Selenite and selenate affect the fatty acid profile in *in vitro* incubated ovine ruminal fluid containing linseed oil

M. Czauderna[1], J. Kowalczyk[1], M. Marounek[2,3]

[1]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland
[2]Institute of Animal Science, Prague-Uhříněves, Czech Republic
[3]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

ABSTRACT: The influence of selenite (Se^{IV}) or selenate (Se^{VI}) added to ovine ruminal fluid containing linseed oil (LO) on the profile of fatty acids (FA), particularly conjugated linoleic acid (CLA) isomers, was investigated. The ruminal fluid was incubated *in vitro* at 39°C under CO_2 either alone (the control fluid) or with LO (3.3 mg/ml) or with a combination of LO with either a low (0.167 μg/ml) or high (1.67 μg/ml) level of Se as Se^{IV} or Se^{VI}. LO added to ruminal fluids also provides an extra source of energy. The tubes with the examined fluids were removed after 0, 6, 12, 18, or 24 h of *in vitro* incubation and then analyzed to determine the FA levels. The lower and higher concentration of Se^{IV} in the fluids with the LO revealed negligible effect on the concentration of the sum of the CLA isomers (ΣCLA) in the fluid compared with the fluid with LO alone. The addition of a higher amount of Se^{IV} to the fluid containing LO usually decreased the concentration of ΣCLA compared with the fluid containing the lower concentration of Se^{IV} and LO. The concentration of *c9t11c15*C18:3 (cLNA) in the fluids with LO, irrespective of the presence of extra Se, increased throughout the incubations, although the addition of Se^{IV} or Se^{VI} to the fluids containing LO numerically reduced the increase of the concentration of cLNA compared with the fluid with LO alone. The concentration sum of the C18:1 isomers (ΣC18:1) in the control fluid numerically decreased throughout the incubations, while LO added to the fluid increased the concentration of ΣC18:1 throughout the incubations. LO added to the fluid, irrespective of the presence of Se^{IV} or Se^{VI}, significantly increased the concentration of ΣC18:1 compared with the control fluid and the fluids with Se^{IV} or Se^{VI}. The concentrations of C16:0 and C18:0 in the control fluid and the fluids containing Se^{IV} or Se^{VI} numerically increased throughout the incubations and were usually lower than in the fluids containing LO without or with Se^{IV} or Se^{VI}. The concentration of C18:3n-3 decreased throughout the incubation of the fluids containing LO, irrespective of the presence of Se^{IV} or Se^{VI}. LO added to the fluids, irrespective of the presence of Se^{IV} or Se^{VI}, increased the concentration of C18:2n-6 compared with the control fluid and the fluids with Se^{IV} or Se^{VI}. The higher concentration of Se^{IV} or Se^{VI} in the fluid with LO most efficiently increased the concentration of *c5c8c11c14c17*C20:5 compared with the control fluid or the fluids containing LO, irrespective of the presence of the lower concentration of Se^{IV} or Se^{VI}. LO added to the fluid, irrespective of the presence of Se^{IV} or Se^{VI}, increased the concentration of polyunsaturated FA compared with the control fluid or the fluids containing Se^{IV} or Se^{VI}.

Keywords: vegetable oil; selenium; biohydrogenation; bacterial isomerization; *cis-trans* fatty acids; conjugated linoleic acid isomers

Most vegetable and fish oils added to diets can modify the fatty acid (FA) composition of the body of farm animals (Mir et al., 2002; Raes et al., 2004; Loor et al., 2005; Bernard et al., 2009). Dietary vegetable oils or plant extracts have substantially increased the content of monounsaturated FA

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(MUFA) and polyunsaturated FA (PUFA), including conjugated linoleic acid (CLA) isomers in the bodies of sheep and cows (Aharoni et al., 2004; Niedźwiedzka et al., 2008; Miri et al., 2013). In ruminants, dietary vegetable and fish oils disappear in the rumen stomach compartment through lipolysis to free FA (fFA), followed by biohydrogenation of the released unsaturated FA (UFA) (Burns et al., 2003; Lee and Jenkins, 2011; Buccioni et al., 2012). In the rumen, dietary esterified lipids are hydrolyzed to fFA and glycerol as well as, in small concentrations, to mono- and diglycerides by microbial lipases; these lipases are extra-cellular enzymes assembled in small beads (Jenkins et al., 2008; Buccioni et al., 2012). Monoenoic, dienoic, trienoic *trans* (*t*) FA (*t*-FA) and the positional isomers of dietary FA are produced as intermediates during the biohydrogenation of the UFA released from dietary oils. Isomerization of UFA is one of the steps that make FA less toxic to microorganisms. When biohydrogenation is not completed, intermediates (e.g. *t*-FA or the isomers of dietary UFA) from the incomplete biohydrogenation of the UFA become available for deposition in the ruminal microorganisms and in the tissues of ruminants (Or-Rashid et al., 2007; Niedźwiedzka et al., 2008; Buccioni et al., 2012; Gudla et al., 2012).

Dietary UFA, especially long-chain PUFA (LPUFA), have several positive and negative effects on rumen metabolism that affect the fermentation pattern, protozoal numbers, nutrient digestion, growth efficiency of microorganisms, and kinetics and site of digestion (Burns et al., 2003; Chikunya et al., 2004; Niedźwiedzka et al., 2008; Czauderna et al., 2010, 2012a, b; Buccioni et al., 2012). The nutritional quality of lipids in the edible parts of a ruminant's carcass may be enhanced by dietary manipulation strategies that minimize biohydrogenation of the ingested UFA in the rumen. This strategy has been traditionally achieved by the treatment of protein-rich lipid supplements with formaldehyde (Chikunya et al., 2004). LPUFAn-3, abundantly present in fish oils, may provide a natural protection against biohydrogenation by the ruminal microorganisms (Burns et al., 2003; Chow et al., 2004; Wąsowska et al., 2006a; Buccioni et al., 2012). Recent studies revealed that selenate (Se^{VI}) or selenite (Se^{IV}) changed the contents of the FA, especially the CLA isomers and other conjugated FA, in the ovine ruminal fluid (Sieber et al., 2004; Wąsowska et al., 2006b; Czauderna et al., 2010, 2012a), the liver, muscles, internal organs and adipose tissues of animals (Czauderna et al., 2004a, b, 2007; Korniluk et al., 2006; Niedźwiedzka et al., 2008; Krajewska et al., 2012). Analysis of the selenium (Se) concentration of the microorganisms revealed that the ruminal microbial Se abundance was enriched relative to the level of Se in the diets. This concentration of Se in the ruminal microorganisms was significantly higher than that of the diet (Lyons and Jacques, 2001; Whanger, 2004). Ruminal bacteria are also able to synthesize Se-methionine (Se-Met) and Se-cysteine (Se-Cys), and these Se-amino acids (Se-AA) are then incorporated into the microbial protein. The predominant Se-AA was Se-Cys, when the ruminal microbes were incubated with Se^{IV} or Se^{VI} (Whanger, 2004). Microorganisms can reduce excessive doses of Se-compounds to unabsorbable elemental Se or selenide forms. Interestingly, Se is the essential component of antioxidant enzymes that can decrease the risk of UFA peroxidation (Crespo et al., 1995; Demirel et al., 2004; Suzuki, 2005; Juniper et al., 2008; Navarro-Alarcon and Cabrera-Vique, 2008).

Considering the above, we hypothesized that Se^{IV} or Se^{VI} added to the ruminal fluid with linseed oil (LO) would stimulate the accumulation of UFA, especially the CLA isomers and their precursors, in the incubated fluid with LO. LO added to ruminal fluids also provides an extra source of energy. Therefore, the aim of our studies was to determine the effect of Se^{IV} or Se^{VI} added to the ovine ruminal fluid on the concentration of FA in *in vitro* incubated ruminal fluid containing LO.

MATERIAL AND METHODS

Animals and diets

Eight ruminally fistulated adult sheep received a mixed diet comprising grass hay, barley, molasses, soybean meal, a mixture of minerals and vitamins at 500, 299.5, 100, 91, and 9.5 g/kg dry matter, respectively, fed in equal meals of 500 g at 8.00 and 16.00 h. Ruminal digesta samples were taken from each sheep before feeding in the morning, and they were kept at 39°C and strained through linen cloth before use in incubations of the ruminal fluid.

