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Net nutrient absorption and liver metabolism in lactating dairy cows fed supplemental dietary biotin

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The effect of feeding supplemental biotin on net absorption and metabolism of nutrients by the portal-drained viscera (PDV; the gut, pancreas, spleen and associated fat) and liver of lactating dairy cows was measured. Three cows in early to mid-lactation catheterised for measurements of net nutrient absorption and metabolism by the PDV and liver were fed a total-mixed ration with or without supplemental biotin at 20 mg/day using a switch-back design (ABA v. BAB) with three 2-week periods. There were no effects of feeding biotin on dry matter intake (22.2 kg/day), milk yield (29.5 kg/day) or milk composition. There was also no effect of feeding biotin on net release of glucose by the liver, net liver removal of glucose precursors (propionate, alanine, lactate) or net liver release of β-hydroxybutyrate. Feeding biotin increased net PDV release of ammonia. Reasons for the response are not certain, but a numerical increase in net PDV release of acetate suggests that rumen or hindgut fermentation was altered. Results of the present study do not support the hypothesis that supplemental biotin increases liver glucose production in lactating dairy cows.

Keywords: biotin, dairy cows, lactation, liver, portal-drained viscera

Introduction

Biotin is synthesised by rumen micro-organisms and thus has not been considered an essential ingredient in ruminant diets. Textbooks state unequivocally that the 'evidence indicates that supplementary feeding (of B-complex vitamins) is of little value unless some unusual condition prevails' (Church, 1979). This view has now been challenged as regards to the potential benefit of supplemental B-complex vitamins in lactating dairy cows (Girard, 1998). It is well documented that long-term biotin supplementation can have positive effects on hoof health in pigs (Webb *et al.*, 1984) and horses (Comben *et al.*, 1984). More recently, studies in lactating dairy cows have consistently demonstrated a positive effect of supplemental biotin on hoof growth, integrity, and the incidence of lameness (Midla *et al.*, 1998; Fitzgerald *et al.*, 2000; Hedges *et al.*, 2001; Bergsten *et al.*, 2003). In some of these studies (Midla *et al.*, 1998; Bergsten *et al.*, 2003) and others (Bonomi *et al.*, 1996; Zimmerly and Weiss, 2001; Majee *et al.*, 2003), supplemental biotin also increased milk yield, although the effect was not consistent across all studies (Fitzgerald *et al.*, 2000; Rosendo *et al.*, 2004). Although improved hoof health would ultimately benefit both dry-matter intake and milk yield, the response in milk yield to supplemental biotin is more rapid than improvements in hoof integrity, and can occur without changes in DMI (Zimmerly and Weiss, 2001), suggesting a metabolic effect.

Ruminants normally absorb little glucose from the small intestine, thus glucose requirements are met primarily by liver production (Reynolds, 1995). Biotin is a cofactor for two carboxylase enzymes required for glucose synthesis in the liver, pyruvate carboxylase and propionyl-CoA carboxylase. In nonruminants, biotin deficiency has been associated with an inhibition of gluconeogenesis and reduced blood glucose levels (Bender, 1999). The effect of supplemental biotin on milk yield has been attributed to an effect on rumen fermentation and digestion, glucose production in the liver, or metabolic effects on nutrient partitioning and milk synthesis (Zimmerly and Weiss, 2001). The objective of the present study was to determine the effect of supplemental biotin on net absorption and liver metabolism of glucose, glucose precursors and other products of digestion and gut metabolism.

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Material and methods

Animals

Three Holstein × Friesian cows in their third, fourth or fifth lactation were used. Cows were 36, 71 or 174 days *post partum* and 670 ± 9 kg body weight at the beginning of the first experimental period. At the end of the study they averaged 661 ± 9 kg body weight. Cows were surgically prepared with a rumen fistula and catheters for measurement of net absorption and metabolism of nutrients by the portal-drained viscera (PDV: the tissues of the gastrointestinal tract, pancreas, spleen and associated fat) and liver as described by Huntington *et al.* (1989). Surgeries were conducted from 25 to 31 months prior to the beginning of the present study; therefore, cows had completed two entire lactations and a number of previous studies of nutrient metabolism (Reynolds *et al.*, 1998, 1999 and 2000; Benson *et al.*, 2002) between surgery and the present study. Cows were housed, fed and milked in individual standings employing head halters or neck yokes allowing continuous access to water and trace mineralised salt blocks. Cows were milked twice daily at 0600 and 1700 h, weighed weekly and allowed grazing when not on experiment. All procedures used were licensed under the Animal Scientific Procedures Act (1986) and cows were under the routine care and inspection of a herd veterinarian and a Home Office Veterinary inspector.

