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Effect of different levels of supplied cobalt on the fatty acid composition of bovine milk

Inger J. Karlengen[1]*, Ole Taugbøl[1], Brit Salbu[2], Are H. Aastveit[3] and Odd M. Harstad[1]

[1]*Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, PO Box 5003, N-1432 Ås, Norway*
[2]*Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, Ås, Norway*
[3]*Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway*

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Abstract

In previous studies, administration of high amounts of Co decreased the proportion of MUFA in bovine milk. The present study was conducted to examine the amount of Co needed to obtain this effect. High-yielding dairy cows (*n* 4), equipped with ruminal cannulas, were used in a 4 × 4 Latin square design study. The basal diet consisted of concentrate mixture (9 kg/d) without added Co and grass silage (*ad libitum*). The following four levels of Co were administrated as cobalt acetate dissolved in distilled water: no Co (treatment 1, T1); 4·0 mg Co/d (T2); 380 mg Co/d (T3); 5300 mg Co/d (T4). Each period lasted for 18 d, including 11 d of treatment. During the treatment periods, the solutions were continuously infused into the rumen. Milk yield and milk concentration of fat, fatty acids (FA), protein, lactose, Co, Zn, Fe and Cu were determined. Blood plasma was analysed with respect to FA, Co, Zn, Fe and Cu. Feed intake and total tract digestibility of feed components were also determined. There was a linear effect of increasing the level of Co on milk FA composition. The effects of Co on FA composition in blood were insignificant compared with the effects on milk. In milk fat, the concentration of *cis*-9-18 : 1 was reduced by as much as 38 % on T4 compared with T1. Feed intake and milk yield were negatively affected by increasing the Co level.

Key words: **Δ9-Desaturase: Milk fat: Cobalt: Dairy cows**

The bovine milk fat fraction is generally characterised by a low concentration of unsaturated fatty acids (FA)[1]. The FA *cis*-9-18 : 1 is the unsaturated FA with the highest concentration, accounting for 20–30 % (w/w) of the total FA[2]. The concentration of *cis*-9 FA in bovine milk is determined by their supply to the udder and by the extent of desaturation of their corresponding saturated FA caused by the action of Δ9-desaturase (stearoyl-CoA desaturase) in the mammary gland[3]. In previous studies, large amounts of Co (1·6–3·5 g/d administrated orally or 175 mg/d injected intravenously) produced a reduction in the concentration of MUFA in bovine milk[4–8]. Large amounts of Co might also affect the proportions of some PUFA in milk fat[4]. In those experiments, the supplied Co was present in different physico-chemical forms (Co-EDTA or cobalt acetate) and different methods of administration were used (*per os*, intraruminal or intravenous). However, in all those studies, the concentration of Co given was as high as 50–150 mg/kg diet DM. That level of Co is very high compared with the dietary requirement for Co (0·11 mg Co/kg DM) set for dairy cows[9], and even compared with the maximum tolerance level of 25 mg Co/kg feed DM set for cattle[10]. From a human health perspective, a lower concentration of unsaturated FA in milk and milk products is undesirable[11]. Thus, the main objective of the present experiment was to examine the effects of increasing Co levels on milk FA composition.

Materials and methods

Animals, experimental design, feeds, feeding, treatments and feed sampling

A total of four cows of the Norwegian red cattle breed, three in their second and one in its fourth lactation, weighing 649 (SEM 39) kg, averaging 89 (SEM 15) d in milk and yielding 34·5 (SEM 2·5) kg/d of milk at the start of the experiment, were used for the present study. All cows were equipped with ruminal cannulas (100 mm inner diameter; Bar Diamond, Inc.). They were housed in individual tie stalls and had free access to water. The care and handling of cows conformed to the laws and regulations controlling experiments with live animals in Norway (the Animal Protection Act of 20 December

Abbreviations: FA, fatty acid; FAME, fatty acid methyl ester; ICP-OES, inductively coupled plasma optical emission spectrometry; T1, cows supplemented 0 mg Co/d; T2, cows supplemented 4 mg Co/d; T3, cows supplemented 380 mg Co/d; T4, cows supplemented 5300 mg Co/d.

* **Corresponding author:** I. J. Karlengen, fax +47 64965101, email inger.johanne.karlengen@umb.no

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1974 and the Animal Protection Ordinance Concerning Experiments in Animals of 15 January 1996).

The experiment was designed as a 4 × 4 Latin square with four treatments, four lactating cows and four periods. Each period lasted for 18 d, and was divided into a treatment period of 11 d (days 1–11) and a post-treatment period of 7 d (days 12–18). The basal diet consisted of a concentrate mixture without extra Co (Table 1) plus grass silage, mainly prewilted timothy and meadow fescue. The grass silage was ensiled with a formic acid-based additive, GrasAAT Lacto, containing 780 g formic acid/kg, 20 g lactose/kg and 70 g NH_3/kg (Addcon Group GmbH). All cows were fed the same amount of concentrate (9 kg/d), and the amounts were held constant throughout the study. The concentrate was offered in three equal meals at 06.00, 14.00 and 22.00 hours. The grass silage was given *ad libitum*. Representative samples of the feeds were taken daily and composited to one representative sample of the concentrate and one representative sample of the silage per period. Feed refusals were removed and recorded daily before morning feeding. Daily feed intakes were measured throughout the experiment. The treatments included four levels of Co: basal diet plus distilled water (no added Co, treatment 1, T1); 4·0 mg Co/d (T2); 380 mg Co/d (T3); 5300 mg Co/d (T4). The concentrations of Co in the concentrate and silage were 0·26 and 0·19 mg/kg DM, respectively (Table 1), resulting in a Co concentration of approximately 0·22 mg/kg DM in the total diet with T1. In T2, the amount of Co given was comparable with (slightly above) the level normally supplied when using a commercial concentrate mixture in Norway. The Co levels in T3 and T4 were approximately 100- and 1000-fold this level, respectively. T4 was approximately 50 % above the level reported to affect desaturase indices in earlier experiments[7,8].

