

**NUTRITIONAL AND TECHNOLOGICAL FACTORS IN ORDER TO OBTAIN
FUNCTIONAL FOOD ENRICHED WITH PUFA OMEGA 3 AND CLA AT SHEEP:
A REVIEW**

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Abstract

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The nutritional quality of animal products is a very important parameter, especially with regard to the links between food and health, a vital research field today. The international medical-scientific world now holds saturated fatty acids partially responsible for some diseases, especially those of a cardio-vascular nature, and ewes milk is particularly rich in these fatty acids (Ney, 1991; Strata, 2000; Martini, 2004). An increasing interest in enhancing polyunsaturated fatty acids (PUFA) Omega 3 (C 18:3, EPA, C 22:3, C 22:5 și DHA) and CLA (conjugated linoleic acids) content in food products is attributed to its potential anticarcinogenic, antidiabetic, antiobesity, antiatherogenic, and immunomodulatory functions in experimental animal models. CLA is synthesized in the rumen from linoleic acid or from the endogenous conversion of t-11 C 18:1 in the mammary gland by desaturase [Ip, S., et al. 2004]. More than a dozen isomers of CLA have been detected in foods of ruminant origin, of which c-9, t-11 comprising 80 to 90% (Khanal R., et al. 2004). The dairy products characteristics depend on a large number of factors linked to animal management systems: sheep breed, climatic conditions, feeding, stage of lactation, etc (Claps S. et. al. 2007; Mierlita, D., et al. 2009). A host of factors appear to affect the PUFA Omega 3 and CLA content in milk, meat, and other food products from various species of ruminant, animal breed, which could be broadly classified into diet, and post-harvest related factors. Although food products from sheep are the richest sources of PUFA Omega 3 and CLA for humans, it is possible to enhance the Omega 3 and CLA content of foods by supplementing fats, oils, and oilseeds can and, concentrate in the diet. The PUFA Omega 3 and CLA content in milk or meat varies from a 2 -11% respectively 1 – 3%.

Place and importance of poly-unsaturated fatty acids in maintaining the human health

In the human nutrition, fats have a bad reputation because they are associated with obesity, cardiovascular diseases and many other serious disorders. However, the researches initiated by *McLean* within the famous Harvard University showed that the poly-unsaturated fatty acids (PUFA) include a special category of fats named Omega – 3 fatty acids and conjugated linoleic acid (CLA = Conjugated Linoleic Acid) that are essential for the development of the human body and that must be provided through food because they are not synthesized in the body.

The family of the conjugated linoleic acid (CLA) designates a complex mixture of both isomerides and position isomerides that result in the

hydrogenation of the linoleic acid due to the microorganisms in the rumen (*Gunstone F.D., 2002*). The most important isomeric CLA is the acid rumenic, which represents over 90% of the milk's CLA (*Bougnoux P. et al., 2004*). CLA is available in the human food, mainly in the meat and milk of the ruminants.

The interest in Omega – 3 poly-unsaturated fatty acids and CLA compounds is due to their multiple physiological features that are manifested in the human nutrition:

- to **prevent cardiovascular diseases** (arterosclerosis, myocardial infarction, brain vascular accidents) (*Uany et al. 2000; Mena et al 2001; Millward 2003*), by reducing the thrombocytes' tendency to agglutinate at the level of the blood vessels avoiding the formation of atheroma plaquette and the appearance of thrombosis.

The statistics showed that the peoples of the nations inhabiting the shores of some water bodies or rivers live longer and their heart and blood vessels are healthier, because they consume fish that contain a high content of Omega – 3 PUFA almost every day.

M.Larocque et al. (2003) mentions that the patients that have had a diet rich in Omega – 3 poly-unsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) presented 50% less cases of lethal and non-lethal myocardial infarction, and in the case of the patients that have already suffered one myocardial infarction and that have a rich diet in Omega -3 PUFA and CLA, the cases of deceases decreased with 29%.

- to **protect and maintain the integrity of vascular endothelia**. By cholesterol esterification in the body with saturated and monosaturated hydrogenated fatty acids (of trans type), LDL is formed (bad cholesterol) and if the esterification is made with both Omega – 3 poly-unsaturated fatty acids and CLA results HDL (good cholesterol). Omega – 3 PUFA and CLA stimulate the oxidation of the bad cholesterol and diminish significantly the ratio LDL – HDL (*Bougnoux 1999, Ip 2004*).