Diets

Cows were fed a basal total-mixed ration containing 300, 200, and 500 g per kg dry matter (DM) of dehydrated lucerne, grass silage, and cereal-based concentrates (Table 1), respectively. Based on the measured chemical composition of ingredients (Table 2) the total mixed ration DM fed averaged 178 g crude protein, 158 g starch, 369 g neutral-detergent fibre and 232 g acid-detergent fibre per kg DM. Daily rations were fed as 24 equal meals provided hourly to minimise post-prandial fluctuations in nutrient absorption and metabolism. Refusals were removed at 0730 h and feeding of a day's ration began at 0800 h.

Table 1 *Formulation of the total mixed ration fed*

Ingredient	g/kg DM
Dehydrated, chopped lucerne[1]	300.0
Grass silage	200.0
Wheat feed (mids)	191.7
Ground barley	142.9
Ground maize	47.8
Molassed sugar-beet shreds	47.8
Soya-bean meal, solvent extracted, 48% CP	52.7
Sodium bicarbonate	4.9
High phosphorus mineral mix[2]	12.2

[1] 'Hippoluz', Dengie Crops Ltd., Southminster, UK.
[2] Labelled as containing 180 g calcium (as dicalcium phosphate and limestone), 120 g phosphorus (as dicalcium phosphate), 50 g magnesium (as magnesium oxide and magnesium phosphate), 1.5 g copper (as copper sulphate), 0.015 g selenium (as sodium selenite), 380 000 IU vitamin A, 80 000 IU vitamin D3, 550 IU vitamin E per kg.

Table 2 *Composition (g/kg dry matter) of the forages and concentrate mixture fed*

Component	Grass silage	Lucerne	Concentrates
Nitrogen	25.8	26.4	30.8
Starch	ND[†]	ND	316.7
Neutral-detergent fibre	485.1	491.7	248.7
Acid-detergent fibre	315.9	402.2	95.5
Ash	83.0	108.0	74.9

[†] Not determined.

Treatments

Cows were fed 20 mg supplemental biotin daily in 500 g ground wheat or 500 g ground wheat as a control in a switch-back design with three 2-week periods (ABA *v.* BAB). Two cows received the control treatment in the first period, and one received the biotin treatment first. The control wheat carrier used was derived from the same batch used as the carrier for the biotin. To reduce the risk of mistakes in feeding supplements the biotin supplement included trace amounts of CrO_3 to impart a green colour. Supplements were added to the total mixed ration daily and for the duration of the study basal ration DM offered was held at 97% of *ad libitum* intake for the week immediately preceding the first period of the study. This was to ensure complete consumption of the biotin supplement and minimise variation in liver and PDV metabolism due to changes in intake.

Measurements

Milk yield and DM intake were measured daily throughout the study. During the last week of each period forage and concentrate samples were obtained daily, analysed for DM content and then composited, dried at 60 °C, ground and analysed for chemical composition by a commercial laboratory (Natural Resources Management, Ltd., Bracknell, UK). Any refusals were weighed and analysed for DM content. Milk samples were obtained over the last 7 days of each period, treated with preservative (1 mg/ml potassium dichromate; Lactabs, Thompson and Capper, Runcorn, UK) and stored at 4 °C until analysed for crude protein, fat and lactose content by infrared spectroscopy (Foss Electric, Ltd, UK).

Measurements of blood flow and net nutrient metabolism by the PDV, liver and total-splanchnic (PDV plus liver) tissues were obtained on the last day of each period using methods described previously (Huntington *et al.*, 1989; Reynolds *et al.*, 2003). Eight simultaneous blood sample sets (20 ml) from the mesenteric artery and portal and hepatic veins were obtained hourly beginning at 0730 hours during continuous mesenteric vein infusion of sterile ρ-aminohippurate (PAH; a marker for blood flow measurements) solution. Anaerobic blood samples (2 ml) were also obtained for immediate analysis of pO_2, pCO_2 and pH (Reynolds *et al.*, 2003). Blood samples were immediately placed on ice and kept chilled until processed, analysed or frozen. Plasma concentrations of PAH, glucose, and

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L-lactate were obtained as soon as possible using assays adapted for use on a discrete analyser (Cobas-MIRA, Roche, Welwyn Garden City, UK) as described (Reynolds *et al.*, 2003). Fresh blood samples were analysed for packed cell volume by micro-centrifugation and haemoglobin using a colorimetric assay and blood O_2 and CO_2 content calculated as described by Reynolds *et al.* (2003). Blood sample aliquots were pooled by sample site for each cow observation ($n = 9$), deproteinised, neutralised, and stored frozen in aliquots at $-85\,^{\circ}C$ until assayed for concentrations of ammonia, L-alanine, L-glutamate, L-glutamine and ß-OH-butyrate as described by Reynolds *et al.* (2003). Additional pools of blood samples were analysed for volatile fatty acid (VFA) concentrations as described by Reynolds *et al.* (2003).