Table 1. Ingredient and chemical composition of the concentrate mixture and grass silage

	Concentrate mixture	Grass silage
Ingredient composition (g/kg)		
Oats	200	
Cane molasses	50	
Rapeseed meal	50	
Barley	423	
Soyabean meal	50	
SoyPass®*	100	
Bran	50	
Soyabean oil	20	
Fat†	30	
Limestone meal	5·5	
Monocalcium phosphate	7·0	
NaCl	5·0	
Magnesium oxide	4·0	
Mineral premix‡	5·0	
Vitamins	0·5	
DM (g/kg)§	877	273
Chemical composition (g/kg DM)		
Crude protein	188	151
Starch	392	–
NDF	178	515
Fat	82	36
Ash	67	80
Minerals (mg/kg DM)		
Co	0·26	0·19
Zn	27	127
Fe	166	130
Cu	6·8	21·2

NDF, neutral-detergent fibre.
* Supplied by Borregaard Ligno Tech.
† Containing 920 g stearic acid/kg; Aarhus Karlshamn Sweden AB.
‡ Supplied by Norsk Mineralnæring. Containing per kg feed: Cu (copper(II) sulphate), 15 mg; Se (sodium selenite), 0·25 mg; Zn (zinc sulphate), 65 mg; I (calcium iodate), 2 mg; Mn (manganese(II) sulphate), 30 mg.
§ Uncorrected because the content of volatile substances was not determined.

Co was given as cobalt acetate ($C_4H_6CoO_4.4H_2O$) from Honeywell Speciality Chemicals dissolved in distilled water. The solutions were continuously infused through plastic tubes into the rumen during the entire treatment period, using a peristaltic pump (Cenco Instruments MIJ N.V.) at a rate of approximately 2·3 litres/d. Representative samples of the different Co solutions were taken for each period before the treatment started.

Milk recording and sampling

The cows were milked and yields recorded by use of a Tru-Test Milk Meter (Tru-Test Distributors Limited), twice daily (at 06.30 and 16.30 hours), throughout the entire study. Milk samples from both morning and afternoon milking were collected on days 1, 2, 3, 5, 8, 11, 12, 13, 15 and 18, and a 70 ml aliquot from each day was preserved with 2-bromo-2-nitropropane-1,3-diol and stored at 4°C until analysis of fat, protein, lactose and urea. Non-preserved milk samples (20 ml aliquot) from each day were used for analysis of FA composition, and samples (10 ml aliquot) collected on days 11 and 18 were used for the analysis of concentrations of Co, Fe, Cu and Zn. These samples were kept frozen (−20°C) until analysis.

Faeces and urine collection and sampling

Of the treatments, T2 (corresponding to 0·4 mg Co/kg concentrate mixture which is within the normal range in Norwegian concentrate mixtures) and T4 (expected to depress milk desaturase indices significantly[4,7,8]) were examined more closely. Accordingly, faeces and urine from cows on T2 and T4 were collected separately for 72 h, starting at 08.00 hours on day 8. Faeces were collected in steel containers located under the back of the cows, whereas urine was collected through tubes that were attached to the cows and led into plastic containers located next to the containers for faeces. Both urine and faeces were collected and weighed three times per 24 h, at 14.00, 22.00 and 08.00 hours. To prevent NH_3 from evaporating, 500 ml of H_2SO_4 (1 M) were added to the urine cans before starting and after each emptying. The collected faeces and urine were stored in plastic buckets at 4°C. After 24 h of collection, urine and faeces were carefully blended before representative samples for analysis were taken out.

Blood sampling

Blood samples were also taken to further investigate the effects of Co. Samples were taken from cows on T2 and T4 on days 11 and 18. The samples for FA analysis

(5 ml heparin-containing tubes) were withdrawn from the milk vein at 08.00, 10.00 and 12.00 hours. Additional samples (10 ml heparin-containing tubes), withdrawn from the jugular vein at 10.00 hours were analysed for the content of Co, Fe, Cu and Zn. Blood was centrifuged at 500 ***g*** for 20 min and plasma was stored at −20°C until analysis.

Chemical analysis

Feeds were analysed for DM and ash according to Malkomesius & Nehring[12], and neutral-detergent fibre was analysed according to Mertens *et al.*[13]. Crude protein (Kjeldahl-N × 6·25) was analysed according to Association of Official Analytical Chemist method 985.13[14] with the following modifications: samples were digested with 15 ml H_2SO_4, 3·5 g K_2SO_4 and 0·4 g $CuSO_4$, boiled for 45 min at 420°C and 25 ml of distilled water were added after cooling. Analysis of starch was performed according to McCleary *et al.*[15], and crude fat was analysed after hydrolysis with petroleum diethyl ether on an Accelerated Solvent Extractor (ASE200) from Dionex.

Samples of feed for analyses of Co, Fe, Zn and Cu were pretreated according to the following procedure: dried feed (1 g) was accurately weighed into a MLS-Milestone Ultra Clave III Teflon digestion vessel (Mikrowellen Labor Systeme GmbH), and 5 ml of concentrated ultrapure HNO_3 and 5 ml Milli-Q® water (18 MΩ cm) were added to the vessel. A known concentration of an yttrium standard solution was added as a yield monitor. The system was closed, loaded with nitrogen to 140 bars, and the mixture was heated to 250°C for 30 min. The samples were then transferred to beakers and diluted with Milli-Q® water (18 MΩ cm) to a volume of 50 ml. Then, the concentrations of Fe, Co, Zn and Cu were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Perkin-Elmer Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer (Perkin-Elmer Instruments), equipped with a standard torch and a Perkin-Elmer AS 93^+ Autosampler. The Optima 5300 DV is a simultaneous ICP-OES instrument with an echelle polychromator and a segmented array charge-coupled detector. The determination of Fe was based on the 238·204 nm line, Co on the 228·616 nm line, Zn on the 206 nm line and Cu on the 327·393 nm line. The limits of detection were defined as ten times the standard deviation of ten blank samples, and were 0·02, 0·06, 0·07 and 0·05 μg/g for Co, Zn, Fe and Cu, respectively. The concentrations of Co in the Co solutions were determined by ICP-OES as described for feed, but without any pretreatment. Faeces were analysed for DM, ash, neutral-detergent fibre, crude protein, starch and crude fat as described for feed.

