.

Canola oil contains about 59% C18:1n-9, 21% C18:2n-6 and 13% C18:3n-3 while the proportion of SFA is about 7% (Lin *et al.*, 2013). Palm oil contains 44% C18:1n-9, 10% C18:2n-6, and about 40% C16:0 (Siew and Ng, 2000). Based on the FA

profile, it was hypothesized that a blend of palm oil and canola oil would enhance the beneficial UFA in chevon without disrupting rumen microbial metabolism.

Dietary fat can influence fat deposition in ruminants (Zinn, 1989b; Bock *et al.*, 1991; Marinova *et al.*, 2001). The deposition and distribution of fat play a vital role in the quality and commercial value of ruminant carcasses (Marinova *et al.*, 2001; Bas *et al.*, 2005). Goats deposit more internal fat and less subcutaneous, inter and intramuscular fats compared with sheep and cattle (Casey *et al.*, 2003; Tshabalala *et al.*, 2003). The deposition of more internal fats is economically and energetically expensive and represents a waste of dietary energy (Tshabalala *et al.*, 2003; Webb *et al.*, 2005). A poor subcutaneous fat cover decreases grading of goat carcasses and could instigate carcass evaporative losses (Tshabalala *et al.*, 2003). In addition, a low intramuscular fat is responsible for the low juiciness and tenderness of chevon (Sheradin *et al.*, 2003).

Conjugated linoleic acid (CLA) has been identified as a potent modulator and repartitioning agent in fat metabolism (Qi *et al.*, 2014; Malinska *et al.*, 2015). CLA can be synthesized in the rumen by the BH of C18:2n-6 and C18:3n-3 (Bauman *et al.*, 2003) or synthesized endogenously in the tissue by the action of Δ -9 desaturase on C18:1 *trans*-11 which is a mutual intermediate product of BH of C18:1n-9, C18:2n-6 and C18:3n-3 (Shingfield *et al.*, 2012). Based on the FA composition, it was proposed that the blend of canola oil and palm oil would affect lipid metabolism and body fat partitioning in Boer crossbred bucks.

Dietary supplementation of unsaturated fats, if not stabilized, can instigate oxidative stress in animals (Andrews *et al.*, 2006) and could predispose the meat to lipid oxidation (Nute *et al.*, 2007). Lipid oxidation could instigate protein oxidation (Bekhit *et al.*, 2013). Both lipid and protein oxidation can have negative effects on the physicochemical properties, safety, nutritive value and shelf life of meat (Falowo *et al.*, 2014; Ponnampalam *et al.*, 2014). Thus, attenuating lipid and protein oxidation to maintain product quality is essential. Compared with lipid oxidation (Nute *et al.*, 2007; Karami *et al.*, 2013), the effects of dietary fat on protein oxidation, myofibrillar protein profile and antioxidant enzyme activities in ruminant meat remain obscure.

Dietary incorporation of antioxidant-rich vegetable oils in animal diets has been suggested as an economical and effective strategy for curbing *postmortem* oxidative deterioration and an alternative strategy for enhancing these beneficial nutrients in human diets (Kang *et al.*, 2001). Canola oil contains about 0.53-0.97% plant sterols and about 700-1200 ppm tocopherol (Lin *et al.*, 2013). Palm oil is the richest natural plant source of lipid-soluble antioxidants such as carotenoids, vitamin E and ubiquinone (Oguntibeju *et al.*, 2009). Thus, due to the high antioxidant contents of palm and canola oils, it was hypothesized that a blend of canola oil and palm oil would preclude lipid and protein oxidation in UFA-enriched chevon.

In cognizance of the need to enhance bioactive lipids in ruminant meat at a low cost without compromising rumen metabolism and meat quality, this study was initiated to examine the effects of blend of canola oil and palm oil on rumen metabolism, growth performance, carcass traits and meat quality in goats. The research will support the delivery of healthier chevon that responds to consumers' expectation as well as underpinning economic benefits in terms of delivering a "value-added" chevon to improve nutritional value and deliver "functional" benefits to consumers.

The specific objectives of the study were:

1. To examine the effects of blend of canola oil and palm oil and forage to concentrate ratio on *in vitro* rumen fermentation and apparent biohydrogenation of oleic, linoleic and linolenic acids.
2. To determine the nutrient intake and digestibility, rumen metabolism, growth performance, serum lipids and biochemical parameters in goats fed blend of canola oil and palm oil.
3. To assess the carcass profile, body fat partitioning, meat yield and fatty acid composition of adipose tissues in goats fed blend of canola oil and palm oil.
4. To determine the fatty acid profile and antioxidant status of muscles and offal and physicochemical properties, myofibrillar protein profile and sensory attributes of *longissimus lumborum*, *semimembranosus*, *infraspinatus* and *gluteus medius* muscles in goats fed blend of canola oil and palm oil.

Presentation of the thesis

The current study is partitioned into eight chapters. The first two chapters discussed the framework of the experimental research. Chapter 1 provides the rationale for the focus of the research. Chapter 2 presents the review of literature covering the nutritional significance of meat and its implication for human health, factors affecting the FA composition of ruminant meat, fat metabolism, importance of goats in livestock production, and the effects of dietary fat on rumen metabolism, carcass traits and meat quality. Chapter 3 through 6 present the experimental works for this study. Chapter 7 describes the major findings and highlights the practical importance. Chapter 8 presents the summary, conclusions and recommendations for future studies.

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