
UNIVERSITI PUTRA MALAYSIA

# *RUMEN FERMENTATION, FATTY ACID PROFILES, MEAT QUALITY AND ADIPOGENESIS RELATED GENE EXPRESSION OF GOAT AND SHEEP FED WITH HIGH CONCENTRATED DIET*

CANDYRINE SU CHUI LEN

IPTSM 2016 3

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By

**CANDYRINE SU CHUI LEN**

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

**December 2016**

## DEDICATION

*To my late grandmother (Anna Chong Kui Fah), who always tell me to 'belajar bagus- bagus di KL, jangan suka- suka ikut orang jalan sini-sana'.*

*May you rest in peace with the Heavenly Father (1925- 2013).*

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

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The small ruminant (goats and sheep) industry in Malaysia can be described as slow growing as compared to the demand for the products. On the other hand, ruminant products are known to contain high amount of saturated fatty acids (SFA) which are often implicated as a source of modern-day diseases. To date, limited comparative studies have been carried out to characterize differences between goats and sheep, hence the two species are often assumed to be similar and thus raised and managed similarly. To meet the local demand, there is a need to improve the productivity of the small ruminant industry, by optimizing the growth protential of each animal species; and at the same time, to produce healthier meat and meat products. It is hypothesized that the feeding of high concentrate diet supplemented with linseed oil as a source of polyunsaturated fatty acids (PUFA), could improve the growth rate of the animals and at the same time, fortifying the meat and products with health beneficial PUFA. The primary objectives of this thesis were to co-compare rumen fermentation characteristics, growth performance, meat quality attributes and fatty acids profile in tissues of goat and sheep fed high concentrate fattening diet, with and without supplementation of PUFA; and to infer the differences in fatty acids profile due to the dietary treatments and animal species (goats *vs* sheep) through the expression of adipogenesis related genes. Three experiments were conducted to achieve the above objectives. In the first experiment, *in vitro* rumen fermentation characteristics in goats and sheep with or without PUFA added to the substrate were evaluated. Results showed superior fermentation characteristics in goats with higher *in vitro* gas production rate ($P<0.001$), accompanied by higher volatile fatty acids (VFA) production and *in vitro* organic matter digestibility ($P<0.05$). The higher population of total bacteria and two major cellulolytic bacteria (*Ruminococcus albus and Butyrivibrio fibrisolvens)* in goat compared to sheep also indicates higher digestive capability in goats. The addition of 4% linseed oil to the substrate did not show any negative effect on fermentation characteristics nor the VFA production, suggesting that the level oil was appropriate.

Experiment 2 consisted of a 100-day feeding trial to determine the growth performance, digestibility and meat quality attributes of goats and sheep fed fattening diet with or without oil supplementation, in a 2 species x 2 diets factorial experiment with 6 animals (replicates) per treatment. Results of the study showed that irrespective of diet, sheep had better body weight gain (BWG) and feed efficiency (FE) compared to goats. Overall, supplementation of linseed oil at 29 g/day improved BWG by about 65 g/day (45% improvement over the control) and FE by 15% (5.01 *vs* 5.93). There was no species difference (except for crude protein) and no effect of dietary treatments on apparent digestibility. Goat meat had better quality attributes ($P$<0.001), including lower lipid oxidation and higher water holding capacity than sheep. Linseed oil supplementation did not affect meat lipid oxidation, indicating that the level of supplementation was appropriate.

The fatty acids profile in various tissue, *in vivo* rumen fermentation and expression of adipogenesis related genes from the above feeding trial are reported in Chapter 5 with the objective of determining whether differences exist in fatty acids profile in goats and sheep fed similar diets and to elucidate the possible mechanisms for any differences exist. Results of the study showed that there were significant differences in the fatty acids profile in the *longissimus dorsi* (LD) muscle, subcutaneous (SC) fat and liver tissue between species and between dietary treatments. Although no species differences were observed in the total SFA, MUFA and PUFA content in the LD muscle, several individual fatty acids were significantly different between species, including higher n-3 PUFA in LD muscle of goats. Sheep had higher total SFA content but lower UFA in the SC fat and liver tissue. Linseed oil supplementation increased the linolenic acid content by 4.5 to 6.1 folds in the tissues samples analyzed suggesting oil supplementation provides a viable approach to enrich meat and ruminant products with essential PUFA.

