Effects of administration of three different by-pass lipids on growth performance, rumen activity and feeding behaviour of beef cattle

Stefano L. Vandoni, Vittorio Dell’Orto, Carlo A. Sgoifo Rossi
Dipartimento di Scienze e Tecniche veterinarie per la Sicurezza alimentare, Università di Milano, Italy

Abstract
A study was carried out on beef cattle to compare three different by-pass lipids administration in relation to their intake, performance, bunk behaviour and rumen fermentation characteristics. Ninety-six Charolaise males were subdivided into three groups. Each group was fed a specific diet including differentiated sources of rumen by-pass fats. The first group received 500 g/head/day of calcium salts (CaS), the second and the third received 420 g/head/day of hydrogenated fatty acids (HF) and triglycerides (AL), respectively. The three by-pass fats differ in fatty acids composition, chain length and mean particle size. AL group had average daily gain significantly greater (P=0.0005) than animals fed calcium soaps. No difference was observed between AL and HF groups (P=0.08) and between HF and CaS groups. Final body weights of AL animals were significantly higher (P=0.005) than CaS group. There were no differences between AL group and HF group and between HF group and CaS group. Average dry matter intake (DMI) was higher (P<0.05) in AL and HF groups compared with CaS group. Feed conversion rate was better for AL and CaS groups (P<0.05) compared with HF group. Evaluating their behaviours in approaching feed, the animals seemed to prefer hydrogenated fats flavour as regard to calcium soaps. No differences were highlighted in ruminal pH and ruminal volatile fatty acids composition among the three groups. When high dosage of fat is included into a beef cattle diet, the administration of hydrogenated triglycerides, characterized by a pleasant flavour and a small mean particle size, is likely to be more suitable than adding calcium soaps or hydrogenated free fatty acids, probably thanks to its higher DMI and feed conversion rate, respectively. With regard to the latter higher rate, it could be only supposed that it is related to the smaller mean particle size of hydrogenated triglycerides.

Materials and methods
Three diets, including different sources of rumen by-pass fats (RBF) and given as total mixed ration (TMR), were administered to three different groups of beef cattle every morning at 09.00 h. During the experimental period one group received an average amount of 500 g/head/day of calcium salts of long chain fatty acids (CaS), the second (HF) and third (AL) groups received an average amount of 420 g/head/day of hydrogenated free fatty acids (FFA) and hydrogenated triglycerides, respectively. Chemical characteristics of diets are reported in Table 1.

Animals and experimental design
Ninety-six Charolaise males (536.8±58.8 Kg live weight) were used. The animals were divided in three groups of 32 subjects each, and allocated to one of the three dietary treatments. At the beginning of the experiment the animals were housed in slatted pens according to their live weight (LW). Each group was split into four pens containing eight animals each. The animals did not leave their respective pen for the whole study period.

During one month before the experimental period beginning, all groups received the same free-fats diet, so that cattle were not used to any specific fat flavour.

Animal bunk behaviour
Animal bunk behaviour was registered daily for 10 consecutive days prior to day 0, for 7 consecutive days after trial start and every 6 days thereafter. Mean numerousness of animal at manger immediately after feed administration and animal feeding approach were recorded by six specifically instructed operators (two for each group, changing group every day). Feeding behaviours were evaluated considering two characteristic behaviours that bulls usually show when they approach an unpleasant or new flavour in feed, i.e. sniffing and digging the diet before eating it. After feed administration, a fifteen-minute check of
### Table 1. Diet composition.

| Chemical characteristics | AL Diet* | HF Diet* | CaS Diet* |
|--------------------------|----------|----------|-----------|
| Concentrate*             | 17.82%   | 17.82%   | 17.78%    |
| Corn                     | 10.89%   | 10.89%   | 10.86%    |
| Beet pulp                | 9.90%    | 9.90%    | 9.88%     |
| Straw                    | 3.96%    | 3.96%    | 3.95%     |
| Corn silage              | 54.40%   | 54.46%   | 54.32%    |
| Vitamin-mineral premix   | 0.64%    | 0.64%    | 0.64%     |
| Rumen by-pass fats       | 2.32%    | 2.32%    | 2.57%     |