Chemicals

The fatty acid methyl ester (FAME) standards, sodium selenite (Se^{IV}) and sodium selenate (Se^{VI}) were purchased from Sigma-Aldrich Co. (St. Louis,

Table 1. Concentration of important fatty acids in linseed oil added to *in vitro* incubated ruminal fluids

Fatty acids	Concentration (μg/g)
C8:0	26.0
C10:0	35.3
C12:0	9.62
C14:0	18.0
C16:0	1 405
C18:0	872
*c5c8c11c14c17*C20:5 (C20:5n-3)	97
*c9c12c15*C18:3 (αLNA)	5 892
c6c9c12 C18:3 (γLNA)	894
c9c12 C18:2 (LA)	4 962
*c9*C18:1	4 169
*c6*C18:1	539
ΣSFA	2 413
ΣMUFA	4 718
ΣPUFA	11 845

LA = linoleic acid, αLNA = α-linolenic acid, γLNA = γ-linolenic acid, SFA = saturated fatty acids, MUFA = mono-unsaturated fatty acids, PUFA = poly-unsaturated fatty acids

USA). A CLA isomer mixture (97–99%), *cis-9,trans-11*CLA (*c9t11*CLA) (95–97%), and *t10c12*CLA (95–97%) were supplied by Larodan Fine Chemicals AB (Malmö, Sweden). LO was provided by APA Polska (Kobylnica near Poznań, Poland). The fatty acid composition of the LO added to the incubated ruminal fluids is shown in Table 1. Dichloromethane (DCM), KOH, NaOH, Na_2SO_4, and concentrated HCl were purchased from POCH S.A. (Gliwice, Poland). Acetonitrile, methanol, and *n*-heptane (99%, GC) were supplied by Lab-Scan (Dublin, Ireland). The other reagents were of analytical grade. The water used for the preparation of the mobile phases and chemical reagents was prepared using an Elix™ water purification system (Millipore, Toronto, Canada).

Incubations with ruminal fluid *in vitro*

Strained ruminal fluids were incubated *in vitro* either alone or with a combination of LO and selenium as Se^{IV} or Se^{VI} to determine the interactions between the metabolisms of LO, Se^{IV}, Se^{VI}, and the main MUFA and PUFA in the ruminant diet. All *in vitro* experiments were performed on four different days using samples withdrawn from eight different sheep. In general, 1 ml of strained ruminal fluid was added under CO_2 to 0.2 ml of water solution containing either alone (the positive control groups) or with a combination of LO, a low (L) or high (H) level of Se^{IV} or Se^{VI} (Table 2). The negative control group (RF) was the combination of 1 ml of strained ruminal fluid and 0.2 ml of water. The final volumes of all incubated *in vitro* ruminal fluids were always 1.2 ml. All *in vitro* incubation with ovine ruminal fluids were performed in Pyrex tubes (120 × 11 mm).

The tubes with the examined ruminal fluids were incubated at 39°C. The tubes were removed after 0, 6, 12, 18 or 24 h of *in vitro* incubation, heated to inactivate ruminal microorganism enzymes for 9–10 min in a block heater at 100°C and stored at –20°C before being submitted for the determination of the FA concentration. The free FA were extracted and analyzed as described below. Samples of the original strained ruminal fluids were stored at –20°C for later protein analysis.

Table 2. Scheme of *in vitro* experiments on ovine ruminal fluids[1]

Group	Additive	Additive concentration	*n*
RF	water	–	5
Se^{IV}L	selenite	0.167 μg/ml	5
Se^{IV}H	selenite	1.67 μg/ml	5
LOSe^{IV}L (E)	selenite linseed oil	0.167 μg/ml 3.3 mg/ml	5
LOSe^{IV}H (E)	selenite linseed oil	1.67 μg/ml 3.3 mg/ml	6
Se^{VI}L	selenate	0.167 μg/ml	5
Se^{VI}H	selenate	1.67 μg/ml	5
LOSe^{VI}L (E)	selenate linseed oil	0.167 μg/ml 3.3 mg/ml	6
LOSe^{VI}H (E)	selenate linseed oil	1.67 μg/ml 3.3 mg/ml	6
LO	linseed oil	3.3 mg/ml	6

Se^{IV} = selenite, Se^{VI} = selenate, LO = linseed oil, E = experimental group, L = low level (0.2 μg), H = high level (2.0 μg), RF = negative control group, *n* = ovine ruminal fluid samples collected from *n* sheep at different times and individually analyzed

[1]1 ml of ovine ruminal fluids was added to 0.2 ml of water solution containing either solely LO (4 mg), Se^{IV} or Se^{VI} at different levels (L, H), or their mutual combinations

Fatty acid extraction and preparation of fatty acid methyl esters

One millilitre of each of the incubated ruminal fluids was placed in each vial and treated with a mixture of 1 ml of 4M KOH in water and 2 ml of 1M KOH in methanol. Next, 50 µl of the internal standard (IS) in chloroform (17 mg C19:0/ml) was added to the obtained mixture. The resulting mixture was flushed with argon (Ar) for ~ 4 min. The vial was then sealed, and the mixture was vortexed and heated under Ar at 95°C for 10 min, cooled for ~ 10 min at room temperature, and sonicated for 10 min. The resulting mixture was protected from light and stored in a sealed vial under Ar at room temperature overnight. Next, 3 ml of water was added to the hydrolyzate, and the solution was again vortexed. The obtained solution was acidified with 4M HCl to ~ pH 2, and the free FA was extracted four times with 3 ml of DCM. The extraction was repeated 4 times using 3 ml of *n*-hexane. The *n*-hexane layer was combined with the DCM layer, and then, the resulting organic phase was dried with ~ 0.1 g of Na_2SO_4. The organic solvents were removed under a stream of Ar at room temperature. The obtained residue was stored at –20°C until FA methylation.

Preparation of fatty acid methyl esters and element analysis

Two ml of 2M NaOH in methanol were added to the residue while mixing, and the resulting mixture was then flushed with Ar and reacted at 40°C for 1 h. After cooling the mixture to 4°C, 2 ml of 25% BF_3 in methanol were added, flushed with Ar, and heated at 40°C for 1 h. To the cooled reaction mixture, 5 ml of water were added, and then the FAMEs were extracted with 5 ml of *n*-hexane. The supernatant was transferred to a GC vial.

Analytical equipment

The analyses of all the FAMEs were performed on a gas chromatograph Shimadzu GCMS-QP2010 Plus EI equipped with a BPX70 fused silica capillary column (120 m × 0.25 mm i.d. × 0.25 µm film thickness) (SHIM-POL A.M., Warsaw, Poland), a quadrupole Mass Selective Detector 5973N (Agilent Technologies Inc., Santa Clara, USA), and an injection port. Helium as the carrier gas operated at a constant pressure (223.4 kPa) and a flow rate of 1 ml/min. The injector and MS detector temperatures were maintained at 200 and 240°C, respectively. The total FAME profile in 1 µl sample at a split ratio of 5 : 1 was determined using the column temperature gradient programme. The oven temperature was programmed as follows: initially 70°C for 4 min, increasing by 12°C/min to 150°C, held for 6 min, programmed at 8°C/min to 168°C, held for 27 min, programmed at 0.75°C/min to 190°C, held for 10 min, programmed at 1.8°C/min to 210°C, held for 15 min, programmed at 6°C/min to 234°C, held for 4 min, programmed at 6°C/min to 236°C, and held for 20 min. The FAME identification was validated based on the electron impact ionization spectra of the FAMEs and compared with authentic FAME standards and the NIST 2007 reference mass spectral library.

Statistical analyses

Statistical analyses were performed using the Statistica software package (StatSoft, Version 10, 2010). Statistical analyses of the effects of LO, Se^{IV}, and Se^{VI} on the CLA isomers and other FA in incubated ruminal fluids were conducted using the non-parametric Mann-Whitney *U* test. The results are presented as the means of the individually analyzed ruminal fluids. Mean values in the columns with different superscripts are significantly different at $^{a,b}P < 0.05$ and $^{A,B}P < 0.01$, while differences at $^{\alpha,\beta}P = 0.1$ are indicated as tendencies.

