Nutritional approaches to improve the fatty acid profile of milk fat in sheep and goats

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The trend of sheep and goat milk in 70 years (estimation on FAO data, 2017)

World sheep milk

13.1 Mtons; +25.6%

+7.8%/y

+3.8%/y


World goat milk

28 Mtons; +52.1%

+9.5%/y

+4.9%/y


Pulina et al., 2017 – ADSA annual meeting - Pittsburg
The demand for sheep and goat milk has grown worldwide over the years

- used to produce cheese and other traditional dairy products
- drinking milk and yogurt
- as component of infant formulas
- …nutraceutical product?
Sheep and goat milk are used for formula and follow-on formula for infants in different parts of the world. In European Union, goat milk can be used for formula and follow-on formula for infants (Directive 2013/46/EU).
Milk and dairy products of small ruminants have a high nutritional value...as sources of high-quality dietary proteins and fats
GOOD NEWS!

1. Intake of total fat and each type of fat (Saturated, MUFA and PUFA) was associated with lower risk of total mortality.
2. Higher saturated fat intake was associated with lower risk of stroke.
3. Total fat and saturated and unsaturated fats were not associated with risk of myocardial infarction or cardiovascular disease mortality.

Fatty acids beneficial for health in milk

**Alpha linolenic acid (C18:3 n3)**
decrease incidence of cardiovascular disease

prevention of dementia
*Yamagishi et al., 2017 - Clin. Nutr.*

**Branched chain FA (BCFA)**
anti-inflammatory properties in animal model

**Odd chain FA (OCFA)**
C15:0 and C17:0 markers of dairy fat intake in humans
*Albani et al., 2017*

inversely associated with CHD
*Khaw et al., 2012 - PLOS; Jenkins et al., 2015 - Molecules*

inversely associated with incidence of type 2 diabetes
Fatty acids beneficial for health [3]

**Vaccenic acid**

- suppresses intestinal inflammation
  (Jacome-Sosa et al. 2016 - J. Lipid Res.)
- does not affect cholesterol metabolism adversely compared to elaidic acid
  (Krogager et al. 2015 – Immunity)

**Conjugated Linoleic Acid**

- improve bone formation
  *(Kim et al., 2013 J Nutr Biochem.)*
- improve immune function
  *(Bassaganya-Riera et al., 2012 - Clin Nutr.)*
- glycemia control
  *(Carvalho et al., 2012 - Vasc Health Risk Manag.; Herrera-Meza et al., 2013 - Int J Food Sci Nutr.)*
- reduce body fat
  *(Onakpoya et al., 2012 - Eur J Nutr.)*
- anti-atherosclerosis activity
  *(Sofi et al., 2009; Pintus et al., 2012 – Br. J. Nutr.)*
- anticancer properties
  *(Ip et al. 1999 – Brit. Nutr.; Song et al. 2006 - Prostate J.; Chen et al. 2003 - World J. Gastroent.)*
## CLA isomer distribution in ruminant milk

