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Source of excess nitrogen affects nutrient partitioning in lactating ewes

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ABSTRACT

This experiment tested the hypothesis that the form of surplus nitrogen (ammonia or amino acids) affects nutrient partitioning in lactating ruminants.

Nine, abomasally-cannulated, 4 year old Coopworth ewes (66.4 ± 5.9 kg liveweight), suckling twin lambs were offered a basal diet designed to meet their requirements for ME and MP in 12 equal portions at 2 h intervals for two periods of five weeks. During both periods, ewes were allocated to one of three groups; continuous abomasal infusion of 2 l water/day (control), infusion of 150 g sodium caseinate (20g N) dissolved in 2 l water or 85 g ammonium bicarbonate (15 g N) in 2 l water. Liveweight change of ewes and lambs, ewe milk production and composition and nitrogen balances were measured.

Treatments had no significant effect on ewe dry matter intake, *in vivo* dry matter digestibility or ewe liveweight change. Relative to control, casein infusion increased milk production (33%) and lamb liveweight gain (by 26% from 132 to 166 g/day) but had no effect on milk composition. In contrast ammonia infusion decreased milk production (15%), reduced milk protein concentration (from 63 to 56 g/kg), increased milk fat concentration (from 66 to 75 g/kg) and reduced lamb liveweight gain (from 132 to 66 g/day). The N apparently utilised (dietary N + infusate N – faecal N – urine N) was apportioned (milk N : body N) in ratios of 0.86 : 0.14, 1.0 : 0 and 0.52 : 0.48 for control, casein and ammonia infusions respectively.

It is concluded that excess N in the form of ammonia diverted protein from milk production to body retention, thus the form of surplus N may affect nutrient partitioning in lactating ewes.

Keywords: protein; nitrogen; ewes; lactation; nutrient partitioning.

INTRODUCTION

Lactating dairy cows in New Zealand produce fewer milk solids, lose less liveweight in early lactation and have higher reproductive success than their overseas counterparts for which concentrate rations constitute a higher, and pasture a lower proportion of daily intake.

A high crude protein (200-300g crude protein/kg dry matter, CP/kgDM; Moller *et al*., 1993, Rusdi and van Houtert, 1997) is a feature of spring pasture. The high and rapid rumen degradability (Beever *et al.,* 1986) of this protein leads to rates of rumen ammonia production greater than the potential for microbial protein synthesis (Cruickshank *et al.,* 1985). The resulting increased rumen ammonia concentration induces transfer of ammonia to the liver where, at a cost of 15-50 kJ ME/g N, it is converted to urea and excreted in urine (Martin and Blaxter, 1965). The second "cost" of urea synthesis is for amino acids which are the source of about 50% of the N in urea. Recently, Lobley and Milano (1997) have suggested fewer amino acids per mol of ammonia are needed for urea synthesis when liver NH_3 concentration is high than when an excess of amino acids exists.

The energy and amino acid requirements for ureagenesis divert nutrients away from milk protein synthesis or draw them from body reserves. High concentrations of blood urea nitrogen have been associated with reductions in milk production in some but not all cases (Westwood *et al.,* 1998). On the other hand, for diets formulated with high crude protein content and of lower rumen degradability but high digestibility, surplus N is more likely to be in the form of amino acids. Deamination of surplus amino acids leaves carbon skeletons as a potential energy source for ureagenesis but involves a higher obligatory requirement for amino acids as a precursor of urea than when ammonia is the source of excess nitrogen (Lobley and Milano, 1997). This may represent a bigger challenge to body protein status than milk synthesis. The cost of, and challenge to, milk production and body energy and protein reserves may be different if surplus N is in the form of ammonia rather than amino acids.

An experiment was designed to test the hypothesis that the source of excess N (NH_3 or amino acids) affects nutrient partitioning between milk synthesis and the body of lactating ruminants using lactating ewes suckling twin lambs as the experimental model.

METHODS

Nine lactating, twin-suckling, 4-year-old Coopworth ewes (66.4 ± 5.9 kg liveweight), were individually penned in metabolism crates and allocated to one of three abomasal (via abomasal cannula) infusions in an experiment of two periods (I and II) each of 5 weeks. Daily infusions were: 150 g sodium caseinate (20 g N) in 2 l water; 85 g ammonium bicarbonate (15 g N) in 2 l water; or 2 l water as the control. Isonitrogenous treatments were not specifically used because the fate of the infused N was unpredictable. One ewe in each treatment group remained on that treatment in Period I and II, and the remaining ewes changed groups between periods in a partial crossover design. On day 1 and 2 of each period, infusions were made at 0.33 and 0.66 concentration respectively with full concentration from day 3. Actual volume infused daily was recorded.

