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EFFECT OF DIFFERENT FAT SOURCES ON *IN VITRO* DEGRADATION OF NUTRIENTS AND CERTAIN BLOOD PARAMETERS IN SHEEP

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This study was designed to determine the effects of calcium salt of palm oil fatty acids (CS), hydroxyethylsoyamide (HESA), butylsoyamide (BSA) and soybean oil (SO) on degradation of crude protein and fibre *in vitro*, and on the blood plasma lipid parameters *in vivo*. Five mature wethers (body weight 75 kg) were fed five diets in a 5 × 5 Latin square experiment. The control diet consisted of 50% meadow hay and 50% concentrate with no added fat. The control diet was supplemented with CS, HESA, BSA, or SO. Fat was added at 3.5% of dietary dry matter (DM). The final ether extract content of the ration was near 6%. Each period lasted 20 days. Fat supplements, except HESA, consistently decreased the *in vitro* DM disappearance of soybean meal as compared to control. In contrast to the effect of other treatments, crude protein degradation was greatest in the test tubes with inocula obtained from sheep fed diet with HESA. Fat supplements equally inhibited the DM and fibre breakdown of alfalfa pellet. CS and HESA seemed to be less detrimental to *in vitro* fermentation of neutral detergent fibre (NDF) than BSA and SO. All fat supplements increased blood plasma triglyceride, cholesterol and total lipid content. Plasma concentration of cholesterol and total lipid was highest with SO. The inclusion of CS in the diet increased 16:0, while all fat supplements increased plasma 18:0 and decreased 16:1 and 18:1 fatty acid content. Plasma 18:2n-6 was not changed by feeding CS and SO. However, compared to the control diet, 18:2n-6 increased with 12 and 41% in plasma fatty acids when sheep were fed HESA and BSA, respectively. The results showed that plasma concentration of linoleic acid was enhanced more when the amide was synthesised from butylamine than when from ethanolamine.

Key words: Fatty acid, soybean oil, oleamide, *in vitro* degradation, sheep

During early lactation, dairy cows are often forced to draw on body reserves to satisfy energy requirements. For this reason, the addition of fat sources to the diet may be useful to overcome limitations in energy supplies. Fat supple-

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mentation can increase the energy intake of dairy cows by more than 5% (Palmquist and Conrad, 1978). According to Palmquist (1988), 15–20% of the metabolisable energy (ME) requirement can be met by feeding protected fats. This corresponds to a dietary fat content of 7–8%, which is higher than what is considered detrimental.

The need for protecting fat sources can be explained by the chemical structure of free fats. The antimicrobial effect of lipids in the rumen greatly resembles the cytotoxic effect of fatty acids on the membrane function of eukaryotic cells (McCarthy, 1961; Borst et al., 1962). Long-chain fatty acids readily attach to lipid bilayers in biological membranes. At least ten different ways have been identified by which fatty acids can alter biological membrane function (Gutknecht, 1988). Ruminal lipids may similarly inhibit fermentation by partitioning into the microbial membrane and then disrupting its function (Gruber and Low, 1988). The free carboxyl group of fatty acids arising from the hydrolysis of lipids plays an important role in the inhibitory effect exerted on microorganisms. It was demonstrated by Jenkins (1987) *in vitro* and by Legay-Carmier and Bauchart (1989) *in vivo* that the effect of fatty acids on ruminal fermentation could be markedly diminished by substituting a given functional group for the carboxyl group of fatty acids. This is proved by experimental results obtained with different fatty acid derivatives. The Ca salts of long-chain fatty acids (Jenkins and Palmquist, 1982; Lebzien et al., 1992; Enjalbert et al., 1994; Chouinard et al., 1998; Schmidt et al., 2000), fatty alcohols (Czerkawski et al., 1966), fatty acyl amides (Fotouhi and Jenkins, 1992; Jenkins, 1999), and triglycerides (Chalupa et al., 1984) inhibit ruminal fermentation less than free fatty acids (FFA) do.

The objective of these experiments was to study the effects of fat protection based upon amide formation. This choice of subject was justified by the fact that *in vitro* fermentation studies to be presented in this paper had not been performed with amides previously. We wished to compare the effects and the degree of protection of the amides formed with those of Ca salts of fatty acids widely used in the practice. The changes of fatty acid composition of the blood plasma was also analysed to confirm the protected status of additional fat sources.