- to prevent the obesity and its associated diseases due to their poor fat-formation capacity,. A study made on a group of volunteers (men and women) showed that the food supplementation with Omega – 3 PUFA and CLA for 12 weeks diminished the content of the lipids in the body with 4.1% (*Chardigny et al. 2000, Daiman et al. 2003*). The feed supplementation of lab animals (mice) with 1.3 g CLA (isolated from the cow milk) determined an increased modification of the body's chemical composition by stimulating the development of the muscles and reducing the quantity of fats in the body (*L. Rossant 2003*).

- to significantly diminish the development of the stomach, breast and skin tumors due to the **anticarcinogenic and antioxidant potential** of both Omega – 3 PUFA and CLA (*Parodi 2006*). These fatty acids enter into the

structure of the cell membranes' phospholipids where they act as antioxidant. The oxidative degradation at the level of cell membranes is considered the cause of the tumoral development; thus, Omega – 3 PUFA and CLA prevent the appearance of cancer due to its antioxidant action.

The capacity of Omega – 3 PUFA and CLA to inhibit the chemically-induced carcinogenesis (tumoral development) was proved in numerous experiments made on lab animals, the experimental lots presenting significantly less tumors in comparison with the witness lot, with 47% in the case of the breast cancer - *Scimeca Ip. 2004*; with 32-60% in the case of the stomach and colon cancer - *Santos et al. 2004*; *Chouinard et al. 2003*; *Baer et al. 2004*; *Abu-Gazaleh et al. 2004*; with 33% in the case of lung cancer – *Rose 2006*. In these experiments, Omega – 3 PUFA isolated from the fish fat and CLA isolated from the cow milk have been used.

- ***imuno-modulating action***; Omega – 3 PUFA and CLA stimulate the imunogenesis processes (especially IgA and IgM) and regulate the tolerance to glucose and hyperinsulinemia at diabetics (*Sugano 2004*).

- to ***intervene in the development of the children's nervous cells***. Knowing that there is a great quantity of Omega – 3 PUFA and CLA in the brain, an improvement of the brain blood flow and a greater quantity of serotonin in the brain of the piglets fed normally was noticed (serotonin stimulates the development of the axons and dendrites).

It is remarkable that the cow milk contains nine times less Omega – 3 PUFA and CLA in comparison with the human milk, *Rossant L. (2003)* explaining, this way, the better development of the brain and the higher level of intelligence in breast-fed children. A similar conclusion may be drawn from *Andrew's* researches (2004) who noticed that a low intake of Omega – 3 PUFA and CLA cause the increase of the depressive states (e.g. in New Zealand, 6% of the population suffer of depression in comparison with 1% in the case of the Japanese population, where the intake of Omega – 3 PUFA and CLA is four times greater).

In this context, the enrichment of the animal food products in Omega – 3 PUFA and CLA, respectively the achievement of the so-called “functional food products” (as *A. Meluzzi* named them in 2004) must represent a priority in the modern researches in Animal Science. Thus, at the Conference of the European Federation of Animal Sciences (2004), J.F. Hacquette highlighted the importance and in the same time the possibility to enrich the animal food products in Omega – 3 PUFA and CLA through genetic and nutritional methods.

Factors affecting PUFA Omega 3 and CLA (conjugated linoleic acid) content in milk and meat sheep.

Most studies referring to the factors that influence the structure and the polyunsaturated fatty acids (PUFA) content of the animal fat, were made on poultry and taurines; there were few research made on sheep

By resuming the data mentioned in the specialized literature results the fact that the factors that can influence the production, the structure and the PUFA content of existing fats in meat and milk on ruminants can be classified as follows:

I. Factors depending on the animal (***genetic and physiological factors***): species, breed, suckling stage, age and individuality.

The research regarding the structure and the polyunsaturated fatty acids (PUFA) content of the fat in ruminants aimed mainly young taurin subject to fattening, but the research made by *Patnowska SD et al. (2002)* demonstrated the fact that the fat from young ovines subject to fattening contains 20,6% more polyunsaturated fatty acids (PUFA) Omega – 3 in comparison to the fat from young taurin and 15,8% more PUFA Omega – 3 in comparison to the fat from kids, and the polyunsaturated fatty acids content is 14,6-18,3% lower (determinations were made m. longissimus dorsi).

