Influence of feeding system, stage of lactation and genetic types on ?9-desaturase activity in caprine milk

Impemba G., Cifuni G.F., Di Trana A.

in

Priolo A. (ed.), Biondi L. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.).
Advanced nutrition and feeding strategies to improve sheep and goat

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 74

2007
pages 147-152

Influence of feeding system, stage of lactation and genetic types on Δ^9-desaturase activity in caprine milk

G. Impemba*, G.F. Cifuni and A. Di Trana***
*Dip. Scienze delle Produzioni Animali, Università degli Studi della Basilicata, 85100 Potenza, Italy
**Istituto Sperimentale per la Zootecnia, 85050 Bella, Potenza, Italy

SUMMARY – The objectives of the present study were to evaluate the influences of feeding system, stage of lactation and genetic types on the product/substrate ratio of fatty acids (C10:1/C10:0; C12:1/C12:0; C14:1/C14:0; C16:1/C16:0; C18:1/C18:0; CLA/C18:1t11) and on the Desaturation Index (DI) as indicators of Δ^9-desaturase activity in caprine milk fat. Experiment 1: Four groups of lactating goats were fed with the following diets: natural pasture (P), pasture plus maize and broad beans (PMB), pasture plus barley and chickpeas (PBC), and hay plus mixed grains (HG). The product/substrate ratios and DI were affected ($P<0.01$) by the nutritional treatments. In the PMB fed goats C10:1/C10:0 and C18:1/C18:0 ratios were higher than those observed in the other groups. The highest values for C14:1/C14:0; C16:1/C16:0; CLA /C18:1t11 ratios and DI were observed in P feeding system. Experiment 2: Milk samples from lactating goats were collected every fifteen days starting 85 DIM till 175 DIM. The stage of lactation significantly affects C10:1/C10:0, C14:1/C14:0, C16:1/C16:0 and C18:1/C18:0 ratios. No differences were found for CLA/C18:1t11 ratio and DI during lactation. Experiment 3: Milk samples were obtained from four different genetic types of goats (Maltese, Red Syrian, Girgentana and Cashmere) in mid lactation. Genetic type did not affect product/substrate ratios except for C10:1/C10:0; C12:1/C12:0 ratio. The Cashmere genetic type exhibited higher Δ^9-desaturase activity for C10:1/C10:0 and C12:1/C12:0 ratios than other breeds. In conclusion, product/substrate ratios and DI, parameters that indirectly measured Δ^9-desaturase activity, were affected by feeding system; only some ratios were influenced by stage of lactation; the genetic types affect only C10:1/C10:0; C12:1/C12:0 ratios.

Keywords: Δ^9-desaturase, goat milk, feeding system, stage of lactation, genetic types.

RESUME – *"Influence du système alimentaire, du stade de lactation et de différents types génétiques sur l'activité Δ^9-désaturase dans le lait de chèvre". Les objectifs de cette étude étaient d'évaluer les influences du système alimentaire, du stade de lactation et de différents types génétiques sur le rapport produit/substrat des acides gras (C10:1/C10:0; C12:1/C12:0; C14:1/C14:0; C16:1/C16:0; C18:1/C18:0; CLA/C18:1t11) et sur l'Index de Désaturation (ID) utilisé comme indicateur d'activité de la Δ^9-désaturase dans le gras du lait de chèvre. Expérience 1 : quatre groupes comparables de chèvres en lactation ont été alimentés selon les modalités suivantes : pâturage naturel (P), pâturage plus maïs et féverole (PMB), pâturage plus orge et pois chiche (PBC), foin et grains mélangés (HG). Les rapports produit/substrat et l'index de désaturation (ID) ont été affectés ($P<0,01$) par les traitements alimentaires. Dans le groupe PMB ont été enregistrées, pour le rapport C10:1/C10:0 et le rapport C18:1/C18:0, les teneurs les plus élevées en comparaison aux autres groupes. Les teneurs les plus élevées pour les rapports C14:1/C14:0; C16:1/C16:0; CLA/C18:1t11 et ID ont été observées dans le groupe P. Expérience 2 : les échantillons de lait de chèvre ont été collectés tous les quinze jours entre 85 et 175 jours de lactation. Le stade de lactation a influencé de manière significative les rapports suivants : C10:1/C10:0, C14:1/C14:0, C16:1/C16:0 C18:1/C18:0. Aucune différence n'a été trouvée pour CLA / C18:1t11 et ID au cours de la lactation. Expérience 3 : les échantillons de lait ont été obtenus de quatre types génétiques différents de chèvres (Maltais, Syrien, Girgentana et Cachemire) en milieu de lactation. Les types génétiques n'ont pas affecté de manière significative les rapports produit/substrat à l'exception de C10:1/C10:0 et C12:1/C12:0. Le type génétique Cachemire a montré l'activité la plus élevée de la Δ^9-désaturase pour les rapports C10:1/C10:0 et C12:1/C12:0 par comparaison aux autres types génétiques. En conclusion, les rapports produit/substrat et ID, paramètres qui sont capables de prédire l'activité de la Δ^9-désaturase, ont été influencés de façon significative par le système alimentaire, et seulement quelques rapports ont été influencés par le stade de lactation et les types génétiques.*