RESULTS

The effect of Se^{IV} and Se^{VI} on the level of CLA isomers and their precursors in ruminal fluids

Our previous investigations documented that ruminal microorganisms in ovine ruminal fluids survive and are active for 24 h of *in vivo* incubation (Wąsowska et al., 2006a, b; Czauderna et al., 2012a). As observed from the obtained results (Tables 3 and 4), the concentrations of the individual CLA isomers and ∑CLA are below the limits

Table 3. Effects[1] of two levels of Se^{IV} and Se^{VI} (L, H) on the concentration of *t11*C18:1, *c11*C18:1, *c9t11c15*C18:3, and CLA isomers (μg/ml) in incubated ruminal fluids containing linseed oil

	*t11*C18:1 (TVA)	*c11*C18:1	*c9t11c15*C18:3 (cLNA)	CLA isomers				ΣCLA isomers[2]
				c9t11	*t10c12*	*c9c11*	*t9t11*	
Incubation time 0 h								
RF	11.7^{A}	0.55^{a}	0[3]	0	0	0	0	0
$Se^{IV}L$	11.5^{A}	0^{b}	0	0	0	0	0	0
$Se^{IV}H$	12.3^{A}	0.90^{a}	0	0	0	0	0	0
$LOSe^{IV}L$	10.0^{A}	0^{b}	0	0	0	0	0	0
$LOSe^{IV}H$	10.9^{A}	0^{b}	0	0	0	0	0	0
$Se^{VI}L$	11.7^{A}	0^{b}	0	0	0	0	0	0
$Se^{VI}H$	13.9^{A}	1.2^{a}	0	0	0	0	0	0
$LOSe^{VI}L$	11.0^{A}	0^{b}	0	0	0	0	0	0
$LOSe^{VI}H$	9.8^{A}	0^{b}	0	0	0	0	0	0
LO	10.6^{A}	0^{a}	0	0	0	0	0	0
Incubation time 6 h								
RF	13.3^{A}	0^{a}	0^{A}	0^{A}	0^{a}	0^{A}	0.82^{Aa}	1.82^{A}
$Se^{IV}L$	13.9^{A}	0^{a}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$Se^{IV}H$	12.6^{A}	0^{a}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$LOSe^{IV}L$	105^{Ba}	3.1^{b}	50^{Bab}	22.7^{B}	1.2^{b}	9.2^{Ba}	7.0^{B}	60^{Bb}
$LOSe^{IV}H$	90^{Ba}	6.4^{c}	49^{Ba}	14.3^{B}	2.6^{b}	9.7^{Bb}	16.5^{B}	43^{Ba}
$Se^{VI}L$	12.8^{A}	0^{a}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$Se^{VI}H$	11.7^{A}	0^{a}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$LOSe^{VI}L$	97^{Ba}	7.7^{c}	55^{Bab}	15.5^{B}	2.3^{b}	13.2^{B}	19.1^{B}	50^{B}
$LOSe^{VI}H$	104^{Ba}	10^{a}	46^{Ba}	17.5^{B}	1.5^{b}	13.1^{B}	14.8^{B}	46^{B}
LO	99^{Ba}	8.7^{c9}	57^{Bb}	18.8^{B}	3.2^{b}	16.9^{B}	18.3^{B}	57^{B}
Incubation time 12 h								
RF	16.1^{A}	0^{A}	0^{A}	0^{A}	0^{a}	0^{A}	1.17^{Aa}	1.17^{Aa}
$Se^{IV}L$	16.8^{A}	0^{A}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$Se^{IV}H$	18.1^{A}	0^{A}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$LOSe^{IV}L$	216^{Cb}	9.5^{Bc}	86^{Bc}	4.5^{d}	10.0^{B}	13.5^{B}	27.4^{B}	55^{Bb}
$LOSe^{IV}H$	174^{Cb}	9.5^{Bc}	73^{Bbc}	10.9^{B}	3.9^{d}	8.5^{Ba}	26.5^{B}	50^{Bab}
$Se^{VI}L$	17.6^{A}	0^{A}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$Se^{VI}H$	17.4^{A}	0^{A}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$LOSe^{VI}L$	162^{Cb}	3.8^{Bb}	75^{Bbc}	10.7^{B}	4.6^{d}	13.5^{B}	25.5^{B}	54^{B}
$LOSe^{VI}H$	171^{Cb}	9.7^{Bc}	72^{Bbc}	10.9^{B}	6.1^{d}	10.7^{B}	28.4^{B}	56^{B}
LO	199^{BCb}	16.4^{Bd}	98^{Bc}	$11.\ 2^{B}$	4.0^{d}	15.9^{Bb}	32.5^{B}	64^{B}
Incubation time 18 h								
RF	15.1^{A}	0^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0^{A}	0^{A}
$Se^{IV}L$	17.8^{A}	1.52^{Ba}	0^{A}	0^{A}	0^{a}	0^{A}	0^{A}	0^{A}
$Se^{IV}H$	18.1^{A}	5.1^{Bbc}	0^{A}	0^{A}	0^{a}	0^{A}	0^{A}	0^{A}
$LOSe^{IV}L$	292^{Cc}	10.1^{Bc}	83^{Bbc}	13.8^{B}	5.4^{d}	19.6^{Ba}	26.2^{B}	65^{Bb}
$LOSe^{IV}H$	217^{Cbc}	12.2^{Bcd}	68^{Bab}	8.0^{B}	3.6^{d}	6.4^{Bb}	27.0^{B}	45^{Ba}
$Se^{VI}L$	16.8^{A}	0^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0^{A}	0^{A}
$Se^{VI}H$	18.7^{A}	0^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0^{A}	0^{A}
$LOSe^{VI}L$	234^{Cc}	13.3^{Bd}	89^{Bc}	11.3^{Ba}	5.0^{d}	12.8^{B}	29.0^{B}	58^{Bab}
$LOSe^{VI}H$	201^{Cbc}	8.8^{Bc}	79^{Bab}	8.8^{Bb}	4.9^{d}	7.6^{Bc}	27.1^{B}	48^{Ba}
LO	231^{Cc}	16.2^{Bd}	93^{Bc}	14.3^{Ba}	2.9^{b}	17.4^{B}	28.6^{B}	63^{Bb}

Table 3 to be continued

	*t11*C18:1 (TVA)	*c11*C18:1	*c9t11c15*C18:3 (cLNA)	CLA isomers				∑CLA isomers[2]
				c9t11	*t10c12*	*c9c11*	*t9t11*	
Incubation time 24 h								
RF	13.8^{A}	1.53^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0.99^{Aa}	0.99^{Aa}
$Se^{IV}L$	18.7^{A}	1.61^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$Se^{IV}H$	18.8^{A}	0^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{Ab}
$LOSe^{IV}L$	262^{Ccd}	15.8^{Bd}	96^{Bc}	1.5^{Ba}	3.8^{bd}	10.5^{Ba}	28.3^{B}	54^{Bb}
$LOSe^{IV}H$	251^{Ccd}	12.7^{Bcd}	62^{Bab}	10.1^{Bb}	2.7^{b}	8.6^{B}	24.4^{B}	46^{Ba}
$Se^{VI}L$	15.5^{A}	0^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{A}
$Se^{VI}H$	18.4^{A}	0^{Aa}	0^{A}	0^{A}	0^{a}	0^{A}	0^{Ab}	0^{A}
$LOSe^{VI}L$	258^{Ccd}	8.5^{Bc}	98^{Bc}	11.6^{Ba}	4.1^{d}	10.1^{Ba}	27.3^{B}	53^{Bd}
$LOSe^{VI}H$	236^{Cc}	12.4^{Bcd}	75^{Bbc}	8.6^{Bb}	3.0^{bd}	5.1^{Bb}	21.7^{B}	38^{Bc}
LO	292^{Cd}	11.3^{Bcd}	98^{Bc}	10.6^{Bab}	1.9^{b}	11.4^{a}	21.9^{B}	46^{B}

LO = linseed oil, Se^{IV} = selenite, Se^{VI} = selenate, L = low level (0.2 μg), H = high level (2.0 μg), cLNA = rumelenic acid, RF = negative control group (reference fluid – *in vitro* incubated 1ml of ovine ruminal fluid with 0.2 ml of water), CLA = conjugated linoleic acid

[1]means in columns at the same incubation time with different superscripts significantly differ at $^{a,b}P < 0.05$ or at $^{A,B}P < 0.01$, while differences at $^{\alpha,\beta}P = 0.1$ are indicated as tendencies; all results are mean values obtained from the individual analyses of ruminal fluid samples collected at different times, statistical analyses of parameter changes were also performed at different times of collection

[2]concentration sum of all assayed CLA isomers in *in vitro* incubated ovine ruminal fluids

[3]below quantification limit

of quantification in the control ruminal fluids (RF) and the fluids containing Se^{IV} or Se^{VI}. The lower concentration of Se^{IV} in the fluids with LO revealed negligible influence on the concentration of ∑CLA in the fluid compared with the fluid with LO. The addition of the higher amount of Se^{IV} to the fluid containing LO numerically or statistically decreased the concentration of ∑CLA in the fluid compared with the fluid containing LO and the lower level of Se^{IV}. The accumulation of *t10c12*CLA in the fluid with LO and Se as Se^{IV} or Se^{VI} increased throughout the incubations. The concentration of *c9t11*CLA in the fluids with LO, irrespective of the presence of Se^{IV} or Se^{VI}, positively correlated with the index values of *c9t11*CLA formation ($index_{c9t11}$) via the bacterial geometrical isomerization of *c9c12*C18:2 (linoleic acid). The concentration of *t11*C18:1 (TVA) in all examined fluids usually increased throughout the *in vitro* incubations. In contrast, the addition of Se^{IV} or Se^{VI} to the fluids with LO numerically or statistically decreased the concentration of *c11*C18:1 in the fluids in comparison with the fluids with LO incubated from 6 to 18 h.