The result of the *in vivo* rumen fermentation characteristics explained for the differences in the fatty acids profile between species. The higher production of total VFA and acetic acid (primary precursor in fatty acid synthesis) in sheep suggest higher lipogenesis activity, and explained for the higher SFA content. The higher population of *B. fibrisolvens* in sheep than goats, which on the other hand had higher population of total methanogens and methanobacteriales, explained for the higher biohydrogenation intermediate in sheep.

Relative expressions of adipogenesis related genes were used to elucidate the differences in the fatty acids profile between species and dietary treatment. The study found that, irrespective of diet, sheep had higher expression of fatty acid synthase gene (FASN) gene and also higher Acetyl-CoA carboxylase gene (ACACA) with oil supplementation. The higher expression of the above genes led to higher production of SFA content (especially palmitic acid) in the SC fat and liver tissue in sheep. Stearoyl-CoA desaturase (SCD) gene, which responsibles for the unsaturation of SFA, was

lower in sheep than goats and was further downregulated with the addition of oil, which explains for the lower mono-unsaturated fatty acids (MUFA) content in sheep and in the oil supplemented group. On the other hand, peroxisome proliferator-activated receptor alpha (PPARα), which serves as the transcriptional factor for many lipogenic genes, is negatively correlated with ACACA and FASN genes. Therefore the downregulation of PPARα gene was accompanied by upregulation of ACACA and FASN genes in sheep and the opposite in goats, accounting for the different SFA production between species.

Result of this thesis suggests that high concentrate feeding supplemented with PUFA (linseed oil at 4% level) is an applicable approach to improve the growth performance of small ruminants, and to produce healthier meat with beneficial fatty acids profile, without detrimental effect on rumen fermentation or meat quality attributes. Species differences in fatty acids profile were partly influenced by the various lipogenic genes expression and therefore, dietary manipulation of adipogenesis related genes expression (nutrigenomics) can be a useful tool especially in reducing the SFA content, in order to produce healthier ruminant meat and meat products.

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

**PENAPAIAN RUMEN, PROFIL ASID LEMAK, KUALITI DAGING DAN EKPRESI GEN BERKAITAN PENGHASILAN LEMAK PADA KAMBING DAN BEBIRI DENGAN PEMAKANAN TINGGI KONSENTRAT**

Oleh

**CANDYRINE SU CHUI LEN**

**Disember 2016**

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Industri ruminan kecil (kambing dan bebiri) di Malaysia boleh digambarkan dengan pertumbuhan yang perlahan berbanding dengan permintaan terhadap produk tersebut. Di samping itu, produk ruminan diketahui umum mempunyai kandungan asid lemak tepu (SFA) yang tinggi dan selalu dikaitkan sebagai sumber penyakit hari-moden. Sehingga kini, bilangan kajian yang membandingkan perbezaan antara kambing dan berbiri adalah terhad, maka kedua-dua spesis ternakan tersebut dianggap sama dan dibesarkan dan diurus dengan sama. Untuk memenuhi permintaan tempatan, maka terdapat keperluan untuk meningkatkan produktiviti industry ruminant kecil ini, dengan mengoptimumkan potensi pertumbuhan setiap spesis ternakan tersebut, dan pada masa yang sama, menghasilkan daging dan produk daging yang lebih sihat. Hipotesis kajian adalah pemakanan tinggi konsentrat, ditambah dengan minyak biji rami sebagai sumber asid lemak poli-tak tepu (PUFA), boleh meningkatkan kadar pertumbuhan binatang dan pada masa yang sama, menambahbaik daging dan produk dengan PUFA yang baik untuk kesihatan. Objektif utama tesis ini adalah untuk membandingkan ciri-ciri penapaian rumen, prestasi pertumbuhan, sifat-sifat kualiti daging dan profil asid lemak dalam tisu kambing dan bebiri di bawa pemakanan tinggi konsentrat, tanpa penambahan dan dengan penambahan PUFA; dan untuk merungkai perbezaan profil asid lemak mekanisasi yang bertanggungjawab atas perbezaan profil asid lemak yang disebabkan oleh pemakanan dan spesis binatang (kambing *vs* berbiri). Tiga eksperimen telah dijalankan untuk mencapai objektif-objektif tersebut. Dalam eksperimen yang pertama, ciri-ciri penapaian rumen *in vitro* dalam kambing dan bebiri, tanpa penambahan dan dengan penambahan PUFA pada substrat telah dikaji. Kajian menunjukan kelebihan ciri penapaian pada kambing dengan kadar penghasilan gas *in vitro* yang lebih tinggi ($P$<0.001), diikuti dengan kandungan asid lemak meruap (VFA) dan kebolehcernaan bahan organik *in vitro* yang lebih tinggi. Populasi jumlah bakteria dan juga dua bakteria utama dalam penghadaman selulosa (*Ruminococcus albus dan Butyrivibrio fibrisolvens)* yang lebih tinggi pada kambing berbanding bebiri

