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Article *in* Journal of Animal and Feed Sciences · January 2006

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Journal of Animal and Feed Sciences, 15, Suppl. 1, 2006, 97–100

The influence of selenium on the level of linoleic acid isomers in incubated ruminal fluid*

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ABSTRACT

Ovine ruminal fluids were incubated *in vitro* at 39°C under CO_2 either alone or with a combination of linoleic acid (LA) (1.67 g/l), a low (0.167 µg/ml) or high (1.67 µg/ml) level of selenite (Se(IV)), or selenate (Se(VI)). The high level of Se(IV) resulted in a decrease of CLA isomer accumulation in *in vitro* incubated cultures with LA. Low and high levels of Se(VI) in ruminal fluids elevated the content of CLA isomers and *trans*-vaccenic acid in fluids incubated with LA.

KEY WORDS: selenate, selenite, CLA isomers, linoleic acid, linseed oil, ovine ruminal fluid

INTRODUCTION

Recent studies showed that n-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) isomers possess potential health benefits, such as anticarcinogenic and antidiabetogenic properties or delaying the onset of atherosclerosis. Studies indicated that dietary PUFA is directly incorporated in the rumen bacteria, isomerized to other geometric and positional isomers, metabolized into CLA isomers (Raes et al., 2004), biohydrogenated to *trans*-vaccenic acid and finally to stearic acid. In recent studies was found that the PUFA levels in lipids of animals and in membranes of microorganisms were positively correlated with the concentration of selenite and selenate in the diet.

Considering the above, it seemed reasonable to study the influence of selenite (Se(IV)) and selenate (Se(VI)) on the content of CLA isomers and *trans*-vaccenic acid (TVA; *trans-9*C18:1) in *in vitro* incubated ruminal fluids of sheep.

* Supported by the Marie Curie Training Site, 'Mass School'
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MATERIAL AND METHODS

Eight ruminally fistulated adult sheep received a mixed diet comprising grass hay, barley, molasses, soyabean meal, and vitamins and minerals, 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, kept at 39°C and strained through linen cloth before use.

Linoleic acid (LA), TVA, CLA isomer methyl ester standards, sodium selenite (Se(IV)) and sodium selenate (Se(VI)) were from Sigma (UK).

Strained ruminal fluid was incubated either alone or with a combination of LA and two concentrations of Se(IV) or Se(VI). One ml of strained ruminal fluid was added under CO_2 to tubes containing one of the following: 0.2 ml of distilled water ($RF_{control}$); 0.1 ml of water and 0.1 ml of 20 μg/ml Se(IV) or Se(VI) (the high Se level: Se-H; 1.67 μg Se/ml); 0.01 ml of 20 μg/ml Se(IV) or Se(VI) (the low Se level: Se-L; 0.167 μg Se/ml); 0.1 ml of 20 mg/ml LA and 0.1 ml of water ($LA_{control}$); 0.1 ml of 20 mg/ml LA and 0.1 ml of 20 μg/ml Se(IV) or Se(VI) (the high Se level); 0.1 ml of 20 mg/ml LA, 0.09 ml of water and 0.01 ml of 20 μg/ml Se(IV) or Se(VI) (the low Se level). The tubes were incubated at 39°C. All experiments were carried out on four different days using samples withdrawn from four different sheep.

The methods of hydrolysis, derivatization and fractionation were as described previously (Christie, 2003). FA-MEs were separated and quantified using a gas chromatograph (Model 6890, Agilent Technologies, UK) equipped with a FID (Wąsowska et al., 2006).

RESULTS

The influence of the low (L) and high (H) concentrations of Se(IV) and Se(VI) in ruminal fluids on the CLA isomers and TVA compositions in *in vitro* incubated fluids is shown in Table 1. The concentrations of all CLA isomers were usually below detection limits in incubated ruminal fluids without (RF) and with Se(IV) or Se(VI) at both concentrations. Addition of Se(VI) numerically or statistically decreased the concentrations of individual CLA isomers, as well as the sum of CLA isomers in ruminal fluids in comparison with fluid containing only LA. The higher amount of Se(IV) added to the ruminal fluids resulted in a decrease in the accumulation of CLA isomers in incubates with LA. The concentrations of formed *t10c12*CLA and *t9t11*CLA increased in fluids with LA as the duration of incubation increased, regardless of Se(IV) additions. These isomers accumulated, whereas the *c9t11*CLA formed during 6 h incubation disappeared throughout the incubations of fluids with LA regardless of Se(IV) at two levels. The *c9c11* isomer formed during 6 h of incubation remained almost the

Table 1. Effects[1] of two concentrations (low, L; high, H) of Se(IV) and Se(VI) on the concentration of CLA isomers and *trans*-vaccenic acid (TVA) (μg/ml) in incubated ruminal fluids ($RF_{control}$)