**Dry matter basis**

| Ingredient            | AL   | HF   | CaS  |
|-----------------------|------|------|------|
| CP, %                 | 14.43| 14.41| 14.52|
| NDF, %                | 33.89| 34.32| 33.31|
| ADF, %                | 20.78| 20.83| 20.65|
| EE, %                 | 6.33 | 6.19 | 6.23 |
| NFC, %                | 41.11| 39.50| 40.41|
| Starch, %             | 32.67| 32.70| 32.74|
| Ash, %                | 4.95 | 5.58 | 5.53 |
| Meat, UF/kg           | 1.04 | 1.04 | 1.04 |

**Chemical composition**

- AL = Hydrogenate triglycerides; CaS = Calcium soaps; HF = Hydrogenated free fatty. DM = 88.24%; UF/kg DM 1.02; CP 28.91%; CF 13.1%; EE 3.80; NFC 84.55:11.45.

**Iodine number**

| Fats:Ca                     | AL   | HF   | CaS  |
|-----------------------------|------|------|------|
| 8:0                         | 0.61 | 0.09 | 0.04 |
| 10:0                        | 0.51 | 0.09 | 0.05 |
| 12:0                        | 4.38 | 0.96 | 0.55 |
| 14:0                        | 2.25 | 1.65 | 1.32 |
| 15:0                        | 0.05 | 0.07 | 0.06 |
| 16:0                        | 38.06| 49.30| 47.90|
| 17:0                        | 0.12 | 0.12 | 0.10 |
| 18:0                        | 32.39| 43.59| 45.1 |
| 20:0                        | 0.67 | 0.34 | 0.32 |
| 22:0                        | 0.39 | 0.04 | 0.05 |
| 24:0                        | 0.13 | 0.03 | ---  |
| 16:1                        | ---  | ---  | 0.22 |
| 17:1                        | ---  | ---  | 0.03 |
| 18:1                        | 0.40 | 3.64 | 35.87|
| 20:1                        | ---  | ---  | 0.13 |
| 18:2                        | 0.04 | 0.08 | 8.58 |
| 18:3                        | ---  | ---  | 0.27 |

*AL = Hydrogenate triglycerides; CaS = Calcium soaps; HF = Hydrogenated free fatty acids.

### Table 2. Chemical and physical characteristics of AL, HF and CaS.

| Chemical characteristics | AL* | HF* | CaS* |
|--------------------------|-----|-----|------|
| Fats:Ca                   | --- | --- | 84.55:11.45 |
| Iodine number             | 5   | 13.1| 26.3 |
| % of fatty acids          |     |     |      |
| 8:0                       | 0.61| 0.09| 0.04 |
| 10:0                      | 0.51| 0.09| 0.05 |
| 12:0                      | 4.38| 0.96| 0.55 |
| 14:0                      | 2.25| 1.65| 1.32 |
| 15:0                      | 0.05| 0.07| 0.06 |
| 16:0                      | 38.06| 49.30| 47.90 |
| 17:0                      | 0.12| 0.12| 0.10 |
| 18:0                      | 32.39| 43.59| 45.1 |
| 20:0                      | 0.67| 0.34| 0.32 |
| 22:0                      | 0.39| 0.04| 0.05 |
| 24:0                      | 0.13| 0.03| ---  |
| 16:1                      | --- | --- | 0.22 |
| 17:1                      | --- | --- | 0.03 |
| 18:1                      | 0.40| 3.64| 35.87 |
| 20:1                      | --- | --- | 0.13 |
| 18:2                      | 0.04| 0.08| 8.58 |
| 18:3                      | --- | --- | 0.27 |

*AL = Hydrogenate triglycerides; CaS = Calcium soaps; HF = Hydrogenated free fatty acids.

### Animal performance measurement

Animals were weighed individually (PM 1500 super, Marechalle-Pesage, France) at 07:00, before morning feed administration, the day before starting fat administration (day 0) and 48 days after the beginning of the trial (day 48).

