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BRIEF COMMUNICATION: Plasma amino acid profiles of lactating dairy cows fed fodder beet and ryegrass diets

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Introduction

In pastoral systems, N intake by dairy cows generally exceeds requirements (Kolver 2000). Feeds with low N concentration can 'dilute' the amount of N eaten by grazing dairy cattle, and ultimately reduce N pollution of the environment. Use of fodder beet (FB) crop has increased substantially in New Zealand because of its high DM yield potential. Fodder beet also has low N content in the bulb of the plant (0.8 to 1.5% of DM; Heuzé et al. 2015) which is useful for diluting dietary N content. However, these low N concentrations could result in deficiencies of specific amino acids (AA), potentially limiting milk production.

This paper reports the effect on plasma AA concentrations of partial replacement of ryegrass pasture with FB in the diet of lactating dairy cows. This information will provide insights on how low-N diets could impact nutrient supply to dairy cattle.

Materials and methods

All animal manipulations described here were approved by the Ruakura Animal Ethics Committee (Approval 13490).

Dairy cows (n=16) in mid-late lactation (March 2015) were fed diets consisting of 100% ryegrass pasture (RG) or RG with FB. Both the bulbs and tops of FB were offered, at a DM ratio of about 75:25. The RG composition was consistent with expectations for a Waikato summer pasture: 191 g/kg crude protein (CP); 517 g/kg neutral detergent fibre (NDF) and 99 g/kg of ash in the DM. Fodder beet bulbs contained 71 g/kg CP, 107 g/kg NDF and 43 g/kg ash, whilst FB leaves contained 147 g/kg CP, 334 g/kg NDF and 222 g/kg ash.

The cows were adapted to diets that included 0, 20, 40 and 60% FB on a DM basis (n=4 for each diet) for 14 days before being housed in metabolism stalls as part of a larger study to measure nitrogen balance and digestibility over a six-day intensive measurement period. During adaptation, two of the cows in the 60% FB group developed acidosis and had to be removed from the experiment. The two remaining cows in that group had the allocation of FB reduced to about 50% of the DM offered. For the purposes of analysis, the final data structure based on actual intakes consists of four cows fed RG ("RG" group), four cows fed 23% FB ("LoFB" group) and six cows fed 45% FB ("HiFB" group). The cows were fed at about 0730 and 1530 h daily and had free access to water at all times.

Blood samples were collected via coccygeal venepuncture from all cows on day 6 of the nitrogen-balance period, at 0700, 1100 and 1400 h. Samples were collected into evacuated tubes (Vacutainer, Beckton-Dickinson) and plasma harvested after centrifugation at 4000 *g* for 20 minutes. Samples were frozen at -20°C until analysed for concentrations of AA (µmol/L) as described by van der Linden et al. (2013). Concentrations of individual AA were expressed relative to the total AA assayed.

Data was analysed using a mixed model that included the fixed effect of dietary treatment, sampling time and their interaction, and the random effect of cow. The variance-covariance matrix type was modelled as unstructured. Contrasts were generated to assess differences between diets with and without FB, and also between the two levels of inclusion of FB in the diet. Significant differences are declared when P <0.05. All analyses described were conducted using the Mixed procedure in SAS v 9.3 (SAS Institute Inc. Cary, NC, USA).

Results and discussion

Total AA concentrations were 1859, 1867 and 2024 µmol/L for RG, LoFB and HiFB respectively (P=0.36). Diets with FB reduced (P<0.05) the proportion of arginine, citrulline and ornithine in total AA. Cows in the HiFB group had significantly greater proportion of Gly in the total AA, as indicated by the significant contrast between FB groups. Inclusion of FB in the diet tended (P<0.10) to reduce asparagine and increase aspartate proportions in total AA, whilst HiFB tended to reduce isoleucine (Table 1).

The concentration of total AA in the venous plasma measured in this study is comparable to previous reports for ryegrass-fed dairy cows fed similar amounts of N, albeit at a different stage of lactation (Pacheco-Rios 2000). While AA concentrations have been extensively described for lactating dairy cows fed total-mixed rations (Pacheco-Rios, 2000), there is a less information on the circulating AA profile of dairy cows fed fresh temperate pasture.

