

## Comparison of the Fatty Acid Composition of the Longissimus Dorsi Muscle of Kids, Lambs and Calves Produced under Iranian Transhumant Production System

Research Article

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### ABSTRACT

Transhumant production system (TPS) is a type of extensive livestock production practiced by transhumant pastoralists in which indigenous livestock breeds are mainly fed a pasture-based diet. The hypothesis tested in this work was whether differences existed between fat samples from the different species in respect to ratios of n-6/n-3, and polyunsaturated fatty acids to saturated fatty acids. To test our hypothesis, fatty acids (FAs) composition of the longissimus dorsi muscle of kids (n=10), fat-tailed lambs (n=10), and calves (n=10) produced in a pasture-based system were determined. All animals were indigenous intact male and randomly selected from nomads in the Zagros mountains (Noorabad, Lorestan province). The live body weight of kids, lambs and calves were  $21 \pm 6$ ,  $27 \pm 4$ , and  $158 \pm 35$  kg respectively. Meat samples were analyzed either without (lean meat) or mixed with 30% of sirloin subcutaneous fat (fat meat). Results showed that saturated FAs (as percentage) in kids meat was lower than those in lambs and calves (41.4 vs. 46.2 and 47.4%  $P=0.02$ ). Kid meat had higher  $\alpha$ -linolenic (C18:3 n-3), eicosapentaenoic acid (C20:5 n-3), docosapentaenoic acid (C22:5 n-3), but lower undesirable FAs (C16:0+C14:0) and n-6/n-3 ratio in comparison with lambs and calves. In conclusion, goat meat produced under TPS conditions, compared with lambs and calves, showed more promising healthy source of FAs for human nutrition.

**KEY WORDS** omega-3, red meat, ruminants, transhumant pastoralists.

### INTRODUCTION

Transhumant production system (TPS) is practiced in Iran predominantly in the Zagros mountains where livestock are mainly raised in a pasture-based feeding (Badripour, 2004). The annual migration of nomads' takes place from mountainous cold rangelands towards the warmer plains at the beginning of autumn, with the reverse movement in the spring. In transhumant system animal husbandry, the stock comprise on average 48% sheep, 47% goats, 3% cattle and 2% draught animals (Badripour, 2004). Nomadic and trans-

humant pastoralists own 13.6, 8.3 and 0.25 million heads sheep, goats and cattle, respectively and they produce about 20% of red meat production (e.g. 800 thousand metric ton) in the country (Organization for Nomadic People of Iran, 2008). Red meat production under extensive systems (such as TPS) is different from that of intensive production systems in many aspects including management, breeds, feeding regimes, animal activity, and environmental condition (Zervas and Tsiplakou, 2011). In TPS, ruminants are usually finished on diets containing high proportions of green forages which might produce meat with more desir-

able fatty acids compositions than that of intensive production systems (Fincham *et al.* 2009; Daley *et al.* 2010; Howes *et al.* 2015).

Red meat is a good dietary source of essential amino acids, minerals, vitamins, and fatty acids (FAs) (Williams, 2007), however, its consumption is under question mainly due to unbalanced ratio of n-6 to n-3 FA, and ratio of polyunsaturated FA (PUFA) to saturated FA (SFA) (Binnie *et al.* 2014). These unhealthy nutritional facts of red meat could be to some extent improved by dietary inclusion of green grass in animal daily ration (French *et al.* 2000; Nuernberg *et al.* 2005; Fincham *et al.* 2009). There are opportunities in extensive production systems in which ruminants are fed green forage in which enhancement of desirable FAs compositions might occur (Nuernberg *et al.* 2005; Talpur *et al.* 2008; Fincham *et al.* 2009; Ponnampalam *et al.* 2014; Howes *et al.* 2015; Kiani and Fallah, 2016). In TPS, ruminants are finished on diets containing high proportions of green forages, thus they might produce meat with more desirable FAs compositions. To our knowledge, information on fatty acid composition of red meat produced in Iranian transhumant production system practicing by nomads is lacking. The hypothesis tested in this work was whether differences existed between fat samples, with respect to n-6/n-3, and PUFA/SFA ratios, from the goats, sheep and cattle reared under TPS. To test our hypothesis, we chose the *longissimus dorsi* (LD) muscle to compare FAs composition and ratios of n-6/n-3 and PUFA/SFA in kids, lambs, and calves produced under TPS.