All ewes were offered, in 12 equal portions per day, a basal pelleted diet supplying 930 g organic matter, 145 g crude protein and 11.2 MJME/kg DM at a level designed to provide 0.8 x metabolisable energy and adequate metabolisable protein for lactating ewes.

The lambs (approximately 1 week old at the start of the experiment) were housed in metabolism crates adjacent to their dams and suckled 3 x per day for 10 minutes each time. In Period II, lambs were offered up to a maximum of 200 g/day fresh weight of a diet containing 0.59, 0.40 and 0.01 (DM basis) proportions of barley, lucerne chaff and sodium bicarbonate respectively.

Ewes and lambs were weighed weekly and rate of liveweight change was calculated from the regression of liveweight against time for each individual.

Milk yield was determined weekly. Ewes were injected with 2 ml (i.v.) of oxytocin immediately before suckling for 5-10 min. Any residual milk was withdrawn by hand. After six hours and a further 2 ml oxytocin, ewes were hand-milked and the volume recorded. Milk samples were retained, stored at –20°C for subsequent compositional (fat, protein and lactose) analysis by standard milk laboratory methods (Meadow Fresh Foods, Canterbury Ltd). The gross energy content of milk was calculated from its composition (Sebek and Everts, 1993).

Nitrogen balance of ewes and *in vivo* digestibility of the diet were measured in week 3 of each period. Collection of faeces and urine was made over 5 days. Urine was collected in 400-500 ml (99.8% pure) acetic acid. While lambs had access to ewes (for suckling), contamination of ewe faeces and urine by that from lambs was prevented by fitting lambs with disposable diapers (Treasures, Auckland). A proportion (0.1) of the daily output of faeces and urine was retained for each ewe and stored at –20°C for later analysis of dry matter (oven drying at 95°C for 48 h), organic matter (8 h at 550°C in a muffle furnace) and nitrogen content (Kjeldahl method using Tecator Kjeltec 1035 Analyser). Metabolisable energy concentration of the diet was estimated from the equations of Alderman (1985).

The effects of infusion treatment (3) and previous treatment were used as fixed effects in an analysis of variance using REML procedures (Genstat 5, Release 4.1, Lawes Agricultural Trust, 1997). Previous treatment was coded 0 for all ewes for Period I and 1, 2, 3 for Period II respectively for control, casein and ammonia treatments.

The protocol for the experiment was approved by the Lincoln University Animal Ethics Committee.

RESULTS

All ewes completed both periods and results for the mean dry matter, digestible organic matter and N intake are shown in Table 1. Infusate (145 ± 4.40g Na caseinate, 85.0 ± 1.8g NH_4HCO3) contributed only 6% and 4% respectively to total dry matter intake of the casein and ammonia treatments. Infusion treatment did not significantly (P>0.05) affect total feed intake although there was a trend to a slight reduction (8%) in mean dry matter intake (g/kg$W^{0.75}$/day) with the ammonia infusion.

TABLE 1: Total dry matter intake, apparent organic matter digestibility, digestible organic matter intake and nitrogen balance of ewes receiving abomasal infusions of water, casein or ammonia.

	Infusion			
	Control	Casein	Ammonia	S.E.D.
Total dry matter intake(g/kg$W^{0.75}$/d)	103	104	95.6	5.67
Apparent organic matter digestibility (g/kg)	757^{b}	835^{a}	769^{b}	19.1
Digestible organic matter intake(g/kg$W^{0.75}$)	74.6	70.2	69.2	7.62
N balance				
N intake (g/kg$W^{0.75}$/d)				
diet	2.50	2.10	2.27	0.24
infusion	0	0.94	0.62	NA
total	2.50	3.04	2.89	0.25
N loss (g/kg$W^{0.75}$/d)				
faeces	0.93	0.87	0.70	0.12
urine	0.83^{B}	1.26^{A}	1.24^{A}	0.11
N absorbed (g/kg$W^{0.75}$/d) (intake – faeces)	1.58^{b}	2.16^{a}	2.18^{a}	0.18
N apparently utilised (g/kg$W^{0.75}$/d) (absorbed – urine)	0.75	0.90	0.94	0.15
N in milk (g/kg$W^{0.75}$/d)	0.58^{a}	0.93^{b}	0.48^{a}	0.22
N retention (g/kg$W^{0.75}$/d)	0.09^{b}	0.00^{b}	0.44^{a}	0.12