Analysis of the FA composition of total lipids in plasma was performed as described by Taugbøl *et al.*[7]. For the determination of Fe, Co, Zn and Cu, 3–5 ml of plasma were accurately weighed into a MLS-Milestone Ultra Clave III Teflon digestion vessel (Mikrowellen Labor Systeme GmbH), and samples were then pretreated and analysed for Fe, Co, Zn and Cu using ICP-OES as described for feed.

Analyses of fat, protein, lactose and urea concentration of milk were performed using an infrared milk analyser (MilkoScan 6000; Foss Electric). Milk lipids were extracted with ethanol, diethyl diethyl ether and petroleum diethyl ether (International IDF standard no. 1D: Milk Determination of Fat Content, Reference Method). The extracted lipids were methylated according to Kramer *et al.*[16] with $NaOCH_3$ and HCl–methanol. FA methyl esters (FAME) were quantified using gas–liquid chromatography (6890N; Agilent Technologies) with a split/splitless injector, an (7683B) automatic sampler and flame ionisation detection. FAME were separated with a CP-select CB for FAME (200 m × 0·25 mm inner diameter × 0·25 μm film thickness) fused silica capillary column (Varian Inc.), with the procedure described by Taugbøl *et al.*[7]. FA peaks were identified using a 37-component FAME mix and *trans*-11-18 : 1 from Supelco. Additional standards for conjugated linoleic acid isomers were obtained from Natural ASA. Theoretical response factors for flame ionisation detectors have been used for all FAME[17,18]. For the determination of Fe, Co, Zn and Cu, 5 ml of milk were accurately weighed into a Teflon digestion vessel (MLS-Milestone Ultra Clave III; Mikrowellen Labor Systeme GmbH), and

Table 2. Effect of cobalt level supplied on proportions of plasma fatty acids (FA) and calculated desaturase indices (DI) at the last day of cobalt administration (day 11)

(Mean values with their standard errors, *n* 4)

	T2	T4	SEM	*P*
FA composition (g/100 g FA)				
14 : 0	0·77	0·79	0·072	0·574
15 : 0	0·48	0·49	0·020	0·381
16 : 0	7·61	7·46	0·172	0·151
cis-9-16 : 1	0·79	0·87	0·036	0·313
17 : 0	0·51	0·45	0·029	0·043
18 : 0	12·01	11·87	0·342	0·817
cis-9-18 : 1	6·18	5·49	0·143	0·053
cis-11-18 : 1	0·23	0·26	0·008	0·091
cis-9,12-18 : 2	35·09	36·82	0·715	0·337
cis-6,9,12-18 : 3	0·97	0·52	0·043	0·068
cis-9,12,15-18 : 3	7·32	8·34	0·284	0·238
22 : 0	0·26	0·26	0·019	0·655
cis-8,11,14-20 : 3	1·54	1·01	0·199	0·011
cis-5,8,11,14-20 : 4	1·04	1·09	0·059	0·024
23 : 0	0·46	0·49	0·027	0·392
cis-8,11,14,17-20 : 4	0·77	0·60	0·054	0·172
cis-5,8,11,14,17-20 : 5	0·99	0·97	0·073	0·393
24 : 0	0·50	0·51	0·030	0·919
cis-15-24 : 1	0·49	0·48	0·022	0·730
cis-7,10,13,16,19-22 : 5	0·64	0·67	0·011	0·358
cis-4,7,10,13,16,19-22 : 6	3·24	3·33	0·047	0·203
SFA*	22·60	22·33	0·443	0·737
MUFA†	7·70	7·10	0·138	0·049
PUFA‡	51·60	53·34	0·819	0·373
DI				
DI *cis*-9-16 : 1	0·09	0·10	0·003	0·273
DI *cis*-9-18 : 1	0·34	0·32	0·007	0·281
DI *cis*-6,9,12-18 : 3	0·03	0·01	0·001	0·060
DI *cis*-5,8,11,14-20 : 4	0·40	0·53	0·025	0·057

T2, cows supplemented 4 mg Co/d; T4, cows supplemented 5300 mg Co/d.
* SFA = 14 : 0 + 15 : 0 + 16 : 0 + 17 : 0 + 18 : 0 + 22 : 0 + 23 : 0 + 24 : 0.
† MUFA = *cis*-9-16 : 1 + *cis*-9-18 : 1 + *cis*-11-18 : 1 + *cis*-15-24 : 1.
‡ PUFA = *cis*-9,12-18 : 2 + *cis*-6,9,12-18 : 3 + *cis*-9,12,15-18 : 3 + *cis*-8,11,14-20 : 3 + *cis*-5,8,11,14,17-20 : 5 + *cis*-8,11,14,17-20 : 4 + *cis*-5,8,11,14,17-20 : 5 + *cis*-7,10,13,16,19-22 : 5 + *cis*-4,7,10,13,16,19-22 : 6.

Table 3. Effect of cobalt level supplied on proportions of milk fatty acids (FA) and calculated desaturase indices (DI) at the last day of cobalt administration (day 11)

(Mean values with their standard errors, *n* 4)