Mots-clés : *Δ^9-désaturase, lait de chèvre, système alimentaire, stade de lactation, types génétiques.*

Introduction

The enzyme Δ^9-desaturase is responsible for adding a *cis* double bound on the 9th carbon of fatty acids with chain lengths of 10 to 18 atoms of carbon. The activity of Δ^9-desaturase can be indirectly

measured from the product/substrate ratios of fatty acids dependant on the enzyme (Lock and Garnsworthy, 2003). Kay *et al.* (2002) proposed that the presence of C10:1c9, C12:1c9 and C14:1c9 in milk fat is almost solely dependent on the Δ^9-desaturase activity. Lock and Garnsworthy (2003) suggested that the C14:1/C14:0 ratio is the best indicator of Δ^9-desaturase activity due to all C14:0 being produced via *de novo* synthesis in the mammary gland, therefore, Δ^9-desaturase is responsible for the presence of C14:1 in milk fat. Polland *et al.* (1980) proposed that Δ^9-desaturase, preferentially, acts on longer fatty acids (C16:0 and C18:0 and *trans* fatty acids). Griinari *et al.* (2000) observed that about 75% of CLA in milk fat is produced in mammary gland by Δ^9-desaturase from C18:1t11. Factors affecting Δ^9-desaturase activity have not been extensively studied in dairy goats. It seems that genotype, stage of lactation (Kelsey *et al.*, 2003) and nutrition (Lock and Garnsworthy, 2003) may play a significant role in the regulation of Δ^9-desaturase activity. The Desaturation Index (DI), that represents a estimation of Δ^9-desaturase activity takes into account product/substrate of C14, C16 and C18 fatty acids (Malau-Aduli *et al.,* 1997). The objectives of the present study were to evaluate the influences of feeding system, stage of lactation and genetic types on the product/substrate ratios of fatty acids and on Desaturation Index as indicators of Δ^9-desaturase activity in caprine milk fat.

Material and methods

The study was conducted in an experimental farm at 360 m a.s.l. (40°'N; 15°E) in Southern Italy.

Experiment 1: The trial was conducted during spring season (April) on multiparous goats in mid lactation (96±6 DIM). Forty Red Syrian goats were allocated to four groups (10 goats for group) that were fed according to the following feeding regimen: (i) natural pasture (17.8% CP, 3.1% EE, 40.0% NDF) (Group P); (ii) natural pasture plus 550 g DM/head/day of maize and broad-beans (15.4% CP, 3.3% EE, 15.0% NDF) (Group PMB); (iii) natural pasture plus 550 g DM/head/day of barley and chickpeas (16.2% CP, 3.1% EE, 15.8% NDF) (Group PBC); and (iv) hay (10.0% CP, 2.8% EE, 48.9% NDF) plus 550 g/head/day of mixed grains (15.0% CP, 3.0% EE, 15.6% NDF) (Group HG). Goats were acclimatised to dietary treatments for 10 days and then a milk sample was collected from all goats.

Experiment 2: We used eight multiparous Red Syrian goats soon after the kids were weaned. This study began in April (85 DIM) and ended in July (175 DIM). Milk samples were obtained every fifteen days at 85, 100, 115, 130, 145, 160 and 175 days of lactation. The goats were kept indoors and fed with hay *ad libitum* plus 470 g DM/head/day of commercial supplement. The chemical composition (% DM) of feed was: hay (10.0% CP, 2.8% EE, 48.9% NDF) and commercial supplement (CP 14%, 3.1% EE, NDF 18%).

Experiment 3: In this trial, we used four different genetic types of multiparous goats: lactating Maltese (n = 12;), Red Syrian (n = 20), Girgentana (n = 12) and Cashmere (n = 12). All goats were allowed to graze on a natural pasture and they received hay *ad libitum* plus 470 g DM/head/day commercial supplement. Milk sample was collected from all goats on the daily milkings (88±7 DIM) on the same day.

