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SELENO-CYSTINE AFFECTS THE FATTY ACID PROFILE IN *IN VITRO* INCUBATED OVINE RUMINAL FLUID CONTAINING α-LINOLENIC ACID

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ABSTRACT

The influence of seleno-cystine ($CySe_2$) added to ovine ruminal fluids containing α-linolenic acid (αLNA) on the profile of fatty acids (FA) was investigated. Fluids were incubated in vitro at 39°C under CO_2 either alone (RF) or with αLNA (1.67 mg/ml) or with a combination of αLNA with either a low (1.34 µg/ml) or high (3.33 µg/ml) level of Se as $CySe_2$. Fluids were removed after 0, 6, 12, 18, 24 hrs of incubation and then analyzed to determine FA levels. αLNA added to the fluids without/with $CySe_2$ decreased the C18:0 concentration for incubation at all times from 6 hrs compared with the RF or the fluid containing $CySe_2$. αLNA added to the fluids without/with $CySe_2$ decreased the biohydrogenation yield to C18:0. $CySe_2$ added to the fluids decreased the C18:0 concentration and the index of the biohydrogenation to C18:0 compared with the RF. The higher concentration of $CySe_2$ in the fluids with αLNA reduced the accumulation of trans11C18:1 for incubation at all times from 18 hrs compared with the fluids with αLNA, irrespective of the presence of the lower concentration of $CySe_2$. The lowest concentration of trans11C18:1 in the fluids with αLNA and the higher concentration of $CySe_2$ correlated with the lowest yield of the isomerization of αLNA into cis9trans11cis15C18:3 and the lowest yield of the initial biohydrogenation of cis9trans11cis15C18:3 to trans11cis15C18:2 in the fluids containing αLNA and the higher concentration of $CySe_2$. $CySe_2$ added to the fluids with αLNA decreased the ratio of polyunsaturated FA to saturated FA for incubation at all times from 12 hrs compared with the fluids containing αLNA. $CySe_2$ in the fluids without/with αLNA reduced the FA sum in the fluids.

Keywords: Selenium, α-Linolenic acid, Ovine ruminal fluids, Fatty acids, Biohydrogenation, Isomerization.

Contribution/ Originality

Our original study documents that $CySe_2$ added to the ovine ruminal fluids, irrespective of the presence of αLNA, affects concentrations of fatty acids, the capacity of the bacterial isomerases and the biohydrogenation yield of unsaturated fatty acids in *in vitro* incubated ruminal fluids compared with the control fluid.

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1. INTRODUCTION

A large proportion of selenium (Se) in feedstuffs is present in organic compounds, as seleno-cysteine (CySe) and especially as seleno-methionine (Se-Met), and forms a part of the amino acid structure of protein molecules in the feed [1, 2]. Rumen microorganisms can incorporate CySe and Se-Met into their own proteins, but can also reduce an excess of dietary Se-compounds into inorganic forms (like selenide or Se^{o}) that are largely unavailable to the ruminant [3, 4]. Ruminal microbial Se concentrations were enriched relative to the concentration of Se in rations. Indeed, the concentration of Se was significantly higher than that of the ration, whether considered relative to diet dry matter (average of 46-fold), nitrogen (average of 11.3-fold) or sulfur abundance (average of 26-fold) [5]. Moreover, the enrichment of Se in microbial cells (2-78-fold) was greater than the enrichment of either nitrogen (average of 4-fold) or sulfur (average of 1.6-fold). Ruminal microorganisms reduce much of dietary inorganic Se (as selenite or selenate) to unabsorbable elemental Se or inorganic selenide forms. Bacteria are also able to synthesize Se-Met and CySe, and then these Se-amino acids (Se-AA(s)) are incorporated into microbial proteins. Thus, dietary Se finds its way into a form metabolizable by ruminants, and so the way to improve the healthiness of ruminant meat and milk by increasing the concentration of CySe and Se-Met [6, 7].

Recent investigations demonstrated that n-3 polyunsaturated fatty acids (PUFA) like α-linolenic acid (αLNA) and long-chain PUFA (LPUFA) possessed also several potential health benefits, including cancer prevention, decreased atherosclerosis, improved immune response and altered fatty acids (FA) and protein metabolism [8-11]. Numerous *in vitro* and *in vivo* studies documented that ruminant dietary C18-PUFA like linoleic acid (LA) or αLNA are direct incorporated into the ruminal bacteria, isomerized to other geometrical and positional isomers, metabolized into conjugated linoleic acid (CLA) isomers [8, 10, 12-14], as well as biohydrogenated to *trans11*C18:1 (*t11*C18:1) and finally to C18:0. Indeed, C18-PUFA, especially LPUFA, have toxic effects on cellulolytic bacteria and protozoa, act against ruminal lactate producers thereby favouring propionate producers [15-17]. Interestingly, unsaturated fatty acids (like αLNA, LA or *cis9*C18:1) inhibited bacterial enoyl-acyl carrier protein reductase, an essential component of bacterial fatty acid synthesis [18]. Unsaturated fatty acids (UFA) are more toxic than saturated fatty acids (SFA) and can inhibit fermentation in the rumen more intensively [19]. UFA are especially toxic to G^{+} bacteria, whereas G^{-} bacteria are less sensitive to fatty acids at the same concentration [20]. Biohydrogenation is a microbial pathway in ruminal contents designed to reduce unsaturation of lipids found within plant matter, and is likely an evolutionary adaptation to protect the microbial population from antimicrobial effects of unsaturated fatty acids (UFA) [8, 13]. Current studies indicated that *t11*C18:1, the intermediate biohydrogenation product of αLNA and LA formed in a rumen, may also be of benefit in the prevention of cancer due to endogenous conversion of *t11*C18:1 to *cis9,trans11*CLA (*c9t11*CLA) in mammalian tissues [21-24]. In our recent studies it was found that the concentration of UFA in a body of animals, as well as in membrane of microorganisms cells were positively correlated with the Se level in a diet [25-28]. Indeed, Se is an integral component of antioxidant enzymes (e.g. glutathione

