*Research Articles*

# Fatty Acid Composition in Adipose Tissues of Goats and Sheep Reared on either Grazing or Confinement Regimen

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## Abstract

**Essential fatty acids play a part in many metabolic processes such as immune system, and cardiovascular system. A lower ratio of omega-6 to omega-3 fatty acids or increased omega-3 fatty acids in human diet was more desirable in reducing the risk of the chronic diseases. Hence, the objective of this study was to determine fatty acid composition from goat and sheep adipose tissues with two different rearing regimens, grazing and confinement. Ten young male crossbred goats and sheep were equally divided into two groups by their species reared on either grazing or confinement. Analysis of data as a 2 x 2 factorial combination in Completely Randomized Design showed that different species did affect fatty acid compositions and no significant differences were found by either rearing regimens in fatty acid composition in perirenal adipose tissues. The ratio of the saturated to unsaturated fatty acid was significantly higher ($P<0.01$) in goats than that found in sheep. But, the effects of rearing and species-rearing interaction were not observed on this ratio. Moreover, there was significantly higher ($P<0.05$) α-linolenic acid (C18:3 ω3) in sheep adipose tissue than goats in both rearing regimens. Thus, ω6/ω3 ratio tended to be lower in sheep than goats. Perirenal adipose tissues from goat and sheep contained 4.02 % and 4.57 % polyunsaturated fatty acids (PUFA), respectively.**

**Keywords:** fatty acid composition, goats, sheep, grazing and confinement

# องค์ประกอบของกรดไขมันในเนื้อเยื่อไขมันของแพะและแกะที่มีการเลี้ยงแบบปล่อยแทะเล็มและขังคอก

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## บทคัดย่อ

กรดไขมันชนิดจำเป็นมีความสำคัญในกระบวนการเมแทบอลิซึมของระบบต่างๆของร่างกาย เช่น ระบบภูมิคุ้มกัน และระบบหมุนเวียนเลือด มีการศึกษาพบว่าอาหารที่มีสัดส่วนของกรดไขมันชนิดโอเมก้า 6 ต่อกรดไขมันชนิดโอเมก้า 3 (ω6/ω3 ratio) ในระดับที่ต่ำช่วยลดปัจจัยเสี่ยงต่อการเกิดโรคเรื้อรังต่างๆได้ ดังนั้นการศึกษาในครั้งนี้จึงมีวัตถุประสงค์เพื่อเปรียบเทียบชนิดและปริมาณของกรดไขมันในเนื้อเยื่อไขมันรอบไตของแพะและแกะหนุ่มเพศผู้ระหว่างการเลี้ยงแบบปล่อยแทะเล็มและขังคอก โดยแพะและแกะชนิดละ 10 ตัว ถูกแบ่งออกเป็นสองกลุ่ม สัตว์ทั้งสองชนิดถูกแบ่งเลี้ยงแบบปล่อยแทะเล็มและขังคอก กลุ่มละเท่าๆกัน จากการวิเคราะห์ข้อมูลโดยจัดทรีตเมนต์แบบพหุคูณ 2 x 2 ในการทดลองแบบสุ่มสมบูรณ์ แสดงให้เห็นว่าชนิดของสัตว์มีผลต่อชนิดและปริมาณของกรดไขมัน โดยสัดส่วนกรดไขมัน ชนิด ω6/ω3 ในแพะนั้นสูงกว่าแกะอย่างมีนัยสำคัญ ($P<0.01$) ในขณะเดียวกันพบว่ารูปแบบของการเลี้ยงนั้นไม่มีนัยสำคัญทางสถิติต่อชนิดและปริมาณของกรดไขมัน กรดไขมันจำเป็นชนิดแอลฟ่าไลโนเลนิก (C18:3 ω3) ในเนื้อเยื่อไขมันแกะสูงกว่าแพะ ($P<0.05$) ทั้งสองรูปแบบการเลี้ยงและมีแนวโน้มว่าสัดส่วนกรดไขมันชนิด ω6/ω3 ในแกะต่ำกว่าแพะ เนื้อเยื่อไขมันรอบไตแพะและแกะประกอบไปด้วยกรดไขมันชนิดไม่อิ่มตัวสายยาว (polyunsaturated long chain fatty acids, PUFA) 4.02 % และ 4.57% ตามลำดับ