The structure and the PUFA content of fat in the carcass of young ovines is influenced by the genotype. *Felton E.D. (2004)* mentions the fact that PUFA proportion in meat and subcutaneous fat is higher at Suffolk breed in comparison to Romney and Rambouillet, and *Enser M (2001)* finds a better quality fat at Welsh sheep in comparison to British Milkshoop. *Zs. Rozsa Varsegi et al. (2003)* get to the conclusion that the best fat regarding PUFA Omega – 3 and CLA content is at Merinos German and Suffolk breed.

Specialized literature does not mention papers from which results the influence of factors depending on the animal PUFA Omega – 3 and CLA content and structure from milk fat at sheep, but these kinds of research were made on milk cows (*Murphy J. 2001; Lawless P. 2003; Kelsey et al. 2003; Martin J. et al. 2002; Mierlita D et al. 2005*). The highest quantity of Omega – 3 and CLA in milk was registered at Montbeliardes breed (18,4 mg/g), and the lowest quantity at Bruna and Jersey breed (7,2-6,4 mg/g) (*Kelsey et al 2003*).

II. **Nutritional factors:** fodder type, structure and type of food ratio, fat supplement and saturation level.

The nutritional facts have the highest importance in handling PUFA Omega – 3 and CLA content and structure of animal fat (*Bencini-1997; Pulina-2000; Cooper-2004; Khanal-2003; Duckett-2001*).

In the fat from fodder plants unsaturated fatty acids, especially

unsaturated fatty linoleic and linolenic acids prevail, that on the level of rumen, under the action of enzymes, the microsymbiont products are hydrogenated. Over 50% of PUFA Omega – 3 from herbs are turned into stearic acid (saturated fatty acid prevailing in animal fat) (*Kay et al. 2003*).

The presence of food concentrate in the young ovines fodder subjected to fattening reduces the hydrogenation process in the rumen and increases the polyunsaturated fatty acids content (*Pannampalam et al. 2001*). In comparison with barley, the corn has a better influence on PUFA Omega – 3 and CLA content and structure of fat from muscular and adipose tissue (*Duket et al. 2004*). A similar effect has the lucerne hay on rousers (*Wachira et al. 2003*). Introduction of food concentrates and hay in suckling lambs alimentation led to an increase of fat proportion in the carcass, but the level of PUFA Omega – 3 and CLA increased within fat structure, from 5,43% to 7,24% (*Okeudo et al. 2004*).

Research regarding the utilization of fats as source of energy in the alimentation of young ovines subjected to fattening certifies the fact that fats having a low level of saturation can significantly improve PUFA Omega – 3 and CLA content and structure of fats. The highest level of PUFA Omega – 3 was found in the case of fish oil utilization (*Wachira et al. 2002*), but *Pannampalam et al. 2003* mention the fact that fish oil reduces ingestion DM and production performances. Among vegetal oils, seed oil has the highest level of PUFA Omega – 3 in meat, their level being 256% higher in comparison with witness lot (*Cooper S.L. 2004*).

Saponification of fats with a low saturation level has proved to be more efficient in protecting the polyunsaturated fatty acids against ruminal biohydrogenation and their transfer into the carcass fat (*Martz et al. 2005*). PUFA Omega – 3 and CLA content of carcass fat significantly increases if the fat supplement in the food is accompanied by 200 UI/day vitamin E addition (*Elmore 2002*).

Regarding the influence of nutritional factors on production, on PUFA Omega – 3 and CLA content and structure of milk fat, the research made on suckling sheep is relatively little (*Martini M. et al. 2004; Gargouri A et al 2005; Perez A et al. 2007*), and aim mainly at the modification of the production and structure in fatty acids of milk fat.

Most studies were made on milk cows. Research made by *Sloniewski K et al. (2004); Mierlita D. et al. (2004)* led to the conclusion that the milk from the cows kept on the pasture has a higher nutritional value in comparison with the milk obtained in house, because it has a significantly higher polyunsaturated fatty acids Omega – 3 and CLA content and a lower saturated fatty acids content. *Dhiman T.R. (2004) and Barbano S. (2005)* mention the fact that during pasturing period PUFA content of milk is higher in April – May (by 13,3-17,02 %) in comparison with September.

This modification of structure in fatty acids of milk fat, depending on the type of feeding or during pasturing period, dues to the level of cellulose from food, if we take into account *Palmquist et al. (2002)* conclusions who mention the fact that saturated fatty acids from milk result mainly from acetic acid (final product of rumenal and cellulose fermentation) whereas as the unsaturated ones (mono and polyunsaturated Omega – 3 and CLA) result mainly from plasmatic triglycerides (unsaturated fatty acids that escaped from rumenal biohydrogenation).