The amount of TMR administered and orts were recorded daily for each pen. The experimental conditions did not allow recording individual feed intake. Feed conversion rate (FCR) was evaluated considering average daily gain (ADG) and dry matter intake (DMI).

### Diet sampling and analysis

Samples of TMR diets were collected at the beginning of experimental period. Chemical composition of diets was determined following the European Union standard methods for starch (European Commission, 1999), ether extract (European Commission, 1998) and ash (European Commission, 1981). Determination of neutral detergent fibre (NDFom) and acid detergent fibre (ADFom) were performed according to Van Soest et al. (1991) with amylase and sodium sulphite and expressed exclusive of residual ash. Nitrogen content was determined using the official Italian method.

By-pass fats were analyzed for iodine number (UNI EN ISO 3961:1999) and fatty acid composition (ISO 5508:1990). Fats:calcium ratio of calcium soaps was determined using official European Community method (European Commission, 1998).

### Fats mean particle size analysis

Particle size distribution of fat supplements was determined by mechanical shaking the fats through dry sieves. The mean particle size was calculated according to ANSI/ASAE S319.3 feb.2003/ASABE Standard 2006.

### Rumen liquor sampling and analysis

Rumen fluid samples (~300 mL) were collected approximately 11 hours after feeding on d 0 and d 48 of the experimental period. Samples were collected from 12 bullocks per group with a ruminal probe (SELEKT, Pharmalett, Kolding, DK). The first 500 mL of collected fluid were discarded to avoid saliva contamination. Sample collection was performed in the pens to ensure minimal animal stress. Rumen fluid pH was measured immediately after collection with a digital portable pH meter (Hishi023, Hanna Instruments, Padova, Italy). An aliquot of 4 mL of each rumen fluid sample was preserved with 1 mL of a solution consisting of 2 g/L mercuric chloride, 2 g/L orthophosphoric acid, and 0.002 mg/L 4-methylvaleric acid, and frozen immediately (Juany, 1982). Volatile fatty acids (VFA) (C2-C5) were subsequently determined using gas chromatography (Fussell and McCalley, 1987). Due to high toxicity of mercuric chloride, it was treated and disposed as special waste, according to National laws in force. A second aliquot (4 mL) of each rumen fluid sample was collected and preserved with 4 mL 0.2 N HCl (Channey and Marbach, 1982) and frozen. The concentration of ammonia nitrogen (N-NH3) was subsequently determined using the Kjeldhal method (Italian Government, 2004).

### Statistical analysis

Statistically significant differences between
treatments were determined by analysis of variance (ANOVA) using the GLM procedure (SAS, 2001).

For the analysis of live weight and average daily gain data, each animal was treated individually and data were adjusted by covariance analysis using initial weight as a covariate.

The general model for weight and average daily gain was:

\[ Y_{ijkl} = \mu + T_i + bX_j + B_k(T_i) + \varepsilon_{ijkl} \]

Where:
- \( Y_{ijkl} \) = the dependent variable;
- \( \mu \) = overall mean;
- \( T_i \) = effect of treatment \( i (i=1,\ldots,3); \)
- \( X_j \) = effect of starting weight \( j (j=1,\ldots,96); \)
- \( b \) = coefficient of regression;
- \( B_k(T_i) \) = the fixed effect of pen within treatment; \( \Sigma \) = residual error.

Since there was no evidence (\( P>0.05 \)) of starting weight effect and of pen within treatment effect for ADG, these terms were not included in the statistical model.

Due to impossibility of recording individual feed intake, statistical analysis of feed intake and FCR were carried out considering a pen as an experimental unit using the GLM procedure of SAS (SAS, 2001). The general model for feed intake and FCR was:

\[ Y_{ijk} = \mu + T_i + B_j + e_{ijk} \]

Where:
- \( Y_{ijk} \) = the dependent variable, \( \mu \) = the overall mean; \( T_i \) = the fixed effect of the \( i \)th treatment; \( B_j \) = the fixed effect of the \( j \)th box; and \( e_{ijk} \) = the random residual error.