**คำสำคัญ :** องค์ประกอบของกรดไขมัน แพะ แกะ การเลี้ยงแบบปล่อยแทะเล็มและขังคอก

## Introduction

Human requires essential fatty acids, linoleic acid (C18:2 ω6) and α-linolenic acid (C18:3 ω3), from the food. However, an increased omega-6 fatty acid and decreased omega-3 fatty acid intake, promote the pathogenesis of cardiovascular disease, inflammatory and autoimmune disease (Simopoulos, 2006), whereas increased essential omega-3 fatty acid (a low omega-6/ omega-3 ratio) exert suppressive effects of those pathogenesis. A ratio of 2-3 to 1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5 to 1 had a beneficial effect on patients with asthma. Consequently, a lower ratio of omega-6 to omega-3 fatty acids in animal products was more desirable in reducing the risk of many of the chronic diseases (Simopoulos, 2002).

Consumers 's demand for food products of superior healthy quality has renewed interest in modifying the fatty acid composition of meat (Tanaka, 2005). Indeed, ruminant products with a high level of saturated fatty acid (SFA) *per se*, however, meat from grass-fed animals was often higher in omega-3 than meat from the corresponding grain-fed animals. The omega-6 to omega-3 ratio of grass-fed beef was about 2:1, making it a more useful source of omega-3 than grain-fed beef, which usually has a ratio of 4:1 (de Lorgeril et al., 1994). Commercially available lamb was almost always grass-fed, and subsequently higher in omega-3 than other common meat sources. At present the consumers request for the products from the goats and sheep greater than the past because of their tasty and high benefit for human health. Due to the ingesting behavior of both species, sheep were more grazer consuming mostly lower quality grasses, while browsers such as goats ingest high nutritious grasses and shrubs. This different behavior may influence fatty acid composition in their body tissues, especially adipose tissues. Indeed, perirenal adipose tissues could be used to determine the content of fatty acids distribution in their muscles (Bouchard et al., 1993) and when considering goats and sheep adipose tissue fatty acid ratio was not known yet. And it could be expected that differences in rearing regimens and species or its interaction might influence fatty acid composition in adipose tissue. Therefore, the aim of the present experiment was to compare fatty acid composition in young male goats and sheep adipose tissues reared on either grazing or confinement regimen under conventional rearing management in Thailand.

## Materials and Methods

### Animals

Ten young male crossbred goats and ten young male crossbred sheep, aged 6 months, were used in a 3-month feeding study at the Suwanvajokkasikit Animal R&D Institute, Kamphaeng Saen Campus, Kasetsart University, Nakhon Pathom, Thailand. The five animals as their species were housed in one pens (2.5 m x 3.5 m) with a concrete floor. There were two pens per specie. The pens were located in a barn in which the temperature was not controlled (30-35 $^{o}$C).

### Rearing regimens and diets

Either two grouped goats or two grouped sheep were randomly divided into two rearing regimens, grazing and confinement. Accordingly, there were 4 experimental treatments in a 2 x 2 factorial combination in Completely Randomized Design with two species (goats and sheep) and two rearing regimens (grazing and confinement). Each group was fed the same amount of commercial concentrate twice a day (0.5 kg/head/day), according to the feeding standard recommended by the NRC (1981, 1985). All animals were offered fresh Para grass as a roughage for *ad libitum*. Goats and sheep in grazing groups were allowed to graze at 08.30 to 17.30 h, which a grazing area was 10 acres. The confinement groups of both species were fed by cut and carry forage system. Tap water was provided for *ad libitum* consumption for all groups.

**Collection and Preservation of samples**

The animals were fasted overnight before they were killed by Islamic-Halal method at the end of experiment. About 30 grams of adipose tissue around the kidneys were collected and stored in a sealed plastic bag at -20 °C (Folch et al., 1957) until fatty acid composition analysis. Concentrate and roughage (pasture grazed) of either confinement or grazing groups were randomly collected, dried (60 °C, for 72 hr) and subsequently, ground through a sieve 1 mm for chemical analysis.

**Chemicals Analysis**

**Weende analysis of diets**

Nitrogen contents were determined by the macro-Kjeldahl method (Association of Official Analytical Chemists, AOAC, 2001) using Kjeltec™, Foss Tecator technology; a factor of 6.25 was used to convert nitrogen content into crude protein. Crude fat and fiber contents of the both concentrate and roughage were analyzed according to the AOAC (1984) method using Soxtec™ and Fibertec™, respectively. The samples were ashed at 480 °C for 6 hr for ash analysis. The accuracy of each assay run was found to be within ±3 % deviation from the target value of in house hay reference standard. The combined within- and between-run precision of the determinations was <3.0% (coefficient of variation). Chemicals analyzed of diets were shown in Table 1.