In comparative digestive-physiological studies performed with rumen-cannulated sheep the effects of oil, protected lipid (Ca salts of fatty acids) and amide supplementation were studied. The objective was to determine the effects that any form of vegetable fat supplementation exerts on the *in vitro* breakdown of dry matter, protein and fibre by rumen microorganisms and on certain blood lipid parameters.

Materials and method

Fat supplements

Two different fatty acyl amides were synthesised by mixing amines with soybean oil (SO) as described by Jenkins (1997). The process involved reacting soybean oil with butylamine to form butylsoyamide (BSA). Substituting ethanolamine for butylamine in the reaction with soybean oil yielded hydroxyethylsoyamide (HESA). The final amide products were examined by thin-layer chromatography (Bilyk et al., 1991) to confirm the absence of triglyceride, which indicated complete conversion of soybean oil to amides. Other fat supplement was calcium salt of palm oil fatty acids (Magnapac™, Norel S.A., Spain) for CS diet. Major fatty acids in the soybean oil, amides and Ca salt of palm oil fatty acids (Magnapac) were determined by gas chromatography (GC) after a conversion of the fatty acids to methyl esters (Husvéth et al., 1982).

Experimental animals

The inoculum for *in vitro* study was obtained from five rumen-fistulated adult, 2 years old Hungarian merino wethers (average body weight: 75 ± 2.4 kg). The rumen cannulas were installed 3 months prior to the study. The animals were housed in pens where water and salt were freely available. Animal research procedures were approved by the Animal Use and Care Administrative Advisory Committee of the Municipal Veterinary Service for Animal Protection (No. of permission: 114-3/2000).

Feeding

Sheep were fed a ration comprising 50% meadow hay and 50% concentrate mixture (41.1% extracted sunflower meal, 30% corn, 14% barley, 14% oat, 0.5% mineral and vitamin premix, 0.4% limestone). Chemical composition of concentrate and meadow hay is given in Table 1. The animals were fed at a restricted level (50 g dry matter/kg body weight$^{0.75}$) and the diets were offered in two equal portions at 08:00 and 16:00 h. The fat supplementation was made to achieve 6% of the total ether extract content in the ration. The forage to concentrate ratio (50:50) and the fat content of the diets in the experiment were similar to those of the rations fed to high-yielding cows. Fat supplements were added to the concentrate portion before each meal and mixed thoroughly. Amides were melted before being mixed with the concentrate.

Table 1

Chemical composition of diets

Composition	Concentrate	Meadow hay
Dry matter (DM), %	91.2	88.9
Ash, % of DM	5.7	8.3
Crude protein, % of DM	21.5	8.5
Ether extract, % of DM	2.8	1.7
Crude fibre, % of DM	10.9	41.2
NDF, % of DM	25.2	73.2
ADF, % of DM	14.3	47.7
NEm, MJ/kg DM	7.7	4.6
MPE[1], g/kg DM	111.2	72.9
MPN[2], g/kg DM	138.6	48.9

[1]MPE: Metabolisable protein dependent on energy supply; [2]MPN: Metabolisable protein dependent on N supply

Experimental protocol

According to the different treatment five experimental diets were fed to the animals:

Control (no added fat)

Calcium salt of palm oil fatty acids-supplementation (CS diet)

Soybean oil supplementation (SO diet)

Hydroxyethylsoyamide supplementation (HESA diet)

Butylsoyamide supplementation (BSA diet)

The trials were carried out in a 5 × 5 Latin square design with 20-day periods. During each period, sheep were adapted to their diets for 14 days followed by a sampling period. Rumen fluid samples as inocula for the *in vitro* investigation were taken according to the timetable described below.

Collection day of experimental period	15th	16th	17th	18th	19th
Animals serving as inoculum source	1 and 5	2 and 4	1 and 3	4 and 5	2 and 3

In vitro *fermentation*

The *in vitro* system used was essentially that of Tilley and Terry (1963). Two substrates were used to determine the degradation rate of nutrients after 24-h incubation. High rumen degradable extracted soybean meal was used to investigate N degradation. *In vitro* digestibility of fibre was also determined by using alfalfa pellet as substrate. In this case, of the different fibre fractions, neutral detergent fibre (NDF) content was measured. The degradation rate of dry matter

was investigated with both substrates. Sixteen tubes each were simultaneously incubated per each animal and each substrate.