All milk samples were stored at -20°C until fatty acids analysis. Milk lipids were extracted with chloroform and methanol (Bligh and Dyer, 1959). Fatty acids (FAs) in milk fat were converted into methyl esters and separated by gas chromatography (Varian 3800 with CP 8410 auto injector) equipped with a FID detector and a 60m x 0.25 mm (id) cyanopropyl polysiloxane (DB 23, J & W) fused silica capillary column. Operating conditions were previously reported (Di Trana *et al.,* 2004 b). The fatty acids peaks were identified using mixture of standard fatty acids (Sigma). The individual standard references of CLA isomers (*cis*-9*, trans*-11 97 % and *trans*-10*, cis*-12 3%) and *trans*-11 C18:1 was obtained from Larodan (Malmö, Sweden). FAs were expressed as percentage of fatty acid methyl ester (FAME). The Δ^9-desaturase activity has been calculated as product/substrate ratios of C10:1/C10:0; C12:1/C12:0; C14:1/C14:0; C16:1/C16:0; C18:1/C18:0 and CLA/C18:1t11 according to Thomson *et al.,* (2003). The Desaturation Index (DI) was calculated according to the formula (DI=100*(∑C14:1;C16:1;C18:1/∑C14:1;C16:1;C18:1;C14:0;C16:0;C18:0) of Malau-Aduli *et al.* (1997).

The statistical analysis of product/substrate ratios and DI were carried out by ANOVA procedure using SYSTAT statistical package (Systat, 1992) with a mono factorial model for Experiment 1 (effect of diet) and for Experiment 3 (effect of breed). The statistical analyses of Experiment 2 was carried

out by ANOVA procedure repeated measures with respect to days of lactation. Mean values were compared by Fisher's LSD test.

Results and discussion

Experiment 1

The product/substrate ratios of specific fatty acids are reported in Table 1. A significant effect of feeding system on C10:1/C10:0; C14:1/C14:0; C16:1/C16:0; C18:1/C18:0; CLA/C18:1t11 product/substrate ratios and on DI was found. The maximum C10:1/C10:0 ratio was detected in PMB group and the minimum in PBC group. The C14:1/C14:0 ratio was higher (P<0.01) in P group than other groups. This ratio (C14:1/C14:0) has been indicated as the optimal indicator of Δ^9-desaturase activity because all of the C14:0 in milk fat is produced via *de novo* synthesis in the mammary gland, consequently desaturation is the only source of C14:1 (Lock and Garnsworthy, 2003). The increase of Δ^9-desaturase activity in P group could be linked to the nutritional and hormonal control of Δ^9-desaturase activity (Ntambi, 1995). This trial was realised during the spring season when the fresh grass of pasture is rich in soluble carbohydrate which can stimulate insulin secretion. Insulin, indeed, seems to increase Δ^9-desaturase activity (Enser, 1979) and this observation may explain the increase in Δ^9-desaturase activity observed in P group. Pasture lipids were also rich in linolenic acid (Di Trana *et al.,* 2004a) which is considered the principal precursor of C18:1t11 (Harfoot and Hazlewood, 1988). The C16:1/C16:0 ratio ranged between 0.020 (P) and 0.015 (HG) indicating that there was a significant (P<0.001) difference between groups of goats that were fed fresh grass or dry forage. No difference was found between PMB and PBC groups. The feeding system significantly affected (P<0.01) the C18:1/C18:0 ratio; the lower product/substrate ratio was observed in PBC group than other groups. The C16:1/C16:0 and C18:1/C18:0 ratios could be affected by double origin of these FAs as diet and adipose tissue metabolism. No significant difference of CLA/C18:1t11 ratio was found between groups that fed fresh grass with or without concentrate supplementation (PMB, PBC and P). Di Trana *et al*. (2004c) observed a significant effect of concentrate supplementation type on some FAs and CLA. The lower content of polyunsaturated fatty acids in hay in comparison to pasture (Di Trana, not published data) could, partly, explain the higher CLA/C18:1t11 ratio in HG group. The DI was higher (P<0.001) in P (31.76) and PMB (30.75) than HG (28.64). Thomson *et al.* (2003) found effect of feeding regimen on product/substrate ratios in cows fed either pasture alone or pasture plus ruminally protected oilseed. Moreover, C10:1/C10:0, C14:1/C14:0, C16:1/C16:0 and CLA/C18:1t11 ratios were not affected by feeding regimen when cows utilized full fat rapeseed in comparison to pasture.