Animal bunk behaviour was not statistically analyzed, thus only incidence of animals showing a specific behaviour are reported.

### Results and discussion

Experimental diets are shown in Table 1; they only differed as to the kind of RBF administered. Fatty acid composition of dietary fat sources is reported in Table 2. AL and HF were similar in fatty acids composition, while CaS contained a higher percentage of unsaturated fatty acids (45.1% CaS vs. 0.44% AL vs. 3.72% HF).

The particle size distribution for the fat sources used in our experiment is shown in Table 3. The three RBF differ in their particle size. In particular, AL was characterized by a mean particle size of 0.183 mm ranging from 0.63 to 0.125. HF and CaS had similar mean particle size (0.54 vs. 0.58 mm), and differ in their sieve distribution. HF ranged from 1 to 0.125 mm, while CaS had size bigger than 1 mm for 41.6% of all particles.

Bunk behaviour analysis (Figures 1, 2 and 3) of the week prior to day 0 did not indicate

---

### Table 3. Particle size of the three rumen-bypass fats.

| Sieve size | AL* | HF* | CaS* |
|------------|-----|-----|------|
| 500**.84, mm | --- | --- | 6.25 |
| 2 | --- | --- | 15.32 |
| 1 | --- | 4.85 | 19.69 |
| 0.8 | 31.50 | 8.57 |
| 0.63 | 25.43 | 6.87 |
| 0.4 | 26.42 | 14.46 |
| 0.25 | 9.24 | 12.01 |
| 0.125 | 2.27 | 10.93 |
| Bottom | 0.28 | 5.89 |
| Mean particle size, mm | 0.183 | 0.542 | 0.584 |

*AL = Hydrogenate triglycerides, CaS = Calcium soaps, HF = Hydrogenated free fatty acids. Weight of fat particles remaining on the screen after sieving divided by total weight of fat particles X 100. °Calculated according to ANSI/ASAE S319.3 Feb. 2003/ASABE Standard 2006.

---

Figure 1. Animals not present at manger after feed administration.

Figure 2. Animals sniffing ration.

---
Numerousness of animals not at manger immediately after feed administration was similar for HF and CaS groups and differed in a marginal way for AL group. Similarly, no animal showed specific behaviours in approaching feed, like sniffing or digging. As expected, after the RBF supplementation the animals showed a sort of wariness, demonstrated by an increased number of them sniffing and digging ration before eating. As a consequence of lower acceptability of calcium soaps compared with other fats sources (Allen, 2000), sniffing and digging behaviours were highly manifested in CaS group, particularly during the first week, in which a maximum of 50% of the animals showed them. The same behaviours counted less than 10% of the animals in AL group and less than 20% in HF group. After an adaptation period, the incidence of such behaviors decreased to less than 10% in AL and HF group but not in CaS group.

Numerousness of animals not at manger after feed administration confirm ed the results obtained from the evaluation of feeding approach behaviors. In fact, numerousness of animals not present at manger increased to a maximum of 80% in CaS group, while in AL and HF groups it got 35% and 45% respectively. The different acceptability of diets persisted also after day 19, when AL and HF groups showed an incidence of animals not at manger lower than 15%, while in CaS group it was higher than 30-35%.

The effects of the administration of different sources of rumen by-pass fats on growth performance are reported in Table 4. AL group had ADG significantly greater (P=0.0005) than animals fed calcium soaps. No difference was observed between AL and HF groups (P=0.08) and between HF and CaS groups. Final body weights of AL animals were significantly higher (P=0.005) than CaS group. There were no differences between AL and HF groups and between HF and CaS groups. Final body weights of AL animals were significantly higher (P=0.005) than CaS group. There were no differences between AL and HF groups and between HF and CaS group. Average DM was higher (P<0.05) in AL and HF groups compared with CaS group. Feed conversion rate was better for AL and CaS groups (P<0.05) compared with HF group.