Soybean meal and alfalfa pellet were ground through a 1-mm screen. A 1-g sample of each of the two substrates was weighed into 100-ml glass tubes. Thirty-two ml of McDougall's artificial saliva (McDougall, 1948) was then dispensed to the test tubes and rumen inocula were finally added to the flasks. Ruminal contents were obtained 3 h after the morning feeding from the sheep fed as described previously. The rumen fluid was strained through six layers of cheesecloth under a stream of O_2-free CO_2. Eight-millilitre rumen inocula were transferred to the tubes. All solutions were kept at 39 °C, and saturated with CO_2 prior to dispensing. Tubes were sealed immediately with gas-release stoppers and placed in a water bath at 39 °C (0 hour). Flask contents were mixed by gentle swirling every 60 min except between hours 8 to 24 of the assays. At the end of the incubation (24 h) the whole contents of the flasks were transferred into centrifuge tubes and the samples were immediately centrifuged at $2{,}000 \times g$ for 20 min. The supernatant was removed and the residue was transferred to the glass container and dried at 50 °C for 48 h. Association of Official Analytical Chemists (AOAC, 1990) methods were used to analyse centrifuged samples for dry matter (DM) and crude protein (CP). Neutral detergent fibre (NDF) analysis was conducted by the method of Van Soest and Robertson (1985).

Blood sample collection and analysis

Blood samples were taken by jugular puncture on the last day of each period (20th day) at 3 h after feeding. Determination of total cholesterol (TCh) and triglyceride (TG) concentration in the Na-heparin blood plasma was performed by means of Cobas-Emina Autoanalyser (Roche, Switzerland), while total lipid was determined using the method of Zöllner and Kirsch (1962). The changes in the proportion of fatty acids were also studied by a gas-chromatographic method. The lipids from the plasma were extracted with a 2:1 (v/v) mixture of chloroform and methanol. The lipid extracts were converted to fatty acid methyl esters by transesterification using sulphuric acid in methanol (Husvéth et al., 1982). The fatty acids were identified by the comparison of retention times with known external standard (FAME Mix: Cat. No. 47885-U; Supelco Inc., Bellefonte, USA), and quantified by means of a Shimadzu C-RGA integrator to calculate weight percentages of fatty acid methyl esters.

Statistical analysis

Analysis of variance was conducted using the General Linear models procedure of SPSS™ (SPSS Version 7.5, 1996, SPSS Inc., Chicago, Illinois, USA). The data were statistically evaluated using ANOVA and Student's *t*-test. The level of statistical significance was declared at $P \leq 0.05$ level.

Results

Fatty acid composition of different fat supplements determined by gas chromatography is shown in Table 2. Heating the soybean oil and amines at 70 °C to produce fatty amides did not effect fatty acid composition. Linoleic acid was the primary and oleic acid was the secondary fatty acid in both the oleamide products and the soybean oil. Other fatty acids (16:0; 18:0 and 18:3) were present at 10% or less. Fatty acid composition of Magnapac is also shown in Table 2. Unlike soybean oil and amides, Magnapac contains higher percentage of palmitic (16:0) and oleic acids (18:1).

Table 2

Fatty acid composition of the soybean oil, oleamide supplements and Magnapac (g/100 g total fatty acid)

	16:0 Palmitic acid	18:0 Stearic acid	18:1n-9 Oleic acid	18:2n-6 Linoleic acid	18:3n-3 Linolenic acid
Soybean oil	9.69	3.96	27.33	52.37	6.65
HESA[1]	9.56	4.05	27.62	52.48	6.29
BSA[2]	10.74	4.27	27.23	50.98	6.78
Magnapac[3]	49.40	4.42	36.89	8.94	0.35

[1]HESA: hydroxyethylsoyamide; [2]BSA: butylsoyamide; [3]Magnapac: Ca salt of palm oil fatty acids

In vitro *fermentation*

Fat supplements, except HESA, consistently decreased *in vitro* DM disappearance of soybean meal compared with control (Table 3). BSA treatment had no significant effect on CP digestion of soybean meal. Both CS and SO administration resulted in a lower CP degradation. In contrast to the effect of other treatments, CP degradation was greatest in the test tubes with inocula obtained from sheep fed diet with HESA. Fat supplements equally inhibited the DM and NDF breakdown of alfalfa pellet. CS and HESA seemed to be less detrimental to *in vitro* fermentation of NDF than BSA and SO.

Blood parameters

All fat supplements increased triglyceride, cholesterol and total lipid (Table 4) content of blood plasma. Plasma concentration of cholesterol (3.47 mmol/l) and total lipid (3.2 g/l) was highest with SO. Highest blood triglyceride (0.39 mmol/l) was observed in the sheep fed CS diet.