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Composition (g/100g CLA)</th>
<th>Bovine</th>
<th>Caprine</th>
<th>Ovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-8, trans-10</td>
<td>&lt;0.01-1.70</td>
<td>&lt;0.01</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>cis-9, trans-11</td>
<td>65.6-88.9</td>
<td>62.1-75.1</td>
<td>80.0-80.9</td>
<td></td>
</tr>
<tr>
<td>cis-11, trans-13</td>
<td>&lt;0.01-0.23</td>
<td>0.16-0.69</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>cis-12, trans-14</td>
<td>&lt;0.01-1.06</td>
<td>0.00-0.13</td>
<td>1.69-1.83</td>
<td></td>
</tr>
<tr>
<td>trans-7, cis-9</td>
<td>2.63-9.49</td>
<td>4.57-11.7</td>
<td>5.96-6.08</td>
<td></td>
</tr>
<tr>
<td>trans-8, cis-10</td>
<td>&lt;0.01-2.33</td>
<td>1.85-3.48</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>trans-9, cis-11</td>
<td>&lt;0.01-3.93</td>
<td>&lt;0.01-4.21</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>trans-10, cis-12</td>
<td>&lt;0.01-1.61</td>
<td>&lt;0.01-0.90</td>
<td>0.55-0.57</td>
<td></td>
</tr>
<tr>
<td>trans-11, cis-13</td>
<td>0.06-9.33</td>
<td>0.22-0.48</td>
<td>2.14-2.38</td>
<td></td>
</tr>
<tr>
<td>trans-6, trans-8</td>
<td>&lt;0.01-1.40</td>
<td>0.12-1.91</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>trans-7, trans-9</td>
<td>0.02-2.80</td>
<td>0.42-1.08</td>
<td>0.40-0.42</td>
<td></td>
</tr>
<tr>
<td>trans-8, trans-10</td>
<td>0.19-0.67</td>
<td>0.36-1.47</td>
<td>0.34-0.42</td>
<td></td>
</tr>
<tr>
<td>trans-9, trans-11</td>
<td>1.31-3.23</td>
<td>2.99-5.77</td>
<td>1.40-1.60</td>
<td></td>
</tr>
<tr>
<td>trans-10, trans-12</td>
<td>0.31-1.40</td>
<td>0.76-4.16</td>
<td>0.53-0.85</td>
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<tr>
<td>trans-11, trans-13</td>
<td>0.89-6.00</td>
<td>0.58-1.14</td>
<td>3.04-3.18</td>
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<tr>
<td>trans-12, trans-14</td>
<td>0.35-3.55</td>
<td>0.72-1.90</td>
<td>1.90-2.20</td>
<td></td>
</tr>
<tr>
<td>trans-13, trans-15</td>
<td>&lt;0.01-0.16</td>
<td>&lt;0.01</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

Shingfield et al., 2007
The c9t11-CLA isomer is the main isomer in total CLA family in milk fat

Partial chromatogram from: Nudda et al. 2005
Ruminant dairy products are the main source of CLA in human diet

CLA content in milk of different species

CLA concentrations are the highest in milk from ruminants

Adapted from Jahreis et al., 1999; Roy and coll., 2005; Banni and Martin, 1998, Sebedio & Christie (eds.)
RUMEN BIOHYDROGENATION

Linoleic acid (n6) (18:2 cis9cis12)

Stearic acid (C18:0)

Vaccenic acid (C18:1 trans11)

Alpha-linolenic (n3) (18:3 c9c12c15)

CLA cis9trans11

(C18:1 trans11)

CLA cis9, trans11

(C18:3 cis9trans11cis15)
In the mammary gland part of vaccenic acid (C18:1 t11) is desaturated into c9t11 CLA by D9-desaturase enzyme.

It was estimated that ∼80% of milk fat c9t11 CLA originated from VA.

Mosley et al., 2006 - J. Nutr. 136:570-575
alpha-linolenic acid (C18:3\(\omega-3\)) the main FA in “green feeds”

<table>
<thead>
<tr>
<th>g/100g of fat</th>
<th>Concentrate</th>
<th>Silage</th>
<th>Pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>17.9</td>
<td>24.0</td>
<td>12.9</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.3</td>
<td>2.9</td>
<td>1.0</td>
</tr>
<tr>
<td>C18:1 c9</td>
<td>21.4</td>
<td>6.3</td>
<td>2.0</td>
</tr>
<tr>
<td>C18:2 (\omega6)</td>
<td>39.3</td>
<td>14.5</td>
<td>10.6</td>
</tr>
<tr>
<td>C18:3 (\omega3)</td>
<td>3.0</td>
<td>46.2</td>
<td>60.4</td>
</tr>
</tbody>
</table>

more grass…
more CLA and omega3 in milk
in Mediterranean areas the C18:3 n3 and CLA content in sheep milk is higher during the months of greater pasture availability

in Mediterranean areas the C18:3 n3 and CLA content in SHEEP MILK is higher during the months of greater pasture availability

c9,t11 CLA in sheep and goat milk produced from January to June-July in Italy and in Greece

