Use of Cobalt-Acetate to Estimate the Endogenous Synthesis of Milk *cis*-9 *trans*-11 18:2 in Dairy Ewes Fed Linseed Oil

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Administration of cobalt-acetate may represent a low cost alternative to other more expensive and involved methods used to estimate the endogenous synthesis of milk *c*9*t*11-18:2 (a conjugated linoleic acid isomer; Shingfield *et al* 2008; Frutos *et al* 2014). Cobalt inhibits the enzyme stearoyl-CoA desaturase (SCD), which is responsible for the conversion of *t*11-18:1 to *c*9*t*11-18:2 in body tissues (Palmquist *et al* 2005). Using Co-acetate or sterculic acid, Δ9-desaturation was estimated to account, respectively, for 51 or 74% of the *c*9*t*11-18:2 found in ewe milk (Bichi *et al* 2012; Frutos *et al* 2014), these inconsistent results being probably due to differences in diet composition rather than in methodological approaches. Thus, although *t*11-18:1 is produced as a major intermediate with diets rich in either 18:2n-6 or 18:3n-3, the latter minimizes the amount of milk *c*9*t*11-18:2 coming from ruminal origin (Loor *et al* 2005), which may affect the relative contribution of Δ9-desaturation to milk *c*9*t*11-18:2 secretion.

The aim of this study was therefore to examine, through oral administration of Co-acetate, the endogenous synthesis of milk *c*9*t*11-18:2 in sheep receiving a diet enriched in 18:3n-3.

Twelve Assaf ewes fed a TMR (forage:concentrate ratio 50:50) supplemented with 2% linseed oil were allocated to 2 experimental groups and received an oral drench supplying either 0 (Control) or 9 mg of Co (as Co-acetate)/kg of liveweight.day. Treatments were administered in 3 equal doses at 8 h intervals, for 6 days. On days 0 (i.e., before Co-administration) and 6, milk production was recorded and samples were collected for analysis of fat content and fatty acid (FA) composition (Frutos *et al* 2014). Administration of cobalt had no effect on milk yield or milk fat content ($P > 0.10$) but decreased ($P < 0.01$) milk Δ9-desaturation ratios, consistent with an inhibition of SCD (Table 1). Changes in the content of *c*9-10:1, *c*9-12:1 and *c*9-14:1 to Co were used as an indication of incomplete inhibition and allowed to estimate that 92% of milk *c*9*t*11-18:2 was endogenously synthesized. Cobalt had negligible effects on other milk FA.

Table 1. Effect of oral administration of cobalt on milk yield (kg/d), and fat content (%), major fatty acid (FA) groups (g/100 g total FA) and Δ9-desaturation ratios in milk of lactating ewes

	Control	Cobalt	s.e.d.		Control	Cobalt	s.e.d.
Milk yield	2.09	2.08	0.098	*c*9-10:1/10:0	0.038[a]	0.022[b]	0.0013
Milk fat content	6.03	5.79	0.281	*c*9-12:1/12:0	0.020[a]	0.009[b]	0.0009
<C16 FA	35.91	35.02	1.138	*c*9-14:1/14:0	0.021[a]	0.010[b]	0.0010
C16 FA	23.50	23.95	0.707	*c*9-16:1/16:0	0.034[a]	0.017[b]	0.0014
>C16 FA	40.37	41.25	1.289	*c*9-17:1/17:0	0.338[a]	0.232[b]	0.0235
PUFA n-3	1.25	1.17	0.073	*c*9-18:1/18:0	1.841[a]	1.216[b]	0.0730
PUFA n-6	2.38	2.34	0.069	*c*9*t*11-18:2/*t*11-18:1	0.505[a]	0.257[b]	0.0677

[a,b]Different superscripts within a row indicate significant differences (P<0.05).

Comparison of the estimate of 92% obtained in ewes fed a diet enriched in 18:3n-3 with previous estimates in sheep fed non-supplemented diets (51%; Frutos *et al* 2014) suggests that this high proportion could be related to higher supply of *t*11-18:1 and lower of *c*9*t*11-18:2 of ruminal origin. Basal diet composition would therefore be a major determinant of the relative contribution of Δ9-desaturation to milk *c*9*t*11-18:2 content.

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