	T1	T2	T3	T4	SEM	Linear*	Quadratic*	Cubic*
FA composition (g/100 g FA)								
4 : 0	4·43	4·46	4·52	4·87	0·131	0·010	NS	NS
6 : 0	2·51	2·49	2·54	2·15	0·095	<0·001	NS	NS
8 : 0	1·43	1·39	1·43	1·04	0·085	<0·001	NS	NS
10 : 0	2·96	2·90	2·98	2·15	0·189	<0·001	NS	NS
12 : 0	3·20	3·11	3·18	2·28	0·199	<0·001	NS	NS
14 : 0	11·35	11·42	11·47	10·60	0·148	<0·001	NS	NS
cis-9-14 : 1	0·77	0·64	0·62	0·18	0·055	<0·001	NS	NS
15 : 0	0·97	1·01	0·97	0·97	0·031	NS	NS	NS
16 : 0	25·33	26·17	25·35	26·37	0·901	NS	NS	NS
cis-9-16 : 1	0·77	0·70	0·69	0·35	0·050	<0·001	NS	NS
17 : 0	0·40	0·43	0·42	0·50	0·009	<0·001	NS	0·037
18 : 0	14·59	15·35	15·59	24·91	0·724	<0·001	NS	NS
trans-9-18 : 1	0·19	0·18	0·18	0·18	0·004	NS	NS	NS
trans-10-18 : 1	0·31	0·30	0·30	0·32	0·011	NS	NS	NS
trans-11-18 : 1	1·30	1·38	1·32	1·41	0·066	NS	NS	NS
cis-9-18 : 1	20·66	19·40	19·90	12·89	0·650	<0·001	NS	NS
cis-11-18 : 1	0·27	0·26	0·26	0·31	0·017	0·044	NS	NS
cis-9,12-18 : 2	1·29	1·32	1·27	1·53	0·066	0·005	NS	NS
cis-9,12,15-18 : 3	0·41	0·42	0·42	0·54	0·022	0·002	NS	NS
20 : 0	0·18	0·19	0·19	0·29	0·009	<0·001	NS	NS
cis-9,*trans*-11-18 : 2*	0·51	0·49	0·47	0·23	0·027	<0·001	NS	NS
cis-5,8,11,14-20 : 4	0·06	0·06	0·05	0·05	0·007	NS	NS	NS
cis-5,8,11,14,17-20 : 5	0·05	0·04	0·04	0·03	0·003	<0·001	NS	NS
cis-4,7,10,13,16,19-22 : 6	0·007	0·007	0·006	0·007	0·0005	NS	NS	NS
SFA†	67·22	68·74	68·45	75·84	0·755	<0·001	NS	NS
MUFA‡	22·47	21·00	21·47	13·72	0·734	<0·001	NS	NS
PUFA§	1·81	1·85	1·79	2·16	0·084	0·005	NS	NS
DI								
DI 14 : 1	0·06	0·05	0·05	0·02	0·004	<0·001	NS	0·034
DI 16 : 1	0·03	0·03	0·03	0·01	0·002	<0·001	NS	NS
DI 18 : 1	0·59	0·56	0·56	0·34	0·018	<0·001	NS	NS

T1, cows supplemented 0 mg Co/d; T2, cows supplemented 4 mg Co/d; T3, cows supplemented 380 mg Co/d; T4, cows supplemented 5300 mg Co/d.
* May include small amounts of other conjugated 18 : 2 isomers.
† SFA = 4 : 0 + 6 : 0 + 8 : 0 + 10 : 0 + 12 : 0 + 14 : 0 + 15 : 0 + 16 : 0 + 17 : 0 + 18 : 0 + 20 : 0.
‡ MUFA = *cis*-9-14 : 1 + *cis*-9-16 : 1 + *cis*-9-18 : 1 + *cis*-11-18 : 1.
§ PUFA = *cis*-9,12-18 : 2 + *cis*-9,12,15-18 : 3 + *cis*-5,8,11,14-20 : 4 + *cis*-5,8,11,14,17-20 : 5 + *cis*-4,7,10,13,16,19-22 : 6.

samples were then pretreated and analysed for Fe, Co, Zn and Cu as described for feed.

Calculations

Feed intake was calculated as the difference between the feed given and the refusals collected before morning feeding the following day for three consecutive days (days 9–11) in each period. Milk yield was calculated as the mean of the yield measured for three consecutive days (days 9–11). Days 9–11 were used to ensure that the effects of Co on desaturase indices in milk fat were achieved[4–8]. Production of fat, protein and lactose was calculated from the average milk yield on days 9–11, multiplied by the analysed concentration of fat, protein and lactose, on day 11. The amount of Co administered per d was calculated as the product of the average amount of solution infused between days 1 and 11 and the analysed concentration of Co in the solutions. Total tract digestion of feed components was calculated as the difference between their average intake for days 6–9 and their average faecal excretion on days 8–10 for cows given treatments T2 and T4. Desaturase indices were calculated as follows: (product of the desaturase)/(product of the desaturase + substrate of the desaturase). Δ9-Desaturase indices were calculated for three pairs of FA in milk (*cis*-9-14 : 1–14 : 0, *cis*-9-16 : 1–16 : 0 and *cis*-9-18 : 1–18 : 0) and two pairs of FA in plasma (*cis*-9-16 : 1–16 : 0 and *cis*-9-18 : 1–18 : 0; *cis*-9-14 : 1 was not detected in plasma). Desaturase indices for Δ6-desaturase and Δ5-desaturase were also calculated in plasma. Δ6- and Δ5-Desaturase indices were calculated as (*cis*-6,9,12-18 : 3)/(*cis*-6,9,12-18 : 3 + *cis*-9,12-18 : 2) and (*cis*-5,8,11,14-20 : 4)/(*cis*-5,8,11,14-20 : 4 + *cis*-8,11,14-20 : 3), respectively.

Statistical analysis

Data were analysed as a Latin square using ANOVA with the MIXED procedure of the Statistical Analysis Systems statistical software package version 9.1 (SAS Institute). The following model was used to analyse the data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \varepsilon_{ijk},$$

where μ is the general mean; α_i is the random effect of a cow; β_j is the effect of treatment; δ_k is the effect of period; and ε_{ijk} is the experimental error. Linear, quadratic and cubic contrasts