juga menunjukkan keupayaan pencernaan yang lebih tinggi pada kambing. Penambahan minyak biji rami pada kadar 4% pada substrat tidak menunjukkan sebarang kesan negatif terhadap ciri penapaian atau produksi VFA, lalu mencadangkan penggunaan minyak pada kadar tersebut adalah berpatutan.

Eksperimen 2 adalah eksperimen pemakanan secara *in vivo* selama 100 hari untuk menguji kadar pertumbuhan, kebolehcernaan dan sifat-sifat kualiti daging pada kambing dan bebiri di bawa pemakanan pengemukkan, tanpa penambahan dan dengan penambahan minyak, dalam 2 spesis binatang x 2 jenis pemakanan, eksperimen faktorial dengan 6 binatang (replica) untuk setiap kumpulan rawatan. Hasil kajian menunjukkan bahawa tanpa mengira jenis pemakanan, bebiri mempunyai kenaikan berat badan (BWG) dan nisbah penukaran makanan (FCR) yang lebih baik berbanding kambing. Secara keseluruhan, penambahan minyak biji rami pada kadar 29 g/hari telah menambahbaik BWG sebanyak 65 g/hari (45% penambahbaikan berbanding kumpulan kawalan) dan FE sebanyak 15% (5.01 *vs* 5.93). Tiada perbezaan spesies (kecuali protin kasar) dan tiada kesan jenis pemakanan terhadap kebolehcernaan ketara makanan. Daging kambing mempunyai sifat-sifat kualiti daging yang lebih baik ($P$<0.001), termasuk kadar pengoksidaan lipid yang lebih rendah dan keupayaan menyimpan air yang lebih tinggi berbanding daging bebiri. Penambahan minyak biji rami pada makanan tidak mengganggu kadar pengoksidaan lipid, lalu mencadangkan bahawa kadar penambahan minyak tersebut adalah berpatutan.

Profil asid lemak di pelbagai tisu ciri-ciri penapaian rumen *in vivo*, dan ekspresi gen berkaitan penghasilan lemak daripada eksperimen pemakanan di atas telah dilaporkan di Bab 5, dengan objektif untuk menentukan sama ada wujud perbezaan pada profil asid lemak pada kambing dan bebiri di bawa pemakanan yang sama, dan menjelaskan mekanisasi yang berkemungkinan menyebabkan perbezaan tersebut. Hasil kajian menunjukkan perbezaan yang ketara pada profil asid lemak di daging *longissimus dorsi* (LD), lemak bawa kulit (SC) dan tisu hati antara spesis binatang dan antara jenis pemakanan. Walaupun tidak terdapat perbezaan spesis binatang pada jumlah kandungan SFA, asid lemak mono-tak tepu (MUFA) dan PUFA dalam daging LD, beberapa asid lemak adalah berbeza secara ketara antara spesis binatang, termasuklah kandungan n-3 PUFA yang lebih tinggi pada daging LD kambing. Bebiri mempunyai kandungan SFA yang lebih tinggi tetapi rendah dalam kandungan asid lemak tak tepu dalam lemak SC dan tisu hati. Penambahan minyak biji rami dalam makanan telah meningkatkan kandungan asid linolenic sebanyak 4.5 hingga 6.1 kali ganda dalam semua tisu yang dikaji lalu mencadangkan penambahan minyak tersebut merupakan jalan yang nyata untuk menambah baik daging dan produk ruminan dengan PUFA penting.