Values, in a row, with different superscripts differ significantly (lower case, P<0.05, upper case P<0.01).
N.A. = not available
S.E.D.=standard error of a difference

Apparent organic matter digestibility was higher (P<0.05) in casein infused ewes but it did not result in greater DOMI for this group relative to control or ammonia treatments.

The independent removal of pre-treatment effects in the statistical model resulted in "adjusted means". Consequently, the use of adjusted means for dry matter intake and OMD in Table 1, to calculate mean DOMI gives a different value to the adjusted mean estimated by the model from the individual values of DMI and OMD for each sheep.

Dietary N intake of ewes was similar for all treatments (P>0.05). Casein and ammonia infusion increased total N intake by 22 and 16% respectively over the control treatment although the difference in total N intake was not statistically significant (P>0.05). The slightly higher N intake and lower faecal N losses in the casein and ammonia group were reflected in significantly more N absorbed with the casein (+37%) and ammonia (+38%) infusions compared with controls.

Urinary N losses were significantly increased (P<0.01) by N infusions. Urinary N losses equalled 46 and 66% of the N infused for the casein and ammonia infusions respectively. As a consequence of the high urinary N loss, the N available for milk production or body tissue gain increased by only 20 and 25% for casein and ammonia, respectively, above that of the control treatment (statistically non-significant, P>0.05). With the control treatment, N apparently utilised was apportioned 0.86 to milk and 0.14 to N retention. This ratio increased to 1.0:0 for casein infusion and decreased to 0.52:0.48 with ammonia infusion.

Type of infusion had no significant effect on ewe liveweight gain or milk production although relative to the control group, milk production was increased 33% by casein infusion and decreased 15% by ammonia infusion (Table 2).

TABLE 2: Liveweight change, milk production and milk composition of ewes receiving abomasal infusions of water, casein or ammonia and the liveweight change of their suckling lambs.

	Infusion			
	Control	Casein	Ammonia	S.E.D.
Ewes				
Liveweight gain (g/day)	77.6	104	97.5	46.6
Milk yield (kg/d)	1.38	1.84	1.17	0.31
Milk composition (g/kg)				
Protein	62.7^{a}	61.0^{a}	56.3^{b}	2.12
Fat	65.7^{AB}	59.3^{B}	75.1^{A}	5.30
Protein yield (g/d)	87.6^{ab}	110^{a}	65.3^{b}	17.9
Fat yield (g/d)	97.5	115	80.0	21.3
Fat:protein (g/d)	1.05^{ab}	0.95^{b}	1.36^{a}	0.11
Lambs				
Liveweight gain (g/day)	132^{a}	166^{a}	65.9^{b}	21.2

Values, in a row, with different superscripts differ significantly (lower case, $P<0.05$, upper case $P<0.01$).

Source of the additional N had a significant effect on milk composition. Ammonia infusion significantly depressed milk protein concentration from 62.7 (control) to 56.3 g/kg and increased milk fat concentration compared with casein infusion (75.1 vs 59.3 g fat/kg milk). These treatment effects on milk composition translated to a significantly higher (+ 69%) milk protein yield (g per day) in casein- than ammonia-supplemented ewes.

Liveweight gain of lambs suckling ammonia-infused ewes was significantly lower than for lambs from the other two treatment groups.

DISCUSSION

The results of this study strongly suggest that the source of excess nitrogen affects nutrient partitioning in lactating ewes. Abomasal infusions of casein (an amino acid source) and ammonium bicarbonate (a source of ammonia) exceeding the N requirements of ewes (over 60% of the infused N was excreted in urine), changed the proportion of absorbed N which was secreted as milk protein to that retained in the body. The additional amino acids (casein) tended to increase milk and milk protein yield at the expense of N retention in the body. Excess ammonia on the other hand increased the fat:protein ratio in ewes' milk, decreased the proportion of absorbed nitrogen partitioned to lactation and enhanced body N retention.