Hypophagic effect of unsaturated fatty acids of calcium soaps was reported by several authors and may be explainable by: i) a lower acceptability of calcium soaps diet (Ngidi et al. 1990), ii) a reduction of rumen fibre digestion which is responsible for increasing distension of reticulo-rumen, iii) a metabolic regulation of DMI because of the greater absorption of unsaturated compared with saturated long-chain fatty acids (Allen, 2000) or iv) a depressive effect on gastrointestinal motility.

**Table 4. Effects of different sources of rumen by-pass fats on growth performance of heavy beef cattle.**

| Parameter                  | Treatment           | AL group* | CaS group* | HF group* | SE  |
|----------------------------|---------------------|-----------|------------|-----------|-----|
| Starting live weight, kg   |                     | 531.4     | 527.3      | 526.3     | 3.58|
| Final live weight, kg      | 639.1*              | 621.0b    | 625.9ab    | 3.58      |     |
| Average daily gain, kg/d   | 1.96*               | 1.70b     | 1.81ab     | 0.43      |     |
| Average dry matter intake, kg DM/d | 11.40*             | 10.42b    | 11.38ab    | 0.028     |     |
| Feed conversion rate       | 5.86*               | 6.25b     | 6.58b      | 0.2       |     |

AL = Hydrogenate triglycerides, CaS = Calcium soaps, HF = Hydrogenated free fatty acids. *Means with different superscript differ significantly (P<0.05). A,B Means with different superscript differ significantly (P<0.05.)

**Table 5. VFA and N-NH₃ (mmol/L) in ruminal fluid of bulls fed three different sources of rumen by-pass lipids.**

| Parameter      | Day | AL group* | CaS group* | HF group* | SE  |
|----------------|-----|-----------|------------|-----------|-----|
| pH             | 0   | 6.24a     | 6.41a      | 5.98b     | 0.09|
|                | 48  | 6.43      | 6.23       | 6.31      | 0.09|
| Total VFA      | 0   | 88.08a    | 79.21b     | 79.66b    | 2.18|
|                | 48  | 89.68a    | 82.09b     | 85.55ab   | 2.18|
| Acetic acid    | 0   | 60.34     | 55.52      | 54.76     | 1.62|
|                | 48  | 60.34     | 57.07      | 58.09     | 1.62|
| Propionic acid | 0   | 15.81a    | 12.87b     | 14.82a    | 0.63|
|                | 48  | 14.40     | 13.76      | 14.59     | 0.63|
| Isobutyric acid| 0   | 0.64      | 0.59       | 0.57      | 0.04|
|                | 48  | 0.62      | 0.61       | 0.67      | 0.04|
| Butyric acid   | 0   | 9.78      | 8.90       | 8.10      | 0.62|
|                | 48  | 12.87a    | 9.77b      | 10.90b    | 0.62|
| Isovaleric acid| 0   | 0.49      | 0.49       | 0.50      | 0.06|
|                | 48  | 0.51      | 0.49       | 0.45      | 0.06|
| Valeric acid   | 0   | 1.01      | 0.85       | 0.79      | 0.10|
|                | 48  | 0.95      | 0.99       | 1.02      | 0.10|
| N-NH₃          | 0   | 7.91      | 7.80       | 8.01      | 0.14|
|                | 48  | 7.88      | 7.58       | 8.09      | 0.14|

a,b,c Means with different superscript differ significantly (P<0.05). *AL = Hydrogenate triglycerides, CaS = Calcium soaps, HF = Hydrogenated free fatty acids.
In the present study the reported lower acceptability of calcium soaps diet was clearly shown by animal bunk behaviour. As a prove of calcium soaps unpleasant flavour, more than 30% of the animals in CaS group showed sniffing and digging behaviours immediately after fats inclusion in the diet, and these behaviors went on even when AL and HF animals got completely used to the new supplementation.