Keputusan ciri-ciri penapaian rumen *in vivo* menjelaskan perbezaan profil asid lemak antara spesis binatang. Jumlah VFA dan asid asetik (sebagai pelopor utama dalam sintesis asid lemak) yang lebih tinggi pada bebiri, mencadangkan aktivit penghasilan lemak yang lebih tinggi, dan menjelaskan kandungan SFA yang lebih tinggi. Populasi *B. fibrisolvens* yang lebih tinggi pada bebiri berbanding kambing, yang mana mempunyai populasi jumlah methanogens dan methanobacteriales yang lebih tinggi, menjelaskan kandungan hasil perantaraan biohydrogenasi pada berbiri.

Ekspresi relatif gen berkaitan penghasilan lemak digunakan untuk menjelaskan perbezaan profil asid lemak antara spesis binatang dan antara jenis pemakanan. Kajian mendapati bahawa tanpa mengira jenis pemakanan, bebiri mempunyai ekspresi gen asid lemak synthase (FASN) yang lebih tinggi, dan juga ekspresi gen Acetyl-CoA carboxylase (ACACA) yang lebih tinggi dalam pemakanan yang ditambah minyak. Ekspresi kedua gen tersebut yang lebih tinggi, mendorong kepada penghasilan SFA (terutamanya asid palmitik) yang lebih tinggi di dalam lemak SC dan tisu hati dalam bebiri. Gen Stearoyl-CoA desaturase (SCD), yang bertanggungjawab untuk menyah-tepukan SFA, adalah diekspresikan dengan lebih rendah pada bebiri dan diturun regulasi di bawa pemakanan yang ditambah dengan minyak, lalu menjawab kepada kandungan MUFA yang lebih rendah pada bebiri dan juga kumpulan pemakanan yang ditambah minyak. Di samping itu, peroxisome proliferator-activated receptor alpha (PPARα), yang merupakan factor transkripsi untuk pelbagai gen penghasilan lemak, adalah berkadar songsang dengan ACACA dan FASN gen. Maka, penuranan regulasi pada PPARα adalah diikuti dengan kenaikan regulasi gen ACACA dan FASN pada bebiri dan sebaliknya pada kambing; lalu menjawab kepada perbezaan penghasilan SFA antara spesis binatang.

Hasil kajian tesis ini mencadangkan bahawa pemakanan tinggi konsentrat ditambah dengan PUFA (minyak biji rami pada kadar 4%) adalah berguna untuk menambahbaik kadar pertumbahan ruminan kecil, dan untuk menghasilan daging yang lebih sihat dengan profil asid lemak yang bermanfaat, tanpa kesan buruk terhadap penapaian rumen ataupun sifat-sifat kualiti daging. Perbezaan spesis binatang dalam profil asid lemak adalah dipengaruhi secara separa oleh tahap ekspresi gen. Maka dengan itu, manipulasi pemakanan terhadap ekspresi gen berkaitan penghasilan lemak (nutrigenomik) boleh dijadikan alat yang berguna terutamanya dalam menurunkan kandungan SFA, untuk menghasilkan daging dan produk daging yang lebih sihat.