**Measurement of fatty acid composition of adipose tissues**

Samples of perirenal adipose tissue were extracted by chloroform : methanol, 2:1 mixture according to the Folch et al. (1957). Then, the lipid was saponified with 0.5 N methanolic sodium hydroxide and methylated with 14% Borontrifluoride according to the method of Metcalfe et al. (1966). The methyl esters were separated and quantified by gas chromatography (Agilent technologies, helium carrier, 30 meters cross-linked PEG column, 275 °C FID detector) for determination of fatty acid composition.

**Table1** Chemical compositions of the pasture grazed and commercial concentrate.

| Chemical composition (%) | Pasture grazed[2] | Commercial Concentrate[3] |
|---|---|---|
| Dry matter, % of as fed | 26.50 | 94.63 |
| | % of DM | |
| Crude Protein | 7.42 | 14.28 |
| Crude Fiber | 29.38 | 8.14 |
| Crude fat | 1.81 | 4.03 |
| Carbohydrate[1] | 19.64 | 68.60 |
| Ash | 5.04 | 4.58 |

[1] Calculated as : Carbohydrate = % of dry matter - (% of crude protein + % of crude fiber + % of crude fat + % of ash).

[2] The pasture grazed consisted of : Para grass.

[3] The ingredients consisted of : corn meal, cassava meal, broken rice, coconut meal, bone meal, salt, urea, sulfur, vitamin and mineral premix.

**Statistical analysis**

This experiment used a 2 x 2 factorial combination in Completely Randomized Design (CRD). Prior to statistical analysis, the observed parameters were checked for their normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene 's test). For all statistical data calculations were performed using SPSS software version 18.0 for Microsoft Windows (SPSS, 2010) by following the general linear model (GLM) procedure in order to identify the variables effect; species, rearing regimens and its interaction on the observed fatty acid composition and multiple comparison of mean values followed by Duncan's new multiple range test. Pearson's correlation was performed to identify the correlation of individual fatty acids. Throughout, statistical significance was preset at P-value <0.05.

## Results

The fatty acid composition of perirenal adipose tissue was shown in Table 2. Palmitic acid (C16:0) was the most abundant saturated fatty acid and followed by stearic acid (C18:0). And, oleic acid (C18:1) was predominant unsaturated fatty acid found in adipose tissue in both species. The percentage of essential fatty acids in adipose tissue, linoleic acid (C18:2 ω6) was higher than α-linolenic acid (C18:3 ω3) for all combination treatments observed. Upon analysis of variance (GLM procedure), it was shown that the effects of species, rearing regimens and its interaction on the percentage of <C18:2 and >C20:0 fatty acids; were failed to reach statistical significance (P>0.05), but only the percentage of α-linolenic acid (C18:3 ω3) was influenced (P<0.05) by the species. The sum of the percentage of saturated fatty acids was not statistically significant (P>0.05) by the effects of species, rearing regimens and its interaction; but only that of species effect was found to influence (P = 0.03) the sum of the percentage of unsaturated fatty acids. There was no rearing effect on the ratio of saturated to unsaturated fatty acid, but the effect of species was significant (P = 0.01). The saturated to unsaturated fatty acid ratio in adipose tissue of sheep was lower than that of goats in both confinement and grazing regimen which could be identified (P<0.05) by Duncan's new multiple range test (Table 2). Because there was significantly higher α-linolenic acid (C18:3 ω3) in adipose tissue of sheep than goats in both rearing regimens which led the ω6/ω3 ratio in goats trended to be higher (P>0.05) than sheep. When considered, the rearing effect, the ratio of saturated to unsaturated fatty acids in grazing group trended to be higher than confinement group. On the other hand, the ω6/ω3 ratio in grazing group trended to be lower than confinement group in both species. However, those ratios were not statistically significant (P>0.05). The individual correlations of fatty acid composition in adipose tissue of both species were shown in Table 3. Palmitic acid (C16:0) was negatively correlated (Pearson's r = -0.473, P<0.05, n=20) with linoleic acid (C18:2 ω6) and this consequence leading the ω6/ω3 ratio was highly correlated (Pearson's r = -0.565, P<0.01, n=20) with palmitic acid.