The research made on suckling sheep established the influence of ratio type and its content in nourishing substances on milk production and its content in fat and protein, without any reference to polyunsaturated fatty acids PUFA Omega – 3 and CLA content and structure of milk fat. Thus, the ratio very rich in concentrated carbohydrate led to a decrease of milk production and fat content; *Pulina and Rassu (2001)* established a relation between milk fat and NDF (*Neutral Detergent Fibre*) from the ratio (fat% = 4,59+0,05 NDF).

Different authors (*Perez H – 2002, Martini M – 2004; Gargouri A. 2005; Chiofalo P. – 2006*) mention the fact that the utilization of fats as an energetic supplement in suckling sheep feeding leads to an increase of the concentration of fats in milk, but in the same time decreases the protein quantity, affecting the efficiency of turning milk into cheese. Research made on milk cows proved the fact that the utilization of by-pass fats (protected against rumenal fermentation) doesn't influence the rumenal function, protein milk content and neither its coagulation properties (*Lundy F.P – 2004; Mason S. – 2004; Pamquist D.L. – 2003; Ryhanen El – 2005*). The protection of fats supplement in feeding against rumenal fermentation can be done by the treatment with calcium, formaldehyde or by high temperatures heating (*Mierlita D. – 2001*). Following the research made on milk cows, *Offer N.W. (2004)* showed the fact that protecting vegetal fats by treatment with calcium is more efficient than formaldehyde or high temperatures heating treatment, for a higher level of polyunsaturated fatty acids Omega – 3 and conjugated linoleic acid (CLA).

CONCLUSION

An increasing interest in enhancing polyunsaturated fatty acids (PUFA) Omega 3 (C 18:3, EPA, C 22:3, C 22:5 si DHA) and CLA (conjugated linoleic acids) content in food products is attributed to its potential anticarcinogenic, antidiabetic, antiobesity, antiatherogenic, and immunomodulatory functions in experimental animal models. CLA is synthesized in the rumen from linoleic acid or from the endogenous conversion of t-11 C 18:1 in the mammary gland by desaturase [Ip, S., et al. 2004]. More than a dozen isomers of CLA have been detected in foods of ruminant origin, of which c-9, t-11 comprising 80 to 90% (Khanal R., et al. 2004). The dairy products characteristics depend on a large number of factors linked to animal management systemsm sheep breed, climatic conditions, feeding, stage of lactation, etc (Claps S. et. al. 2007; Mierlita, D., et al. 2009). A host of factors appear to affect the PUFA Omega 3 and CLA content in milk, meat, and other food products from various species of ruminant, animal breed, which could be broadly classified into diet, and post-harvest related factors. Although food products from sheep are the richest sources of PUFA Omega 3 and CLA for humans, it is possible to enhance the Omega 3 and CLA content of foods by supplementing fats, oils, and oilseeds can and, concentrate in the diet. The PUFA Omega 3 and CLA content in milk or meat varies from a 2 -11% respectively 1 – 3%.