peroxidases) that can decrease the risk of PUFA peroxidation in particular [29, 30]. Moreover, our recent studies revealed that of selenate or selenite changed concentrations of FAs and CLA isomers in particularly in incubated ovine ruminal fluid muscle [23, 24].

Considering the above, we hypothesized that Se-cystine ($CySe_2$) added to the ovine ruminal fluids affected bacterial isomerization of α-linolenic acid (αLNA) and reduced the biohydrogenation of UFA in *in vitro* incubated ruminal fluid. Therefore, the major objective of the current study was to examine the hypothetically effect of $CySe_2$ on the concentration of UFA, especially αLNA, their geometric and positional isomers, in *in vitro* incubated ovine ruminal fluids containing αLNA.

2. MATERIALS AND METHODS

2.1. Animals and Diets

Eight ruminally fistulated adult sheep received a mixed diet comprising grass hay, barley, molasses, soybean meal and 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 [23]. Ruminal digesta samples were taken before feeding in the morning from each sheep. The ruminal fluid was kept at 39°C and strained through linen cloth before use.

2.2. Reagents

$CySe_2$, αLNA and fatty acid methyl ester standards were purchased from Sigma (Poole, Dorset, UK). Other reagents were of analytical grade and were from POCh (Gliwice, Poland). Water used for the preparation of mobile phases and chemical reagents was prepared using an Elix™ water purification system (Millipore).

2.3. Incubations with Ruminal Fluid *in Vitro*

Strained ruminal fluids (SRF) were incubated either alone or with a combination of αLNA and two concentrations of $CySe_2$ to determine the interactions in the metabolism of αLNA. 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 Pyrex tubes (120 x 11 mm) containing 0.2 ml of water solution containing either alone (the positive control groups) or with a combination of αLNA, a low (l) or high (h) level of $CySe_2$ (Table 1). The tubes with the ovine ruminal fluids were incubated at 39°C. The tubes were removed after 0, 6, 12, 18 or 24 hrs of *in vitro* incubation, heated to inactivate ruminal microbial enzymes for 9–10 min in a block heater at 100°C and stored at −20°C before being submitted for the quantification of FA. The FA were extracted, methylated and analyzed as described below.

2.4. Fatty Acid Extraction and Preparation of Fatty Acid Methyl Esters (FAME)

The method of alkaline saponification and extraction was as described previously [24]. Derivatization of the extracted free fatty acids to FAME was carried out using a procedure that

contained mild, base- and acid-catalyzed methylation steps that minimized positional and geometrical isomerization of UFA [24]. FAME were then analysed using gas chromatography (GC) according to Czauderna, et al. [24]. The analyses of all FAME in ruminal fluid samples were performed on a SHIMADZU GC-MS QP2010 Plus EI equipped with a BPX70 fused silica capillary column (120 m x 0.25 mm i.d. x 0.25 μm film thickness; SHIM-POL), a quadrupole mass selective detector (Model 5973N) and an injection port. FAME identification was validated based on the electron impact ionization spectra of FAME and compared with authentic FAME standards and the NIST 2007 reference mass spectra library.

2.5. Statistical Analyses

Statistical analyses were performed using the Statistica software package (StatSoft, Version 10, 2010). Statistical analyses of the effects of αLNA and $CySe_2$ on the concentrations of selected FA in *in vitro* 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 fluid samples. Mean values in the columns with different superscripts are significantly different at $^{a,b}P < 0.05$ and $^{A,B}P < 0.01$.

