Effects of peripartum biotin supplementation of dairy cows on milk production and milk composition with emphasis on fatty acids profile

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Abstract

Forty Holstein dairy cows receiving a 38% concentrate diet based on maize silage were assigned to either a control group, either a biotin group, receiving 20 mg of biotin per day from 15 days before expected calving date and for 120 days after calving. Milk production was measured daily, milk fat content, protein content, urea and somatic cell counts were determined weekly from week 2 to week 17 of lactation. The profile of milk fatty acids was determined at weeks 3 and 10. Plasma glucose and blood beta-hydroxybutyrate were determined before calving and at weeks 1, 2, 3, 5, 7 and 10 of lactation.

Biotin supplementation resulted in an increased milk production in multiparous cows during weeks 2 to 6, but the effect was no more significant between 7 and 17 weeks of lactation. Milk protein percent was decreased by 0.1% in multiparous cows. Milk fat content was not affected by biotin, and milk fat daily production tended to increase during early lactation. In milk fat, biotin supplementation tended to decrease the proportion of fatty acids with less than 16 carbons at week 3, but the daily amount was not affected. Biotin tended to decrease biohydrogenation intermediates, increased C16:1 at week 3, and tended to increase *cis*-9 C18:1 at weeks 3 and 10. After 7 weeks of lactation, biotin tended to increase blood beta-hydroxybutyrate in multiparous cows with values remaining in a normal range, and decreased plasma glucose in primiparous cows. These modifications of plasma parameters, milk protein content and profile of milk fatty acids could be due to a higher lipid mobilisation from adipose tissue driven by the increased milk production.

Keywords: Biotin; Cows; Milk production; Milk composition; Fatty acids

1. Introduction

As a B-vitamin, biotin has long been thought to be provided in sufficient amounts to ruminants by the ruminal microorganisms. However, recent data showed a negative ruminal balance (Santschi et al., 2005; Schwab et al., 2006), and some experiments on lactating dairy cows have shown that long term biotin supplementation can decrease hoof disorders (Midla et al., 1998; Pötzsch et al., 2003) and that peripartum biotin supplementation can positively impact milk production (Zimmerly and Weiss, 2001). This later experiment and

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others demonstrating positive production effects (Bergsten et al., 2003; Majee et al., 2003) were conducted on high producing cows with diets based on maize silage and containing 50% concentrate or more, but no effect of supplemental biotin on milk production was observed on low producing cows receiving a grass-based diet (Fitzgerald et al., 2000), suggesting that the effect of biotin depends on the forage/concentrate ratio and/or the milk yield. Increased glucose production has been suggested to explain the positive effect of biotin supplements on milk production (Zimmerly and Weiss, 2001) because biotin is a coenzyme of pyruvate carboxylase and of propionyl-CoA carboxylase which are key enzymes of neoglucogenesis.

Biotin is also the cofactor of acetyl-CoA carboxylase, the enzyme of the first step of fatty acids synthesis from acetate. This enzyme is rate-limiting for mammary fatty acids synthesis (Mellenberger et al., 1973), and all milk fatty acids with less than 14 carbons and part of fatty acids with 14 and 16 carbons originate from mammary synthesis. Furthermore, biotin is necessary for cellulolytic bacteria (Scott and Dehority, 1965). These bacteria are important for ruminal biohydrogenation of dietary unsaturated fatty acids (Harfoot and Hazlewood, 1988), whose part of intermediates can outflow the rumen and be incorporated into milk lipids. However, the effects of biotin supplementation on the profile of milk fatty acids have not been studied.

The objectives of this study were to investigate the effects of biotin supplementation around parturition on milk production, milk composition with emphasis on milk fatty acids profile, and plasma metabolites.

Table 1
Ingredients and chemical composition of diets

Diets	Pre-calving	Post-calving basal diet	Post-calving average diet [a]
Ingredients (% dry matter)			
Maize silage	36.4	63.4	54.8
Ray-grass hay	42.7	0.0	0.0
Grass silage	3.4	5.9	5.1
Dehydrated alfalfa	1.1	2.0	1.7
Wheat	4.6	8.0	6.9
Fat supplement [b]	1.0	1.7	1.5
Concentrate 1 [c]	10.1	17.6	15.1
Concentrate 2 [d]	0.0	0.0	13.7
Salt	0.1	0.1	0.1
Mineral-vitamin mix [e]	0.7	1.3	1.1
Chemical composition (% dry matter)			
Crude protein	11.4	14.0	15.5
Acid detergent fibre	26.6	21.8	20.7
Starch	12.9	22.5	22.6
Crude fat	3.6	4.8	4.7