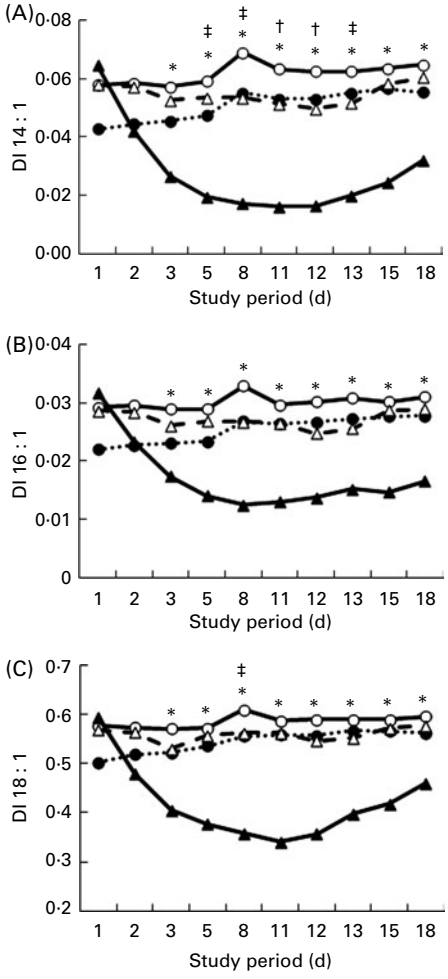

Fig. 1. Temporal changes in milk desaturase indices (DI) for (A) 14 : 1, (B) 16 : 1 and (C) 18 : 1 in response to supplementation of different levels of cobalt during the treatment period (days 1–11) and the depuration period (days 12–18) (n 4). T1, cows supplemented 0 mg cobalt/d (–○–); T2, cows supplemented 4 mg cobalt/d (··●··); T3, cows supplemented 380 mg cobalt/d (–△–); T4, cows supplemented 5300 mg cobalt/d (–▲–). The SE values for desaturase indices were 0·001, 0·001 and 0·007 for 14 : 1, 16 : 1 and 18 : 1, respectively. All three DI had significant treatment × time interactions ($P<0·001$). *There was a significant linear effect of treatment within day ($P<0·05$). †There was a significant cubic effect of treatment within day ($P<0·05$). ‡There was a tendency for cubic effects of treatment within day ($0·05 < P<0·10$).

were tested using the CONTRAST statement of the MIXED procedure. The coefficient matrix generated in PROC IML was added for unequally spaced treatments, making the polynomial contrasts orthogonal. For parameters with repeated measurements within a cow and period (feed intake and milk yield), a first-order autoregressive structure was used as the covariance structure. Parameters with significant treatment × time interactions were tested using the SLICE command. When slice was $P<0·05$, linear, quadratic and cubic contrasts for that respective time were tested using the CONTRAST statement of the MIXED procedure.

For parameters involving only T2 and T4 (blood parameters and digestibility), data were analysed as a replicated 2 × 2 Latin square with rows and columns nested:

$$Y_{ijkl} = \mu + \alpha_{i(l)+}\beta_j + \delta_{k(l)} + \tau_l + \varepsilon_{ijkl},$$

where μ is the general mean; $\alpha_{i(l)}$ is the random effect of cow i within replicate l; β_j is the effect of the jth treatment; $\delta_{k(l)}$ is the effect of period k within replicate l; τ_l is the effect of the lth replicate; ε_{ijkl} is the experimental error. Cow was treated as a random effect, and t tests were used to separate means. The level for statistical significance was defined as $P=0·05$ and $0·05 < P<0·10$ was considered to indicate a tendency of effect. All data are presented as means with their standard errors, unless otherwise stated.

Results

Fatty acid composition and desaturase indices in blood plasma and milk

Proportions of plasma FA and calculated desaturase indices are presented in Table 2. In general, increasing Co supplementation from T2 (4·0 mg/d) to T4 (5300 mg/d) had relatively small effects on plasma FA composition. Of the saturated FA, 17 : 0 was significantly reduced ($P=0·043$), whereas the others were unaffected. The proportion of *cis*-9-18 : 1 tended to be depressed ($P=0·053$), whereas *cis*-11-18 : 1 tended to increase ($P=0·091$). However, the sum of MUFA decreased ($P=0·049$). The effect of Co levels on the proportion of PUFA was not consistent. The plasma concentration of *cis*-6,9,12-18 : 3 tended to be reduced ($P=0·068$) when cows received T4, resulting in a tendency for a lower Δ6-desaturase index ($P=0·060$). Also, the plasma concentration of *cis*-8,11,14-20 : 3 decreased ($P=0·011$). In contrast, *cis*-5,8,11,14-20 : 4 increased slightly ($P=0·024$), resulting in a tendency for an increased Δ5-desaturase index ($P=0·057$).

As expected, plasma FA composition on T4 had, with only a few exceptions, reached the T2 level the last day of the post-treatment period (day 18; data not shown). However, on day 18, *cis*-5,8,11,14,17-20 : 5 was lower on T4 compared with T2 ($P=0·042$), and some other FA also tended to be affected. In contrast to day 11, neither the concentration of MUFA nor the resultant desaturase indices were significantly different between T4 and T2 at day 18.

In contrast to plasma, milk FA composition was highly affected by the treatment (Table 3). Concentrations of all *cis*-9 MUFA (the product FA of Δ9-desaturase) in milk were decreased with increased levels of Co (linear, $P\leq 0·001$), resulting in lower desaturase indices for 14 : 1 (linear, $P<0·001$; cubic, $P<0·05$), 16 : 1 (linear, $P<0·001$) and 18 : 1 (linear, $P<0·001$). The effects on their corresponding saturated FA were not consistent, but the concentration of 18 : 0 was 24·9 g/100 g FA on T4 compared with 15·4 g/100 g FA on T2, representing an increase of >60 % (w/w) (linear, $P<0·001$). Interestingly, and in contrast to *cis*-9 MUFA, the concentrations of the essential FA *cis*-9,12-18 : 2 and *cis*-9,12,15-18 : 3 (substrate FA for Δ6-desaturase) increased (linear, $P<0·01$) with increased levels of Co. However, the concentrations of *cis*-9,*trans*-11-18 : 2 (CLA, the product of Δ9-desaturase) and *cis*-5,8,11,14,17-20 : 5 (EPA, the product of Δ6-desaturase and