3. RESULTS

3.1. The Influence of $CySe_2$ on the Accumulation of Selected Saturated Fatty Acids (SFA) in in Vitro Incubated Ruminal Fluids with α LNA

Although factors altering the microbial population and ruminal fermentation are undoubtedly keys to controlling the yield of the biohydrogenation and synthesis of positional or geometric isomers of fatty acids very few studies have directly associated production of fatty acid isomers and their products of the biohydrogenation in ruminal fluids enriched in Se-compounds [12-14, 23, 24, 31, 32]. Therefore, *in vitro* studies were conducted to determine the effect of $CySe_2$ on the concentrations of selected fatty acids in ovine ruminal fluids containing extra αLNA. The results summarised in Table 2 documented that the addition of αLNA to the incubated ruminal fluids, irrespective of the presence of $CySe_2$, decreased the concentration of C18:0 from 6 to 24 hrs of *in vitro* incubation compared with the control ruminal fluid (RF) or the fluid containing only $CySe_2$, regardless of its concentration. On the other hand, $CySe_2$ added to the ruminal fluids revealed minute influence on the accumulation of C18:0 compared with the RF; indeed, $CySe_2$, in dose dependent manner, slightly reduced the concentration of C18:0 from 12 to 24 hrs compared with the RF. Of interest is the observation that the addition of αLNA to the fluids with $CySe_2$, irrespective of its concentration, slightly stimulated the accumulation of C14:0 from 6 to 24 hrs compared with the RF and other ruminal fluids with αLNA or $CySe_2$. Moreover, αLNA added to the ruminal fluid, irrespective of the presence of $CySe_2$, stimulated the accumulation of C20:0 and C22:0 especially from 18 to 24 hrs of incubation compared with the RF and the fluids with $CySe_2$. On the other hand, all additives in the incubated fluids revealed negligible influence on the concentrations of C15:0 and C16:0 as well as the concentration sum of all assayed SFA (ΣSFA) compared with the RF.

3.2. The Influence of $CySe_2$ on the Accumulation of Selected Mono- and Poly-Unsaturated Fatty Acids in in Vitro Incubated Ruminal Fluids with αLNA

As can be seen from the results summarized in Table 3, all additives in the incubated ruminal fluids revealed negligible influence on the concentrations of *c9*C14:1 and *c9*C16:1 compared with the RF. Similarly, the addition of $CySe_2$ to the fluids enriched in αLNA revealed negligible influence on the concentrations of *c9*C18:1 and *t11*C18:1 (TVA) as well as the concentration sums of Σ*c*MUFA and Σ*t*C18:1 compared with the fluids containing only αLNA. The concentrations of *c9*C18:1, *t11*C18:1 and Σ*c*MUFA were higher from 6 to 24 hrs of incubation in the fluids with $CySe_2$ and αLNA than in the fluids containing only $CySe_2$.

As can be seen from results summarized in Table 4, $CySe_2$ or/and αLNA added to the ruminal fluids affected the concentrations of PUFA in *in vitro* incubated ruminal fluids. The concentration of *t11c15*C18:2 (*tc*C18:2) increased throughout the incubation in the ruminal fluids enriched in αLNA, whereas decreased in the RF and the fluids containing only $CySe_2$. The addition of $CySe_2$ to the fluids with αLNA reduced the concentrations of *tc*C18:2 and *c9t11c15*C18:3 (*ctc*C18:3) from 18 to 24 hrs of incubation compared with the fluids containing only αLNA. The concentrations of *ctc*C18:3, *t9t11c15*C18:3 (*ttc*C18:3) and αLNA were quantitatively detected in the fluids containing αLNA; the addition of $CySe_2$ to the fluids with αLNA revealed negligible influence on the concentrations of *ttc*C18:3 and αLNA as well as on the concentration sum of PUFA (ΣPUFA) compared with the fluids with αLNA. The addition of $CySe_2$ to the ruminal fluids with αLNA slightly decreased the concentration ratio of ΣPUFA to ΣSFA (ΣPUFA/ΣSFA) from 12 to 24 hrs of incubation compared with the fluids with only αLNA. Moreover, $CySe_2$ added to the ruminal fluids without or with αLNA, decreased the concentration sum of all assayed fatty acids (ΣFA) in incubated fluids compared with the RF and the fluids with only αLNA, respectively. As observed from the obtained results (Table 4), αLNA or/and $CySe_2$ added to the ruminal fluids changes the indexes of the initial, intermediate and final biohydrogenation in incubated fluids compared with the RF.

4. DISCUSSION

Wina, et al. [33] documented that the contents of the major ruminal microorganisms, including protozoa, were found to be kept at higher levels than after 24 hrs in *in vitro* incubation. Considering the above, the *in vitro* incubation periods in the current investigation were set to 6, 12, 18 and 24 hrs to determine the influence of $CySe_2$ and/or αLNA on the concentration of FA in incubated ovine ruminal fluids. Interestingly, the concentration of protozoa in incubated fluids was highest at 12 hrs of *in vitro* incubation, whereas only slightly lower (~15%) after 24 hrs of incubation compared with the initial concentration of protozoa [33]. Our studies and other investigations have shown that pH of ruminal fluids containing αLNA slightly decreases after 24 hrs of *in vitro* incubation of ovine fluids; the pH value is reduced by approx. 0.5 [34, 35].