Nudda et al., 2008 - Intern. Symp. SIPZOO

Tsiplakou et al., 2006 - Livest. Sci., 103:74-84.
The content of milk CLA was found higher in sheep than goats under the same pasture condition.
differences in feeding behavior

…. Differences between sheep and goats could be related to differences in feeding behavior (e.g. total intake, selectivity and timing) during grazing:
- goats selected more woody and shrub species and less herbaceous plants than sheep (Osoro et al., 2003; Ferereira et al., 2013)
- when fed on pasture goats had a markedly lower preference for legumes compared to grasses (Fedele et al., 1993)
a short-term cafeteria trials in goats showed a preference for grass flavor than legume flavor

goats have more frequent and smaller meals compared to sheep

Abijaoudé et al., 2000; Gorgulu et al., 2011 - Options Méditerranéen
CLA and C18:3 omega3 increase with pasture intake both in sheep and goats…but the increase is not linear.

*de Renobales et al. 2012 - Food Chemistry, 130: 90–96.*

The CLA in milk vary with the forage species in the pasture

The sulla (*H. coronarium*) based pasture increase markedly the C18:3-n3 in milk

![Bar chart showing C18:3n3 in different plants](chart.png)

Dry summer!
…when grass is not available?

what feeding strategies to increase CLA and omega 3 in milk?

use of vegetable or marine oil
### FATTY ACID PROFILE OF DIFFERENT VEGETABLE OILS

<table>
<thead>
<tr>
<th></th>
<th>soybean</th>
<th>Sunflower (high oleic)</th>
<th>safflower</th>
<th>Linseed</th>
<th>palm</th>
<th>olive</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0-C12:0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.5</td>
<td>9.0</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>2.3</td>
<td>3.7</td>
</tr>
<tr>
<td>C16:0</td>
<td>15</td>
<td>6.5</td>
<td>6.5</td>
<td>7.7</td>
<td>38.4</td>
<td>21.4</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.1</td>
<td>-</td>
<td>trace</td>
<td>0.2</td>
<td>2.3</td>
<td>3.7</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.1</td>
<td>2</td>
<td>3.9</td>
<td>4.6</td>
<td>38.7</td>
<td>4.1</td>
</tr>
<tr>
<td>C18:1c9</td>
<td>22</td>
<td>45.4</td>
<td>16</td>
<td>19.2</td>
<td>4.8</td>
<td>38.7</td>
</tr>
<tr>
<td>C18:1ct11</td>
<td>1.6</td>
<td>-</td>
<td>1</td>
<td>0.6</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>C18:2n6</td>
<td>48.9</td>
<td>46</td>
<td>72</td>
<td>15.8</td>
<td>8.1</td>
<td>19.6</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>7.7</td>
<td>0.12</td>
<td>0.5</td>
<td>50.9</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.7</td>
<td>0.3</td>
<td>0.3</td>
<td>0.9</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>C20:1n9</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Adapted by data of *Secchiari et al. 2003; **Chowdhury et al. 2007; ***Nudda et al., 2013;
CLA in milk of sheep supplemented with SOYBEAN oil (100 g/d per head)

CLA in milk of sheep supplemented with different doses of SUNFLOWER oil

CLA and C18:3 n3 in milk of indoor sheep fed extruded LINSEED

C9t11CLA

C18:3n3

200 g extruded linseed which supplied 70 g/day of fat per head

CLA and C18:3 n3 in milk of indoor goats fed extruded LINSEED

200 g extruded linseed which supplied 70 g/day of fat per head

The extent of CLA increase in GRAZING animals supplemented with vegetable oils is lower than indoor animals.

**GOAT**

Goats grazed 3hrs on pasture (ryegrass + white clover) in May-June. 200 g extruded linseed which supplied 70 g/day of fat per head.