Plasma 16:0 fatty acid content decreased when either HESA or SO was added to the diet (Table 5). The inclusion of CS in the diet increased 16:0. All fat supplements increased plasma 18:0 and decreased 16:1 and 18:1. Both amid supplements decreased plasma 18:3. Plasma 18:2n-6 was not changed by feeding CS

and SO. However, compared to the control diet, feeding HESA increased the ratio of linoleic acid from 32.67 (control) to 36.43% of total fatty acids. The butylsoyamide supplement was even more effective in raising linoleic acid in plasma because BSA further elevated plasma linoleic acid to 45.92%. Although plasma linoleic acid increased in sheep fed HESA diet compared to control, no significant difference was observed between HESA and SO. Therefore the effect of BSA on plasma 18:2 was more pronounced than that of HESA.

Table 3

In vitro degradation of soybean meal and alfalfa pellet

	Control	CS[1]	SO[2]	HESA[3]	BSA[4]
Soybean meal					
Dry matter, %	70.0 ± 2.9^{a}	63.6 ± 3.2^{b}	68.3 ± 2.1^{cd}	69.7 ± 3.4^{a}	67.7 ± 2.9^{d}
Crude protein, %	70.6 ± 3.7^{a}	66.2 ± 1.6^{b}	69.5 ± 3.0^{c}	72.8 ± 3.4^{d}	70.7 ± 3.8^{a}
Alfalfa pellet					
Dry matter, %	38.0 ± 2.8^{a}	34.8 ± 3.0^{b}	36.2 ± 1.8^{cd}	35.8 ± 2.2^{c}	36.6 ± 1.6^{d}
NDF, %	32.9 ± 3.6^{a}	29.5 ± 4.3^{b}	28.3 ± 3.8^{b}	29.5 ± 3.3^{b}	28.4 ± 3.6^{b}

[a,b,c,d]Means within a row with different superscripts are different ($P < 0.05$); [1]CS: Ca salt of palm oil fatty acids (Magnapac); [2]SO: soybean oil; [3]HESA: hydroxyethylsoyamide; [4]BSA: butylsoyamide

Table 4

Effect of fat supplementation on certain blood lipid parameters of wethers

	Control	CS[1]	SO[2]	HESA[3]	BSA[4]
Total lipid, g/l	1.85 ± 0.24^{a}	2.96 ± 0.30^{bc}	3.20 ± 0.30^{c}	2.37 ± 0.33^{de}	2.59 ± 0.40^{e}
Triglyceride, mmol/l	0.18 ± 0.04^{a}	0.39 ± 0.10^{bcd}	0.34 ± 0.14^{ce}	0.34 ± 0.06^{d}	0.28 ± 0.06^{e}
Total cholesterol, mmol/l	1.96 ± 0.26^{a}	3.25 ± 0.40^{bc}	3.47 ± 0.43^{c}	2.66 ± 0.38^{de}	2.77 ± 0.31^{e}

[a,b,c,d,e]Means within a row with different superscripts are different ($P < 0.05$); [1]CS: Ca salt of palm oil fatty acids (Magnapac); [2]SO: soybean oil; [3]HESA: hydroxyethylsoyamide; [4]BSA: butylsoyamide

Discussion

Fats added to the ration of ruminants can greatly disrupt ruminal fermentation, reducing the digestibility of nonlipid energy sources. A ration supplemented with 10% fat may decrease the ruminal degradation of structural carbohydrates by 50% (Ikwuegbu and Sutton, 1982). This reduction in digestion is accompanied by reduced production of methane, hydrogen and VFA, including a lower acetate to propionate ratio (Chalupa et al., 1984). The feeding of untreated fats rich in unsaturated fatty acids was reported to result in diminished acetic

acid and increased propionic acid production (Rohr et al., 1978; Lebzien, 1980; Ikwuegbu and Sutton, 1982). The decrease of acetic acid production may be due to the adverse effect exerted by fats on the surface activity of the microbial cell membrane (Ikwuegbu and Sutton, 1982). The lipid 'coating' theory also attempts to explain reduced acetic acid production by a lipid layer over feed particles that inhibits digestion of cellulose (Rohr et al., 1978; Oslage, 1984). Like the above-mentioned authors, in this experiment we also observed lower degradation of the NDF content of alfalfa pellets. Compared with the control diet, in the experimental groups the degradation of NDF decreased by 3–4% in absolute value. In addition, the *in vitro* degradation of the dry matter of alfalfa pellets was also significantly lower.