Ruminal microorganisms may use the biohydrogenation as a method of defence against toxicity of UFA, especially PUFA (like αLNA or LA). Therefore, the first step of the biohydrogenation consists of the enzymatic bacterial isomerisation, which turns *cis* bonds into

trans bonds. This isomerisation step is rapid compared with the biohydrogenation step [8]. As can be seen from data summarized in Table 2, αLNA added to the ruminal fluids, irrespective of the presence of $CySe_2$, decreased the yield of the final biohydrogenation (fBH) to C18:0. Considering the above, we argue that αLNA decreased the activity of group B bacteria in a rumen [36]. Indeed, group A included the ruminal bacteria that hydrogenate PUFA (e.g. αLNA or LA) into *t11*C18:1 where their effect was thought to end; on the other hand, group B ruminal bacteria were thought to be capable of converting the same PUFA as those of group A bacteria, but they are more capable of biohydrogenating a wider range of fatty acids, ending with C18:0 flow from the rumen [36].

As consequence, the addition of αLNA to the fluids, regardless of the presence of $CySe_2$, revealed negligible influence on the concentration of C14:0, C15:0 and C16:0 in incubated fluids. Indeed, these saturated fatty acids are not the products of the fBH [8]. The current results documented that added $CySe_2$, in dose depended manner, reduced the formation of C22:0 in the fluids containing αLNA compared with the fluids with only αLNA from 18 to 24 hrs of the incubation; so, our results suggested that added $CySe_2$ decreased the capacity of the elongation of saturated fatty acids in the incubated ruminal fluids enriched in αLNA. Our investigations revealed that $CySe_2$ added to the fluids without or with αLNA reduced the synthesis of fatty acids or/and increased their oxidation. Therefore, the concentration of ΣFA was lower in the incubated fluids containing $CySe_2$ without or with αLNA than in the RF or the ruminal fluids with αLNA.

Our current studies documented that the higher concentration of $CySe_2$ in the ruminal fluids enriched in αLNA reduced the accumulation of *t11*C18:1 from 18 to 24 hrs of incubation compared with the fluids containing αLNA, irrespective of the presence of the lower concentration of $CySe_2$ (Table 3); *t11*C18:0 is the product of isomerization and/or the intermediate biohydrogenation of unsaturated fatty acids (C18:2), like *t11c15*C18:2 [25]. Indeed, the higher concentration of $CySe_2$ in the ruminal fluids containing αLNA most efficiently reduced the index of the initial biohydrogenation of *c9t11c15*C18:3 and *t9t11c15*C18:3 to *t11c15*C18:2 from 18 to 24 hrs of incubation (Table 4). Moreover, the lowest concentration of *t11*C18:1 in the fluids with αLNA and the higher concentration of $CySe_2$ well correlated with the lowest yield of the bacterial isomerization of αLNA into *c9t11c15*C18:3 (*ctc*C18:3) and the lowest yield of the initial biohydrogenation of *ctc*C18:3 to *t11c15*C18:2 (*tc*C18:2) in the incubated fluids containing αLNA and the higher concentration of $CySe_2$ (Table 4). Therefore, the concentration of *ctc*C18:3 and *tc*C18:2 in the fluids enriched in αLNA and the higher concentration of $CySe_2$ was lower than in the fluids with αLNA, irrespective of the presence of the lower concentration of $CySe_2$, from 12 to 24 hrs of the incubation. Considering the above results, we argued that $CySe_2$ added to the incubated fluids with αLNA reduced the yield of the bacterial isomerization of αLNA (Table 3) as well as the initial biohydrogenation of *ctc*C18:3 in dose dependent manner from 18 to 24 hrs of the incubation (Table 4). Moreover, we suggest that αLNA added to the incubated fluids, irrespective of the presence of $CySe_2$, reduced the capacity of the final biohydrogenation to C18:0 compared with the RF (Tables 2 and 4). Moreover, $CySe_2$ added to the ruminal fluids decreased the concentration of C18:0 as well as the index of the final biohydrogenation to C18:0 compared with

the RF. Current observations are also consistent with our recent *in vitro* studies that have reported that selenate or selenite added to the ruminal fluids containing αLNA reduced the capacity of the bacterial isomerization of C18-PUFA and lowered the yield of the final biohydrogenation to C18:0 compared with the fluids with only αLNA [24]. Interestingly, αLNA or/and $CySe_2$ added to the ruminal fluids revealed an inconsistent effect on the index values of the intermediate biohydrogenation (Table 4) involved in the formation of *t11*C18:1 (TVA). We suggest that this inconsistent effect may be due to the fact that *t11*C18:1 is the substrate which is consumed during the final biohydrogenation. Generally, the index values of the intermediate biohydrogenation to *t11*C18:1 were higher in the fluids containing αLNA and the higher or lower concentration of $CySe_2$ compared with the ruminal fluids with only αLNA. On the other hand, our results suggest that the higher concentration of $CySe_2$ in the fluids with αLNA usually stimulated the yield of elongation of *c9c12*C18:2 to *c13c16*C22:2 (i.e. the unsaturated fatty acids) compared with the incubated fluids containing only αLNA. Surprisingly, $CySe_2$ added to the ruminal fluids with αLNA decreased the concentration ratio of ΣPUFA to ΣSFA (ΣPUFA/ΣSFA) for incubation at all times from 12 hrs compared with the fluid containing αLNA (Table 4).