The concentration of *c9t11c15*C18:3 (rumelenic acid; cLNA) in the fluids with LO, irrespective of the presence of extra Se^{IV} or S^{VI}, increased throughout the *in vitro* incubations. The concentration of cLNA in the fluids with LO, irrespectively of the presence of Se^{IV} or Se^{VI}, positively correlated with the index values of cLNA formation ($index_{cLNA}$) via the bacterial geometrical isomerization of *c9c12c15*C18:3 (αLNA).

The effect of Se^{IV} and Se^{VI} on the concentration of non-conjugated fatty acids in ruminal fluids

The concentrations of *c9*C18:1 in the control fluid (RF) and the fluids with Se^{IV} or Se^{VI} (irrespective of their concentrations) numerically decreased throughout the *in vitro* incubations (Table 4). The addition of LO to the fluid, irrespective of the presence of Se^{IV} or Se^{VI}, significantly increased the concentration of *c9*C18:1 compared with the RF and the fluids containing Se^{IV} or Se^{VI}. The concentration of *c9*C18:1

Table 4. Effects[1] of two levels of Se^{IV} and Se^{VI} on the concentration of *c9*C18:1 (μg/ml) and index values of *c9t11c15*C18:3 formation ($index_{cLNA}$), the final biohydrogenation of *t11*C18:1 to C18:0 ($index_{C18:0}$), *c9t11*CLA formation ($index_{c9t11}$), and the initial biohydrogenation of *c9t11*CLA and *t9t11*CLA to TVA ($index_{TVA}$) in *in vitro* incubated ovine ruminal fluids containing linseed oil

Group	*c9*C18:1	$Index_{cLNA}$[2]	$Index_{C18:0}$[3]	$Index_{c9t11}$[4]	$Index_{TVA}$[5]
Incubation time 0 h					
RF	11.9^{A}	0	0.897^{a}	0	1.000^{Aa}
$Se^{IV}L$	11.4^{A}	0	0.898^{a}	0	1.000^{Aa}
$Se^{IV}H$	11.5^{A}	0	0.893^{a}	0	1.000^{Aa}
$LOSe^{IV}L$	144^{b}	0	0.923^{b}	0	1.000^{Aa}
$LOSe^{IV}H$	132^{Ba}	0	0.918^{ab}	0	1.000^{Aa}
$Se^{VI}L$	10.6^{A}	0	0.891^{a}	0	1.000^{Aa}
$Se^{VI}H$	11.7^{A}	0	0.883^{a}	0	1.000^{Aa}
$LOSe^{VI}L$	150^{Ba}	0	0.921^{ab}	0	1.000^{Aa}
$LOSe^{VI}H$	147^{Ba}	0	0.925^{b}	0	1.000^{Aa}
LO	151^{Ba}	0	0.924^{b}	0	1.000^{Aa}
Incubation time 6 h					
RF	11.7^{A}	0^{a}	0.892^{A}	0^{A}	0.880^{ABb}
$Se^{IV}L$	12.1^{A}	0^{a}	0.888^{A}	0^{A}	1.000^{A}
$Se^{IV}H$	11.0^{A}	0^{a}	0.891^{A}	0^{A}	1.000^{A}
$LOSe^{IV}L$	203^{Bb}	0.142^{b}	0.581^{B}	0.152^{B}	0.725^{B}
$LOSe^{IV}H$	180^{Bb}	0.156^{b}	0.624^{B}	0.111^{B}	0.745^{B}
$Se^{VI}L$	10.8^{A}	0^{a}	0.897^{A}	0^{A}	0.914^{A}
$Se^{VI}H$	10.6^{A}	0^{a}	0.896^{A}	0^{A}	1.000^{A}
$LOSe^{VI}L$	188^{Bb}	0.171^{b}	0.610^{B}	0.118^{B}	0.736^{B}
$LOSe^{VI}H$	1192^{Bb}	0.139^{b}	0.575^{B}	0.128^{B}	0.763^{B}
LO	194^{Bb}	0.172^{b}	0.617^{B}	0.135^{B}	0.728^{B}
Incubation time 12 h					
RF	9.2^{A}	0^{a}	0.884^{A}	0^{A}	0.932^{Aab}
$Se^{IV}L$	10.2^{A}	0^{a}	0.878^{A}	0^{B}	1.000^{A}
$Se^{IV}H$	10.9^{A}	0^{a}	0.867^{A}	0^{B}	1.000^{A}
$LOSe^{IV}L$	202^{Bb}	0.280^{b}	0.447^{B}	0.089^{B}	0.853^{Ab}
$LOSe^{IV}H$	201^{Bb}	0.243^{b}	0.519^{B}	0.094^{B}	0.823^{B}
$Se^{VI}L$	10.2^{A}	0^{a}	0.872^{A}	0^{B}	1.000^{A}
$Se^{VI}H$	11.0^{A}	0^{a}	0.873^{A}	0^{A}	1.000^{A}
$LOSe^{VI}L$	206^{Bb}	0.247^{b}	0.537^{B}	0.091^{B}	0.843^{Ab}
$LOSe^{VI}H$	206^{Bb}	0.214^{b}	0.509^{B}	0.086^{B}	0.813^{Ab}
LO	213^{Bb}	0.311^{b}	0.502^{B}	0.098^{B}	0.820^{b}
Incubation time 18 h					
RF	6.5^{A}	0^{a}	0.892^{A}	0^{A}	1.000^{A}
$Se^{IV}L$	8.5^{A}	0^{a}	0.879^{A}	0^{A}	1.000^{A}
$Se^{IV}H$	8.7^{A}	0^{a}	0.874^{A}	0^{A}	1.000^{A}
$LOSe^{IV}L$	205^{Bb}	0.302^{b}	0.417^{B}	0.134^{Bb}	0.879^{Ab}
$LOSe^{IV}H$	204^{Bb}	0.242^{b}	0.516^{B}	0.074^{Ba}	0.861^{Ab}
$Se^{VI}L$	8.2^{A}	0^{a}	0.887^{A}	0^{A}	1.000^{A}
$Se^{VI}H$	9.1^{A}	0^{a}	0.873^{A}	0^{A}	1.000^{A}
$LOSe^{VI}L$	211^{Bb}	0.295^{b}	0.490^{B}	0.100^{Bba}	0.853^{Ab}
$LOSe^{VI}H$	212^{Bb}	0.256^{b}	0.537^{B}	0.074^{Ba}	0.848^{Ab}
LO	224^{Bb}	0.318^{b}	0.513^{B}	0.124^{Bb}	0.843^{Ab}

Table 4 to be continued

Group	*c9*C18:1	Index$_{cLNA}$[2]	Index$_{C18:0}$[3]	Index$_{c9t11}$[4]	Index$_{TVA}$[5]
Incubation time 24 h					
RF	5.2^{A}	0^{a}	0.900^{Aa}	0^{A}	0.933^{Aa}
Se^{IV}L	7.4^{A}	0^{a}	0.877^{Ab}	0^{A}	1.000^{A}
Se^{IV}H	7.5^{A}	0^{a}	0.875^{A}	0^{A}	1.000^{A}
LOSe^{IV}L	213^{Bb}	0.338^{b}	0.504^{B}	0.110^{B}	0.868^{Ab}
LOSe^{IV}H	205^{Bb}	0.244^{b}	0.525^{B}	0.099^{B}	0.879^{Ab}
Se^{VI}L	6.3^{A}	0^{a}	0.901^{Aa}	0^{A}	1.000^{A}
Se^{VI}H	8.1^{A}	0^{a}	0.884^{A}	0^{A}	1.000^{A}
LOSe^{VI}L	214^{Bb}	0.345^{b}	0.515^{B}	0.109^{B}	0.869^{Ab}
LOSe^{VI}H	210^{Bb}	0.275^{b}	0.572^{B}	0.081^{B}	0.886^{Ab}
LO	215^{Bb}	0.339^{b}	0.487^{B}	0.100^{B}	0.900^{Ab}

LO = linseed oil, Se^{IV} = selenite, Se^{VI} = selenate, L = low level (0.2 μg), H = high level (2.0 μg), cLNA = rumelenic acid, αLNA = α-linolenic acid, LA = linoleic acid, RF = negative control group, CLA = conjugated linoleic acid, TVA = *t11*C18:1