## MATERIALS AND METHODS

### Animals and meat samples

In total, 30 intact indigenous male animals including 10 Lori goat kids, 10 fat-tailed lambs, and 10 Lori calves; all reared in a pasture-based feeding system by nomads were randomly selected. All animals had free access to their dams' milk even when they were able to graze. The animals had grazed on the same natural ranges at least for two months prior to slaughter day. The ranges were located at Zagros Mountains, near Noorabad, Lorestan province, Iran (34 °02'34.8"N, 48 °17'53.0"E). Predominant plant species of the grazed ranges were ryegrass (*Lolium perenne*), clover (*Trifolium* spp.), and other legumes (such as *Astragalus* spp.). Kids had free access to green leaves of oak trees (*Quercus brantii*). Slaughter age for lambs and kids were 5.5 ± 1 and for calves were 8 ± 2 months. Live body weight (Mean±SD) of the animals averaged 21 ± 6, 27 ± 4, and 158 ± 35 kg for kids, lambs and calves, respectively. All animals were slaughtered in a commercial processing plant (Gholshan Abattoir, Khoramabad, Lorestan) according to the Halal procedure. Slaughter procedure was approved by

the Animal Ethics Committee of Lorestan University. Meat samples (about 30-50 g) were taken from the loin portion of *longissimus dorsi* (between ribs 12<sup>th</sup> and 13<sup>th</sup>) muscles on the left side of the carcasses. Samples were cut off within 2 h after slaughter and were chilled to +4 °C for 24 h. Meat samples were divided into two sub-samples; either without subcutaneous fat (lean meat) or mixed with 30% sirloin subcutaneous fat of each animal (fat meat). Samples were ground by means of a food processor (3×5 s), and stored at -80 °C pending analysis.

### Lipid extraction and methylation

Fatty acid methyl esters (FAME) were determined using the procedures described by (Sukhija and Palmquist, 1988) with some modification. Briefly, 0.1 g of meat sample was weighed (0.0001 g, KERN, Germany) and then was freeze dried. Sample was then placed into screw cap Pyrex culture tube (16×125 mL, Scott glass tube). One mL of heptane was added and mixed. After that, 0.2 mL of sodium methylate (25%) was added, and the tube was put in a 50 °C water bath for 10 min. Sample was then cooled for about 5 °C and 3 mL of freshly made methanolic HCl 10% (prepared by adding 20 mL of acetyl chloride to 100 mL of anhydrous methanol) was added to the sample and the tube was shaken vigorously. The tube was put in steam bath (90 °C) for 30 min and then the tube sample was cooled with ice. Finally, one mL of heptane and three mL of potassium carbonate 10% was added and mixed for one minute using a shaker. The sample was centrifuged (Centrifuge 5415 R; Rotofix 32A, Germany) at 300 g for 5 min. Heptane phase (upper phase) was transferred to the GC vial (1.5 mL) using Pasteur pipette.

### Determination of fatty acid composition

Fatty acid methyl esters were analyzed using a GC (GC-FID; HP 6890 chromatograph, Hewlett-Packard, Avondale, PA, USA) fitted with a flame ionization detector. The GC was equipped with a Chrompack CP-Sil 88 TM fused silica capillary column (100 m×0.25 mm i.d., 0.2 mm film thickness; Varian Inc., Walnut Creek, CA, USA). The injector and detector temperatures were maintained at 280 °C and 300 °C, respectively. Initially, the column temperature was held at 150 °C for 5 min and then increased at 5 °C min<sup>-1</sup> to 180 °C (held for 30 min), then increased at 1 °C min<sup>-1</sup> to 190 °C (held for 5 min) and finally increased at 1 °C min<sup>-1</sup> to 200 °C (held for 35 min). Hydrogen was used as the carrier gas at a flow rate of 1.0 mL min<sup>-1</sup>. Identification of common FAs was accomplished by comparison of sample peak retention times with those of FAME standard mixtures (Supelco<sup>TM</sup>37 component FAME Mix, Supelco-47885-U, Sigma-Aldrich Chemie GmbH, Germany) and by using published chromatograms obtained under similar analytical

conditions. Result for each FA was expressed as percentage of total FAME.