Group	*In vitro* incubation time, h	TVA		CLA isomers								Sum of all CLA isomers	
				c9t11	*t10c12*	*c9c11*	*t9t11*	*c9t11*	*t10c12*	*c9c11*	*t9t11*		
		Se(IV)	Se(VI)	Additive:		Se(IV)		Additive:		Se(VI)		Se(IV)	Se(VI)
$RF_{control}$	0	13	13	0	0	0	0	0	0	0	0	0	0
Se-L	0	15	15	0	0	0	0	0	0	0	0	0	0
Se-H	0	14	14	0	0	0	0	0	0	0	0	0	0
$LA_{control}$	0	14	14	4	1.5	0	0.5[a]	4	1.5	0	0.5	6	6
LASe-L	0	14	13	2.9	0.7	0	0.5	2.4	0	0	0	4	2.4
LASe-H	0	17	13	3.4	0.9	0.6	2.1[a]	3.6	1.2	0	0.5	7	0
$RF_{control}$	6	15[a]	15[a]	0	0	0	0	0	0	0	0	0	0
Se-L	6	14	14	0	0	0	0	0	0	0	0	0	0
Se-H	6	14	14	0	0	0	0	0	0	0	0	0	0
$LA_{control}$	6	48[ab]	48[a]	285[a]	37[ab]	18[a]	45[a]	285	37	18	45	385[a]	386
LASe-L	6	33	42	238	26[b]	14	32	281	31	16	43	309	370
LASe-H	6	33[b]	51	229[a]	21[a]	13[a]	35[a]	257	43	16	46	298[a]	362
$RF_{control}$	12	20[a]	20[a]	0	0	0	0	0	0	0	0	0	0
Se-L	12	18	19	1	0	0	0.5	0.7	0	0	0.5	1.5	1.2
Se-H	12	19	18	0.7	0	0	0.6	0	0	0	0	1.3	0
$LA_{control}$	12	198[a]	198[a]	230	62[a]	22	70	230	62	22	70	384	384
LASe-L	12	101	162	207	38	15	44	260	61	22	72	305	415
LASe-H	12	94	170	154	26[a]	14	51	266	60	22	67	245	415
$RF_{control}$	18	19[a]	19[a]	0	0	0	0	0	0	0	0	0	0
Se-L	18	19	18	0	0	0	0	0	0	0	0.5	0	0.5
Se-H	18	18	21	0	0	0	0	0	0	0	0	0	0
$LA_{control}$	18	446[a]	446[a]	171	70	17	82	171	70	17	82	339[a]	339
LASe-L	18	334	448	165	80	14	67	134	67	15	79	325	295
LASe-H	18	287	375	100	53	13	52	168	71	18	87	217[a]	344
$RF_{control}$	24	18[a]	18[a]	0	0	0	0	0	0	0	0	0	0
Se-L	24	15	17	0	0	0	0	0	0	0	0	0	0
Se-H	24	18	20	0	0	0	0	0	0	0	0	0	0
$LA_{control}$	24	618[a]	618[a]	85	55	22	79[a]	85	55	22	79	241	241
LASe-L	24	564	552	76	66	8	46[a]	121	85	14	94	196	315
LASe-H	24	446	491	75	77	8	57	132	81	19	99	217	332

[1] means in columns at the same incubation time with the same letter are significantly different at [a,b] $P<0.05$ or at [A,B] $P<0.01$; *t, c* - abbreviation for geometrical form: *trans, cis*, respectively

same as in ruminal fluid with LA until 24 h, whereas both levels of Se(IV) in fluids with LA slightly reduced the content of *c9c11*CLA after 18 h incubation.

The influence of two levels of Se(IV) on the concentration of TVA in incubates with LA was similar to the effect of accumulation of *c9t11*CLA. However, a tendency or statistical increase of the decrement of the TVA concentration was observed with increasing the concentration of Se(IV) in ruminal fluids with LA.

To test the differential effects of Se(IV) and Se (VI) on the metabolism of LA and CLA isomer formation and their disappearance rate in ruminal fluids, Se(VI) was incubated in fluids with LA. The effects of low and high levels of Se(VI) on CLA isomer accumulation differed from the influence of Se(IV) on the abundance of CLA isomers in rumen fluids. Se(VI) numerically stimulated formation of *c9t11*CLA, *t10c12*CLA and *t9t11*CLA in incubates with LA after 24 h incubation. The high level of Se(VI) usually more effectively promoted the accumulation of these CLA isomers and the sum of all CLA isomers in incubates with LA. No effect of either level of Se(VI) was noticed on the content of *c9c11*CLA in incubates with LA. Se(VI) numerically lowered the rate of LA metabolism, in addition, disappearance of LA decreased as the level of Se(VI) increased. The content of TVA in incubated ruminal fluids is also numerically lower in fluids with Se(VI) and the high level of Se(VI) resulted in the lowest accumulation of TVA.

CONCLUSIONS

Addition of Se(VI) elevated the concentration of CLA isomers and TVA in incubated ruminal fluids with LA. We hypothesise that Se(VI) is preferentially biohydrogenated by ruminal bacteria and the reduction of Se(VI) to Se(IV) competes with the reduction of double bonds of CLA isomers and other unsaturated fatty acids. Therefore, we could suggest that feeding Se(VI) and LA can improve the nutritive value of products derived from ruminants. In particular, meat, milk and dairy products should contain more CLA isomers derived from ruminal digesta, as well as from endogenous synthesis of conjugated dienes from TVA or *trans*-7C18:1.

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