[a] Basal diet plus average amount of concentrate 2 (4 kg per cow per day).
[b] Calcium salts of palm oil.
[c] Mainly composed of soybean meal and sunflower meal; 44.1% crude protein, 17.4% acid detergent fibre, 2.8% starch and 3.0% crude fat (dry matter basis).
[d] Mainly composed of grain, grain by-products and meals; 24.7% crude protein, 14.2% acid detergent fibre, 23.5% starch and 4.1% crude fat (dry matter basis).
[e] Contained 7% P, 21% Ca, 6% Mg, 1% Na, 300,000 IU/kg vitamin A, 60,000 IU/kg vitamin D3, 700 mg/kg vitamin E, 4500 mg/kg Zn, 3500 mg/kg Mg, 800 mg/kg Cu,70 mg/kg I, 20 mg/kg Co, 15 mg/kg Se.

Table 2
Average amount of concentrate 2 [a] and milk production allowed by net energy of lactation and protein digestible in the intestine carried by the post-calving average diet, calculated using NRC (2001) estimates of dry matter intake

	Primiparous	Multiparous
Average amount of concentrate 2 (kg/day)		
Weeks 2–6	1.5	2.7
Weeks 7–17	2.8	5.5
Milk allowed by net energy for lactation (kg)		
Weeks 2–6	25.9	35.9
Weeks 7–17	35.0	47.6
Milk allowad by protein digestible in the intestine (kg)		
Weeks 2–6	24.8	33.8
Weeks 7–17	33.0	44.7

[a] See Table 1.

2. Materials and methods

2.1. Experimental design and diets

All procedures for this study complied with the *Guide for the Care and Use of Agriculture Animals in Agricultural Research and Teaching* (FASS, 1999). Two groups of 22 Holstein dairy cows (6 primiparous and 16 multiparous) were used. Twelve primiparous cows were affected to one of the groups according to calving date. Thirty-two multiparous cows were affected to one of the two groups of cows according to calving date, parity, milk production, milk fat and protein contents during the previous lactation. During the experiment, 2 multiparous cows were eliminated in each group due to abortion (1 cow), dramatic decrease of production following acute mastitis (1 cow) or serious metabolic problems in the early beginning of lactation (2 cows).

Pre-calving and post-calving diets are presented on Table 1. The basal diet was available *ad libitum*. Additionally, lactating cows individually received a commercial concentrate based on grain, grain by-products and meals, distributed with a computer-controlled feeder. The amount of this concentrate was 0.5 and 1 kg/day for

Table 3
Least square means of milk yield and composition of lactating cows fed control diet or diet supplemented with biotin

	Primiparous		Multiparous		MSE[a]	Significance			
	Control	Biotin	Control	Biotin		Parity	Biotin		Time × biotin
							Primiparous	Multiparous	
Milk yield (kg/day)									
weeks 2–6	28.7	29.6	38.3	42.3	24.0	0.000	0.752	0.037	0.420
weeks 7–17	33.3	32.7	42.3	44.2	24.9	0.000	0.830	0.323	0.975
Milk fat (%)									
Weeks 2–6	4.57	4.71	4.68	4.67	0.35	0.851	0.690	0.949	0.648
Weeks 7–17	3.85	3.86	4.22	4.07	0.22	0.080	0.990	0.419	0.572
Milk protein (%)									
Weeks 2–6	3.02	2.94	3.01	2.90	0.03	0.690	0.495	0.133	0.117
Weeks 7–17	2.96	2.88	2.97	2.87	0.02	0.965	0.310	0.048	0.472
Milk fat (g/day)									
Weeks 2–6	1283	1382	1770	1967	72264	0.000	0.525	0.060	0.721
Weeks 7–17	1269	1246	1779	1794	52870	0.000	0.865	0.865	0.665
Milk protein (g/day)									
Weeks 2–6	849	863	1147	1224	17186	0.000	0.851	0.129	0.141
Weeks 7–17	980	939	1252	1263	15000	0.000	0.573	0.810	0.924
Milk urea (mg/L)									
Weeks 2–6	372	358	356	362	2821	0.732	0.652	0.795	0.900
Weeks 7–17	351	384	377	395	2601	0.303	0.269	0.342	0.282
Somatic cells count (log)									
Weeks 2–6	2.19	1.86	2.00	2.07	0.20	0.965	0.213	0.701	0.285
Weeks 7–17	2.05	1.74	2.06	1.96	0.21	0.480	0.239	0.584	0.903