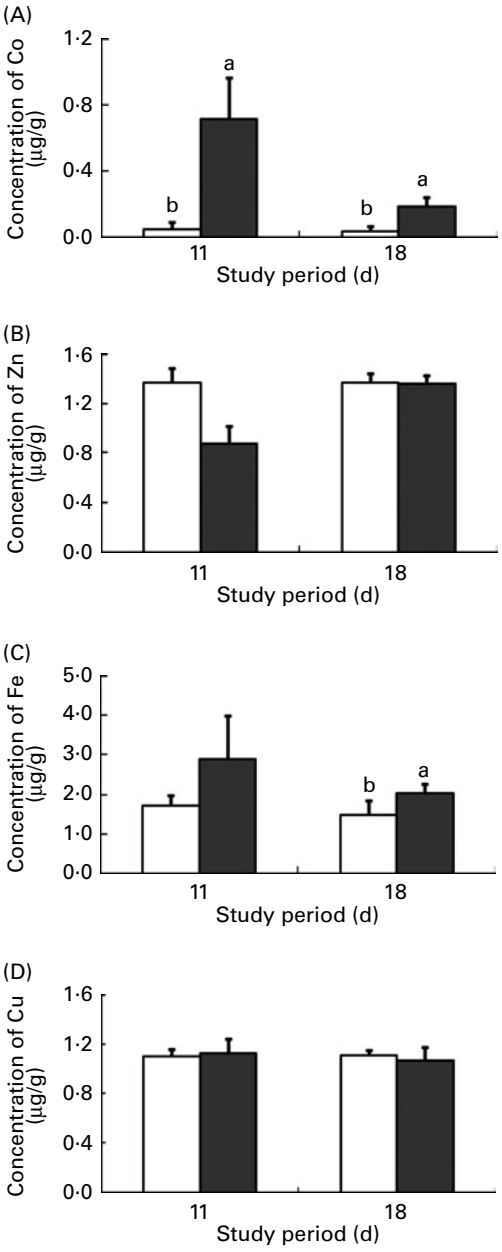

Fig. 2. Concentration of (A) cobalt, (B) zinc, (C) iron and (D) copper in blood plasma the last day of the treatment period (day 11) and the last day of the depuration period (day 18) for cows supplemented with different levels of cobalt. Values are means, with their standard errors represented by vertical bars (n 4). [a,b] Mean values with unlike letters tended to be different ($0{\cdot}05 < P < 0{\cdot}10$). T2, cows supplemented 4 mg cobalt/d (□); T4, cows supplemented 5300 mg cobalt/d (■). For both cobalt and zinc, there were significant treatment × time interactions ($P < 0{\cdot}01$).

Δ5-desaturase) were lower (linear, $P < 0{\cdot}001$). Increasing the level of Co also increased the concentration of 17 : 0 in milk (linear, $P < 0{\cdot}001$; cubic, $P < 0{\cdot}05$). In addition, the lower concentration of FA with chain lengths less than sixteen carbon atoms (linear, $P < 0{\cdot}001$) indicated reduced *de novo* FA synthesis. All FA with chain lengths up to fourteen carbon atoms decreased with increased amounts of Co (linear, $P < 0{\cdot}05$).

Most of the FA (data not shown) and all desaturase indices (linear, $P < 0{\cdot}01$; Fig. 1) in milk were not recovered the last day of the post-treatment period (day 18). However, compared with day 11, the effects were less evident.

Cobalt, iron, copper and zinc in blood plasma and milk

Blood plasma and milk concentrations of Co, Zn, Fe and Cu are shown in Figs. 2 and 3, respectively. There were interesting

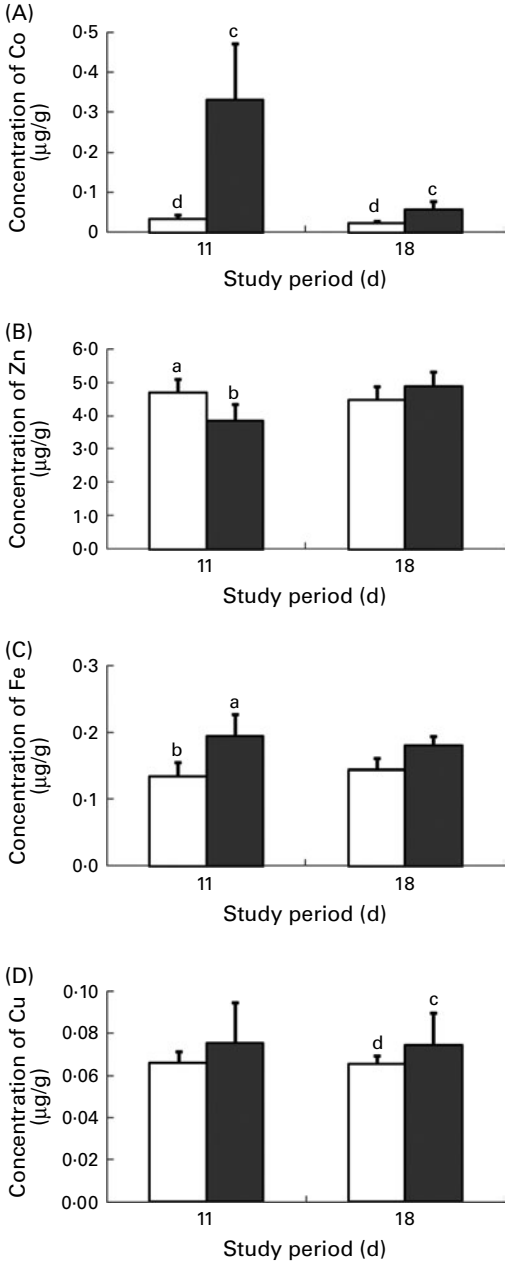

Fig. 3. Concentration of (A) cobalt, (B) zinc, (C) iron and (D) copper in milk the last day of the treatment period (day 11) and the last day of the depuration period (day 18) for cows supplemented with different levels of cobalt. Values are means, with their standard errors represented by vertical bars (n 4). [a,b] Mean values with unlike letters were significantly different ($P < 0{\cdot}05$). [c,d] Mean values with unlike letters tended to be different ($0{\cdot}05 < P < 0{\cdot}10$). T2, cows supplemented 4 mg cobalt/d (□); T4, cows supplemented 5300 mg cobalt/d (■). For both cobalt and zinc, there were significant treatment × time interactions ($P < 0{\cdot}01$).