Calculations

Blood and plasma flow for the portal vein and liver and calculations of net PDV, liver and total splanchnic (PDV plus liver) flux of nutrients and blood gasses were calculated as described (Reynolds *et al.*, 2003). Using venous-arterial differences, a negative flux denotes net removal of a metabolite from blood supply, whilst a positive flux denotes net release of a metabolite into venous blood. Net flux rates represent the sum of unidirectional uptake and release and therefore may underestimate unidirectional uptake and release for many metabolites when tissues as heterogenous as the PDV and liver are concerned. Net liver extraction as a percentage of total supply and net PDV release were also calculated (Reynolds *et al.*, 2003).

Statistical analysis

There were no missing blood samples. Averages for each cow-sampling period were statistically analysed using the mixed procedure of Statistical Analysis Systems Institute (2006) and a model testing fixed effects of period and treatment and random effects of cow and the cow by period interaction as described by Templeman (2004). Data are presented as least squares means with the standard error of the control mean. Because the number of observations is small, $P < 0.10$ is considered significant.

Results

There was no effect of biotin supplementation on body weight, DMI, milk yield or milk composition (Table 3). Blood and plasma flow and packed cell volume were also unaffected by biotin (Table 4). Similarly, arterial concentrations of most metabolites (Table 4) were not affected by biotin supplementation; however, the arterial concentration of O_2 and haemoglobin tended to increase when supplemental biotin was fed ($P = 0.11$ and $P = 0.14$, respectively).

Supplemental biotin increased ($P < 0.10$) net PDV release of ammonia (Table 5), but the net flux of other metabolites across the PDV was not affected. Similarly, the net flux of metabolites measured across the liver and total splanchnic tissues, and the fractional removal of lactate, alanine and ammonia by the liver, were not affected by biotin supplementation There was no effect of supplemental biotin on net liver release of glucose, or removal of the glucose precursors alanine and lactate. Arterial concentrations and net fluxes of VFA across the PDV, liver and total splanchnic tissues (Table 6) and the fractional net extraction of VFA by the liver (Table 7) were not affected by supplemental biotin, although there was a numerical increase in net liver removal of i-valerate ($P = 0.20$) when biotin was added to the diet.

Table 3 *Body weight, dry-matter intake (DMI) and milk yield, composition and component yield in lactating dairy cows fed a diet without (control) or with supplemental biotin*

	Control	Biotin	s.e.	*P*
Body weight (kg)	662	665	26	0.904
DMI (kg/day)	22.3	22.1	1.5	0.901
Milk yield (kg/day)	29.3	29.7	2.3	0.886
FCM[†] (kg/day)	30.0	30.0	2.6	0.989
Protein content (g/kg)	34.6	34.6	0.8	0.957
Fat content (g/kg)	41.5	41.0	1.5	0.744
Lactose content (g/kg)	47.1	47.5	0.5	0.671
Protein output (g/day)	1012	1027	63	0.876
Fat output (g/day)	1219	1209	110	0.922
Lactose output (g/day)	1388	1412	123	0.863

[†] Fat-corrected (40 g/kg) milk yield.

Table 4 *Blood and plasma flows and packed cell volume in lactating dairy cows fed a diet without (control) or with supplemental biotin*

	Control	Biotin	s.e.	*P*
Blood flow (l/h)				
Portal	2037	2004	164	0.883
Liver	2611	2531	125	0.655
Hepatic artery	579	544	337	0.934
Plasma flow (l/h)				
Portal	1469	1426	132	0.804
Liver	1879	1792	70	0.553
Hepatic artery	395	387	211	0.961
Packed cell volume (%)[†]	28.3	28.7	1.4	0.588
Arterial haemoglobin (g/l)	94.5	98.7	4.73	0.135
Arterial concentration (mmol/l)				
Blood				
Alanine	0.248	0.226	0.040	0.551
Glutamine	0.210	0.215	0.015	0.843
Glutamate	0.119	0.121	0.012	0.614
Ammonia	0.274	0.188	0.048	0.449
β-Hydroxybutyrate	0.587	0.593	0.019	0.470
Oxygen	5.351	5.571	0.275	0.106
Carbon dioxide	27.169	26.935	1.190	0.753
Plasma				
Lactate	0.473	0.472	0.079	0.995
Glucose	3.568	3.545	0.047	0.730

[†] Average for arterial and hepatic and portal vein blood.