In a review paper, Allen (2000) reported that increasing concentrations of calcium salts of palm fatty acids (FA) in diets decreased DMI in 11 studies on 24, and 22 out of 24 studies had numerically lower DMI. The same study pointed out that hydrogenated FFA or triglycerides resulted in decreased feed intake in only one experiment and increased feed intake in 2 out of 21 studies. Furthermore, Harvatine and Allen (2006) indicated a decrease of 0.22 kg of DMI using a diet based on a 2.5% (DM basis) calcium soaps supplementation instead of a diet supplemented with 2.5% (DM basis) saturated fatty acids, with no effect either on the time spent by animals to eat or on the number of meals. The same authors hypothesised that intake of unsaturated FA stimulates satiety resulting in a decreased meal size that is not compensated by increased meal number.

Doreau and Ferley (1994) observed that digestibility is lower for longer chain saturated fatty acids compared with polyunsaturated FA, and that mean digestibility coefficients corresponded to 0.77, 0.85 and 0.83 for 18 carbon fatty acids with zero, one and two double bonds, respectively. Our findings seem to show that the higher digestibility of unsaturated FA at small intestine level compared with saturated FA, is not able to compensate the hypophagic effect determined by high calcium soaps administration.

Although degree of saturation plays an important role, digestibility of hydrogenated FFA has been reported to be higher than that of hydrogenated triglycerides with similar degree of saturation, suggesting that lipolysis may limit FA digestion at small intestine level (Elliott et al., 1999). Despite this evidence, AL group showed the same growth performances. As stated above, available reports on the effect and metabolism of by-pass fats on beef cattle are fewer (Elliott et al., 1999; Ngidi et al., 1999; Hightshoe et al., 1991) than dairy cattle. Further investigation is needed to determine the actual stability of by-pass fats in the rumen of beef cattle, which is characterized by low pH, and to understand the intensity of hydrolysis of hydrogenated triglycerides in the rumen itself.

Conclusions

To sum up, feeding beef cattle with hydrogenated triglyceride, characterized by a pleasant flavour and a small mean particle size, is likely to be more suitable than adding calcium soaps or hydrogenated free fatty acids, thanks to its higher dry matter intake and feed conversion rate, respectively. With regard to the latter higher rate, we can only suppose it could be related to the smaller mean particle size of hydrogenated triglycerides.

As stated above, available reports on the effect and metabolism of by-pass fats on beef cattle are fewer (Elliott et al., 1999; Ngidi et al., 1999; Hightshoe et al., 1991) than dairy cattle. Further investigation is needed to determine the actual stability of by-pass fats in the rumen of beef cattle, which is characterized by low pH, and to understand the intensity of hydrolysis of hydrogenated triglycerides in the rumen itself.