[a] Mean square error.

all cows during weeks 1 and 2 of lactation, respectively. Thereafter, it was 400 g for each kg of milk above 30 kg (multiparous cows) or 26 kg (primiparous cows) of daily milk production. This amount could not increase by more than 1 kg per week, and could not be higher than 8 kg/day, whatever the production of the cow. Individual amount of this concentrate was adjusted by ± 0.5 kg/day to achieve similar average amounts for control and biotin groups. The average amount of this concentrate was 4.0 kg/day (4.7 and 2.3 kg/day for multiparous and primiparous cows, respectively), resulting in an average 62/38 forage/concentrate ratio. Milk productions allowed by net energy for lactation and protein digestible in the intestine (INRA, 1989) were calculated using NRC (2001) estimates of dry matter intake and are shown on Table 2.

Biotin (20 mg/day) was mixed with 100 g wheat, and distributed *via* the computer controlled feeders, at the beginning of the first concentrate meal of each day, in order to achieve complete consumption. Biotin was given from 15 days before expected calving date to 120 days after calving.

2.2. Samples and chemical analysis

Samples of diet ingredients were taken four times during the experiment, and frozen until analysis for dry matter, crude protein, acid detergent fibre, starch and crude fat. Milk production was measured daily. Morning and evening milk samples were collected from each cow once a week from week 2 to week 17 of lactation and composited for determination of fat, true protein and urea by infrared analysis (Milkoscan 605, Foss Electric, F-75001 Paris). At weeks 3 and 10, a second sample of milk was sampled for analysis of fatty acids, and freeze-dried (Vitris Freezemobile 25; Vitris Gardiner, NY). Fatty acids were methylated as described by Park and Goins (1994), with acetyl chloride in dry methanol instead of boron trifluoride. Total and *trans*-C18:1 fatty acids profiles were analysed by GLC (Agilent 6890N, equipped with a model 7683 auto injector, Network GC System, Palo alto, California, USA). The column was a fused silica capillary (CPSil88, 100 m × 0.25 mm ID, 0.2 μm film thickness, Chrompack-Varian, Middleburg, Netherlands). Flame ionization detector temperature was maintained at 260 °C and the injector at 255 °C, and a split ratio of 1/50 was used. Hydrogen was the carrier gas with a constant pressure (22.8 psi). The samples were injected in 0.5 μl of hexane. Initial temperature of oven was 60 °C, held for 1 min, increased by 20 °C/min to 150 °C, held at 150 °C for 10 min, increased by 2 °C/min to 175 °C, held at 175 °C for 20 min, increased by

Table 4
Least square means of percentages of fatty acids (other than biohydrogenation intermediates) in the milk fat of cows fed control diet or diet supplemented with biotin