Previous detailed, short-term experiments comparing the effects of amino acid and ammonia overload on ureagenesis (Lobley *et al*; 1995; Luo *et al.,* 1995) have not allowed for measurements of gross changes in animal performance. However, other work independently comparing casein or urea supplementation does support many of the effects measured in this work. For example, of 10 separate reports (reviewed by Malik, 1998) of the effects of post-ruminal casein infusion, 8 recorded increases in milk yield (from 5.6 to 32.3%; 33% in this study); 6 reported no significant effect on milk fat content as in this study; 7 reported significant increases in protein yield from between 9 and 41% which is similar to the 26% (but non-significant) response in milk protein yield to casein infusion in this study.

Inclusion of ammonia or urea in diets of lactating cows (Shukla and Talpada, 1974) and ewes (Tanev, 1971; Farid *et al.,* 1984) reduced milk yield (as in this study), but other studies have shown no such effect (Lines and Weiss, 1996, Moorby and Theobold, 1997) although there is more agreement that treatments increase milk fat concentration.

The changes in milk yield and composition induced by casein and ammonia infusion observed in the present study are thus consistent with the literature.

Partitioning of absorbed N to lactation and body retention from the data gathered in this study relies heavily on estimates of ewe milk yield and the residual error of N balance which is factored into body N by virtue of its calculation by difference.

Lamb liveweight gain (an indirect measure of milk yield) showed significant treatment effects and ranked treatment groups (casein > control > ammonia). Treatment groups ranked in the same order for milk production but the variability between ewes in milk production was large (Table 2) and treatment effects were not significant. Evidence that there was no systematic pre-experimental differences in mean milk production between experimental groups would have had value for interpreting treatment effects on milk yield.

As a direct result of the reduction in N apportioned to milk in ammonia infused ewes, apparent body N retention was much greater in ammonia treated ewes than for control or casein infused ewes, but the difference in ewe liveweight gain between the three groups was not significant. A more direct approach to measuring body composition is required to confirm the treatment effects on partitioning nutrients away from (casein), or to (ammonia) body retention.

There are some anomalies in these data. Unless there had been significant recycling of ammonia as urea to the rumen and the incorporation of this into microbial protein, (which is unlikely given the crude protein content (145 g CP/kg DM) of the diet (Poppi and McLennan, 1995)), it is difficult to accept that the N apparently utilised for synthesis should have been as great (0.94 g N/kg$W^{0.75}$/day) with ammonia infusion as for casein infusion (0.90 g). A proportion of the casein (38%) appears to have satisfied a metabolisable protein deficiency in the control diet group and increased milk yield . Ammonia cannot fulfill this role and would have been expected to result in lower apparently utilised N than for the casein treatment.

Again, subject to the above proviso about urea recycling, it is surprising that the N apparently utilised for milk and body retention should be as high for the ammonia group (0.94 g/kg$W^{0.75}$/day) as for the control group (0.75 g/kg$W^{0.75}$/d). It might be anticipated that at most, N diverted from milk production with ammonia treatment would be retained, with any additional absorbed N excreted in urine. Milk N yield was decreased by 0.10 g N/kg$W^{0.75}$/day but N retention increased by 0.39 g N/day due to the increase in N apparently utilised. The difference (0.28 g N/kg$W^{0.75}$/day) is equivalent to the difference between the observed urine losses of ammonia infused ewes (1.24 g N/day) and the

estimated urine loss (1.45 g N/day). Although urine was collected under acidic conditions it is possible than N losses may have been underestimated and thus body N retention over-estimated in ammonia treated ewes.

We can only speculate on the underlying cause of the differing effect of excess N supplied as casein or ammonia infusions. The nutrient requirements for lactation are generally accepted to take preference over those for maternal body tissues over the first half of lactation. However, excess nitrogen as ammonia absorbed from the rumen (or abomasum) present a toxic risk and the clearance of ammonia may take priority over lactation, thus diverting metabolisable energy and amino acids away from milk synthesis but sparing body tissues. On the other hand, excess absorbed N as less toxic amino acids may not divert nutrients from lactation but require an input of energy and amino acids from body tissue to fuel the urea cycle.

This study has shown that abomasal infusions of excess nitrogen as casein or ammonia have opposite effects on milk yield, milk composition and apparent N retention in body tissues of lactating ewes. These differences need to be confirmed but could have significant implications for the nutrition of lactating ruminants and provide opportunities for improving milk production and/or cow reproduction.

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