References

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.
Bauman, D.E., Perfield, J.W., De Veth, M.J., Lock, A.L., 2003. New perspectives on lipid digestion and metabolism in ruminants. pp 175-189 in Proc. Cornell Nutr. Conf., Ithaca, NY, USA.
Block, E., 2004. Fatty acids for dairy cows: more than just calories. pp. 33-44 in Penn State Dairy Cattle Nutr. Workshop, Grantville, PA, USA.
Chalupa, W., Rickabaugh, B., Kronfeld, D.S., Sklan D., 1984. Rumen fermentation in vitro as influence by long chain fatty acids. J. Dairy Sci., 67:1439-1444.
Channey, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.
Doreau, M., Ferley, A., 1994. Digestion and utilization of fatty acids by ruminants. Anim. Feed Sci. Tech. 43:379-396.
Drackley, J.K., 1999. New perspectives on energy values and supplementation levels of supplemental fats. Adv. Dairy Technol. 11:171-184.
Drackley, J.K., Elliott, J.P., 1993. Milk composition, ruminal characteristics, and nutrient utilization in dairy cows fed partially hydrogenated tallow. J. Dairy Sci. 76:183-196.
Drackley, J.K., Klusmeyer, T.H., Trusk, A.M., Clark, J.H., 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasums of lactating dairy cows. J. Dairy Sci. 75:1517-1526.
Elliott, J.P., Drackley, J.K., Beaulieu, A.D., Aldrich, C.G., Merchen N.R., 1999. Effects of saturation and esterification of fat sources on site and extent of digestion in steers: digestion of fatty acids, triglycerides, and energy. J. Anim. Sci. 77:1919-1929.
Elliott, J.P., Drackley, J.K., Weigel, D.J., 1996. Digestibility and effects of hydrogenated palm fatty acid distillate in lactating dairy cows. J. Dairy Sci. 79:1031-1039.
Enjalbert, F., Nicot, M. C., Bayourthe, C., Vernay, M., Moncoulon, R., 1996. Effects of dietary calcium soaps of unsaturated fatty acids on digestion, milk composition and physical properties of butter. J. Dairy Res. 64:181-195.
European Commission, 1981. Tenth Commission Directive of 25 July 1984 establishing Community methods of analysis for the official control of feeding-stuffs, 84/425/EEC. In: Official Journal, L 246, 30/07/1984, pp 32-35.
European Commission, 1998. Commission Directive of 3 September 1998 establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feeding-stuffs, 98/64/EC, and amending Directive 71/393/EEC. In: Official Journal, L 257, 03/09/1998, pp 14-28.
European Commission, 1999. Commission Directive 99/79/EC of 27 July 1999 amending the third Commission Directive 72/199/EEC of 27 April 1972 establishing Community methods of analysis for the official control of feeding-stuffs. In: Official Journal, L 209, 27/07/1999, pp 23-27.

[Ital J Anim Sci vol.9:e44, 2010] [page 233]
Fussell, R.J., McCalley D.V., 1987. Determination of volatile fatty acids (C2–C5) and lactic acid in silage by gas chromatography. Analyst 112:1213-1216.

Harvatine, K.J., Allen, M.S., 2006. Effects of fatty acid supplements on feed intake, and feeding and chewing behavior of lactating dairy cows. J. Dairy Sci. 89:1104-1112.

Hightshoe, R.B., Cochran, R.C., Corah, L.R., Harmon, D.L., Vanzant, E.S., 1991. Influence of source and level of ruminal escape lipid supplements on forage intake, digestibility, digesta flow, and fermentation characteristics in beef cattle. J. Anim. Sci. 69:4974-4982.

Jenkins, T.C., 1993. Symposium: Advances in ruminant lipid metabolism. J. Dairy Sci. 76:3851-3863.

Jenkins, T.C., Jenny, B.F., 1989. Effect of Hydrogenated fat on feed intake, nutrient digestion, and lactation performance of dairy cows. J. Dairy Sci. 72:2316-2324.

Jenkins, T.C., Palmquist, D.L., 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. J. Dairy Sci. 67:978-986.

Jouany, J.P., 1982. Volatile fatty acids and alcohol determination in digestive contents, silage juice, bacterial cultures and anaerobic fermentor contents. Sci. Aliment. 2:131-144.

Ngidi, M.E., Loerch, S.C., Fluharty, F.L., Palmquist, D.L., 1990. Effects of calcium soaps of long-chain fatty acids on feedlot performance, carcass characteristics and ruminal metabolism of steers. J. Anim. Sci. 68:2555-2565.

Palmquist D.L., 1991. Influence of soured and amount of dietary fat on digestibility in lactating cows. J. Dairy Sci. 74:1354-1360.

Polidori, F. Sgoifo Rossi, C.A., Senatore, M.E., Savoini, G., Dell’Orto, V., 1997. Effect of recombinant bovine somatotropin and calcium salts of long-chain fatty acids on milk from Italian buffalo. J. Dairy Sci. 80:2137-2142.

Sanz Sampelayo, M.R., Martin Alonso, J.J., Perez, L., Gil Extremera, F., Boza J., 2004. Dietary supplements for lactating goats by polyunsaturated fatty acid-rich protected fat. Effects after supplement withdrawal. J. Dairy Sci. 87:1796-1802.

SAS, 2001. User’s Guide Statistics. Version 8.02. SAS Institute, Inc., Cary, NC, USA.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.