Table 1. Effect of feeding system on product/substrate ratios and on Desaturation Index (DI)

Product/substrate ratios	Feeding system				SE	Significance
	P	PMB	PBC	HG		
C10:1/C10:0	0.023^{ab}	0.025^{a}	0.021^{b}	0.024^{ab}	0.001	**
C14:1/C14:0	0.048^{a}	0.040^{b}	0.042^{b}	0.040^{b}	0.001	***
C16:1/C16:0	0.020^{a}	0.017^{b}	0.017^{b}	0.015^{c}	0.001	***
C18:1/C18:0	1.969^{ab}	2.135^{a}	1.752^{b}	2.008^{a}	0.065	**
CLA/C18:1t11	0.518^{ab}	0.475^{a}	0.494^{a}	0.622^{b}	0.030	**
DI	31.756^{a}	30.748^{ac}	29.959^{bc}	28.638^{b}	0.455	***

Means within a row with different superscripts are significantly different (P<0.05).
**: P<0.01; ***: P<0.001.

Experiment 2

The C10:1/C10:0, C14:1/C14:0, C16:1/C16:0 and C18:1/C18:0 ratios were affected by stage of lactation (P<0.01) (Fig. 1). There were no differences for CLA /C18:1t11 ratio and DI during the trial (P>0.05; Fig. 2). A significant increase of C10:1/C10:0 ratio from 85 DIM till 175 DIM was observed.

During lactation, C14:1/C14:0 ratio showed a mean value of 0.04. The lowest C14:1/C14:0 ratio ($P<0.05$) was observed at 130 DIM. The C16:1/C16:0 ratio decrease from 85 DIM till 160 DIM, then increase at 175 DIM. The C18:1/C18:0 ratio increase as lactation progressed. CLA /C18:1t11 ratio ranged between 0.55 and 0.76 indicating that there were no substantial differences during stage of lactation. The DI varying between 27.7 and 32.3 but no significant differences were found during lactation. Kelsey *et al*. (2003) observed a significant effect of the stage of lactation on C14:1/C14:0, C16:1/C16:0, C18:1/C18:0 and CLA /C18:1t ratios in a large number of dairy cows, while no effect of stage of lactation was detected by Stanton *et al*. (1997) on CLA levels in milk fat, product of Δ^9-desaturase. Finally, the effect of the stage of lactation was detected on DI by Secchiari *et al.* (2003) in Italian Friesian and Reggiana cows. During lactation, no substantial changes in fatty acids profile (CLA and monounsaturated FAs) were found in goats fed with hay *ad libitum* and supplement (Di Trana *et al.*, 2004b).

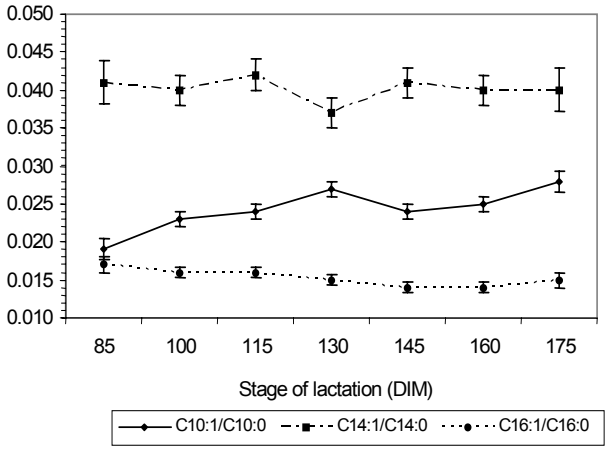

Fig. 1. Product/substrate ratios of fatty acids.

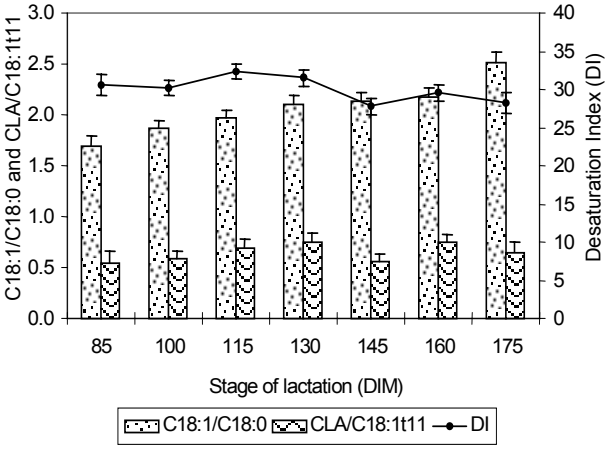

Fig. 2. Product/substrate ratios of fatty acids and DI.