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I certify that a Thesis Examination Committee has met on 8 December 2016 to conduct the final examination of Candyrine Su Chui Len on his thesis entitled "Rumen Fermentation, Fatty Acid Profiles, Meat Quality and Adipogenesis Related Gene Expression of Goat and Sheep Fed with High Concentrated Diet" 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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| | |
|---|---|
| °C | Degrees centigrade |
| µL | Microliter |
| µm | Micrometer |
| *a** | Redness |
| ACACA | Acetyl coenzyme A carboxylase gene |
| ACC | Acetyl- CoA carboxylase enzyme |
| ADF | Acid detergent fiber |
| ADG | Average daily gain |
| *b** | Yellowness |
| *B. fibrisolvens* | *Butyrivibrio fibrisolvens* |
| BW | Body weight |
| BWG | Body weight gain |
| CLA | Conjugated linoleic acids |
| cm | Centimeter |
| CP | Crude protein |
| DM | Dry matter |
| *F. succinogenes* | *Fibrobacter succinogenes* |
| FAS | Fatty acid synthase enzyme |
| FASN | Fatty acid synthase gene |
| FE | Feed efficiency |
| g | Gram |
| *g* | Relative centrifugal force |
| GAPDH | Glyceraldehyde-3-Phosphate Dehydrogenase |
| h | Hour |
| IVOMD | *In vitro* organic matter digestibility |
| Kg | Kilogram |
| L | Liter |
| *L** | Lightness |
| LD | *Longissimus dorsi* |
| m | Meter |
| MDA | Malondialdehyde |
| Messenger RNA | mRNA |
| Mg | Milligram |
| min | Minutes |
| MJ | Megajoules |
| mL | Milliliter |
| mm | Millimeter |
| mM | millimolar |
| mmol | Millimole |
| MUFA | Mono-unsaturated fatty acids |
| NDF | Neutral detergent fiber |
| OM | Organic matter |
| *P* | Probability |
| PPAR | Peroxisome proliferator-activated receptor |
| PUFA | Polyunsaturated fatty acids |
| *R. albus* | *Ruminococcus albus* |
| *R. flavefaciens* | *Ruminococcus flavefaciens* |
| s | Second |

| | |
|---|---|
| SC | Subcutaneous |
| SCD | Stearoyl coenzyme A desaturase |
| SD | Standard deviation |
| SEM | Standard error of mean |
| SFA | Saturated fatty acids |
| Sp | Species |
| TBARS | Thiobarbituric acid reactive substances |
| UFA | Unsaturated fatty acids |
| VFA | Volatile fatty acids |
| WHC | Water holding capacity |

# CHAPTER 1

# INTRODUCTION

Half of the world two billions goats and sheep are found in the Asia-Pacific region, making it the most populated region for goats and sheep in the world (Food and Agriculture Organization, 2014). Goats and sheep farming have been integral parts of agriculture system, contributing to the economy of many developing countries in Asia, including Malaysia. The interest in keeping these animals has increased significantly in the recent years because of their smaller body size and ability to adapt to different harsh environments such as poor quality feed and hot and humid environment compared to cattle and buffaloes. Goats and sheep also have shorter reproductive cycles compared to cattle and buffaloes, making them more reproductively efficient. The Malaysian government also intends to increase the SSL for mutton and chevon to 27.3% through driving modernization in agro-food production by year 2020 (http://www.epu.gov.my). At the moment, the self-sufficiency level (SSL) of mutton and goat meat (chevon) in Malaysia is 11.46% (Table 1.1), lagging far behind compared to poultry meat and eggs which are fully self-sufficient in this country (Department of Veterinary Services, 2015), thus the above scenario provides huge opportunities for growth and development of the small ruminant industry in Malaysia.

**Table 1.1 : Livestock population, outputs, consumptions and self-sufficiency level (SSL, %) of livestock products in Malaysia**

| | **Buffalo** | **Cattle** | **Swine** | **Poultry** | **Goat** | **Sheep** |
|---|---|---|---|---|---|---|
| Population (heads) | 121,504 | 752,032 | 1,828,860 | N.A. | 439,667 | 140,049 |
| | **Beef** | **Pork** | **Poultry meat** | | **Mutton/ chevon** | |
| Outputs (Metric ton) | 50,493 | 215,760 | 1,613.92 $X10^3$ | | 4,367.3 | |
| Consumptions (Metric ton) | 214,866 | 228,030 | 1,544.78 $X10^3$ | | 38,107. 3 | |
| SSL (%) | 23.5 | 94.62 | 104.48 | | 11.46 | |

N.A. = not available
(Source: Department of Veterinary Services, 2015)

The traditional goats and sheep farming system involved animals feeding and grazing on low quality forages with minimal or no feed supplementations (Khan et al., 2014). This low-input low-output system hinders growth, productivity and development of the industry which could not meet the increasing demand for meat and milk from these two small ruminant species. Thus, there is a need to increase production of animal proteins from goats and sheep in this region through various efforts including improved nutrition and feeding management (Aziz, 2010). Morand-Fehr and Sauvant (1987) suggested the incorporation of concentrate in the diets of ruminants to supply

higher energy, proteins, vitamins and minerals to improve feed utilization. Hence, the inclusion of higher proportion of concentrate in the diet can be an alternative in improving the productivity of the animals.