5. CONCLUSION

In conclusion, $CySe_2$, especially the higher concentration of $CySe_2$, reduced the capacity of the biohydrogenation of UFA to C18:0 in the ruminal fluids. Moreover, the higher concentration of $CySe_2$ in the fluids with αLNA most effectively reduced the yield of the bacterial isomerization of αLNA to *c9t11c15*C18:3 and the formation of *t11*C18:1. Our investigations revealed that $CySe_2$ added to the fluids without or with αLNA reduced the accumulation of fatty acids in *in vitro* incubated ruminal fluids.

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Table-1. The scheme of *in vitro* experiments on ovine ruminal fluids[1]

Group	*Additive*	*Additive concentration*	*Number (n) of fluid samples individually analysed in every time*[2]
RF[3]	*Water*	-	*n=6*
L	*αLNA*	*1.67 mg/ml*	*n=6*
Sel	*$CySe_2$*	*1.34 μg/ml*	*n=5*
Seh	*$CySe_2$*	*3.33 μg/ml*	*n=5*
L-Sel	*$CySe_2$* *αLNA*	*1.33 μg/ml* *1.67 mg/ml*	*n=6*
L-Seh	*$CySe_2$* *αLNA*	*3.33 μg/ml* *1.67 mg/ml*	*n=6*

[1] 1 ml of ovine ruminal fluids was added to 0.2 ml of water solution containing either solely α-linolenic acid (αLNA), seleno-cystine ($CySe_2$) at different concentrations (l - low; h - high), or their mutual combinations

[2] n – ovine ruminal fluid samples collected from *n* sheep at different times and individually analyzed. All results in Tables 2-4 are mean values from n fluid samples individually analysed in every time

[3] RF = the negative control group (*in vitro* incubated 1ml of the ovine ruminal fluid with 0.2 ml of water (the control ruminal fluid)

Table-2. Effects[1] of two levels (low, l; high, h) of Se-cystine ($CySe_2$) on metabolism of αLNA and the concentration of selected saturated fatty acids (SFA) and the concentration sum of SFA (ΣSFA) (μg/ml) in *in vitro* incubated ruminal fluids

Group and *in vitro* incubation time, hrs	**C14:0**	**C15:0**	**C16:0**	**C18:0**	**C20:0**	**C22:0**	**ΣSFA**
RF[2] 0	3.8	23.5	66	93	-[3]	4.3	191
L 0	3.4^{a}	24.0^{a}	72^{a}	99^{a}	-	0.7^{Bb}	199^{ab}
Sel 0	3.5^{a}	24.7^{a}	66^{a}	97^{a}	-	4.5^{a}	195^{ab}
Seh 0	3.7^{a}	24.2^{a}	69^{a}	94^{a}	-	4.6^{a}	195^{ab}
L-Sel 0	3.7^{a}	24.6^{a}	74^{a}	100^{a}	-	0.8^{b}	203^{ab}
L-Seh 0	3.7^{a}	24.7^{a}	73^{a}	100^{a}	-	-	202^{ab}
RF 6	4.4	25.9	79	105	0.9	4.9	220
L 6	5.1^{ab}	25.6^{a}	99^{ab}	101^{a}	7.8^{B}	1.4^{b}	239^{abc}
Sel 6	4.5^{a}	24.4^{a}	76^{a}	106^{a}	-	4.7^{a}	216^{abc}
Seh 6	4.6^{a}	24.6^{a}	77^{a}	105^{a}	-	4.7^{a}	216^{abc}
L-Sel 6	5.4^{b}	25.8^{a}	103^{b}	104^{a}	-	-	238^{bc}
L-Seh 6	5.3^{ab}	25.2^{a}	98^{ab}	100^{a}	-	2.7^{b}	231^{abc}
RF 12	5.4	25.6	94	127	1.1	3.2	256
L 12	5.5^{b}	25.3^{a}	102^{ab}	101^{a}	6.3^{B}	2.8^{b}	243^{bc}
Sel 12	5.3^{ab}	24.7^{a}	90^{ab}	121^{b}	1.0^{Aa}	4.2^{a}	246^{bc}
Seh 12	5.3^{ab}	25.0^{a}	91^{ab}	119^{ab}	-	4.4^{a}	244^{bc}
L-Sel 12	5.9^{b}	26.6^{a}	106^{b}	104^{ab}	10.9^{B}	4.4^{a}	258^{bcd}
L-Seh 12	6.4^{b}	25.3^{a}	104^{b}	102^{a}	4.8^{Bb}	2.6^{b}	245^{bcd}
RF 18	5.7	25.1	95	132	1.0	4.5	263
L 18	5.9^{b}	25.8^{a}	105^{b}	100^{a}	4.4^{B}	9.0^{c}	250^{cd}
Sel 18	6.1^{b}	25.6^{a}	98^{ab}	130^{b}	1.0^{Aa}	4.4^{a}	265^{cd}
Seh 18	5.9^{b}	24.7^{a}	96^{ab}	124^{ab}	1.0^{Aa}	4.5^{a}	257^{cd}
L-Sel 18	6.3^{b}	25.2^{a}	107^{b}	104^{ab}	8.4^{B}	6.3^{ac}	257^{cd}
L-Seh 18	6.4^{b}	25.6^{a}	111^{b}	102^{a}	8.9^{B}	6.1^{ac}	260^{cd}
RF 24	5.8	26.9	102	142	2.0	3.8	283
L 24	6.4^{b}	26.3^{a}	106^{b}	101^{a}	6.8^{B}	2.8^{Ac}	260^{cd}
Sel 24	6.2^{b}	25.4^{a}	100^{ab}	135^{b}	1.9^{Aa}	4.1^{b}	273^{cd}
Seh 24	6.0^{b}	24.6^{a}	99^{ab}	131^{ab}	1.8^{A}	3.4^{ab}	266^{cd}
L-Sel 24	6.5^{b}	24.4^{a}	106^{b}	101^{a}	3.7^{ABb}	0.3^{c}	252^{bcd}
L-Seh 24	7.0^{b}	26.5^{a}	111^{b}	106^{ab}	3.9^{ABb}	5.9^{a}	260^{cd}