	Week	Primiparous		Multiparous		MSE[a]	*p*-value
		Control	Biotin	Control	Biotin		Biotin
C4:0	3	4.39	4.29	4.46	4.06	0.90	0.456
	10	3.77	3.55	3.45	3.57	0.30	0.786
C6:0	3	2.62	2.45	2.57	2.36	0.28	0.318
	10	2.52	2.20	2.46	2.47	0.14	0.247
C8:0	3	1.53	1.35	1.42	1.32	0.12	0.261
	10	1.56	1.31	1.57	1.58	0.08	0.228
C10:0	3	2.66	2.20	2.38	2.28	0.45	0.237
	10	3.08	2.59	3.13	3.16	0.411	0.316
C11:0	3	0.16	0.13	0.13	0.11	0.02	0.622
	10	0.15	0.17	0.13	0.20	0.02	0.442
C12:0	3	2.92	2.33	2.67	2.48	0.49	0.123
	10	4.22	3.45	4.30	4.56	0.81	0.422
C13:0	3	0.15	0.11	0.14	0.13	0.00	0.168
	10	0.25	0.19	0.19	0.21	0.01	0.559
C14:0	3	8.71	7.74	8.44	8.09	1.89	0.178
	10	11.24	10.28	11.47	11.49	1.46	0.271
C14:1	3	0.58	0.47	0.60	0.62	0.03	0.453
	10	0.98	0.71	0.93	1.03	0.07	0.386
C15:0	3	0.93	0.77	0.85	0.80	0.04	0.164
	10	1.27	1.14	1.04	1.12	0.08	0.830
C16:0	3	28.03	28.07	29.16	29.00	4.96	0.935
	10	30.65	30.84	31.40	30.01	8.62	0.563
C16:1	3	1.75	2.15	2.12	2.41	0.16	0.019
	10	1.68	1.78	1.87	1.91	0.10	0.550
C17:0	3	0.77	0.81	0.71	0.68	0.02	0.913
	10	0.71	0.70	0.60	0.63	0.01	0.754
C18:0	3	11.22	11.29	10.60	10.35	2.51	0.864
	10	8.67	8.86	8.79	8.32	1.89	0.771
cis-9 C18:1	3	22.18	25.46	23.66	25.48	16.50	0.082
	10	17.97	20.99	17.73	18.80	11.15	0.088
C18:2	3	1.98	1.86	1.95	1.95	0.07	0.543
	10	2.05	1.92	2.08	2.24	0.09	0.877
C18:3	3	0.32	0.34	0.29	0.29	0.00	0.537
	10	0.27	0.28	0.27	0.27	0.00	0.714
C6:0–C15:0	3	20.27	17.56	19.19	18.19	12.20	0.134
	10	25.27	22.05	25.22	25.82	9.44	0.230

[a] Mean square error.

10 °C/min to a final temperature of 225 °C and maintained at 225 °C for 10 min. A second analysis was performed to separate *trans*-13 + 14 C18:1 from *cis*-9 C18:1: initial temperature of oven was 50 °C, increased by 4 °C/min to 190 °C, held at 190 °C for 13 min, increased by 5 °C/min to 225 °C, and held at 225 °C for 8 min. Identification and quantification of peaks was made by comparison with commercial standards when available (Sigma, St. Louis, USA).

Samples of plasma were taken 1 and 2 weeks before expected calving , and 1, 2, 3, 5, 7 and 10 weeks after calving. They were analysed for glucose with a glucose oxidase multilayer slide method (Vitros 250, Ortho-Clinical Diagnistices, Rochester, NY, USA). At the same time, samples of whole blood were taken and analysed for beta-hydroxybutyrate (BHBA) as described by Enjalbert et al. (2001).

2.3. Calculations and statistical analysis

Due to unbalance between groups after elimination of 4 selected animals, milk production of multiparous cows was corrected by covariable adjustment (Morris, 1999) using expected milk production as a preliminary variable. Expected milk production was calculated from the production during the previous lactation using the coefficients proposed by Friggens et al. (1999). Milk fat and protein contents were corrected using corresponding

Table 5
Least square means of percentages of biohydrogenation intermediates in the milk fat of cows fed control diet or diet supplemented with biotin

	Week	Primiparous		Multiparous		MSE[a]	*p*-value
		Control	Biotin	Control	Biotin		Biotin
C18:1							
Total *trans*	3	3.85	3.33	3.18	2.81	1.00	0.210
	10	3.91	4.08	3.77	3.62	1.00	0.974
trans-6+7+8	3	0.35	0.31	0.32	0.28	0.01	0.268
	10	0.36	0.38	0.31	0.35	0.01	0.347
trans-9	3	0.25	0.23	0.25	0.20	0.00	0.092
	10	0.24	0.27	0.22	0.24	0.00	0.177
trans-10	3	0.50	0.42	0.60	0.45	0.24	0.505
	10	0.80	0.90	0.63	0.87	0.35	0.413
trans-11	3	1.45	1.19	0.95	1.03	0.18	0.551
	10	0.91	0.95	1.08	0.75	0.24	0.414
trans-12	3	0.42	0.32	0.32	0.31	0.01	0.062
	10	0.44	0.40	0.38	0.40	0.01	0.643
trans-13+14	3	0.32	0.43	0.39	0.22	0.08	0.763
	10	0.59	0.64	0.63	0.54	0.06	0.621
trans-15	3	0.25	0.19	0.12	0.09	0.02	0.306
	10	0.29	0.28	0.26	0.22	0.01	0.373
trans-16	3	0.28	0.22	0.20	0.20	0.00	0.055
	10	0.25	0.23	0.22	0.22	0.00	0.325
C18:2	3	0.76	0.65	0.57	0.56	0.03	0.347
cis-9,*trans*-11	3	0.76	0.65	0.57	0.56	0.03	0.347
	10	0.70	0.72	0.66	0.58	0.03	0.616

[a] Mean square error.

raw values during the previous lactation. Milk production and composition of primiparous cows were not adjusted.