Experiment 3

The C10:1/C10:0 ($P<0.05$) and C12:1/C12:0 ($P<0.01$) differed significantly amongst the breeds used in this trial (Table 2). The highest values of C10:1/C10:0 and C12:1/C12:0 was observed in Cashmere goats. There were no differences between the four genetic types for the other product/substrate ratios and for DI. In dairy cows a significant effect of breed on product/substrate ratio of C14:1/C14:0; C16:1/C16:0; C18:1/C18:0; CLA/C18:1t11 have been found by Kelsey *et al*. (2003) in a large number of Holstein (n=113) and Brown Swiss (n=106). Several studies have already examined breed effect on CLA content in milk fat that is considered a product of Δ^9-desaturase activity. Lawless *et al.* (1999) compared four breeds of dairy cows and they reported that breed had a small effect on CLA content in milk fat. White *et al.* (2001) found that Holstein cows had a slightly higher level of milk CLA than Jersey cows. However, Secchiari *et al.* (2001) observed no differences of CLA content in milk fat from Garfagnina and Massese sheep breeds fed with the same dietary regimen.

A breed x diet interaction on CLA produced was observed by Whitlock *et al.* (2002) in a study with Holstein and Brown Swiss cows. In agreement with our results Secchiari *et al.* (2003) didn't found effect of breed on DI comparing Italian Friesian and Reggiana dairy cows. Recently Kay *et al.* (2004) suggested that C10:1/C10:0 and C12:1/C12:0 ratios are not the best indicators of the Δ^9-desaturase activity because they observed C10:1 and C12:1 in milk fat of cows following sterculic oil treatment, indicating Δ^9-desaturase activity was not completely inhibited. In all experiments a positive and significant ($P<0.05$) correlation was found between the C14:1/C14:0 and C16:1/C16:0 ratios and between C10:1/C10:0 and C18:1/C18:0 ratios. A significant positive correlation was also found between C18:1/C18:0 and CLA/C18:1t11 ratios in the Experiments 1 and 2.

Table 2. Effect of different genetic types of goats on product/substrate ratios and on DI

Product/substrate ratios	Breed				SE	Significance
	Cashmere	Girgentana	Maltese	Red Syrian		
C10:1/C10:0	0.023[a]	0.018[b]	0.018[bc]	0.019[c]	0.001	*
C12:1/C12:0	0.027[a]	0.022[b]	0.021[b]	0.023[b]	0.001	**
C14:1/C14:0	0.044	0.041	0.047	0.048	0.002	ns
C16:1/C16:0	0.024	0.025	0.014	0.026	0.001	ns
C18:1/C18:0	1.802	1.729	1.753	1.820	0.065	ns
CLA/C18:1t11	0.728	0.732	0.739	0.720	0.011	ns
DI	30.626	33.496	32.526	32.921	0.836	ns

Means within a row with different superscripts are significantly different (P<0.05).
**: P<0.01, *: P<0.05; ns: not significant.

Conclusions

The present study indicate that feeding regimen affect all the product/substrate ratios between milk fatty acids and the DI. The physiological factor (stage of lactation) did not affect the entire range of product/substrate ratios and DI. An effect of breed on Δ^9-desaturase activity was observed only for the C10:1/C10:0 and C12:1/C12:0 ratios; Cashmere goats exhibited the highest value of these ratios. Positive associations between ratios were evident in all experiment.

References

Bligh, E. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol*. 37, 911-917.

Di Trana, A., Cifuni, G.F., Fedele, V., Braghieri, A., Claps, S. and Rubino, R. (2004a). Il sistema alimentare e la stagione influenzano il contenuto di CLA, omega-3 e acidi grassi trans nel latte di capra. *Progress in Nutrition,* 6(2): 109-115.

Di Trana, A., Cifuni, G.F., Impemba, G., Braghieri, A., Fedele, V., Claps, S. and Rubino, R. (2004b). CLA content and fatty acid profile in goat milk during lactation and in different feeding system. In: *8th International Conference on Goats*, Pretoria, South Africa, 4-9 July, [P.012], 38.