[1] means in columns with the different letters are significantly different at $^{(a,b)}P < 0.05$ or at $^{(A,B)}P < 0.01$

[2] RF – *in vitro* incubated ruminal fluids without the additives (the control ruminal fluid)

[3] below the quantification limit (LOQ); LOQ was defined as 10 times the average noise level (LOQ was determined according to Lin and McKeon [37])

Table-3. Effects[1] of two levels (low, l; high, h) of Se-cystine ($CySe_2$) on metabolism of αLNA and the concentration of selected monounsaturated fatty acids (MUFA) and the concentration sums of *c*MUFA (ΣcMUFA)[2] and *t*C18:1 (ΣtC18:1)[3] (μg/ml) in *in vitro* incubated ruminal fluids

Group and *in vitro* incubation time, hrs		*c9*C14:1	*c9*C16:1	*c9*C18:1	*t9*C18:1	*t11*C218:1	ΣcMUFA	ΣtC18:1
RF	0	4.5	4.5	7.7	3.8	1.9	16.7	5.8
L	0	4.4^{a}	1.4^{b}	-[4]	2.9^{a}	-	5.8^{Bb}	2.9^{Ab}
Sel	0	4.4^{a}	4.4^{ad}	7.6^{a}	6.0^{b}	-	16.5^{a}	6.0^{a}
Seh	0	4.6^{ab}	4.6^{ad}	8.2^{a}	3.9^{ab}	2.2^{a}	17.4^{a}	6.1^{a}
L-Sel	0	4.5^{a}	2.5^{ab}	2.8^{Ab}	-	-	9.8^{b}	-
L-Seh	0	4.5^{ab}	2.6^{ab}	2.5^{Ab}	-	-	9.6^{b}	-
RF	6	5.5	5.5	8.4	5.3	2.3	19.4	7.6
L	6	6.9^{ab}	6.1^{cd}	16.1^{Bc}	-	7.3^{b}	29.1^{A}	7.3^{a}
Sel	6	5.3^{ab}	5.2^{cd}	7.3^{a}	7.6^{b}	-	17.8^{a}	7.6^{a}
Seh	6	5.5^{ab}	5.2^{cd}	7.8^{a}	8.2^{bc}	-	18.5^{a}	8.2^{a}
L-Sel	6	7.1^{b}	6.4^{cd}	13.9^{Bc}	-	9.3^{Ab}	27.4^{Ac}	9.3^{a}
L-Seh	6	6.9^{ab}	6.0^{cd}	14.7^{Bc}	3.1^{a}	3.2^{a}	27.6^{Ac}	6.3^{a}
RF	12	6.0	6.4	6.3	6.6	4.0	18.7	10.5
L	12	7.2^{b}	4.8^{ad}	16.8^{Bc}	-	9.2^{b}	28.8^{Cc}	9.2^{a}
Sel	12	5.8^{ab}	6.0^{cd}	5.9^{a}	6.2^{b}	4.2^{a}	17.7^{a}	10.4^{a}
Seh	12	6.0^{ab}	6.1^{cd}	6.8^{a}	7.2^{b}	4.0^{a}	18.9^{a}	11.2^{a}
L-Sel	12	7.5^{b}	6.8^{cd}	17.3^{Bc}	-	9.9^{b}	31.7^{Cc}	9.9^{a}
L-Seh	12	7.2^{b}	6.9^{cd}	16.9^{Bc}	-	9.4^{a}	31.0^{Cc}	9.4^{a}
RF	18	6.2	7.7	2.5	5.9	3.9	16.5	9.8
L	18	7.5^{b}	7.7^{cd}	17.2^{Bc}	2.1^{a}	15.1^{Bc}	32.4^{Cc}	17.2^{Ba}
Sel	18	6.5^{ab}	6.6^{cd}	3.1^{Ab}	6.2^{b}	4.3^{a}	16.2^{a}	10.4^{a}
Seh	18	6.3^{ab}	6.3^{cd}	3.9^{Ab}	6.8^{b}	5.3^{a}	16.5^{a}	12.0^{a}
L-Sel	18	7.6^{b}	7.0^{cd}	17.3^{Bc}	-	15.5^{Bc}	31.9^{Cc}	15.5^{Ba}
L-Seh	18	7.5^{b}	7.1^{cd}	14.4^{Bc}	2.6^{a}	14.1^{Bc}	29.0^{Cc}	16.7^{Ba}
RF	24	6.7	9.2	1.0	6.2	1.1	16.9	7.3
L	24	8.0^{b}	7.0^{cd}	17.0^{Bc}	4.6^{ab}	19.8^{Bc}	32.0^{Cc}	24.4^{Bd}
Sel	24	6.4^{ab}	6.6^{cd}	1.9^{Ab}	3.7^{a}	5.7^{ab}	14.9^{a}	9.3^{a}
Seh	24	6.4^{ab}	6.4^{cd}	2.6^{Ab}	3.1^{a}	6.2^{b}	15.4^{a}	9.2^{a}
L-Sel	24	7.8^{b}	7.5^{cd}	16.3^{Bc}	3.0^{a}	19.8^{Bc}	31.6^{Cc}	22.8^{Bd}
L-Seh	24	8.0^{b}	7.3^{cd}	11.9^{Bc}	15.1^{d}	14.9^{Bc}	27.1^{Ac}	30.0^{Ccd}