### Statistical analysis

Data were analyzed using the MIXED procedures in SAS Software (SAS, 2008). The model considered the fixed effects of meat species (kids, lambs, and calves) and meat type (lean meat vs. fat meat) and the interaction effects. Non-significant interactions were excluded from the model. For all statistical analyses, significance was declared at  $P \leq 0.05$  and trends at  $P \leq 0.10$ , unless otherwise stated. The Fisher's protected least significant difference (LSD) test was used for multiple treatment comparisons using the LSMEANS with letter grouping obtained using SAS pdmix800 macro (Saxton 1998). The residual analysis was carried out to test the model assumptions using the UNIVARIATE procedure with NORMAL and PLOT options.

## RESULTS AND DISCUSSION

### Fatty acid characterizations of red meat produced under TPS

The major FAs of red meat produced in TPS were oleic acid (C18:1), stearic acid (C18:0) and palmitic acid (C16:0) irrespective of the species (Table 1). Stearic and palmitic acid values were numerically similar to those reported in concentrate fed ruminants (Enser *et al.* 1996; Banskalieva *et al.* 2000; Wood *et al.* 2008).

Red meat produced in TPS had relatively less SFAs and more oleic acid (C18:1) than those reported in concentrate fed ruminants (Enser *et al.* 1996; Banskalieva *et al.* 2000; Wood *et al.* 2008). Under TPS condition, animals are generally fed with green forages. Linolenic acid (7 to 37 g), linoleic acid (2 to 10 g), and palmitic acid (3 to 8 g) contribute to the 90% of FAs in forages (Clapham *et al.* 2005). Fatty acids of dietary forages are hydrogenated in the rumen, causing a decrease in linolenic acid and an increase in monounsaturated FAs (MUFAs) (Jenkins *et al.* 2008; Fincham *et al.* 2009).

In the present study, n-6/n-3 ratio in meat produced under TPS was notably lower than those reported for forage-fed and concentrate-fed ruminants.

The low n-6/n-3 ratio in LD muscle found in animals under TPS was mainly due to higher percentage of n-3 fatty acids compared to other reports. For instance, Lori goat reared under TPS in comparison with Pateri goats (Pakistani breed) under traditional feeding system (Talpur *et al.* 2008) produced meat with almost two-fold higher ALA (C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), and docosapentaenoic acid (DPA, C22:5 n-3). In general, inclusion of high quantity of green

forages in ruminant diet positively increased amounts of ALA, and EPA in meat (Nuernberg *et al.* 2005; Bas *et al.* 2005; Fincham *et al.* 2009). Pasture finishing animals produced higher quantities of n-3 fatty acids; consequently improved both the PUFAs/SFAs and n-6/n-3 ratios in comparison with concentrate-fed animals (reviewed by Howes *et al.* 2015). The meat of cattle grazed on pasture had higher amount of ALA and lower linolenic acid (LA; C18:2 n-6) compared to meat from concentrate-fed cattle (Daley *et al.* 2010). French *et al.* (2000) reported that beef cattle on pasture had higher amount of omega-3 FAs, consequently better ratio of n-6/n-3 compared to concentrate-fed ones. Low n-6/n-3 ratio in meat is nutritionally important character because of its relationship with cardiovascular disease mortalities (Simopoulos, 2006). The n-6/n-3 ratio in a healthy diet should be kept below 4.0 (Simopoulos, 2006). Long chain fatty acids such as EPA and DHA have been given much attention due to their biological potency and that they are beneficial to human health. In present study, red meat produced under TPS showed relatively high concentrations of EPA, DHA, and DPA suggesting an alternative source for these important FAs. These findings were in agreement with other reports suggesting that finishing animals in green forage diets enhanced the long chain FAs composition in meat (Howes *et al.* 2015). Therefore, some fatty acid characteristics of red meat produced under TPS such as relatively high concentrations of EPA, DHA, and DPA deserve further investigations.