Di Trana, A. Cifuni, G.F., Fedele, V., Impemba, G., Braghieri, A., Claps, S. and Rubino, R. (2004c). Influenza del tipo di integrazione sul contenuto di CLA e di altri acidi grassi nel latte caprino. *Atti XVI Congresso SIPAOC*, Siena, 29 September - 2 October 2004, pp. 272-273.

Enser, M. (1979). The role of insulin in the regulation of stearic acid desaturase activity in liver and adipose tissue from obese-hyperglycaemic (ob/ob) and lean mice. *Biochem. J*., 180: 551-558.

Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V. and Bauman, D.E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta^9-desaturase. *J. Nutr*., 130: 2285-2291.

Harfoot, C.G. and Hazlewood, G.P. (1988). Lipid metabolism in the rumen. In: *The Rumen Microbial Ecosystem,* Hobson, P.N. (ed.). Ed. Elsevier Science Publications Ltd, London, UK, pp. 285-322.

Kay, J.K., Mackle, T.R., Auldist, M.J., Thomson, N.A. and Bauman, D.E. (2002). Endogenous synthesis and enhancement of conjugated linoleic acid in pasture-fed dairy cows. *Proc. New Zealand Society of Animal Production*, 62: 12-15.

Kay, J.K., Mackle, T.R., Auldist, M.J., Thomson, N.A. and Bauman, D.E. (2004). Endogenous synthesis of *cis*-9,*trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. *J. Dairy Sci*., 87: 369-378.

Kelsey, J.A., Corl, B.A., Collier, R.J. and Bauman, D.E. (2003). The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *J. Dairy Sci*., 86: 2588-2597.

Lawless, F., Stanton, C., L'Escop, P., Devery, R., Dillon, P. and Murphy, J.J. (1999). Influence of breed on bovine milk *cis*-9 *trans*-11 conjugated linoleic acid content. *Livestock Production Science,* vol 62: 43-49.

Lock, A.L. and Garnsworthy, P.C. (2003). Seasonal variation in milk conjugated linoleic acid and Δ^9 -desaturase activity in dairy cows. *Livestock Production Science,* 79: 47-59.

Malau-Aduli, A.E.O., Siebert, B.D., Bottema, C.D.K. and Pitchford, W.S. (1997). A comparison of fatty acid composition of triacylglycerols in adipose tissue from Limousin and Jersey cattle. *Aust. J. Agric. Res*., 48: 715-722.

Ntambi, J.M. (1995). The regulation of stearoyl-CoA desaturase (SCD). *Prog. Lipid Res*., 34: 139-150.

Polland, M.R., Gunstone, F.D., James, A.T., Lindsay, J. and Morris, L.J. (1980). Desaturation of positional and geometric isomers of monoenoic fatty acids by microsomal preparations from rat liver. *Lipids,* 15: 306-314.

Secchiari, P., Mele, M., Serra, A., Buccioni, A., Antongiovanni, M., Ferruzzi, G., Paletti, F. and Andreoli, L. (2001). Conjugated linoleic acid (CLA) content in milk of three dairy sheep breeds. *Progress in Nutrition*, 3: 37-42.

Secchiari, P., Mele, M., Serra, A., Buccioni, A., Paletti, F. and Antongiovanni, M. (2003). Effect of breed, parity, and stage of lactation on milk conjugated linoleic acid content in Italian Friesian and Reggiana cows. *Ital. J.Anim. Sci*., 2(Suppl. 1): 269-271.

Stanton, C., Lawelwss, F., Kjellmer, G., Harrington, D., Devery, J., Connoly, F. and Murphy, J. (1997). Dietary influences o bovine milk cis9trans11 conjugated linoleic acid content. *J. Food Sci.*, 62: 1083-1086.

Systat Statistics (1992). *Staitistics. Version 5.2 Edition*, Evanston, IL.

Thomson, N.A., Chand, A. and Kay, J.K. (2003). Predicting Δ^9-desaturase activity and association with conjugated linoleic acid (CLA) concentration in bovine milk. *Proceedings of New Zealand Society of Animal Production,* 63: 25-30.

White, S.L., Bertrand, J.A., Wade, M.R. and Washburn, S.P. (2001). Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a Total Mixed Ration. *J. Dairy Sci*., 84: 2295-2301.

Whitlock, L.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, R.J., Baer, R.J., Ramaswamy, N. and Kasperson, K.M. (2002). Fish oil and extruded soybeans fed in combination increase conjugated linoleic acid in milk of dairy cows more than when fed separately. *J. Dairy Sci*., 85: 234-243.