**Table 2** Fatty acid composition in perirenal adipose tissues of goats and sheep among their rearing regimens.

| Fatty acid[1] | Goat | | Sheep | | Pooled | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | Grazing | Confinement | Grazing | Confinement | SEM | species | rearing | species x rearing |
| C10:0 | 0.86 | 0.88 | 0.76 | 0.84 | 0.06 | 0.26 | 0.41 | 0.62 |
| C12:0 | 2.36 | 2.30 | 2.08 | 2.10 | 0.36 | 0.51 | 0.96 | 0.91 |
| C14:0 | 11.02 | 11.36 | 11.00 | 11.30 | 0.64 | 0.95 | 0.63 | 0.98 |
| C16:0 | 25.92 | 26.40 | 26.60 | 26.12 | 0.53 | 0.71 | 1.00 | 0.38 |
| C16:1 | 4.36 | 4.52 | 4.12 | 4.36 | 0.15 | 0.20 | 0.20 | 0.79 |
| C18:0 | 26.44 | 25.88 | 25.28 | 24.48 | 0.75 | 0.11 | 0.38 | 0.87 |
| C18:1 | 18.48 | 18.88 | 19.16 | 19.88 | 0.49 | 0.11 | 0.27 | 0.75 |
| C18:2 ω6 | 3.34 | 3.32 | 3.62 | 3.74 | 0.17 | 0.06 | 0.77 | 0.69 |
| C18:3 ω3 | 0.50[b] | 0.42[b] | 0.62[a] | 0.58[a] | 0.05 | 0.02 | 0.27 | 0.71 |
| C20:0 | 0.58 | 0.66 | 0.76 | 0.76 | 0.09 | 0.16 | 0.68 | 0.68 |
| C20:1 | 0.44 | 0.42 | 0.48 | 0.44 | 0.03 | 0.41 | 0.41 | 0.78 |
| C20:2 | 0.28 | 0.18 | 0.28 | 0.30 | 0.04 | 0.20 | 0.39 | 0.20 |
| SFA | 67.18 | 67.48 | 66.48 | 65.60 | 0.66 | 0.07 | 0.66 | 0.38 |
| UFA | 27.40[b] | 27.74[b] | 28.28[ab] | 29.30[a] | 0.53 | 0.03 | 0.22 | 0.53 |
| SFA/UFA ratio | 2.46[a] | 2.44[a] | 2.35[ab] | 2.24[b] | 0.05 | 0.01 | 0.25 | 0.42 |
| ω6/ω3 ratio | 6.90 | 8.38 | 5.98 | 6.80 | 0.82 | 0.15 | 0.18 | 0.69 |

SFA; Saturated Fatty Acids, UFA; Unsaturated Fatty Acids, SEM; Standard Error of the Mean.

[1]Percentage fatty acid methyl ester of total fatty acid methyl esters.

[a,b]Mean values in the same row with different letters were significantly different by species effect (Duncan's new multiple range test, $P<0.05$)

## Discussion

In the present study, the percentages of fatty acid composition were agreed with the ranges found by the experiment of Yeom (2002) and Lee et al. (2000). In Table 2, the present data showing that the contents of ≤C18:1 fatty acids were predominant in adipose tissue of across species and rearing regimens. These may due to they could be *de novo* lipogenesis from ruminal volatile fatty acids (Drackley, 2000). And also, the entering of dietary lipid and microbial lipid through the small intestine which that consists of saturated fatty acids, mainly palmitic acid (C16:0) and stearic acid (C18:0) (Bauman et al., 2003). Indeed, in forage-fed ruminants, dietary lipids consist primarily of galactolipids and other glycolipids that were rich in α-linolenic acid (18:3ω3) and other cereal grains or concentrate ingredients contribute triacylglycerols that were high in linoleic acid (18:2) (Chilliard et al., 2003; Drackley 2000). Hence, it implied that the content of linoleic acid (C18:2 ω6) and α-linolenic acid (C18:3 ω3) found in adipose tissue mainly derived from dietary sources. Moreover, we found that the main *de novo*palmitic acid (C16:0) was negatively correlated (Pearson's r = -0.473, $P<0.05$, n=20) with linoleic acid (C18:2 ω6) without any correlations between α-linolenic acid and >C18:1 fatty acids observed. These facts indicated that the contents of linoleic acid (C18:2 ω6) and α-linolenic acid (C18:3 ω3) found were not derived from *de novo* lipogenesis (Abu Ghazaleh et al., 2002). However, our hypothesis on the difference of regimens (grazing and confinement) influences fatty acid composition in adipose tissue was not proved in this experiment. The reason may due to the fact that both species (goats and sheep) were offered the same amount of concentrate. However, we found that the difference of species (goats and sheep) significantly

influenced essential fatty acid, α-linolenic acid (C18:3 ω3) which confirmed the result of Lee et al. (2000) who reported that the content of linolenic acid was higher in longissimus muscles of sheep than those found in goat. These facts did imply that sheep as a grazer ingested more pasture which rich in linolenic acid content than goat as a browser did across their rearing regimens in this experiment.