### Lean meat versus fat meat

Irrespective of the species, lean meat had higher percentage of myristic acid (C14:0), LA, ALA, arachidonic acid (AA; C20:4 n-6), EPA, and DPA than fat meat (Table 1). Lean meat had higher PUFAs, n-6 FAs, n-3 FAs, and higher ratio of PUFAs/SFAs than fat meat (Table 2). However, no significant differences were found between lean and fat meat in respect to the unhealthy FAs, SFAs, and ratio of n-6/n-3 (Table 2). Furthermore, lean meat showed higher PUFAs/SFAs ratio than fat meat (0.35 vs. 0.18). The value of PUFAs/SFAs found in lean meat produced under TPS conditions was very close to that of desirable value (i.e. >0.4) in a healthy diet (Simopoulos, 2006). The higher PUFAs/SFAs ratio in lean red meat compared to fat meat might be explained by a low capacity of storing long chain FAs in adipose tissue of ruminants (Wood *et al.* 2008). In general, adipose tissue has less phospholipid, but more triglycerides than does muscle tissue. Phospholipids contain 10 times more LA and 8 times more ALA than triglycerides, whereas triglycerides have higher contents of palmitic acid, stearic acid, and oleic acid compared to phospholipids (Scollan *et al.* 2006; Wood *et al.* 2008).

**Table 1** Fatty acids composition (percentage of total fatty acids) of longissimus dorsi muscle either without (lean meat) or with subcutaneous fat (fat meat) in indigenous kids, lambs and calves produced under the same transhumant production system

Items	Species			SEM	P-value	Type meat		SEM	P-value
	Kids	Lambs	Calves			Lean meat	Fat meat		
Lauric acid (C12:0)	0.32	0.35	0.17	0.11	0.50	0.29	0.27	0.10	88.0
Myristic acid (C14:0)	1.77 <sup>b</sup>	2.62 <sup>ab</sup>	3.22 <sup>a</sup>	0.27	< 0.01	2.83	2.24	0.18	0.01
Palmitic acid (C16:0)	20.0 <sup>b</sup>	23.5 <sup>a</sup>	26.0 <sup>a</sup>	0.78	< 0.001	23.0	23.2	0.66	0.84
Margaric acid (C17:0)	1.18	2.06	1.16	0.27	0.06	1.13	1.81	0.23	0.07
Stearic acid (C18:0)	17.7	16.9	16.1	1.22	0.66	16.2	17.6	1.5	0.19
Myristoleic acid (C14:1 n-9 cis)	0.11 <sup>b</sup>	0.69 <sup>a</sup>	0.05 <sup>b</sup>	0.11	< 0.01	0.33	0.23	0.08	0.27
Palmitoleic acid (C16:1 n-9 cis)	2.30 <sup>ab</sup>	1.51 <sup>b</sup>	3.10 <sup>a</sup>	0.40	0.05	1.77	2.84	0.26	< 0.01
Palmitoleic (C17:1 n-9 cis)	0.63	0.76	0.91	0.25	0.73	0.70	0.83	0.16	0.38
Oleic acid (C18:1 n-9 cis)	39.8 <sup>a</sup>	39.8 <sup>a</sup>	35.1 <sup>b</sup>	1.38	0.01	36.1	40.4	1.06	0.05
Linoleic acid (C18:2 n-6 cis)	4.55	5.10	6.53	0.87	0.28	7.19	3.60	0.68	< 0.01
$\alpha$ -Linolenic acid (ALA; C18:3 n-3 cis)	1.81 <sup>a</sup>	0.78 <sup>b</sup>	0.69 <sup>b</sup>	0.16	< 0.001	1.24	0.95	0.11	0.04
Arachidonic acid (AA; C20:4 n-6)	2.04	1.82	1.63	0.44	0.81	2.76	0.90	0.37	< 0.01
Eicosapentaenoic acid (EPA; C20:5 n-3)	1.37 <sup>a</sup>	0.41 <sup>b</sup>	0.25 <sup>b</sup>	0.18	< 0.01	0.85	0.50	0.12	0.02
Docosapentaenoic acid (DPA; C22:5 n-3)	1.44 <sup>a</sup>	0.49 <sup>b</sup>	0.56 <sup>b</sup>	0.19	< 0.01	1.12	0.54	0.14	< 0.01
Docosahexaenoic acid (DHA; C22:6 n-3)	0.26	0.24	0.10	0.08	0.27	0.28	0.11	0.06	0.07