REFERENCES

1. Abu-Gazaleh F.; Lima E.L.; Menezes T.; Fisberg R. (2007) – Fatty acids and cardiovascular diseases: a review. *Rev. Nutr., Campinas*, 15(2): 73-80.
2. Addis, M., A. Cabiddu, G. Pinna, M. Decandia, G. Piredda, A. Pirisi and G. Molle, (2005) – Milk and cheese fatty acid composition in sheep fed mediterranean forages with reference to conjugated linoleic acid cis-9, trans-11. *J. Dairy Sci.* 88: 3443-3454
3. Allred L.; Dhiman T.; Anand R.; Satter L.D. Pariza M.W. (2004) – Conjugated linoleic acid content of milk from cows fed different diets. *J. Dairy Sci.*; 84:2146-2156.
4. Andrade, P.V.D., Schmidely, Ph., (2006) – Influence of percentage of concentrate in combination with rolled canola seed on performance, rumen fermentation and milk fatty acid composition in dairy goats. *Livestock Science*.
5. AOAC, (1990) – Official Methods of Analysis of the AOAC. 15th ed., Association of Official Analytical Chemists, Arlington, VA, USA.
6. Barbano S. Chilliard, Y, Ferlay A.; Loor J; Martini B (2005) – Trans and conjugated fatty acids in milk from cows and goats consuming pasture or receiving vegetable oil or seed. *Ital. J. Anim. Sci.* 1: 243-254.
7. Bocquier F.; Caja G. (2005) – Production et composition du lait de brebis: effets de l'alimentation. *INRA Prod. Anim.* P: 129-140.
8. Buccioni, A., S. Rapaccini, S. Minieri, M. Antongiovanni, (2007) – Quality of lipid fraction in Tuscan sheep cheese (Pecorino Toscano DOP). *Ital. J. Anim. Sci.* Vol. 6 (suppl. 1), p: 539-541.
9. Chilliard, Y., A. Ferlay, J. Rouel, and G. Lamberet (2003) – A review of nutritional and physiological factors affecting goat milk synthesis and lipolysis. *J. Dairy Sci.* 86: 1751 – 1770.
10. Chin, S. F., W. Liu, J. Storkson, Y. L. Ha, M. V. Pariza (1992) – Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogenes. *J. Food Comp. Anal.* 5: 185 – 197.
11. Chouinard P.Y.; Corneau L.; Butler W.R.; Chilliard Y (2007) – Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. *J. Dairy Sci.*, 92: 680-690.
12. Claps, S., Annicchiarico, G., Taibi, L., Cifuni, G.F., Trana, A., Pizzillo, M., (2007) – Effect of sheep breed on milk and cheese characteristics. *Special Issue of the Intern. Dairy Federation 0801/Part 4*, p. 288-290.
13. Cooper S. L.; L.A. Sinclair; R.G. Wilkinson; K.G. Hallett; M. Enser (2004) – Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *Journal Anim. Sci.* 82:1461-1470.
14. Dhiman, T., G. R. Anand, L.D. Satter, and M.W. Pariza (1999) – Conjugated linoleic acid content of milk cows fed different diets. *J. Dairy Sci.* 82: 2146 – 2156.
15. Duckett S; Andrae J.; Owens F.N. (2004) – Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. *J. Anim. Sci.* 85:3353-3360.
16. Enser M; Scollan N.D.; Choi J.; Kurt E. ; Hallett (2007) – Effect of dietary lipid on the content of conjugated linoleic acid (CLA) in beef muscle. *Anim. Sci.* 69:143-146.
17. Gargouri A., (2005) – Production et composition du lait de brebis: effets de l'apport de lipides proteges. *Rev. Elev. Med. vet.*, 58 (3): 183 – 190.
18. INRA, (1989) – Ruminant nutrition. Recommended allowances and feed tables. Institut National de la Recherche Agronomique ed., Paris, France.
19. Ip S., Millward S.; McGuire M.A.; McGuire M.K. (2004) – Conjugated linoleic acid (CLA) and polyunsaturated fatty acids Omega 3: A ruminant fatty acids with beneficial effects on human health. *J. Food Comp. Anal.* 5: 185-197.