Table 5

Effect of different fat sources on blood plasma fatty acid composition (g/100 g total fatty acid) of wethers

Fatty acid	Control	CS[1]	SO[2]	HESA[3]	BSA[4]
Lauric acid 12:0	0.12 ± 0.05	0.06 ± 0.02	0.07 ± 0.04	0.08 ± 0.02	0.08 ± 0.02
Myristic acid 14:0	0.73 ± 0.19	0.77 ± 0.07	0.91 ± 0.31	0.55 ± 0.18	0.67 ± 0.08
Palmitic acid 16:0	15.85 ± 0.79^{a}	21.95 ± 1.17^{b}	14.02 ± 1.25^{de}	12.95 ± 0.61^{cd}	14.81 ± 1.48^{ae}
Palmitoleic acid 16:1	6.15 ± 0.58^{a}	1.34 ± 0.67^{b}	2.82 ± 0.98^{ef}	4.35 ± 1.10^{c}	2.89 ± 0.44^{df}
Stearic acid 18:0	12.65 ± 2.07^{a}	17.82 ± 1.32^{be}	18.60 ± 2.32^{ce}	16.27 ± 1.41^{bc}	15.84 ± 2.25^{bd}
Oleic acid 18:1	27.91 ± 1.25^{a}	23.96 ± 2.09^{b}	24.44 ± 1.82^{bce}	25.56 ± 1.23^{bc}	15.98 ± 1.85^{d}
Linoleic acid 18:2	32.67 ± 2.80^{a}	29.70 ± 3.25^{a}	33.69 ± 4.54^{ab}	36.43 ± 2.51^{b}	45.92 ± 2.97^{c}
Linolenic acid 18:3	0.66 ± 0.74^{bcd}	0.73 ± 0.19^{ad}	0.83 ± 0.29^{d}	0.35 ± 0.07^{b}	0.22 ± 0.07^{c}
Arachidonic acid 20:4	2.32 ± 0.79^{a}	2.63 ± 0.23^{a}	3.86 ± 0.85^{b}	2.64 ± 0.37^{a}	2.88 ± 0.69^{a}
Others	0.94 ± 0.44	1.04 ± 0.22	0.76 ± 0.46	0.82 ± 0.92	0.71 ± 0.40

^{a,b,c,d,e,f}Means within a row with different superscripts are different ($P < 0.05$); [1]CS: Ca salt of palm oil fatty acids (Magnapac); [2]SO: soybean oil; [3]HESA: hydroxyethylsoyamide; [4]BSA: butylsoyamide

Supplementation of the ration with different types of fats exerted dissimilar effects on the *in vitro* breakdown of crude protein. Compared with the control, it can be established that Ca salt of long-chain fatty acids and soybean oil (SO) decreased the degradation of soybean meal protein by 6% and 1.5%, respectively, butylsoyamide (BSA) left it practically unchanged, while hydroxyethylsoyamide (HESA) increased it by 3.1%. Since the objectives of this

experiment did not include monitoring of the changes in other parameters of N metabolism (ammonia concentration of the rumen fluid, efficiency of microbial protein synthesis, duodenal flow of dietary N), evaluation of the obtained data runs into difficulties. A further problem is that even the analysis of data reported in the literature would fail to bring us closer to an accurate interpretation of the effects of dietary fat supplementation on ruminal fermentation and N metabolism. Namely, the relevant data of the literature are rather disparate and conflicting. For instance, Rohr et al. (1978) and Blum et al. (1985) have established that although the energy supply of ruminants can be improved by the incorporation of fats in the ration (Baldwin et al., 1985), fat feeding adversely affects the protein-synthesising activity of the rumen microflora. In the studies of Ohajuruka et al. (1991), none of the forms of fat supplementation (animal or vegetable fat, or Ca salts of fatty acids) affected the efficiency of microbial protein synthesis. Feeding hydrogenated fish oil or calcium salt of fish oil, the *in sacco* degradability of crude protein did not change (Avila et al., 1998). On supplementing the ration with increasing amounts of tallow, Kowalczyk et al. (1977) observed a decrease in ruminal ammonia concentration, and explained it by the direct depressive effect of fat on the proteolytic activity of microorganisms. Quite the contrary, Jilg et al. (1988) are of the opinion that the decrease in the ammonia concentration of the ruminal fluid can be explained by more efficient microbial protein synthesis due to the reduced protozoal numbers.