Results were statistically analysed with SYSTAT (version 9, SPSS Inc, 1998, Chicago), using the model:

$$\text{Variable} = \text{mean} + \text{biotin effect} + \text{parity effect} + \text{parity} \times \text{biotin interaction} + \text{experimental error}.$$

Milk production and composition were analysed separately for early (weeks 2 to 6) and peak and mid (weeks 7 to 17) lactation, using a repeated measures procedure. For milk production, composition and biochemical parameters, because the effect of parity was significant on most parameters, significance of biotin effects was determined separately for primiparous and multiparous cows by contrasts between control and biotin treated cows using the mean square error provided by the model. Milk fatty acids profiles were analysed separately at 3 and 10 weeks of lactation. Because parity had no significant effect on the proportions of fatty acids, only an overall significance of biotin effect was computed.

Table 6
Least square means of desaturase ratios in the milk of cows fed control diet or diet supplemented with biotin

	Week	Primiparous		Multiparous		MSE[a]	*p*-value
		Control	Biotin	Control	Biotin		Biotin
C14:1/C14:0	3	0.066	0.063	0.071	0.077	0.001	0.829
	10	0.087	0.071	0.081	0.089	0.001	0.633
C16:1/C16:0	3	0.063	0.078	0.074	0.084	0.001	0.032
	10	0.055	0.058	0.060	0.064	0.00	0.329
cis-9 C18:1/C18:0	3	1.999	2.279	2.246	2.515	0.217	0.100
	10	2.132	2.454	2.058	2.279	0.242	0.123
cis-9,*trans*-11 C18:2/ *trans*-11 C18:1	3	0.535	0.573	0.611	0.578	0.015	0.957
	10	0.796	0.789	0.723	0.808	0.05	0.600

[a] Mean square error.

Table 7
Least square means of plasma glucose and blood BHBA of lactating cows fed control diet or diets supplemented with biotin

	Primiparous		Multiparous		MSE[a]	Significance of effects		
	Control	Biotin	Control	Biotin		Parity	Biotin	
							Primiparous	Multiparous
Glucose (mmol/L)								
Week−1	3.68	3.63	3.26	3.19	0.15	0.003	0.838	0.611
Weeks 2–5	3.44	3.18	2.94	2.84	0.09	0.000	0.157	0.413
Weeks 7–10	3.49	3.12	3.20	3.11	0.05	0.046	0.006	0.289
BHBA (mmol/L)								
Week−1	0.31	0.39	0.30	0.35	0.04	0.682	0.484	0.525
Weeks 2–5	0.35	0.49	0.40	0.50	0.03	0.602	0.175	0.163
Weeks 7–10	0.40	0.45	0.43	0.52	0.02	0.311	0.547	0.079

[a] Mean square error.

Significance was declared at $P<0.05$, and tendency at $0.05<P<0.15$.

3. Results

3.1. Milk yield and composition

Milk yield was positively affected by biotin supplement in multiparous cows, not in primiparous cows (Table 3). Biotin increased daily milk production of multiparous cows by 4 kg/day during the first 6 weeks of lactation. After 6 weeks, the effect of biotin was not significant any more.

Milk fat content was not affected by biotin, and milk fat yield tended ($P=0.06$) to be higher in multiparous cows receiving biotin than in control cows during the first 6 weeks of lactation. Biotin decreased milk protein content in multiparous cows between weeks 7 and 17, but due to numerically higher milk yield, milk protein yield was similar between control and supplemented cows. Biotin supplements did not affect milk urea or somatic cell counts.

Parity affected milk production and fat and protein yields, and a trend toward an interaction of time by biotin was observed for milk protein in early lactation: compared to control, biotin supplemented cows had a lower milk protein content during weeks 3 to 6, not during week 2 (results not shown).