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Table 5 *Net splanchnic metabolism and liver extraction of nutrients in lactating dairy cows fed a diet without (control) or with supplemental biotin*

	Control	Biotin	s.e.	*P*
Portal drained visceral (mmol/h)				
Blood				
Alanine	90	78	11	0.440
Glutamine	12	−40	51	0.535
Glutamate	11	11	3	0.879
Ammonia	703	945	79	0.080
β-Hydroxybutyrate	265	297	45	0.689
Oxygen	−3672	−3673	303	0.995
Carbon dioxide	1828	1697	533	0.892
Plasma				
Lactate	172	177	18	0.699
Glucose	27	11	18	0.565
Liver (mmol/h)				
Blood				
Alanine	−22	−36	8	0.374
Glutamine	−45	30	55	0.529
Glutamate	39	25	4	0.177
Ammonia	−821	−943	47	0.341
β-Hydroxybutyrate	401	388	147	0.692
Oxygen	−3941	−3842	708	0.873
Carbon dioxide	1449	1567	405	0.642
Plasma				
Lactate	−107	−83	34	0.713
Glucose	694	679	62	0.736
Total splanchnic (mmol/h)				
Blood				
Alanine	65	46	13	0.515
Glutamine	−31	−12	26	0.720
Glutamate	50	36	6	0.211
Ammonia	−114	−4	43	0.345
β-Hydroxybutyrate	668	676	71	0.886
Oxygen	−7572	−7493	613	0.899
Carbon dioxide	3209	3419	261	0.693
Plasma				
Lactate	64	95	30	0.617
Glucose	718	692	51	0.707
Net liver extraction (% of total supply)				
Lactate	10.1	7.9	2.7	0.691
Alanine	3.5	4.6	1.6	0.734
Ammonia	58.6	68.9	5.0	0.369
Net liver removal (% of portal-drained visceral release)				
Lactate	62.0	46.5	18.2	0.676
Alanine	27.4	43.1	15.8	0.631
Ammonia	118.2	99.8	7.0	0.338

Table 6 *Arterial concentration and net flux of blood volatile fatty acids in lactating dairy cows fed a diet without (control) or with supplemental biotin*

	Control	Biotin	s.e.	*P*
Arterial concentration (mmol/l)				
Acetate	2.432	2.591	0.067	0.367
Propionate	0.062	0.06	0.005	0.794
n-Butyrate	0.033	0.03	0.004	0.594
i-Butyrate	0.005	0.006	0.001	0.557
i-Valerate	0.008	0.008	0.001	0.854
n-Valerate	0.002	0.003	0.001	0.317
Portal-drained visceral flux (mmol/h)				
Acetate	3074	3711	468	0.399
Propionate	903	963	118	0.632
n-Butyrate	206	218	29	0.839
i-Butyrate	24	23	3	0.702
i-Valerate	45	51	3	0.444
n-Valerate	37	42	5	0.678
Liver flux, mmol/h				
Acetate	1995	1634	930	0.567
Propionate	−831	−885	124	0.657
n-Butyrate	−144	−160	39	0.737
i-Butyrate	−21	−20	5	0.609
i-Valerate	−39	−47	5	0.194
n-Valerate	−38	−42	5	0.683
Total splanchnic flux (mmol/h)				
Acetate	5062	5180	741	0.983
Propionate	72	77	14	0.836
n-Butyrate	66	52	16	0.506
i-Butyrate	3	3	3	0.933
i-Valerate	7	3	3	0.502
n-Valerate	−1	−1	1	0.665

Discussion

Milk yield

The present study was designed to determine effects of biotin on nutrient absorption and liver metabolism, and not feed intake and milk production. Therefore, DM offered was restricted to just below *ad libitum* and not altered over the course of the study to reduce variation in nutrient absorption and metabolism by splanchnic tissues attributable to DMI. In dairy cows fed ad libitum, supplemental biotin has typically increased milk yield (Bonomi *et al*., 1996; Midla *et al*., 1998; Zimmerly and Weiss, 2001; Bergsten *et al*., 2003; Majee *et al*., 2003) but the effect has not been observed in every study reported (Fitzgerald *et al*., 2000; Rosendo *et al*., 2004). In the present study, the lack of a milk yield response to supplemental biotin may have been due to the restricted DMI of the cows, or the length