In the era of the 21st century, as the standard of living improves, consumers are demanding for healthy and nutritious food. Ruminant meat products (chevon and mutton) are known to contain high amount of fat, especially saturated fatty acids (SFA) which are often associated with modern-day diseases such as cardiovascular problem, obesity and cancer (Turner, 2010), making it less preferred by the consumers. However, despite containing high amount of fat, red meat is a good dietary source of protein, nutritionally dense with essential amino acids, micro-minerals and vitamins (Williamson et al., 2005; Biesalski, 2005). A recent study found that red meat is a good source of anti-inflammatory long chain n-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) (McAfee et al., 2010). The latter has received increasing attentions due to its potential anti-carcinogenic and anti-atherogenic properties (Lock et al., 2005, Hargrave-Barnes et al., 2008), as well as its ability to favorably modulate immune function (Tricon et al., 2004).

The use of various feed supplements in order to enhance the quality of meat products especially in improving the fatty acids profile in red meats have been studied. Alpha-linolenic acid (C18:3n-3), is an n-3 PUFA which has long been reported to be associated with reduced risk of cardiovascular diseases (Hu et al., 1999). It is also the precursor of other long chain n-3 PUFA such as eicosapentaenoic (EPA) and docosahexanoic acid (DHA). These long chain n-3 PUFA are widely known for their association with heart health, beneficial effects towards the central nervous system, retinal function and inflammatory response (Ruxton et al., 2004; Mann et al., 2006, Siddiqui et al., 2008). Linseed oil is a potential commercial source of n-3 PUFA, of which it contained more than 50% of alpha-linolenic acid (Matthews et al., 2000). The supplementation of linseed has been found to be successful in increasing the n-3 PUFA content in broiler chickens (Lopez- Ferrer et al., 2001), pigs (Fontanillas et al., 1997; Rey et al., 2001), lambs (Bessa et al., 2007; Jeronimo et al., 2009), and meat and dairy goats (Marin et al., 2012; Ebrahimi et al., 2013)..

To date, there were only handful of species comparative studies between goats and sheep although interspecies variations in rumen digestion and microbiota have been reported previously (Moon et al., 2010; Lee et al., 2012). The knowledge of putative species-specific differences in fatty acids rise only from indirect comparisons (Loor et al., 2004; Boeckaert et al., 2008a; Toral et al., 2016), due to the scarcity of available data on this subject, particularly in the caprine (Chilliard et al., 2014; Li et al., 2014), most probably because goat is deemed inferior as compared to sheep. In addition, studies on fatty acids profile in goat and sheep has been focusing more on milk and milk products and less attention has been given to meat. Tsiplakou

and Zervas (2008) reported the species differences in fatty acids profile in milk fat of goat and sheep.

Since goats and sheep are economically valuable in the developing countries across Asia including Malaysia, it is relevant that research should focus on achieving better productivity from goats and sheep. This thesis aims at achieving the above with the following hypotheses and objectives:

Hypotheses statements:

1. There are species differences in rumen fermentation, growth, fatty acids profiles and meat quality between goat and sheep under similar high concentrate feeding.
2. Linseed oil supplementation can improve the fatty acids profile in various tissue in goat and sheep especially in the n-3 PUFA content.
3. Differences in the expression of adipogenesis related genes and rumen fermentation characteristics leads to different fatty acids profile between species and between dietary treatments.

Objectives:

1. to determine the *in vitro* rumen fermentation characteristics in goat and sheep incubated with control and linseed oil added substrate;
2. to determine the growth performance, apparent digestibility and meat quality in goat and sheep fed similar diet;
3. to determine the fatty acids profile in liver, muscle and fat tissues, *in vivo* rumen fermentation and the expression of adipogenesis related genes in goat and sheep, under the same high concentrate feeding, with and without supplementation of linseed oil.

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