[1]means in columns at the same incubation time with different superscripts significantly differ at $^{a,b}P < 0.05$ or at $^{A,B}P < 0.01$, while differences at $^{\alpha,\beta}P = 0.1$ are indicated as tendencies; all results are mean values obtained from the individual analyses of ruminal fluid samples collected at different times, statistical analyses of parameter changes were also performed at different times of collection

[2]index of *c9t11c15*C18:3 (cLNA) formation (index$_{cLNA}$) via bacterial geometrical isomerization of *c9c12c15*C18:3 (αLNA) (index$_{c9t11c15C18:3}$ = cLNA/(cLNA + αLNA))

[3]index of the final biohydrogenation of *t11*C18:1 (TVA) to C18:0 (index$_{C18:0}$ = C18:0/(C18:0 + TVA))

[4]index of *c9t11*CLA formation via bacterial geometrical isomerization of *c9c12*C18:2 (LA) (index$_{c9t11}$ = *c9t11*CLA/(*c9t11*CLA + LA))

[5]index of the initial biohydrogenation of *c9t11*CLA and *t9t11*CLA to TVA (index$_{TVA}$ = TVA/(TVA + *c9t11*CLA + *t9t11*CLA))

in the fluids containing LO, irrespective of the presence of Se^{IV} or Se^{VI}, is higher for incubation at all times from 6 h compared with the concentration of *c9*C18:1 in the fluids enriched with LO with or without Se^{IV} or Se^{VI} at 0 h.

The concentration sum of the geometric and positional isomers of C18:1 (ΣC18:1) in the RF and the fluids with Se^{IV} or Se^{VI}, irrespective of the extra Se concentration, varies negligibly throughout the *in vitro* incubations (Table 5). LO added to the fluid considerably stimulated the accumulation of ΣC18:1 throughout the incubation compared with the RF or fluids enriched with Se^{IV} or Se^{VI}. Se^{IV} or Se^{VI} added to the fluid with LO also increased the concentration of ΣC18:1 compared with the RF and the fluids with Se^{IV} or Se^{VI}. The concentrations of C16:0 and C18:0 in the RF and in the fluids containing LO with or without Se as Se^{IV} or Se^{VI} numerically or statistically increased throughout the *in vitro* incubations. The concentrations of C16:0 and C18:0 in the RF or the fluids with Se^{IV} or Se^{VI} were lower than in the fluids containing LO without or with Se as Se^{IV} or Se^{VI}. Se^{IV} or Se^{VI} added to the fluid containing LO had a small and inconsistent effect on the concentrations of C16:0 and C18:0 compared with the fluids with LO.

Linolenic acid (*c9c12c15*C18:3; αLNA) was quantitatively analyzed in the fluids containing LO, irrespective of the presence of Se^{IV} or Se^{VI} (Table 5), whereas in the RF and the fluids with Se^{IV} or Se^{VI}, the concentration of αLNA was below the detection limit. The concentration of αLNA decreased throughout the incubation of the fluids containing LO with or without Se^{IV} or Se^{VI}, irrespective of their concentrations. Linoleic acid (LA) was detected in all examined fluids; the concentration of LA decreased in the RF, the fluids with Se^{IV} or Se^{VI}, and the fluids containing LO with or without Se^{IV} or Se^{VI} throughout the incubations. LO added to the fluids, irrespective of the presence of Se^{IV} or Se^{VI}, significantly increased the concentration of LA compared with the fluids with Se^{IV} or Se^{VI} and the RF. Se^{IV} or Se^{VI} added to the fluid with LO revealed a negligible influence on the concentration of LA in comparison with the fluids with LO.

The level of *t9t12*C18:2 was above the quantification limit only in the fluids containing LO

Table 5. Effects[1] of two levels of Se^{IV} and Se^{VI} on the concentration of C16:0, C18:0, and selected unsaturated fatty acids (mg/ml) in *in vitro* incubated ruminal fluids containing linseed oil

Group	C16:0	*t9t12*C18:2	*c9c12*C18:2 (LA)	αLNA	ΣC18:1[2]	C20:5n-3[3]	C18:0	ΣPUFA
Incubation time 0 h								
RF	68^{a}	0	11.4^{A}	0^{A}	24.1^{A}	1.84^{a}	101^{a}	13.6^{Aa}
$Se^{IV}L$	69^{a}	0	10.7^{A}	0^{A}	23.0^{A}	1.91^{a}	102^{a}	12.6^{Aa}
$Se^{IV}H$	70^{a}	0	11.6^{A}	0^{A}	24.7^{A}	1.83^{a}	103^{a}	13.4^{Aa}
$LOSe^{IV}L$	103^{b}	0	123^{Ca}	328^{Ba}	154^{Ba}	2.82^{b}	120^{a}	454^{B}
$LOSe^{IV}H$	103^{b}	0	114^{Ca}	297^{Ba}	143^{Ba}	2.75^{b}	122^{a}	414^{B}
$Se^{VI}L$	67^{a}	0	10.3^{A}	0^{A}	22.3^{A}	2.06^{a}	95^{a}	12.3^{Aa}
$Se^{VI}H$	72^{a}	0	11.7^{A}	0^{A}	26.8^{A}	2.46^{a}	104^{a}	14.1^{Aa}
$LOSe^{VI}L$	110^{b}	0	128^{Ca}	338^{Ba}	161^{Ba}	3.04^{b}	129^{a}	470^{B}
$LOSe^{VI}H$	101^{b}	0	126^{Ca}	336^{Ba}	156^{Ba}	2.66^{b}	120^{a}	465^{B}
LO	111^{b}	0	130^{Ca}	345^{Ba}	162^{Ba}	3.59^{b}	129^{a}	479^{B}
Incubation time 6 h								
RF	77^{a}	0	8.2^{A}	0^{A}	25.0^{A}	1.81^{a}	111^{a}	11.9^{Aa}
$Se^{IV}L$	80^{a}	0	89^{A}	0^{A}	25.9^{A}	1.81^{a}	110^{a}	10.7^{Aa}
$Se^{IV}H$	74^{a}	0	7.8^{A}	0^{A}	23.6^{A}	1.87^{a}	104^{a}	9.6^{Aa}
$LOSe^{IV}L$	132^{bc}	12^{Aa}	126^{Ca}	301^{Ba}	310^{Bb}	4.03^{c}	145^{a}	553^{B}
$LOSe^{IV}H$	129^{bc}	9^{Aa}	115^{Ca}	264^{Bab}	276^{Bb}	6.88^{c}	149^{b}	487^{B}
$Se^{VI}L$	79^{a}	0	8.1^{A}	0^{A}	23.6^{A}	1.82^{a}	111^{a}	11.1^{Aa}
$Se^{VI}H$	78^{a}	0	8.1^{A}	0^{A}	23.4^{A}	2.32^{a}	109^{a}	10.4^{Aa}
$LOSe^{VI}L$	131^{bc}	11^{Aa}	116^{Ca}	265^{Ba}	292^{Bb}	4.41^{c}	151^{b}	503^{B}
$LOSe^{VI}H$	126^{bc}	10^{Aa}	119^{Ca}	284^{Ba}	296^{Bb}	5.69^{c}	141^{a}	510^{B}
LO	135^{bc}	11^{Aa}	121^{Ca}	274^{Bab}	302^{Bb}	4.11^{c}	160^{b}	524^{B}
Incubation time12 h								
RF	91^{a}	0	4.6^{B}	0^{A}	25.3^{A}	1.72^{a}	123^{a}	7.5^{Aac}
$Se^{IV}L$	89^{a}	0	3.7^{B}	0^{A}	27.0^{A}	1.74^{a}	120^{a}	5.4^{Ac}
$Se^{IV}H$	89^{a}	0	3.7^{B}	0^{A}	29.0^{A}	1.73^{a}	118^{a}	5.4^{Ac}
$LOSe^{IV}L$	144^{c}	23^{B}	102^{Ca}	222^{Bb}	427^{Bc}	5.03^{c}	174^{a}	493^{B}
$LOSe^{IV}H$	149^{c}	16^{B}	105^{Ca}	228^{Bb}	384^{Bc}	8.85^{c}	187^{b}	481^{B}
$Se^{VI}L$	90^{a}	0	4.4^{B}	0^{A}	27.8^{A}	1.68^{a}	120^{a}	6.1^{Aac}
$Se^{VI}H$	91^{a}	0	4.6^{B}	0^{A}	28.4^{A}	2.17^{a}	120^{a}	6.8^{Aac}
$LOSe^{VI}L$	148^{c}	19^{B}	107^{Ca}	230^{Bb}	414^{Bc}	6.81^{c}	201^{b}	494^{B}
$LOSe^{VI}H$	147^{c}	17^{B}	116^{Ca}	262^{Bab}	387^{Bc}	6.79^{c}	177^{b}	529^{B}
LO	154^{c}	22^{B}	103^{Ca}	217^{Bb}	429^{Bc}	4.10^{c}	200^{b}	508^{Ba}
Incubation time 18 h								
RF	90^{a}	0	1.1^{B}	0^{A}	21.6^{A}	1.30^{a}	124^{a}	2.4^{Ad}
$Se^{IV}L$	100^{a}	0	1.7^{B}	0^{A}	27.9^{A}	1.73^{a}	130^{a}	3.4^{Acd}
$Se^{IV}H$	95^{a}	0	1.8^{B}	0^{A}	27.8^{A}	1.86^{a}	126^{a}	3.7^{Acd}
$LOSe^{IV}L$	153^{c}	28^{B}	90^{Cb}	192^{Bb}	507^{Bda}	5.95^{c}	201^{b}	463^{B}
$LOSe^{IV}H$	156^{c}	19^{B}	100^{Cab}	214^{Bb}	$433^{Bcd\beta}$	14.13^{d}	232^{bc}	460^{B}
$Se^{VI}L$	96^{a}	0	2.0^{B}	0^{A}	25.0^{A}	1.68^{a}	132^{a}	3.6^{Acd}
$Se^{VI}H$	99^{a}	0	1.7^{B}	0^{A}	27.8^{A}	2.05^{a}	129^{a}	3.7^{Acd}
$LOSe^{VI}L$	159^{c}	23^{B}	102^{Cab}	213^{Bb}	459^{Bcd}	9.86^{d}	225^{bc}	495^{B}
$LOSe^{VI}H$	158^{c}	20^{B}	110^{Ca}	231^{Bb}	422^{Bcg}	12.43^{d}	233^{bc}	501^{B}
LO	159^{c}	24^{B}	101^{Cab}	200^{Bb}	471^{Bcd}	5.78^{c}	243^{bc}	488^{B}