Table 7 *Net liver extraction of blood VFA as a percentage of net PDV release or total blood supply in lactating dairy cows fed a diet without (control) and with supplemental biotin*

	Control	Biotin	s.e.	*P*
Net liver removal (% of total blood supply)				
Propionate	77.3	79.1	2.9	0.545
n-Butyrate	48.3	54.2	10.4	0.335
i-Butyrate	57.1	52.2	13.7	0.403
i-Valerate	60.3	66.3	9.0	0.335
n-Valerate	88.4	87.0	1.2	0.359
Net liver removal (% of portal-drained visceral release)				
Propionate	91.4	91.6	2.2	0.950
n-Butyrate	66.9	73.1	10.7	0.484
i-Butyrate	87.7	85.4	14.8	0.775
i-Valerate	85.2	92.4	7.7	0.466
n-Valerate	103.3	101.7	1.5	0.614

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of supplementation (14 days) may have been too short to allow for effects on milk yield. However, in a recent study Ferreira (2006) measured an increase in milk yield within 2 days of the start of biotin supplementation in higher yielding (43 kg/day) cows in early lactation, but no effect of biotin on milk yield in lower yielding (23 kg/day) cows in late lactation.

Glucose metabolism

There was no effect of feeding 20 mg/day supplemental biotin for 2 weeks on net liver release or plasma concentrations of glucose in these cows. In the present study there was no effect of supplemental biotin on milk or milk lactose yield, thus no apparent effect on glucose requirement. It is unlikely that in these cows near or well past peak diet intake that the supply of glucose precursors was limiting. Therefore, it is unlikely that liver glucose synthesis was limiting milk production. A positive effect of biotin on glucose synthesis in the liver in non-ruminants may be a consequence of the repletion of a biotin deficiency limiting the activity of gluconeogenic enzymes, rather than a promotion of carboxylase activity *per se* (Bender, 1999).

Reasons for the increase in jugular vein plasma glucose concentration reported by Bonomi *et al.* (1996) and Rosendo *et al.* (2004) are not certain. An increased supply of oxaloacetate via a stimulation of carboxylase activity in the liver would only be used for glucose synthesis if the additional oxaloacetate was required (Reynolds, 1995). The lack of an effect of supplemental biotin on liver lactate, alanine or β-hydroxybutyrate metabolism suggests there was little change in the utilisation of pyruvate, as the net flux of these metabolites across the bovine liver are sensitive indicators of pyruvate and redox status (Reynolds, 1995).

Nitrogen metabolism

Effects of biotin supplementation on ammonia absorption by the PDV were not expected. The magnitude of the increase was much greater than the amount of nitrogen (N) in biotin fed, and was accompanied by a numerical increase in liver ammonia removal. The increase in ammonia absorption when biotin was fed may be attributable to changes in PDV tissue metabolism, fermentation in the rumen or hindgut, or an increase in the cycling of urea N to the gut lumen (via saliva or direct transfer from blood). Urea transfer to the rumen is influenced by a number of factors including fermentable energy and urease activity. Biotin supplementation may have improved microbial energy supply and growth, but this would be expected to decrease net PDV absorption of ammonia as observed when starch was infused into the rumen or abomasum of lactating dairy cows (Reynolds *et al*., 1998).

Volatile fatty acid metabolism

Previous studies in vitro have demonstrated positive effects of biotin on microbial degradation of cellulose, which would cause a relative increase in VFA production (Milligan *et al*., 1967; Baldwin and Allison, 1983). However, apart from numerical increases in net acetate, i-valerate and total VFA release by the PDV, there was no evidence that feeding biotin altered rumen or post-rumen fermentation. Similarly, there was no effect of feeding biotin on rumen VFA proportions (Zimmerly and Weiss, 2001) or total tract fibre digestion (Majee *et al*., 2003).

Conclusions

Feeding supplemental biotin for 2 weeks to lactating dairy cows had no measurable effect on milk production, net glucose production by the liver, or circulating glucose concentrations in blood plasma. This indicates that biotin status in these cows did not limit liver pyruvate and proprionyl-CoA carboxylase function, or that the supply of glucose precursors was not limiting liver glucose production. Surprisingly, feeding 20 mg biotin per day increased net absorption of ammonia N by the PDV. Reasons for these responses are not certain, but a numerical increase in acetate absorption suggests an alteration in rumen fermentation occurred. The results of the present study do not support the hypothesis that feeding supplemental biotin to lactating dairy cows increases glucose production by the liver.

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