20. Janice R.H., 2006 – Dietary fat and cholesterol. Division of Agr, Sci. and Natural Resources-Oklahoma State University.
21. Khanal R.C., Olson K.C., 2004 – Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: a review. *Pakistan Journal of Nutrition* 3(2): 82-98.
22. Kukuk O., Hess B.W., Ludden P.A., Rule D.C. (2001) – Effect of forage/concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. *J. Anim. Sci.* 79: 2233 – 2240.
23. Martini M., C. Scolozzi, D., Gatta, F., Taccini, P., Verita, (2004) – Effects of olive oil calcium soaps and phase of lactation on the fatty acid composition in the milk of Massese ewes. *Ital. J. Anim. Sci.*, 3: 353 – 362.
24. Mele M., A. Buccioni, F. Petacchi, A. Serra, S. Banni, M. Antongiovanni, P. Secchiari (2006) – Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition from Sarda ewes. *Anim. Res.* 55: 273 – 285.
25. Mele M., A. Serra, A. Buccioni, G. Conte, A. Pollicardo, P. Secchiari (2008) – Effect of soybean oil supplementation on milk fatty acid composition from Saanen goats fed diets with different forage:concentrate ratios. *Ital. J. Anim. Sci.*, vol. 7, 297 – 311.
26. Mele, M., A. Serra, G. Conte, A. Pollicardo, M. Del Viva, P. Secchiari (2007) – Whole extruded linseed in the diet of dairy ewes during early lactation: effect on the fatty acid composition of milk and cheese. *Ital. J. Anim. Sci.* Vol. 6 (suppl. 1), p: 560-562.
27. Mierlita D.; F. Beteg, T. Hebein, Gh. Salajan (2004) – Research concerning the ruminal stability of fats treated with Ca salts (by-pass) used in cows fodder and influence on the fermentation and ruminal metabolism. *Buletinul USAMV, Cluj-Napoca, seria Zooteh. Si Bioteh. Agricole*, vol. 57, p: 258-262.
28. Mierliță, D., Maurescu, Cristina; Dărăban, St., Lup, F., (2009) - Effects of Genetic Variability and Week of Lactation for Milk Yield on Milk Fatty Acid Profile in Dairy Ewes. *Bulletin UASVM*, nr. 66 (1-2)/2009.
29. Newman R.E., W.L. Bryden; E. Fleck, (2005) – Dietary n-3 and n-6 fatty acids alter the molecular species profile of avian breast muscle phospholipids. *Journal of Nutrition Asia Pacific*, 10(supl.): 78-84.
30. Ney D.M., (1991) – Symposium: the role of the nutritional and health benefits in the marketing of dairy products. *J. Dairy Sci.* 74: 4002 – 4012.
31. Nudda, A., G. Battacone, S. Fancellu, G. Pulina, (2007) – Seasonal variation of vaccenic acid, conjugated linoleic acid and n-3 fatty acids of goat's milk fat and their transfer to cheese and ricotta. *Special Issue of the Intern. Dairy Federation 0801/Part 4*, p. 336-338.
32. Onneti S.G.; Shaver R.D.; McGuire M.A.; Palmquist D. (2004) – Effect of supplemental tallow on performance of dairy cows fed diets with different corn silage:alfalfa silage rations. *J. Dairy Sci.* 88:632-641.
33. Palmquist D.L.; St-Pierre N.; McClure E (2007) – Tissue fatty acid profiles can be used to quantify endogenous rumenic acid synthesis in lambs. *Journal of Nutrition*, p:2407-2414.
34. Parodi P. (2006) – Conjugated linoleic acid in food. In J. Sebedio, W.W. Christie, and R. Adolf (ed) *Advances in Conjugated Linoleic Acid Research*, vol. 2, pp: 101-121. AOCS Press, Champaign, IL.
35. Perez H., Cavalcanti D.S., Martinez M., Fernandez M. Perez A. (2002) – Olive fatty acids in the diets of Manchega dairy ewes: effects on digestibility and production. *J. Dairy Sci.* 83:3316-3324.
36. Pulina G.; Bencini Roberta (2006) – The quality of sheep milk: a review. *Int. J. of sheep and wool Sci.*, vol 54(3), 182-220.
37. SAS, (1999) – Users Guide: Statistics. SAS Institute Inc., Cary, NC, USA.

38. Schmidely P., D., Sauvant (2001) – Taux butyreux et composition de la matière grasse du lait chez les petits ruminants: effets de l'apport de matières grasses ou d'aliment concentré. *INRA Prod. Anim.*, 14 (5): 337 – 354.
39. Schmidely P.; Sauvant D. (2005) – Fat content yield and composition of milk in small ruminants: effects of concentrate level and addition of fat. *INRA Prod. Animales*, p: 348-353.
40. Secchiari P., Mele, M., Serra, A., Andreotti L. (2001) – Conjugated linoleic acid (CLA) content in milk of three dairy sheep breeds. *Prog. Nutr.* 3 (4): 37 – 42.
41. Stockdale C.R.; Walker G.P.; Wales W.P.; Dalley D.E.; Sheen Z (2006) – Influence of pasture and concentrates in the diet of grazing dairy cows on the fatty acid composition of milk. *J. Dairy Res.* 76:267-276.
42. Strata, A., (2000) – Alimentazione e salute. Scenario attuale e prospettive future. *Prog. Nutr.* 2 (1): 3-11.
43. Tanmahahasamut P., J. Liu, L.B. Hendry, N. Sidell, (2004),: Conjugated linoleic acid blocks estrogen signaling in human breast cancer cells. *J. Nutr.*, 134: 674-680.
44. Tomasz M.; Junkuszew A.; Lipiec A., Lipecka Cz. (2007) – Composition of fatty acid of muscle tissue of lambs fed feedstuff supplemented with flax seeds. *Arch. Tierz.* 49, Special Issue, 181-185.
45. Van Soest, P.J., Robertson, J.B., Lewis, B.A., (1991) – Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583 – 3597.
46. Wachira A.M.; Sinclair L., Wilkinson R.G.; Hallet K.; Enser M.; Wood J.D. (2003) – Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. *J. Agric. Sci.* 135:419-428.