Table 5 to be continued

Group	C16:0	*t9t12*C18:2	*c9c12*C18:2 (LA)	αLNA	ΣC18:1[2]	C20:5n-3[3]	C18:0	ΣPUFA
Incubation time 24 h								
RF	91^{a}	0	0.9^{B}	0^{A}	20.6^{A}	1.45^{Aa}	125^{a}	3.4^{Acd}
$Se^{IV}L$	98^{a}	0	0.6^{B}	0^{A}	28.2^{A}	1.79^{Aa}	133^{a}	2.4^{Ad}
$Se^{IV}H$	99^{a}	0	1.2^{A}	0^{A}	26.3^{A}	1.81^{Aa}	131^{a}	3.0^{Acd}
$LOSe^{IV}L$	163^{c}	27^{B}	93^{Cb}	187^{Bb}	491^{Bcd}	9.77^{d}	266^{bc}	467^{B}
$LOSe^{IV}H$	161^{c}	20^{B}	91^{Cb}	192^{Bb}	469^{Bcd}	11.82^{d}	277^{bc}	423^{B}
$Se^{VI}L$	100^{a}	0	0.6^{B}	0^{A}	21.8^{A}	1.90^{Aa}	141^{a}	2.5^{Ad}
$Se^{VI}H$	101^{a}	0	1.1^{B}	0^{A}	27.0^{A}	2.65^{Aa}	140^{a}	3.7^{Acd}
$LOSe^{VI}L$	162^{c}	31^{B}	95^{Cb}	186^{Bb}	480^{Bcd}	9.26^{d}	275^{bc}	472^{B}
$LOSe^{VI}H$	162^{c}	20^{B}	97^{Cb}	199^{Bb}	458^{Bcd}	10.37^{da}	316^{bc}	440^{B}
LO	157^{c}	27^{B}	95^{Cb}	190^{Bb}	518^{Bd}	$4.46^{c\beta}$	277^{bc}	460^{B}

LO = linseed oil, Se^{IV} = selenite, Se^{VI} = selenate, L = low level (0.2 μg), H = high level (2.0 μg), αLNA = α-linolenic acid (*c9c12c15*C18:3), LNA = linolenic acid, LA = linoleic acid, PUFA = polyunsaturated fatty acids, L = low level, H = high level, RF = negative control group

[1]means in columns at the same incubation time with different superscripts are significantly different at $^{a,b}P < 0.05$ or at $^{A,B}P < 0.01$, while differences at $^{\alpha,\beta}P = 0.1$ are indicated as tendencies; all results are mean values obtained from the individual analyses of ruminal fluid samples collected at different times, statistical analyses of parameter changes were also performed at different times of collection

[2]concentration sum of all assayed C18:1 – *c9*C18:1, *c11*C18:1, *t9*C18:1, and *t11*C18:1

[3]*C5c8c11c14c17*C20:5 (eicosapentaenoic acid)

with or without Se^{IV} or Se^{VI} at all times from 6 h of the incubation (Table 5). The concentration of *t9t12*C18:2 increased throughout the incubation in these fluids. Se^{IV} or Se^{VI} added to the fluids enriched with LO revealed a negligible influence effect on the level of *t9t12*C18:2 compared with the fluids with LO. The concentration of *c5c8c11c14c17*C20:5 (C20:5n-3) was higher in the fluids with LO, irrespective of the presence of Se^{IV} or Se^{VI}, than in the RF or the fluids with Se^{IV} or Se^{VI}. The concentration of C20:5n-3 was elevated especially by the higher concentration of Se^{IV} or Se^{VI} in the fluids with LO at times from 18 h in particular compared with the fluids with LO. LO added to the fluids, irrespective of the presence of extra Se^{IV} or Se^{VI}, significantly increased the concentration of PUFA compared with the RF or the fluids containing Se^{IV} or Se^{VI}. The concentration of PUFA in the RF and the fluids with Se^{IV} or Se^{VI} decreased throughout the incubations. LO added to the fluids, regardless of the presence of Se^{IV} or Se^{VI}, increased the concentration of PUFA compared with the RF or the fluids with Se^{IV} or Se^{VI}.

DISCUSSION

The formation of CLA isomers and their precursors in the ruminal fluids can be explained by the conversion of dietary PUFA through group A ruminal bacteria (Bauman et al., 2003; Sieber et al., 2004; Buccioni et al., 2012). As the first step, *cis,trans* and/or *trans,cis* conjugated FA derived from αLNA, LA or γ-linolenic acid (*c6c9c12*C18:3; γLNA) were formed due to the activity of an isomerase (linoleate isomerase; EC 5.2.1.5) from anaerobic bacterium *Butyrivibrio fibrisolvens.* Low-fibre diets stimulated the activity of other ruminal bacteria, such as *Megasphaera (n.) elsdenii strains* YJ-4 and T81, which are able to produce significant amounts of *t10c12*CLA, but not of some other strains (e.g. strains B159, AW106, and JL1) (Kim et al., 2002, Sieber et al., 2004; Buccioni et al., 2012). The results summarized in Table 5 indicated that Se^{IV} and Se^{VI} added to the fluid with LO impact the capacities of the *cis-12,trans-11* and *cis-9,trans-10* isomerases, which isomerize the *cis-12* and *cis-9* bonds into the *trans-11* and *trans-10* bonds, respectively

(McKain et al., 2010). Indeed, the concentration of *c9t11*CLA in the fluids containing LO and the higher concentration of Se^{IV} or Se^{VI} is usually lower than in the fluid with LO, irrespective of the lower concentration of Se^{IV} or Se^{VI}. These findings are in agreement with the influence of Se^{IV} and Se^{VI} added to the fluids on the index values of *c9t11*CLA formation ($index_{c9t11}$) via the bacterial geometrical isomerization of LA in the fluids; changes in the concentration of *c9t11*CLA in the fluids with LO positively correlated with the changes in the values of $index_{c9t11}$ compared with the fluids containing LO.

The lower concentration of Se^{IV} and particularly Se^{VI} in the fluids with LO usually increased the level of *t10c12*CLA compared with the control fluid and the fluids with LO and the higher concentration of Se^{IV} or Se^{VI}. These results showed that the lower concentration of Se^{IV} and especially Se^{VI} in the fluids stimulated the capacity of the *cis-9,trans-10* isomerase, while the higher concentration of Se^{IV} or Se^{VI} decreased the capacity of the *cis-12,trans-11* isomerase. Similarly, the higher level of Se^{IV} in the fluids reduced the capacity of the bacterial isomerases, which are responsible for the formation of *c9c11*CLA and *t9t11*CLA (Table 5). Se^{VI} added to the fluids with LO decreases the capacity of a bacterial isomerase involved in the formation of *c9c11*CLA; the decrease in the isomerase capacity positively correlated with the concentration of Se^{VI} in the fluids with LO. However, Se^{VI} in the fluid with LO revealed an inconsistent effect on the isomerization involved in the formation of *t9t11*CLA.