3.2. Milk fatty acids profile

Milk fatty acids profiles are presented in Tables 4 and 5. Changes in individual proportions of fatty acids due to biotin supplementation were only observed for C16:1 during week 3, and a trend toward a higher proportion of *cis*-9 C18:1 was observed during both weeks 3 and 10. During week 3, biotin tended to decrease the proportion of C12:0 and the proportions of fatty acids with less than 16 carbons. During week 3, biotin tended to decrease some minor biohydrogenation intermediates (*trans*-9 C18:1, *trans*-12 C18:1 and *trans*-16 C18:1). However, proportions of total or major *trans* fatty acids were not modified. During week 10, no modification of *trans* fatty acids was observed.

Desaturase ratios were decreased for C16:0 at week 3, and tended to be decreased for C18:0 at weeks 3 and 10 (Table 6).

3.3. Plasma glucose and blood beta-hydroxybutyrate

During weeks 7 to 10, biotin supplementation decreased plasma glucose in primiparous cows and tended to increase blood BHBA in multiparous cows (Table 7). Plasma glucose but not blood BHBA was affected by parity.

4. Discussion

Milk production and composition were not affected by biotin supplementation in primiparous cows. Primiparous cows were randomly assigned to control or biotin cows, whereas assignment of multiparous cows took into account milk production and composition during the previous lactation, probably resulting in more similar groups. Moreover, the number of primiparous cows was low in our experiment. Zimmerly and Weiss (2001), measuring the effects of supplemental biotin on milk production, did not indicate different responses between primiparous and multiparous dairy cows.

A positive effect of biotin supplementation was observed in early lactation multiparous cows. This increased production was consistent with the results of Zimmerly and Weiss (2001), who observed a 3.3 kg milk increased production with 20 mg supplemental

biotin in early lactation cows, or the results of Majee et al. (2003), who reported a 1.7 kg increase in an experiment beginning at 46 days of lactation. On the contrary, Rosendo et al. (2004), using 20 mg biotin before calving and 30 mg after calving, did not report any improvement of milk production. Contrary to our observations, in the experiments of Zimmerly and Weiss (2001) and Rosendo et al. (2004), no difference of effect of biotin supplementation according to lactation week was observed. In these experiments, diets contained more than 50% concentrates, compared to an average of 38% in our experiment. The ruminal synthesis of biotin has been reported to be lower *in vitro* when the pH drops (Abel et al., 2001). In our experiment, we can hypothesize that rumen digestion and biotin synthesis were affected by dietary adaptation during the first weeks of lactation, resulting in a strongly positive effect of biotin supplementation, but that, due to the relatively low amount of concentrate, the ruminal outflow of biotin was sufficient to meet cows requirements after peak lactation even in control cows, resulting in a non-significant effect of biotin supplementation.

Because biotin is necessary for the conversion of propionate to glucose, the effect of biotin supplements could be mediated by an increased glucose production. Plasma glucose concentration was not positively affected by biotin supplementation, but plasma glucose may not be a good indicator of glucose availability: a supplemental glucose availability could have been directed toward the mammary gland to support an increased milk production, without increased plasma concentration. This would be consistent with previous experiments on early lactation cows, where authors observed either an increased milk production with a non-significant decrease of plasma glucose (Zimmerly and Weiss, 2001), or lack of effect on production with an increased plasma glucose (Rosendo et al., 2004).

The effects of biotin supplementation on milk protein were negative in multiparous cows when milk protein was expressed as a percentage of milk yield, and tended to be positive during weeks 2 to 6 when milk protein production was considered. This demonstrates that biotin had no negative effect on milk protein synthesis, but that a dilution effect was observed, resulting in a 0.1% decrease of milk protein percent. Decreased milk protein content in dairy herds can be due to insufficient energy supply; however in our experiment, during weeks 7 to 17, net energy allowances were over requirements (see Table 2) for both control and biotin supplemented cows. Previous experiments showed no effect of biotin on milk protein percent and a positive effect on milk protein yield (Zimmerly and Weiss, 2001; Majee et al., 2003), or no effect on both these parameters (Rosendo et al., 2004). In these experiments, dietary crude protein was over 17% of dry matter. In our experiment, the dietary supply of protein was lower, and the protein digestible in the intestine supply just met the requirement of biotin supplemented multiparous cows, which could have been limiting for milk protein synthesis. Whereas biotin could be involved in urea synthesis (Hartwell et al., 2001), biotin supplementation did not affect milk urea concentration.