Reduced N intake by replacing RG pasture with FB has the potential to reduce the portal absorption of ammonia (NH_3), supported by reports by Firkins and Reynolds (2005) who described a positive correlation between N intake and NH_3 absorption. The increase in dietary fermentable

Table 1 Effect of replacing ryegrass (RG) with low (LoFB: 23% of dry matter) or high (HiFB: 45% of dry matter) proportions of fodder beet on plasma concentrations of amino acids (AA) in dairy cows in mid-late lactation. Units are mol/100 mol total AA assayed[1]

Amino acid	RG	LoFB	HiFB	SED[2]	P Diet effect	P Contrast RG ≠ FB	P Contrast LoFB ≠ HiFB
Non-essential AA (NEAA)							
Alanine	11.1	11.1	11.0	0.82	0.99	0.97	0.92
Asparagine	1.2	1.1	1.0	0.10	0.08	0.08	0.17
Aspartate	0.2	0.2	0.3	0.03	0.09	0.12	0.14
Citrulline	3.4	2.8	2.2	0.42	0.04	0.03	0.17
Glutamine	14.2	14.9	12.0	1.07	0.03	0.42	0.02
Glutamate	1.7	2.0	2.0	0.24	0.48	0.25	0.71
Glycine	12.0	11.9	17.1	1.83	0.02	0.15	0.01
Ornithine	3.7	3.4	3.1	0.28	0.10	0.07	0.32
Proline	2.6	2.9	2.7	0.20	0.41	0.27	0.38
Serine	4.5	5.0	6.0	0.68	0.12	0.13	0.18
Tyrosine	2.9	3.0	2.9	0.16	0.73	0.95	0.43
Essential AA (EAA)							
Arginine	4.6	4.2	3.7	0.23	< 0.01	< 0.01	0.02
Histidine	2.7	2.6	2.5	0.24	0.56	0.35	0.71
Isoleucine	5.0	5.3	4.5	0.32	0.06	0.65	0.02
Leucine	5.8	5.3	5.1	0.53	0.34	0.19	0.63
Lysine	4.1	4.1	4.3	0.32	0.85	0.69	0.72
Methionine	1.1	1.3	1.1	0.09	0.34	0.31	0.25
Phenylalanine	2.7	2.5	2.6	0.26	0.84	0.57	0.82
Threonine	4.3	4.6	4.9	0.65	0.60	0.41	0.66
Valine	12.0	11.7	11.3	0.87	0.67	0.51	0.61
BCAA3	22.8	22.3	20.8	1.58	0.37	0.39	0.38
EAA4	42.3	41.6	39.8	2.04	0.45	0.67	0.61
NEAA5	50.5	52.2	54.8	2.30	0.18	0.17	0.25

[1]includes both proteinogenic and non-proteinogenic AA.
[2]SED = Standard error of difference (pooled).
[3]BCAA= branched-chain AA (Ile, Leu, Val).
[4]EAA = proteinogenic essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val). Arg is considered conditionally essential.
[5]NEAA=proteinogenic non-essential AA (Ala, Asn, Asp, Gln, Glu, Gly, Pro, Ser, Tyr).

carbohydrates in FB may further reduce the amount of NH_3 absorbed across the rumen wall, because of the potential for increased N capture in microbial protein (Lapierre & Lobley 2001). Thus, it is reasonable to expect a reduced need for hepatic ureagenesis for NH_3 disposal (Lobley & Milano, 1997) as the amount of FB in the diet is increased. The lower concentrations of arginine and citrulline measured in the plasma of cows in the LoFB and HiFB treatments are compatible with reduced ureagenesis via the ornithine cycle, since these AA are synthesised as intermediaries of this cycle. The long-term impact of these changes requires further examination, because recent reviews have proposed that some of the 'non-essential' AA affected by feeding of FB in our study (e.g., arginine) play a major role as regulators of metabolism in humans and animals (Hou et al. 2015). For example, intravenous supplementation of arginine in pregnant sheep has been associated with increases in foetal brown-fat reserves and increased birth weight of ewe lambs (McCoard et al. 2013), mediated in part through changes in placental development and function (van der Linden et al. 2015). These results suggest that future research needs to assess the effects of feeding FB to pregnant cows on the development and survival of their calves.

The interest in reducing N intake as a means of reducing N excretion to the environment needs to take into consideration the effect on the overall N economy of the ruminant. Concentrations of free circulating AA are an outcome of all metabolic processes involving N, namely inputs such as absorption in the gut, endogenous synthesis and outputs such as oxidation and protein synthesis (Lobley 1993). Based on this premise, increases in the concentration of free AA may result from increase in inputs or reduction of outputs and vice versa. While we cannot identify the underlying causes of changes in AA concentrations observed in this study, it provides evidence that feeding FB results in significant changes in the N economy of the lactating dairy cow. The causes of the changes in circulating AA observed in this short-term experiment and their long-term consequences need to be understood if FB is to be recommended as an alternative feed for dairy herds to help mitigate losses of N to the environment.

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