**SHEEP**

Sheep grazed 3 h/d reygrass pasture + 400 g/d of alfalfa hay + 900 g/d of concentrate containing 100 g Linseed/kgDM.


CLA and C18:3 n3 in milk of sheep fed whole LINSEED

Whole untreated seed did not change significantly milk CLA content

Effect of Linseed on milk CLA in sheep and goats

CLA-sheep +156%

CLA-goat +119%

C18:3-sheep +135%

C18:3-goat +175%

Zhang et al., 2006a
Corredí et al., 2016
Cabiddu et al., 2017
Bodas et al., 2010
Zhang et al., 2006b
Mele et al., 2011
Manzo et al., 2011
Gómez-Cortés et al., 2014

2006
Nudda et al., 2008
Nudda et al., 2013
Enami et al., 2016
Bernard et al., 2016
Chilliard et al., 2003
Bernard et al., 2009
Effect of soybean and sunflower on milk CLA in sheep and goats

The concentrations of CLA increased with sunflower and soybean oil in both species.
Effect of marine oil supplementation to sheep

... Despite being poor in CLA precursors, marine oil is effective in increasing milk VA and CLA through the inhibition of the reduction of VA to 18:0 by rumen bacteria.

\[ \text{c9, t11 CLA} \]

Interaction between fish oil and plant oils in goats

$c_{9}, t_{11}$ CLA (g/100 g of fat)

When pooling data from different experiments, the milk CLA content increased as the content of fat supplemented in the diet rose.

**SHEEP**

\[ y = 0.02x + 0.67 \]
\[ R^2 = 0.78 \]

**GOATS**

\[ y = 0.017x + 0.77 \]
\[ R^2 = 0.58 \]
CLA in milk is transferred to cheese and ricotta

**SHEEP**

**GOATS**

<table>
<thead>
<tr>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
</tr>
</thead>
</table>

| March | Apr   | May | June |


Nudda et al. 2007 - J. Dairy Sci. (Suppl. 1)
### Mean composition of fatty acid (l% of fat) in bulk milk in Sardinia

<table>
<thead>
<tr>
<th></th>
<th>SHEEP</th>
<th>COW</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>69.1</td>
<td>70.4</td>
</tr>
<tr>
<td>UFA</td>
<td>30.9</td>
<td>29.2</td>
</tr>
<tr>
<td>MUFA</td>
<td>25.5</td>
<td>25.7</td>
</tr>
<tr>
<td>PUFA</td>
<td>5.5</td>
<td>3.05</td>
</tr>
<tr>
<td>C18:1 t11</td>
<td>2.43</td>
<td>-</td>
</tr>
<tr>
<td>C18:3 n3</td>
<td>0.92</td>
<td>-</td>
</tr>
<tr>
<td>CLA c9,t11</td>
<td>1.16</td>
<td>-</td>
</tr>
</tbody>
</table>

Milkoscan FT6000 (Foss, Denmark)

data from: Associazione Regionale Allevatori della Sardegna
**FA_GC vs FTIR**

**Goodness of fit statistics**

<table>
<thead>
<tr>
<th>FA</th>
<th>CORREL</th>
<th>RMSEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>0.895</td>
<td>1.729</td>
</tr>
<tr>
<td>MUFA</td>
<td>0.891</td>
<td>1.635</td>
</tr>
<tr>
<td>PUFA</td>
<td>0.901</td>
<td>0.627</td>
</tr>
</tbody>
</table>

Macciootta et al. (2017) ADSA meeting
Health claims might affect the dairy industry…

Unfortunately, the evidences are not sufficient to EFSA to give positive opinion on the CLA health claim that “reduce blood cholesterol" and therefore this claim has not been authorized by the European Commission.
STUDIES ON HUMANS WITH CLA ENRICHED SHEEP CHEESE
Effects of a dairy product (pecorino cheese) naturally rich in cis-9, trans-11 conjugated linoleic acid on lipid, inflammatory and haemorheological variables: A dietary intervention study