[1] means in columns with the different letters are significantly different at $^{(a,b)}P < 0.05$ or at $^{(A,B)}P < 0.01$; all abbreviations as in Table 2

[2] ΣcMUFA = *c9*C14:1 + *c9*C16:1 + *c9*C18:1

[3] Σ*t*C18:1 = *t9*C18:1 + *t11*C18:1 (TVA)

[4] below the quantification limit (LOQ)

Table-4. Effects[1] of two levels (low, l; high, h) of Se-cystine ($CySe_2$) on metabolism of αLNA and the concentrations of *t11c15*C18:2 (*tc*C18:2), *c9t11c15*C18:3 (*ctc*C18:3), *t9t11c15*C18:3 (*ttc*C18:3), *c13c16*C22:2 (C22:2), the concentration sums of PUFA (ΣPUFA) and all assayed fatty acids (ΣFA), the concentration ratio of ΣPUFA to ΣSFA (ΣPUFA/ΣSFA) and the indexes of the initial, intermediate and final biohydrogenation in *in vitro* incubated ruminal fluids

Group and *in vitro* incubation time, hrs		*tc*C18:2	*ctc*C18:3	*ttc*C18:3	αLNA	C22:2	ΣPUFA	ΣPUFA/ΣSFA	ΣFA	Index$_{tcC18:2}$[3]	Index$_{TVA}$[4]	Index$_{C18:0}$[5]
		μg/ml	μg/ml	μg/ml	mg/ml	μg/ml	mg/ml	μg/ml	μg/ml			
RF	0	8.8	-[2]	-	-	1.7	0.011	0.055	224	1.000	0.182	0.980
L	0	19.0^{Ab}	5.6^{Aa}	-	1.68^{Aa}	2.1^{ab}	1.71^{Bc}	8.60^{Bc}	1921^{Aa}	0.774^{A}	-	1.000^{a}
Sel	0	10.7^{Aa}	-	-	0.01^{B}	2.0^{ab}	0.014^{Aa}	0.073^{Aa}	232^{B}	1.000^{B}	-	1.000^{a}
Seh	0	11.4^{Aa}	-	-	-	2.0^{ab}	0.013^{Aa}	0.069^{Aa}	232^{B}	1.000^{B}	0.163^{Aa}	0.977^{a}
L-Sel	0	19.3^{Ab}	6.3^{Aa}	-	1.66^{Aa}	2.1^{ab}	1.70^{Bc}	8.35^{Bc}	1908^{Aa}	0.754^{A}	-	1.000^{a}
L-Seh	0	19.2^{Ab}	6.4^{Aa}	-	1.62^{Aa}	2.7^{ab}	1.66^{Bc}	8.22^{Bc}	1872^{Aa}	0.748^{A}	-	1.000^{a}
RF	6	6.6	-	-	-	2.0	0.009	0.039	256	1.000	0.263	0.978
L	6	22.5^{Ab}	516^{Bb}	72^{Aa}	1.03^{Ab}	2.8^{b}	2.26^{Bc}	9.48^{Bc}	2539^{Ab}	0.037^{Ca}	0.246^{Bb}	0.932^{b}
Sel	6	7.5^{Aa}	-	-	-	2.4^{ab}	0.010^{Aa}	0.046^{Aa}	251^{B}	1.000^{B}	-	1.000^{a}
Seh	6	7.6^{Aa}	-	-	-	2.4^{ab}	0.010^{Aa}	0.047^{Aa}	253^{B}	1.000^{B}	-	1.000^{a}
L-Sel	6	23.8^{Ab}	488^{Bb}	92^{abc}	1.02^{Ab}	2.6^{ab}	2.25^{Bc}	9.45^{Bc}	2526^{Ab}	0.040^{Ca}	0.281^{Bc}	0.918^{b}
L-Seh	6	22.3^{Ab}	502^{Bb}	82^{ab}	0.95^{Ab}	2.4^{ab}	2.19^{Bc}	9.51^{Bc}	2458^{Ab}	0.037^{Ca}	0.124^{Ad}	0.969^{b}
RF	12	2.7	-	-	-	2.5	0.006	0.025	292	1.000	0.598	0.970
L	12	46.1^{Dd}	491^{Bb}	111^{abc}	0.87^{Ab}	1.5^{a}	2.17^{Bc}	8.95^{Bc}	2456^{Ab}	0.071^{Cb}	0.166^{Aa}	0.917^{b}