Moreover, we found that the species effect influenced the sum of percentage of unsaturated fatty acids significantly by accounting for the percentage of polyunsaturated fatty acid (PUFA; the sum of percentage of C18:2, C18:3 and C20:2) in goats and sheep respectively contained of 4.02 % and 4.57 % PUFA. However, these results were controversy with the results of Lee et al. (2000), who reported that PUFA were contained 5.5 % and 4.6 %, respectively, in their adipose tissues, and the explanation for this was not known. In our study, the ratio of saturated to unsaturated fatty acids and all combined treatments was lower in sheep than goats. However, the ω6/ω3 ratio was not that of fashion.

From our outcome of the present study, there was significantly higher α-linolenic acid (C18:3 ω3) in adipose tissue of sheep than that of goats in both rearing regimens. Thus, the trend of ω6/ω3 ratio in sheep was lower than goats. Therefore, it seems that lamb was suitable to consume for preventing risk of consumer health problems compared with goat meat concerning on their fatty acid composition.

**Table 3** Pearson's correlation coefficient of fatty acid composition in perirenal adipose tissues of goats and sheep.

| Items | C10 | C12 | C14 | C16 | C16:1 | C18 | C18:1 | C18:2 ω6 | C18:3 ω3 | C20 | C20:1 | C20:2 | SFA | UFA | SFA/UFA | ω6/ω3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C10 | 1 | | | | | | | | | | | | | | | |
| C12 | -.208 | 1 | | | | | | | | | | | | | | |
| C14 | -.175 | .872** | 1 | | | | | | | | | | | | | |
| C16 | .343 | -.470* | -.221 | 1 | | | | | | | | | | | | |
| C16:1 | .663** | -.210 | -.009 | .459* | 1 | | | | | | | | | | | |
| C18 | .337 | -.586** | -.772** | .131 | .023 | 1 | | | | | | | | | | |
| C18:1 | .074 | -.308 | -.233 | -.213 | .172 | .105 | 1 | | | | | | | | | |
| C18:2 ω6 | -.105 | .325 | .311 | -.473* | -.356 | -.362 | .106 | 1 | | | | | | | | |
| C18:3 ω3 | -.213 | -.255 | -.207 | .312 | -.235 | -.143 | -.266 | .353 | 1 | | | | | | | |
| C20 | -.200 | .374 | .562** | -.058 | .170 | -.740** | .013 | .461* | .295 | 1 | | | | | | |
| C20:1 | -.326 | .121 | -.069 | -.323 | -.419 | .147 | .202 | .010 | -.036 | -.104 | 1 | | | | | |
| C20:2 | -.507* | .375 | .400 | -.605** | -.410 | -.415 | .106 | .487* | -.060 | .296 | .371 | 1 | | | | |
| SFA | .430 | .280 | .333 | .478* | .331 | .179 | -.383 | -.267 | -.212 | -.076 | -.116 | -.377 | 1 | | | |
| UFA | 124 | -.212 | -.103 | -.243 | .220 | -.052 | .936** | .378 | -.089 | .243 | .152 | .228 | -.385 | 1 | | |
| SFA/UFA | .078 | .290 | .220 | .367 | -.039 | .104 | -.880** | -.404 | -.024 | -.231 | -.152 | -.319 | .691** | -.932** | 1 | |
| ω6/ω3 | .125 | .372 | .312 | -.565** | -.002 | .028 | .321 | .128 | -.853** | -.133 | .126 | .299 | .062 | .260 | -.173 | 1 |

SFA; Saturated Fatty Acids, UFA; Unsaturated Fatty Acids.

** Correlation was statically significant at the P value < 0.01 level (2-tailed, n = 20).

* Correlation was statically significant at the P value <0.05 level (2-tailed, n = 20).

## Conclusion

The species effect, goat and sheep did affect fatty acid compositions. There was a significant higher the saturated to unsaturated fatty acids ratioin goats than sheep. But, the effects of rearing and species-rearing interaction were not observed on this ratio. Moreover, there was significantly higher α-linolenic acid (C18:3 ω3) in sheep adipose tissue than goats in both rearing regimens.

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