Table 4. Effect of cobalt level supplied on feed intake, milk production, chemical composition, and production of fat, protein, and lactose

(Mean values with their standard errors, *n* 4)

	T1	T2	T3	T4	SEM	Linear	Quadratic	Cubic
DM intake (kg/d)								
Grass silage	13·3	13·2	12·9	11·2	0·68	<0·001	NS	NS
Concentrate	7·9	7·8	7·9	7·8	0·07	NS	NS	NS
Total	21·2	21·0	20·8	19·0	0·71	<0·001	NS	NS
Milk production								
Yield (kg/d)	31·6	30·9	32·5	26·6	1·40	<0·001	NS	NS
Composition (g/kg)								
Fat	44·0	39·9	41·5	40·0	2·32	NS	NS	NS
Protein	31·8	31·6	31·6	31·6	0·52	NS	NS	NS
Lactose	46·2	46·8	46·4	47·0	0·76	NS	NS	NS
Production (g/d)								
Fat	1373	1235	1350	1050	77·8	0·013	NS	NS
Protein	999	973	1028	837	37·9	0·001	NS	NS
Lactose	1455	1443	1517	1252	68·8	0·010	NS	NS

T1, cows supplemented 0 mg Co/d; T2, cows supplemented 4 mg Co/d; T3, cows supplemented 380 mg Co/d; T4, cows supplemented 5300 mg Co/d.

differences between T4 and T2 for some of these parameters. Compared with T2, cows on T4 produced milk with a higher concentration of Fe ($P=0·014$), but a lower concentration of Zn ($P=0·002$). Parallel effects were found in blood plasma, but were not statistically significant ($P>0·10$). The amounts of Zn and Fe excreted in milk were not different ($P>0·05$), but the excretion of Zn tended to be reduced with T4 ($P=0·076$). The concentrations of Co, Fe, Zn and Cu in plasma and milk were not significantly different between T4 and T2 at day 18. However, the concentration of Co in plasma and milk, and its excretion in milk (data not shown) at day 18 still tended to be higher with T4 than with T2 ($P=0·089$, $P=0·057$ and $P=0·064$, respectively). Moreover, at day 18, the concentration of Fe in plasma and Cu in milk tended to be higher with T4 than with T2 ($P=0·074$ and $P=0·070$, respectively).

Feed intake, diet digestibility and milk production

Silage intake and total DM intake were depressed by increasing amounts of Co (linear, $P<0·01$; Table 4). The negative effect on DM intake seemed to be reversed immediately after Co administration was ended on day 11 (Fig. 4). Digestibility of the main diet nutrients did not differ between T2 and T4, but organic matter digestibility was slightly reduced ($P=0·042$) with T4 (Table 5). Due to the negative effect of T4 on grass silage intake, the amount of protein digested per d was lower on T4 compared with T2 ($P=0·008$), and there was a similar tendency for the amount of digested fat ($P=0·071$).

In line with the negative effect on feed intake, milk yield was depressed with increased amounts of Co (linear, $P<0·001$; Table 4). The effect of Co on milk yield seemed to occur after approximately 1 week of Co treatment (Fig. 4). However, the interaction between treatment and time was not statistically significant. In contrast to milk yield, milk concentrations of fat, protein and lactose were not affected by the treatment. However, as a consequence of the reduced milk yield, production of fat, protein and lactose was reduced with increased amounts of Co (linear, $P<0·05$, $P<0·01$ and $P<0·05$, respectively).

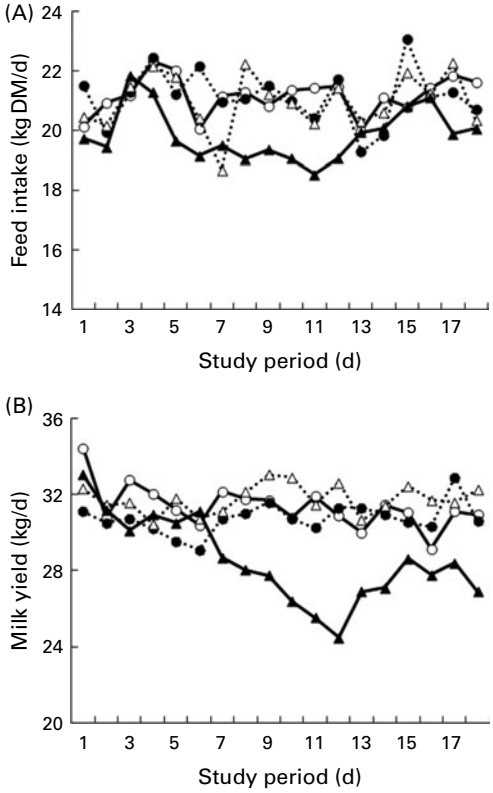

Fig. 4. Temporal changes in (A) feed intake and (B) milk yield in response to supplementation of different levels of cobalt during the treatment period (days 1–11) and the depuration period (days 12–18) (*n* 4). T1, cows supplemented 0 mg cobalt/d (–○–); T2, cows supplemented 4 mg cobalt/d (··●··); T3, cows supplemented 380 mg cobalt/d (··△··); T4, cows supplemented 5300 mg cobalt/d (–▲–). The SE values were 0·600 and 0·910 for feed intake and milk yield, respectively. Interactions between treatment and time were not significant ($P>0·10$).

Table 5. Effect of supplied cobalt level on total tract digestion coefficients of feed ingredients

(Mean values with their standard errors, *n* 4)

	T2	T4	SEM	*P*
DM	0·761	0·749	0·008	0·122
Organic matter	0·768	0·757	0·008	0·042
NDF	0·743	0·715	0·007	0·207
Crude protein	0·761	0·742	0·011	0·257
Fat	0·803	0·806	0·010	0·859
Starch	0·994	0·995	0·002	0·696

T2, cows supplemented 4 mg Co/d; T4, cows supplemented 5300 mg Co/d; NDF, neutral-detergent fibre.