Of the blood parameters studied, total lipid, triglyceride and cholesterol concentrations increased in all groups as a result of fat supplementation. These data are in good agreement with the results of earlier experiments. Astrup et al. (1976) and Palmquist and Conrad (1978) also observed an increase in total lipid concentration following fat supplementation. Supplementation of the ration with fat increased both the triglyceride content (Marty and Block, 1990) and the cholesterol concentration (Sharma et al., 1978; Wrenn et al., 1978; Marty and Block, 1990; Hightshoe et al., 1991) of the blood plasma.

Different forms of fat supplementation, with the exception of Ca salt of long-chain fatty acids (CS), did not markedly affect the ratio of saturated and unsaturated fatty acids, while on the individual fatty acids it exerted an influence of different magnitude and direction. Calculating the total percentage amounts of saturated and unsaturated fatty acids from the data of Table 5, in the control group the proportion of saturated fatty acids proved to be 29.35%. As a result of feeding CS, that proportion rose to 40%, while supplementation of the diet with SO, HESA and BSA did not markedly alter the amount of saturated fatty acids, as values similar to those of the control group were found (33.67%, 29.9% and 31.4%, respectively). In accordance with the above-mentioned changes, the proportion of unsaturated fatty acids naturally decreased from the control level of 69.7% to 58.3% in the group fed the CS diet while it did not change in case of oil or amide supplementation (SO: 65.7%; HESA: 69.3%; BSA: 68%). The rise of

the proportion of saturated fatty acids in the CS group was due to the fatty acid composition of this protected fat (Table 2). Namely, Magnapac is a CS produced from palm oil, in which the proportion of palmitic acid is nearly 50%; in addition, the C18 unsaturated fatty acids present in palm oil are likely to be less resistant to ruminal biohydrogenation processes, which led to a significant increase in the proportion of stearic acid. This saturation process must have caused the significant rise in the proportion of C18:0 in the case of the SO diet and the diets supplemented with the amides formed from it (HESA and BSA). At the same time, amide formation protected linoleic acid against ruminal biohydrogenation processes to some extent. Gas chromatographic determinations revealed that SO as well as HESA and BSA produced from the former contained more than 50% C18:2 (Table 2). At the same time, the linoleic acid concentration of the blood was almost identical in animals fed the SO diet as in those fed the control ration, which indicates a high degree of ruminal biohydrogenation. Fatty acid composition of the blood plasma supports this hypothesis, since the highest proportion of stearic acid was measured in the sheep fed the SO diet. It seems that conversion of FFA to fatty acyl amides effectively resists biohydrogenation, which led to higher plasma C18:2. The concentration of linoleic acid showed the greatest increase after feeding the BSA diet. Several experiments have proved that amide formation products increase the blood plasma level of linoleic acid. In an experiment with wethers fed a diet supplemented with 5% BSA, Jenkins (1995) measured 30% higher linoleic acid levels in the blood plasma. Jenkins et al. (1996) found that linoleic acid, as a percentage of total fatty acids, increased in plasma and milk taken from dairy cows fed BSA. In their comparative studies Jenkins and Thies (1997) measured significantly elevated plasma linoleic acid levels after both HESA and BSA supplementation. However, at variance with our results, the above authors found that HESA increased the linoleic acid level more than did BSA. They found that the amide bond in HESA was more easily broken postruminally than in BSA, which would account for its higher 18:2 in plasma. As in this experiment we did not study the small intestinal digestion of amides, the available data do not allow us to provide a satisfactory explanation for the disparate results obtained. Evaluation of the data would have been facilitated if comparative data had been available not only on the postruminal degradation of the two different amide preparations but also on their stability in the rumen. Jenkins and Thies (1997) studied the loss of 18:2 from *in vitro* cultures supplemented with 5% 18:2 or 10% HESA. They found that the disappearance rate of 18:2 was reduced by 61% when the substrates contained added HESA compared to added linoleic acid. At the same time, they did not study the rate of 18:2 loss from BSA cultures, and thus no information is available on the ruminal biohydrogenation of that preparation.

The changes found in the fatty acid composition of the blood plasma are clearly favourable from the point of view of human nutrition, as the amount of

the biologically more valuable unsaturated fatty acids, including the essential linoleic acid, increased. Polyunsaturated fatty acids are important constituents of cell membranes and act as precursors to a variety of compounds important for metabolism, such as prostaglandins, leukotrienes and lipoxins (Husvéth, 1994).

The marked increase in the proportion of plasma linoleic acid indicates that BSA is more protected and more efficiently resists ruminal biohydrogenation. Therefore, this preparation can have a favourable effect on the fatty acid composition of food sources produced by ruminants, such as milk or meat.

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