The means within the same row with at least one common letter, do not have significant difference (P>0.05). SEM: standard error of the means.

**Table 2** Means of unhealthy, saturated, unsaturated and the ratio of n-6/n-3 fatty acids of longissimus dorsi muscle either without (lean meat) or with subcutaneous fat (fat meat) of indigenous kids, lambs, and calves produced under the same transhumant production system

Items	Species			SEM	P-value	Type meat		SEM	P-value
	Kids	Lambs	Calves			Lean meat	Fat meat		
Unhealthy fatty acids (C14:0 + C16:0)	24.0 <sup>b</sup>	27.6 <sup>b</sup>	32.4 <sup>a</sup>	1.22	0.001	27.1	28.9	0.92	0.14
Saturated fatty acids (SFAs)	41.4 <sup>a</sup>	46.2 <sup>b</sup>	47.4 <sup>b</sup>	1.35	0.02	43.6	46.4	1.08	0.08
Monounsaturated fatty acids (MUFAs)	45.1	44.1	41.6	1.67	0.36	41.5	45.7	1.17	< 0.01
Polyunsaturated fatty acids (PUFAs)	13.3	9.38	10.9	1.69	0.29	14.6	7.80	1.28	< 0.01
n-6 fatty acids	7.54	7.1	8.87	1.26	0.60	10.6	5.05	1.00	< 0.01
n-3 fatty acids	5.1 <sup>a</sup>	1.96 <sup>b</sup>	1.60 <sup>b</sup>	0.49	< 0.001	3.52	2.23	0.33	< 0.01
PUSAs / SFAs ratio	0.33	0.21	0.24	0.04	0.20	0.35	0.18	0.04	< 0.01
n-6 / n-3 ratio	1.5 <sup>c</sup>	3.5 <sup>b</sup>	6.0 <sup>a</sup>	0.42	< 0.001	3.46	3.93	0.31	0.23

PUSAs: polyunsaturated fatty acids and SFAs: saturated fatty acids.

The means within the same row with at least one common letter, do not have significant difference (P>0.05). SEM: standard error of the means.

### Comparisons among three species (kids vs. lambs and calves)

Red meat of kids showed several nutritional merits over the meat of lambs and calves. Sum of SFAs in kids' meat was lower than that in lambs and calves (Table 2). Lower SFAs in kids could be partly explained by higher biohydrogenation in goats compared to the other ruminants. Tora *et al.* (2016) reported an interspecies variation in rumen microbiota and rumen biohydrogenation between dairy goats and cows. They reported more rumen biohydrogenation and higher SFAs concentration (in particular, 16:0 and 18:0) in rumen fluid of goats compared with cows. However, in the present study, kid meat had significantly lower palmitic acid (C16:0) than both calves and lambs (Table 1).