Sel	12	1.3^{Cc}	-	-	-	2.3^{ab}	0.004^{Ab}	0.015^{Ab}	277^{B}	1.000^{B}	0.762^{C}	0.967^{b}
Seh	12	2.5^{Cc}	-	-	-	2.4^{ab}	0.005^{Ab}	0.020^{Aab}	279^{B}	1.000^{B}	0.620^{C}	0.967^{b}
L-Sel	12	55.2^{Dd}	505^{Bb}	132^{bcd}	0.84^{Ab}	2.4^{ab}	2.22^{Bc}	8.61^{Bc}	2520^{Ab}	0.080^{Cc}	0.152^{Aa}	0.913^{b}
L-Seh	12	43.2^{Dd}	457^{Bb}	112^{ab}	0.84^{Ab}	2.0^{ab}	2.07^{Bc}	8.46^{Bc}	2356^{Ab}	0.070^{Cb}	0.179^{Aa}	0.916^{b}
RF	18	0.9	-	-	-	2.4	0.003	0.013	293	1.000	0.807	0.971
L	18	159^{Be}	520^{Bb}	121^{abc}	0.68^{Ab}	1.9^{ab}	2.17^{Bc}	8.68^{Bc}	2468^{Ab}	0.199^{D}	0.087^{D}	0.869^{c}
Sel	18	0.9^{Cc}	-	-	-	2.3^{ab}	0.003^{Ab}	0.012^{Ab}	295^{B}	1.000^{B}	0.825^{CD}	0.968^{b}
Seh	18	-	-	-	-	2.3^{ab}	0.002^{Ab}	0.009^{Ab}	287^{B}	-	1.000^{E}	0.960^{b}
L-Sel	18	147^{Bef}	451^{Bbc}	127^{bcd}	0.78^{Ab}	1.6^{a}	2.13^{Bc}	8.30^{Bc}	2439^{Ab}	0.203^{Dd}	0.095^{A}	0.870^{c}
L-Seh	18	111^{Bf}	435^{Bc}	131^{bcd}	0.77^{Ab}	2.4^{ab}	2.06^{Bc}	7.91^{Bc}	2365^{Aab}	0.164^{De}	0.113^{Ad}	0.879^{c}
RF	24	-	0.5	-	-	1.7	0.003	0.012	311	-	1.000	0.993
L	24	241^{Be}	438^{Bbc}	159^{Bcd}	0.62^{Ab}	2.2^{ab}	2.11^{Bc}	8.13^{Bc}	2426^{Ab}	0.287^{Df}	0.076^{A}	0.836^{c}
Sel	24	-	-	-	-	1.7^{ab}	0.002^{Ab}	0.006^{Ab}	299^{B}	-	1.000^{E}	0.960^{b}
Seh	24	-	-	-	-	1.9^{ab}	0.002^{Ab}	0.007^{Ab}	292^{B}	-	1.000^{E}	0.955^{b}
L-Sel	24	212^{Bef}	403^{Bbc}	161^{Bd}	0.61^{Ab}	1.4^{a}	2.00^{Bc}	7.93^{Bc}	2309^{Aab}	0.274^{Df}	0.086^{Ad}	0.837^{c}
L-Seh	24	205^{Bef}	371^{Bc}	168^{Bd}	0.74^{Ab}	2.4^{ab}	2.08^{Bc}	8.00^{Ac}	2398^{Ab}	0.274^{Dg}	0.068^{Ae}	0.877^{c}

[1] means in columns with the different letter are significantly different at $^{(ab)}P < 0.05$ or at $^{(AB)}P < 0.01$; all abbreviations as in Table 2
[2] below the quantification limit (LOQ)
[3] the index of the initial biohydrogenation of c9t11c15C18:3 (ctcC18:3) and t9t11c15C18:3 (ttcC18:3) to t11c15C18:2 (tcC18:2) (index$_{tcC18:2}$ = tcC18:2/(tcC18:2 + ctcC18:3 + ttcC18:3)
[4] the index of the intermediate biohydrogenation of t11c15C18:2 to t11C18:1 (TVA) (index$_{TVA}$= t11C18:1/(t11C18:1+t11c15C18:2)
[5] the index of the final biohydrogenation of t11C18:1 to C18:0 (index$_{C18:0}$ = C18:0/(C18:0+t11C18:1)