The results discussed above are in agreement with the yield of the biohydrogenation to TVA being dependent on the presence and the concentration of Se^{IV} or Se^{VI} in the fluids containing LO such that a higher concentration of Se^{IV} or Se^{VI} in the fluids with LO usually resulted in a larger decrease in the concentration of TVA compared with the lower concentration of Se^{IV} or Se^{VI} in the fluids containing LO (Table 3). Indeed, the concentration of the substrates (i.e. *c9t11*CLA and *t9t11*CLA), which were consumed during the biohydrogenation to TVA, decreased as the concentration of Se^{IV} or Se^{VI} increased in the fluids with LO. Similarly, Se^{IV} or Se^{VI} added to the fluids with LO usually slightly decreased the index values of the final biohydrogenation of TVA to C18:0 ($index_{C18:0}$) and the concentration of C18:0 in the fluids (Tables 4 and 5) compared with the fluids containing LO.

The higher concentration of Se^{IV} or Se^{VI} in the fluid with LO decreased the capacity of a bacterial isomerase, which is responsible for the formation of cLNA, and decreased the index values of cLNA formation ($index_{cLNA}$) via the bacterial geometrical isomerization of αLNA compared with the fluids with LO (Table 4). In contrast, the lower concentration of Se^{IV} or Se^{VI} in the fluids with LO less efficiently reduced the capacity of a bacterial isomerase and the values of the $index_{cLNA}$ compared with the higher concentration of Se^{IV} or Se^{VI} in the fluids with LO. These observations are consistent with the effect of the higher concentration of Se^{IV} or Se^{VI} in the fluids containing LO on the decrease in the capacity of a bacterial isomerase, which is responsible for formation of *c9t11*CLA, and the decrease in the index values of *c9t11*CLA formation via the bacterial geometrical isomerization of LA (Table 4). Therefore, we argue that this effect, especially the higher concentration of Se^{IV} or Se^{VI} in the fluid with LO, can be observed *in vivo,* resulting in a decreased duodenal flow of the CLA isomers, particularly *c9t11*CLA. Our current results showed that the lower capacity of the *cis-12,trans-11* and *cis-9,trans-10* bacterial isomerases, which in a rumen isomerized dietary LA and αLNA to *c9t11*CLA and *t10c12*CLA, respectively (McKain et al., 2010), is caused by Se^{VI} added to the lambs' diets, irrespective of the presence of LO. Similarly, the higher concentration of Se^{IV} or Se^{VI} in the fluid with LO decreased the capacity of bacterial isomerization of C18-PUFA, which is responsible for the formation of *t9t12*C8:2, compared with the fluid with LO, irrespective of the presence of lower concentration of Se^{IV} or Se^{VI} (Table 4).

We suggest that the increase in the concentration of αLNA in the fluid containing LO and especially with the higher concentration of Se^{IV} or Se^{VI}, compared with the fluid with LO (Table 4), may be due to the lower capacity of the *cis-12,trans-11* and *cis-9,trans-10* bacterial isomerases and/or the higher yield of bacterial lipolysis, which is responsible for the release of free αLNA from esters. Indeed, various bacterial strains of *Butyrivibrio fibrisolvents* and *Anaerovibrio lipolytica* are able to hydrolyze ester bonds. Lipase activity also occurs in the ciliatae protozoa; however, the contribution of this enzyme is smaller than that from the ruminal bacteria (Bessa et al., 2009; Kim et al., 2009; Buccioni et al., 2012).

Our hypothesis relating to bacterial lipolysis confirmed the impact of Se^{IV} and Se^{VI} added to

the fluid, irrespective of the presence of LO, on the concentration of C20:5n-3 (Table 5). Indeed, during incubation, the concentration of C20:5n-3 is higher in the fluid with Se^{IV} or Se^{VI} compared with the RF; moreover, the C20:5n-3 accumulation increased as the concentration of Se^{IV} or Se^{VI} increased in the fluid. These findings confirm our model in which bacterial lipolysis is stimulated by Se^{IV} or Se^{VI} added to fluids, irrespective of the presence of LO. Our results showed that the yield of microbial lipolysis increased with the increasing concentration of Se^{IV} or Se^{VI} added to the fluid with or without LO. The results summarized in Tables 3–5 suggest that Se^{IV} and Se^{VI} added to the fluid, irrespective of the presence of LO, also reduced the yield of the biohydrogenation of C20:5n-3 in the fluid. This decrease in the yield of the biohydrogenation of C20:5n-3 in the fluid increased as the concentration of Se^{IV} or Se^{VI} increased in the fluids with or without LO. The relative increase in the concentration of C20:5n-3 in the fluids with LO and Se, especially as Se^{IV}H or Se^{VI}H, was higher than the relative increase in the concentration of αLNA in the fluids with LO and the higher concentration of Se^{IV} or Se^{VI}. This effect should be observed in *in vivo* investigations, resulting in an enhanced duodenal flow of αLNA or C20:5n-3 (Niedźwiedzka et al., 2008; Czauderna et al., 2010, 2012b; Krajewska et al., 2012). Our observations are also consistent with recent *in vivo* and *in vitro* studies that have reported that the ruminal biohydrogenation of C20:5n-3 and other LPUFAn-3 was significantly lower compared to the ruminal biohydrogenation of C18-PUFA (e.g., LA or αLNA) (Dohme et al., 2003; Chow et al., 2004). During incubation of the fluids, the total concentration of C18-MUFA (∑C18:1) increased in the fluid with LO, irrespective of the addition of Se^{IV} or Se^{VI}. Especially with the addition of the higher concentration of Se^{IV} or Se^{VI} to the fluid with LO, the concentration of ∑C18:1 decreased compared with the fluid with LO. This observation is in line with the changes in the values of the biohydrogenation indexes (i.e. $index_{C18:0}$ and $index_{TVA}$) and the changes in the yield of the initial biohydrogenation of *c9t11*CLA and *t9t11*CLA to TVA and the final biohydrogenation to C18:0 (Table 3). The higher concentration of Se^{IV} or Se^{VI} in the fluid with LO usually decreased the concentration of TVA, *c9*C18:1, and *c11*C18:1 compared with the fluid with LO, irrespective of the presence of a lower concentration of Se^{IV} or Se^{VI}.

CONCLUSION

Our current studies demonstrated that Se^{IV} and Se^{VI} in the ruminal fluids affect the capacity of the bacterial isomerases that are responsible for the formation of CLA isomers, particularly *c9t11*CLA, *t10c12*CLA, *c9c11*CLA, and *t9t11*CLA. Moreover, Se^{IV} or Se^{VI} added to the ruminal fluid enriched with LO slightly lowered the capacity of biohydrogenation.

Our investigations revealed that the higher concentration of Se^{IV} or Se^{VI} in the ruminal fluid with LO particularly elevated the accumulation of health-promoting fatty acids (i.e. αLNA and C20:5n-3) in fluid. These observations are consistent with our previous studies, in which Se^{VI} added to a diet enriched with LO stimulated the accumulation of LPUFAn-3 in the muscles of lambs compared with lambs fed a diet containing LO.

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REFERENCES

Aharoni Y., Orlov A., Brosh A. (2004): Effects of high-forage content and oilseed supplementation of fattening diets on conjugated linoleic acid (CLA) and *trans* fatty acids profiles of beef lipid fractions. Animal Feed Science and Technology, 117, 43–60.

Bauman D.E., Griinari J.M., Corl B.A. (2003): Nutritional regulation of milk fat synthesis. Annual Review of Nutrition, 23, 203–227.

Bernard L., Bonnet M., Leroux C., Shingfield K.J., Chilliard Y. (2009): Effect of sunflower-seed oil and linseed oil on tissue lipid metabolism, gene expression, and milk fatty acid secretion in alpine goats fed maize silage-based diets. Journal of Dairy Science, 92, 6083–6094.

Bessa R.J.B., Maia M.R.G., Jerónimo E., Belo A.T., Cabrita A.R.J., Dewhurst R.J., Fonseca A.J.M. (2009): Using microbial fatty acids to improve understanding of the contribution of solid associated bacteria to microbial mass in the rumen. Animal Feed Science and Technology, 150, 197–206.

Buccioni A., Decandia M., Minieri S., Molle G., Cabiddu A. (2012): Lipid metabolism in the rumen: new insights on the lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. Animal Feed Science and Technology, 174, 1–25.