Kids had the lowest unhealthy FAs (C16:0+C14:0) and the highest desirable FAs (C18:0, C18:1, C18:2, C18:3, C20:5 and C22:5) among the three species. The content of desirable FAs in kids' meat in the present study (76%) was in range of reported 61 to 81% (Banskalieva *et al.* 2000). Stearic acid contributed to 43%, 37% and 34% of sum of SFAs in kids, lambs, and calves meat, respectively. Stearic acid is known to have neutral effect on diet-related disease risk (Hunter *et al.* 2010; Katan *et al.* 1994). In fact, undesirable FAs and SFAs increased plasma cholesterol concentration (Grundy and Denke, 1990; Katan *et al.* 1994; Astrup *et al.* 2011). Therefore, high stearic acid and low undesirable FAs in kid meat are considered as healthy nutritional merits.

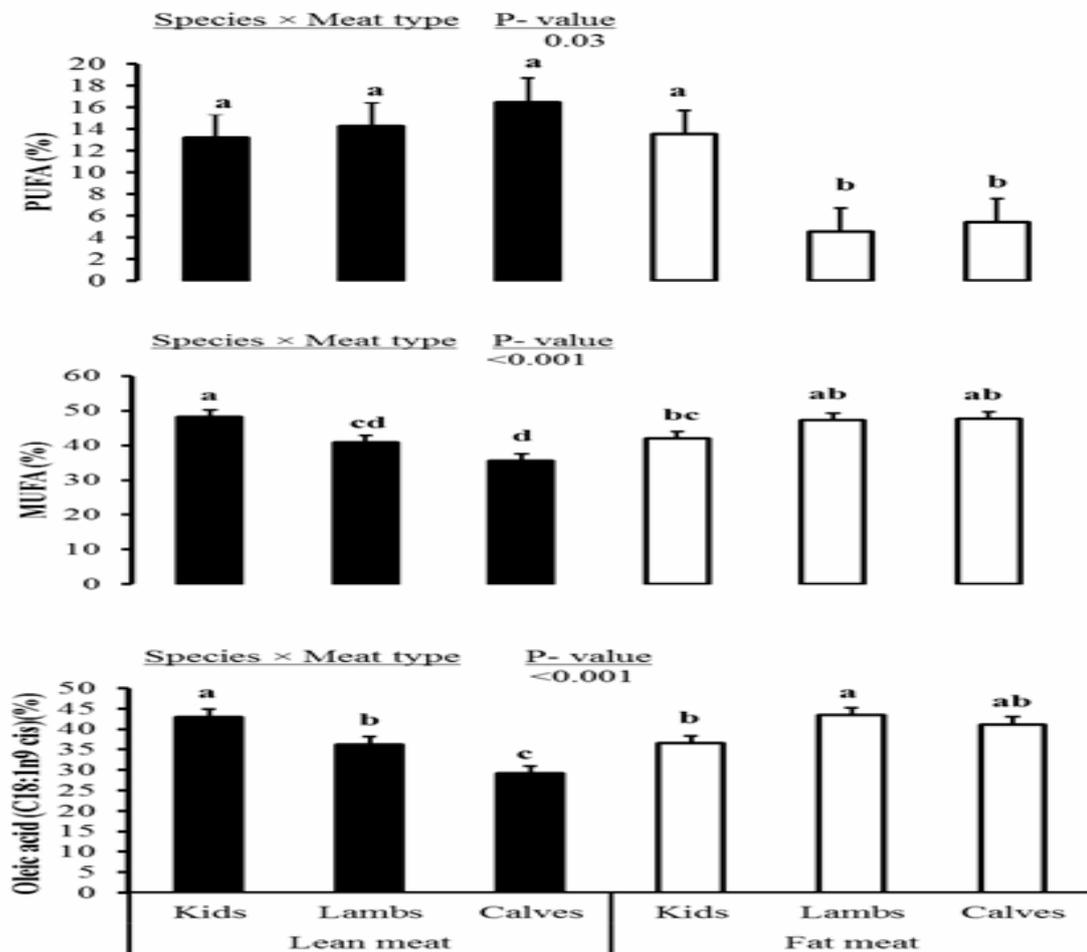
Kids had higher ALA, EPA and DPA than both calves and lambs (Table 1). Consequently, the sum of n-3 FAs in kid's meat was two-fold more than those in calves and lambs (Table 2). The main source of variations in the FAs profile of red meat is animal nutrition. In the present study, animals grazed same range; however, due to goats feeding behavior, kids also used green leaves of oak trees (*Quercus brantii*). The leaves of oak trees are tanniferous feeds. Vasta *et al.* (2009) found the highest UFAs concentrations in ruminal fluid from lambs supplemented with tannins, following to an increase in PUFAs and a decrease in SFAs levels in intramuscular fat. Similarly, the highest levels of LA, ALA and the sum of PUFAs in lamb muscle were found in the diet with the largest amount of tannins (Willems *et al.* 2014). Grazing animals on diverse pastures in mountainous areas accumulated more biohydrogenation intermediates in the rumen and an elevated PUFAs content