F. Sofi a,b,d,e,*, A. Buccioni c,e, F. Cesari a, A.M. Gori a,d,e, S. Minieri c,e, L. Mannini a, A. Casini b,e, G.F. Gensini a,d, R. Abbate a,e, M. Antongiovanni c,e

a Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Italy
b Regional Agency of Nutrition, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy
c Dipartimento di Scienze Zootecniche, Facoltà di Agraria, University of Florence, Italy
d Don Carlo Gnocchi Foundation, Onlus IRCCS, Impruneta, Florence, Italy
e Centro Interdipartimentale per la Ricerca e la Valorizzazione degli Alimenti (CeRA), University of Florence, Italy
Effects of Pecorino naturally rich in cis-9, trans-11 CLA on atherosclerotic biomarkers

200 g of cheese/week

Pecorino cheese
1.5% of CLA

Control cheese
0.2% of CLA

Effects on:

pro-inflammatory interleukine-6 and 8 (IL-6 and IL-8) and tumor necrosis factor (TNF)

involved in the origin of atherosclerosis

Sofi et al. 2010 Nutr Metab Cardiovasc Dis.
Intake of 200 g/week of Pecorino naturally rich in cis-9, trans-11 CLA reduced pro-inflammatory parameters of 35-40%.

Sofi et al. 2010 Nutr Metab Cardiovasc Dis.
Sheep cheese naturally enriched in α-linolenic, conjugated linoleic and vaccenic acids improves the lipid profile and reduces anandamide in the plasma of hypercholesterolaemic subjects

Stefano Pintus, Elisabetta Murru, Gianfranca Carta, Lina Cordeddu, Barbara Batetta, Simonetta Accessu, Danila Pisticci, Sabrina Uda, Maria Elena Ghiani, Marcello Mele, Pier Lorenzo Secchiaroli, Guido Almerighi, Paolo Pintus and Sebastiano Banni
Control cheese (CTRL) or CLA-enriched cheese (CLA) obtained by supplementing the diet of dairy ewes with extruded linseed

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>20.5</td>
<td>16.0</td>
</tr>
<tr>
<td>C18:1 cis 9</td>
<td>18.6</td>
<td>20.9</td>
</tr>
<tr>
<td>CLA c9,t11</td>
<td>0.84</td>
<td>2.5</td>
</tr>
<tr>
<td>18:3 n3 (ALA)</td>
<td>0.6</td>
<td>2.1</td>
</tr>
<tr>
<td>18:1 t11 (VA)</td>
<td>1.7</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Intake for 3 weeks of 90 g/d Pecorino with CLA (2.5%) decrease plasma total cholesterol and LDL by 10% in hypercholesterolemic patients

Control Cheese  CLA = 0.8%
CLA-enriched Cheese CLA = 2.5%

Intake of 90 g/d of enriched cheese Pecorino caused a strong and significant decrease in the plasma concentrations (of about 40 %) of the endocannabinoid called anandamide (AEA) with respect to both baseline and control cheese treatment.

Plasma concentrations of endocannabinoids: (A) anandamide (AEA) and (B) 2-arachidonoylglycerol (2-AG) at baseline (BASE) and after consumption of 90 g/d of the control cheese (CTRL) or enriched cheese (ENCH).

Conclusion

• FA composition of the milk of sheep and goats can be largely modified and improved by feeding

• Sheep and goats have a different behavior on pasture that could affect differently CLA, VA and omega3 content in milk.

• The responses of milk CLA to supplementation with various types of vegetable oils is different between sheep and goats

• Different feeding strategies should be used for sheep and goats to design the “required” milk FA profile
Conclusion

• Sheep seem to respond to the utilization of pasture with a larger increase in beneficial FA compared to goats.

• More research is needed to find the best forage species for goats grazing on pasture?

• More studies on human by using small ruminant dairy are necessary to support the health properties of CLA-enriched dairy products.

• …If approved by EU, the health claims of CLA in the dairy products label could help profitability of sheep and goat industries.
fat is good for health but is important to design milk with fat quality that does better!