Burns P.D., Engle T.E., Harris M.A., Enns R.M., Whittier J.C. (2003): Effect of fish meal supplementation on plasma and endometrial fatty acid composition in nonlactating beef cows. Journal of Animal Science, 8, 2840–2846.

Chikunya S., Demirel G., Enser M., Wood J.D., Wilkinson R.G., Sinclair L.A. (2004): Biohydrogenation of dietary n-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep. British Journal of Nutrition, 91, 539–550.

Chow T.T., Fievez V., Moloney A.P., Raes K., Demeyer D., De Smet S. (2004): Effect of fish oil on *in vitro* rumen lipolysis, apparent biohydrogenation of linoleic and linolenic acid and accumulation of biohydrogenation intermediates. Animal Feed Science and Technology, 117, 1–12.

Crespo A.M., Reis M.A., Lanca M.J. (1995): Effect of selenium supplementation on polyunsaturated fatty acids in rats. Biological Trace Element Research, 47, 335–341.

Czauderna M., Kowalczyk J., Niedźwiedzka K.M., Wąsowska I., Pająk J.J. (2004a): The effect of selenium and linseed oil on growth of sheep and content of selected fatty acids in *M. longissimus dorsi*. Journal of Animal and Feed Sciences, 13, 303–306.

Czauderna M., Kowalczyk J., Niedźwiedzka K.M., Wąsowska I., Pająk J.J., Bulska E., Ruszczyńska A. (2004b): The effect of linseed oil and selenium on the content of fatty acids and some elements in the liver and selected tissues of sheep. Journal of Animal and Feed Sciences, 13, 103–106.

Czauderna M., Kowalczyk J., Korniluk K. (2007): Effect of dietary conjugated linoleic acid mixture and selenized yeast on concentrations of selected fatty acids and mineral elements in rats. Archives of Animal Nutrition, 61, 135–150.

Czauderna M., Rozbicka-Wieczorek A., Krajewska K.A., Kowalczyk J. (2010): Influence of linseed oil and selenate supplemented diet on the concentration of fatty acids and Se, Zn, Fe, Ca and Mg in muscles and liver of sheep. In: Migdał W., Cilev G., Živković B., Jukna V. (eds): Intramuscular Fat and Histological Structure of Meat. Polish Society of Food Technologists – Małopolska Branch, Kraków, Poland, 21–29.

Czauderna M., Kowalczyk J., Wallace J.R. (2012a): Selenite and selenate affected the fatty acid profile in *in vitro* incubated ovine ruminal fluid containing linoleic acid. Journal of Animal and Feed Sciences, 21, 477–492.

Czauderna M., Kowalczyk J., Marounek M. (2012b): Dietary linseed oil and selenate affect the concentration of fatty acids in selected tissues of sheep. Czech Journal of Animal Science, 57, 389–401.

Demirel G., Wachira A.M., Sinclair L.A., Wilkinson R.G., Wood J.D., Enser M. (2004): Effects of dietary n-3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. British Journal of Nutrition, 91, 551–565.

Dohme F., Fievez V., Raes K., Demeyer D. (2003): Increasing levels of two different fish oils lower ruminal biohydrogenation of eicosapentaenoic and docosahexaenoic acid *in vitro*. Animal Research, 52, 309–320.

Gudla P., AbuGhazaleh A.A., Ishlak A., Jones K. (2012): The effect of level of forage and oil supplement on biohydrogenation intermediates and bacteria in continuous cultures. Animal Feed Science and Technology, 171, 108–116.

Jenkins T.C., Wallace R.J., Moate P.J., Mosley E.E. (2008): Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. Journal of Animal Science, 86, 397–412.

Juniper D.T., Phipps R.H., Ramos-Morales E., Bertin G. (2008): Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribution and meat quality in beef cattle. Journal of Animal Science, 86, 3100–3109.

Kim Y.J., Liu R.H., Rychlik J.L., Russell J.B. (2002): The enrichment of a ruminal bacterium *(Megasphaera elsdenii* YJ-4*)* that produces the *trans*-10,*cis*-12 isomer of conjugated linoleic acid. Journal of Applied Microbiology, 92, 976–982.

Kim E.J., Huws S.A., Lee M.R.F., Scollan N.D. (2009): Dietary transformation of lipid in the rumen microbial ecosystem. Asian-Australasian Association of Animal Societies, 22, 1341–1350.

Korniluk K., Czauderna M., Kowalczyk J., Mieczkowska A., Taciak M., Leng Ľ. (2006): Influence of dietary conjugated linoleic acid isomers and selenium on growth, feed efficiency, and liver fatty acid profile in rats. Journal of Animal and Feed Sciences, 15, 131–146.

Krajewska K.A., Rozbicka-Wieczorek A.J., Kowalczyk J., Czauderna M. (2012): Dietary linseed oil and selenate affect the concentration of fatty acids and selenium in the spleen, pancreas, and kidneys of lambs. Journal of Animal and Feed Sciences, 21, 285–301.

Lee Y.-J., Jenkins T.C. (2011): Biohydrogenation of linolenic acid to stearic acid by the rumen population yields multiple intermediate conjugated diene isomers. The Journal of Nutrition, 141, 1445–1450.

Loor J.J., Ueda K., Ferlay A., Chilliard Y., Doreau M. (2005): Intestinal flow and digestibility of *trans* fatty acids and conjugated linoleic acids (CLA) in dairy cows fed a high-concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. Animal Feed Science and Technology, 119, 203–225.

Lyons T.P., Jacques K.A. (eds) (2001): Science and technology in the feed industry. In: Proc. of Alltech's 17th Annual Symposium. Nottingham, UK, 309–415.

McKain N., Shingfield K.J., Wallace R.J. (2010): Metabolism of conjugated linoleic acid and 18:1 fatty acids by ruminal

bacteria: products and mechanisms. Microbiology, 156, 579–588.

Mir P.S., Mir Z., Kuber P.S., Gaskins C.T., Martin E.L., Dodson M.V., Elias Calles J.A., Johnson K.A., Busboom J.R., Wood A.J., Pittenger G.J., Reeves J.J. (2002): Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response intravenous glucose challenge in high percentage Wagyu, Wagyu × Limousin and Limousin steers fed sunflower oil containing diets. Journal of Animal Science, 80, 2996–3004.

Miri V.H., Tyagi A.K., Ebrahimi S.H., Mohini M. (2013): Plant extract enhanced ruminal CLA concentration, *in vitro*. Journal of Animal and Feed Sciences, 22 (in press).

Navarro-Alarcon M., Cabrera-Vique C. (2008): Selenium in food and the human body: A review. Science of the Total Environment, 400, 115–141.

Niedźwiedzka M.K., Kowalczyk J., Czauderna M. (2008): Influence of selenate and linseed oil on fatty-acid and amino-acid profiles in the liver, muscles, fat tissues and blood plasma of sheep. Journal of Animal and Feed Sciences, 17, 328–343.

Or-Rashid M.M., Odongo N.E., McBride B.W. (2007): Fatty acid composition of ruminal bacteria and protozoa, with emphasis on conjugated linoleic acid, vaccenic acid, and odd-chain and branched-chain fatty acids. Journal of Animal Science, 85, 1228–1234.

Raes K., De Smet S., Demeyer D. (2004): Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. Animal Feed Science and Technology, 113, 199–221.

Sieber R., Collomb M., Aeschlimann A., Jelen P., Eyer H. (2004): Impact of microbial cultures on conjugated linoleic acid in dairy products – review. International Dairy Journal, 14, 1–15.

Suzuki K.T. (2005): Metabolomics of selenium: Se metabolites based on speciation studies. Journal of Health Science, 51, 107–114.

Wąsowska I., Maia M., Niedźwiedzka K.M., Czauderna M., Ramalho Ribeiro J.M.C., Devillard E., Shingfield K.J., Wallace R.J. (2006a): Influence of fish oil on ruminal biohydrogenation of C18 unsaturated fatty acids. British Journal of Nutrition, 95, 1199–1211.

Wąsowska I., Czauderna M., Kowalczyk J., Wallace R.J. (2006b): Impact of selenite and selenate on conjugated linoleic acid isomers in incubated ruminal fluid. Journal of Animal and Feed Sciences, 15, 97–100.

Whanger P.D. (2004): Selenium and its relationship to cancer: an update. Review article. British Journal of Nutrition, 91, 11–28.

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Corresponding Author

Prof. dr. hab. Marian Czauderna, Polish Academy of Sciences, The Kielanowski Institute of Animal Physiology and Nutrition, 05-110 Jabłonna, Poland
Tel. +482 276 553 370, fax +482 276 553 302, e-mail: m.czauderna@ifzz.pan.pl