Discussion

The main objective of the present study was to examine the effects of increasing Co levels on milk FA composition. Earlier studies[6] have shown that the ratio between products and substrates for Δ9-desaturase stabilised after 6 d of Co infusion; to be certain of this effect, we therefore planned a treatment period of 11 d. As shown in Fig. 1, the response to Co levelled off before the treatment ended for desaturase indices of *cis*-9-14 : 1 and *cis*-9-16 : 1; however, for *cis*-9-18 : 1, no conclusion could be drawn. When the present experiment was planned, no published results on the time required for recovery of milk FA composition after Co administration were available. The depuration period of 7 d was obviously too short, because desaturase indices did not level off at the end of the depuration period (Fig. 1). A parallel effect on Co concentration in blood plasma and milk supports these results (Figs. 2 and 3). In a study published later by Shingfield *et al.*[5], it has been shown that the ratio between products and substrates for Δ9-desaturase in bovine milk did not level off 5 d after terminating the Co infusion. Data from two cows changing diets from T4 to T2 (in the present study) showed that desaturase indices seemed to level off at approximately the 11th day on T2, corresponding to 18 d after Co infusion was ended on T4 (data not shown).

Overall, the results clearly showed a linear effect of Co level on milk proportions of FA and desaturase indices in bovine milk. The addition of 5300 mg Co/d (T4) resulted in considerably lower Δ9-desaturase indices in milk, which is in line with other studies[4–8]. Besides affecting desaturase indices for even-numbered FA, Co infused into the rumen also increased the concentration of 17 : 0 in milk. This might be a result of reduced desaturation of 17 : 0 to *cis*-9-17 : 1 in the mammary gland. Because the concentration of *cis*-9-17 : 1 in milk was not determined in the present experiment, this cannot be confirmed. However, this is probably not a consistent effect, because Shingfield *et al.*[5] found no effect of infusing Co into the rumen on the concentration of 17 : 0 in bovine milk.

The National Research Council[10] set the maximum tolerable level of Co at 25 mg/kg diet DM, which is considerably lower than the highest level (approximately 280 mg/kg DM) in the present study. Reduced feed intake, which is one of the known signs of Co toxicity[19–21], was observed with increasing amounts of Co. Some of the physiological effects of high Co levels that probably contribute to toxicological signs in animals are the dual effects on haem metabolism: high levels of Co enhance erythropoietin synthesis through a complex mechanism that results in polycythaemia[22], and induce the synthesis of haem oxygenase, the rate-limiting enzyme of haem degradation in the liver, kidney and other tissues[23–27]. However, in the study conducted by Shingfield *et al.*[5] as well as by Karlengen *et al.*[4], using administration of 51 and 150 mg Co/kg DM, respectively, the treatment had no effect on feed intake or milk yield, even though the level of Co supplied was considerably higher than what is considered as the maximum tolerable level[10].

The Co requirement for ruminants is set at 0·11 mg/kg DM[9]. With the concentrations of 0·19 mg/kg DM in the silage and 0·26 mg/kg DM in the concentrate used in the present study, there was a surplus of Co even on the unsupplemented diet (T1). Norwegian commercial concentrate mixtures for dairy cows normally contain Co at levels comparable with T2. In addition, cows are often given Co-containing mineral supplements. Consequently, the intake of Co is in most practical situations considerably higher than the requirement of 0·11 mg/kg DM[9], but, judged from the present results, still with negligible effects on FA composition.

The present results showed only minor and, with a few exceptions, non-significant effects of Co on desaturase indices in plasma (Table 2). These results are in agreement with those of other investigators[7,8], and indicate that the mammary gland is the major site for Δ9-desaturation during lactation in ruminants. A decrease in the expression of Δ9-desaturase in adipose tissue[28] considerably up-regulated the Δ9-desaturase expression in the mammary gland[29], and an inactive Δ9-desaturase in ruminant liver[30] supports this key role of the mammary gland for FA desaturation during lactation. Nevertheless, the plasma concentration of MUFA was slightly reduced with T4, indicating that Co, in principle, has effects in other tissues similar to those in mammary tissues.

Recent results[4] indicate that the effect of Co on bovine milk FA composition is not caused by affecting the production of the Δ9-desaturase enzyme in the udder. It is therefore speculated that a reduction in desaturation products is caused by a decrease in Δ9-desaturase enzyme activity. However, the mechanisms behind the reductions in desaturation products when excess Co is given are not fully understood. Different minerals have been reported to affect Δ9-desaturase[31–35], and an indirect effect of Co on Δ9-desaturation cannot be excluded. Because excess Co tended to decrease plasma desaturase indices for Δ6-desaturase and increase plasma desaturase indices for Δ5-desaturase, a common mechanism for the inhibition of Δ9-desaturase and Δ6-desaturase, but not Δ5-desaturase, is suggested. Both Δ6-desaturase and Δ5-desaturase differ from Δ9-desaturase by containing a fused cytochrome *b*5 domain at their N-terminus, which plays a role as an electron donor during desaturation[36], and microsomal cytochrome *b*5 is essential for the activity of Δ9-desaturase[37,38]. However, microsomal cytochrome *b*5 also plays an important role in the process of Δ6-desaturation[39]. The predicted amino acid sequence of Δ5-desaturase contains all of the structural characteristics present in Δ6-desaturase[40], but a potential role of microsomal cytochrome *b*5 in

the process of Δ5-desaturation has, to our knowledge, not been investigated. Cytochrome *b*5 is a haemoprotein[41], and Karlengen *et al.*[4] suggested that an induction of haem oxygenase due to high amounts of Co may cause reduced Δ9-desaturation. An induction of haem oxygenase can possibly affect the desaturation process by releasing haem Fe from cytochrome *b*5, and might therefore represent a site of inhibition for both Δ9-desaturase and Δ6-desaturase. The increased concentration of Fe in milk and the numerically higher concentration of Fe in plasma found when cows were on the highest amount of Co might strengthen this theory. However, more investigation is needed to confirm this hypothesis.

In conclusion, increasing the amount of additional Co from 0 to 5300 mg/d significantly reduced the concentration of MUFA, and increased the concentration of SFA in milk fat. These changes in milk fat composition are in line with a decrease in desaturation in the mammary gland. Adding such high amounts of Co to the diet also reduced feed intake and milk yield.

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