in inter-muscular fat. This suggested that rumen biohydrogenation was partially inhibited and PUFAs were further desaturated and elongated when animals were grazed on the botanically diverse pasture (Lourenco *et al.* 2007).

Fat meat in kids contained a high percentage of PUFAs (Figure 1), and high ratio of PUFAs/SFAs (0.34) regardless to the type of meat. Fat meat of kids contained lower oleic acid than lean meat whereas fat meat of lambs and calves had higher values compared to lean meat (Figure 1).

Monounsaturated fatty acids in lean meat compared to fat meat was lower in kids, while in calves lean meat had less MUFAs compared with fat meat. Polyunsaturated fatty acids in lean meat and fat meat of kids did not differ, whereas in lambs and calves lean meat had higher PUFAs compared to fat meat (Figure 1).

Lean meat of lambs and calves contained two-fold more PUFAs compared to fat meat.



**Figure 1** Significant interaction effects between species (kids, lambs and calves) and meat type (without or with subcutaneous fat) on oleic acid, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) of longissimus dorsi muscle presented as percentage of total fatty acid proportions.

<sup>a, b and c</sup> Bars in the same chart with a common superscript letter do not differ significantly ( $P < 0.05$ ).

The high percentage of PUFA in fat meats of kids in comparison with lambs and calves is interesting since the PUFAs subcutaneous fat in ruminants is generally low (approximately 0.15). Unsaturated fatty acids of dietary lipids are mainly converted to SFAs through bacterial lipolysis and biohydrogenation in the rumen (Jenkins *et al.* 2008). In general, adipose tissue has less phospholipid, but more triglycerides than does muscle tissue.

Phospholipids contain more PUFAs (LA and ALA) than triglycerides, whereas triglycerides have higher contents of C16:0, C18:0 and C18:1 compared to phospholipids (Wood *et al.* 2008) presumably because the capacity for incorporation of PUFA into phospholipids is limited. Feeding ruminants a diet rich in PUFAs promotes fat deposition in the phospholipid fraction, and SFAs, when in excess, are deposited and stored in the triglyceride fraction. Compared to grass, mixed leguminous pastures produced significantly higher proportions of linoleic acid and  $\alpha$ -linolenic acid in the abomasum and subcutaneous fats (Lourenco *et al.* 2007) suggesting greater duodenal flow of PUFAs. The feeding behavior of kids under TPS condition (eating tanniferous feeds) and lower rumen biohydrogenation might have led to more by-pass and intestine absorption of PUFAs consequently storing extra PUFAs in subcutaneous fat.

## CONCLUSION

Red meat produced under TPS conditions showed desirable nutritional merits in terms of fatty acid profile. Transhumant production system enriches the meat quality characteristics in terms of PUFAs. Under TPS condition, kids showed several nutritional merits over the lambs and calves with higher ALA, EPA and DPA. Furthermore, lower unhealthy FAs (C16:0+C14:0) and n-6/n-3 ratio was found in kids compared to lambs and calves presumably due to kid different feeding behaviour and rumen biohydrogenation. This study suggests that under TPS, goats might produce more promising healthy red meat compared to sheep and cattle.

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