Whatever the lactation period and parity, milk fat percent was not affected by biotin supplementation, which is consistent with the observations of Zimmerly and Weiss (2001), Majee et al. (2003) and Rosendo et al. (2004). Because biotin increased milk yield in early lactation multiparous cows, milk fat yield was significantly increased by biotin supplementation. Biotin is the transporter of carboxyl groups for the acetyl-CoA carboxylase, the enzyme responsible of the initiation of fatty acids synthesis. In the mammary gland, this synthesis produces a variable part of C16:0, a large part of C14:0 and nearly all fatty acids with less than 14 carbons except C4:0 which can be synthesised in part by non-malonyl-CoA mechanisms (Chilliard et al., 2000). In our experiment, at week 3, the proportions of C6:0 to C15:0 fatty acids tended to be lower in biotin supplemented cows, and the daily production of C6:0 to C15:0 (321 and 341 g/day for control and biotin multiparous cows, respectively; $P=0.47$; calculated as suggested by Glasser et al., 2007) did not differ. This means that biotin supplementation did not increase mammary synthesis of fatty acids, even when milk production was improved, which suggests that biotin was not a limiting factor for acetyl-CoA carboxylase activity. At week 10, biotin modified neither the production of milk fat nor the proportion of C6:0 to C15:0 in milk.

Biotin tended to increase C16:1/C16:0 and *cis*-9 C18:1/C18:0 ratios in milk fat of multiparous cows. Such an increased ratio could be interpreted as reflecting a higher Δ-9 desaturase activity in the mammary gland. However, biotin is not a cofactor of this enzyme, and its potential effect on mammary desaturation has never been studied. An increased mammary Δ-9 desaturase activity should have been expected to also result in higher C14:1/C14:0 and *cis*-9,*trans*-11 C182/*trans*11C18:1 ratios; lack of effect on these ratios and on all desaturase ratios in primiparous cows makes it doubtful an effect of biotin on mammary desaturation. Lipids from adipose tissue contain about 2.5 times more *cis*-9 C18:1 than C18:0 (Rukkwamsuk et al., 2000), whereas arterial lipids uptaken by the mammary gland in

cows whose energy requirements are met contain two times less *cis*-9 C18:1 than C18:0 (Enjalbert et al., 1998). This would result in a *cis*-9 C18:1/C18:0 ratio in plasma lipids that increases in parallel with fat mobilisation, so that our observed trend toward increased *cis*-9 C18:1/C18:0 milk fat ratio with biotin supplementation could be related to an increased fat mobilisation in multiparous cows. Moreover, the C16:1/C16:0 ratio is higher in adipose tissue (Rukkwamsuk et al., 2000) than in milk fat, so that an important fat mobilisation could also explain the trend toward higher C16:1/C16:0 ratio observed in milk from biotin supplemented cows. Such an increased body fat mobilisation in biotin supplemented cows would be consistent with a higher difference between their actual production (see Table 3) and the dietary allowances (see Table 2).

The proportion of some minor biohydrogenation intermediates tended to be lowered by biotin supplementation at week 3. A dilution of blood fatty acids originating from digestion by fatty acids from adipose tissue could explain this effect, which was no more observed at week 10. Overall, the effects of supplemental biotin on the biohydrogenation intermediates was low, suggesting no significant effect on ruminal biohydrogenation, so that a lower biohydrogenation of dietary *cis*-9 C18:1 was probably not a possible explanation for the trend toward a higher proportion of this fatty acid in milk fat of multiparous cows.

A stronger fat mobilisation from adipose tissue would be expected to result in higher blood BHBA values: a trend toward higher values was observed in biotin treated cows than in control cows from week 7, when BHBA concentrations reached their maximal values. However, values remained far below the 1.2 mmol/L usually considered as the cut-off point to discriminate between healthy and subclinically ketotic cows (Enjalbert et al., 2001).

In our experiment, biotin supplementation did not modify milk somatic cell count. This cannot be compared with the results of Fitzgerald et al. (2000), who reported that milk somatic cell counts were lowered, but after several months of biotin supplementation in low producing dairy cows.

5. Conclusion

Peripartum biotin supplementation resulted in improved milk production in early lactation multiparous cows, without effect on milk fat percent but a decreased milk protein percentage after 7 weeks of supplementation. Milk fatty acids proportions tended to be modified in early lactation with more *cis*-9 C18:1 and less C6:0 to C15:0 fatty acids. These results suggest that increased milk production due to biotin is associated with an improved lipid mobilisation from adipose tissue.

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