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in

Lindberg J.E. (ed.), Gonda H.L. (ed.), Ledin I. (ed.).
Recent advances in small ruminant nutrition

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 34

1997
pages 149-153

Contribution of a short term *in vitro* method to formulate dairy goat diets

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SUMMARY - The aim of this study was to relate data obtained in lactating dairy goats to those obtained using a short term *in vitro* method. Two experiments were conducted: in the first, four groups of 8 goats received one diet containing either lucerne hay or maize silage as a forage and a concentrate either rich in starch or rich in cellwalls. In the second, ammonia and volatile fatty acid production was measured after 6 hours of *in vitro* incubation of each diet ingredient. *In vitro* results were used to calculate by additivity theoretical ammonia concentrations and intake and available carbon fluxes for each goat. Uraemia was correlated with the calculated ammonia concentration, and urinary nitrogen excretion was more closely related to calculated ammonia intake than to uraemia. The carbon flux available for *de novo* milk fatty acid synthesis estimated from *in vitro* C_2 and C_4 VFA production was highly correlated to *in vivo* C flux incorporated into short and medium chain milk fatty acids. Thus, *de novo* milk fatty acid synthesis and nitrogen excretion might be predicted from *in vitro* data.

Key words: Dairy goats, carbon flux, ammonia, milk fatty acid synthesis, nitrogen secretion, methods to formulate diets.

RESUME - *"Contribution de la méthode* in vitro *à court terme pour formuler les régimes de chèvres laitières". La finalité de cette étude a été de mettre en rapport les données obtenues chez des chèvres laitières en lactation avec celles que l'on a obtenues en utilisant une méthode* in vitro *à court terme. Deux expériences ont été menées : dans la première, quatre groupes de huit chèvres ont reçu un régime contenant soit du foin de luzerne ou de l'ensilage de maïs comme fourrage, et un concentré soit riche en amidon soit riche en parois végétales. Dans la deuxième expérience, la production d'ammoniac et d'acides gras volatils a été mesurée après 6 heures d'incubation* in vitro *pour chaque ingrédient du régime. Les résultats* in vitro *ont été utilisés pour calculer par additivité les concentrations théoriques en ammoniac ainsi que l'ingestion et les flux du carbone disponible pour chaque chèvre. L'urémie a été corrélée avec la concentration calculée en ammoniac, et l'excrétion d'azote urinaire a été mise en relation plus étroite avec l'ingestion calculée d'ammoniac qu'avec l'urémie. Le flux du carbone disponible pour la synthèse* de novo *d'acides gras du lait, estimée à partir de la production* in vitro *d'acides gras volatils de C_2 et C_4, é tait fortement corrélée au flux* in vivo *de C incorporé dans les acides gras du lait de chaîne courte et moyenne. Ainsi, la synthèse* de novo *d'acides gras du lait et l'excrétion d'azote pourraient être prédits à partir de données* in vitro*.*

Mots-clés : *Chèvres laitières, flux de carbone, ammoniac, synthèse des acides gras du lait, sécrétion d'azote, méthodes pour la formulation de régimes.*

Introduction

It is well-known that diets of different composition influence milk production and nitrogen excretion from dairy ruminants, because they do not ferment at the same rates and extents and do not produce the same quantities of end products, such as volatile fatty acids or ammonia. It is very difficult to measure their *in vivo* production. Therefore, this study was conducted to test the relationships between volatile fatty acids and ammonia production in a short term *in vitro* method and *in vivo* data obtained in lactating dairy goats.

Material and methods

The *in vitro* method used was derived from that proposed by Vérité and Demarquilly (1978) to estimate protein fermentability of feeds. 15 g of the test substrate (ingredient or forage) was incubated in an Erlenmeyer flask with 150 g of solid rumen contents, 150 ml of filtrated rumen juice and 300 ml of artificial saliva, the composition of which was given by Durand *et al.* (1988). The donor cows were fed with hay and concentrate as for the *in sacco* method described by Michalet-Doreau *et al.* (1987). After 6 hours of incubation, the Erlenmeyer was emptied, and the fluid was filtrated on a nylon bag cloth. The ammonia content and volatile fatty acid concentrations and composition were measured in each filtrate.

The *in vivo* data were obtained from 32 dairy goats. Two compound feeds given separately with two forages were compared: lucerne hay (L) and maize silage (M) according to a 2 x 2 factorial design with 8 goats in each of the 4 groups. The concentrates had the same nutritive value, but one of them was rich in cellwalls (C), and the other one rich in starch (S). Their composition is given in Table 1. The study began in the 12^{th} week after parturition and lasted 12 weeks. Goats were fed with 400 g concentrate/kg of milk above maintenance for hay diets and with 400 g concentrate/kg of milk above maintenance and a production of 800 g of milk for silage diets. Forages were given *ad libitum*. Forage and concentrate offered and refusals were individually weighed. Blood samples were taken from the jugular vein before the morning meal to measure plasma uraemia. Milk yield, milk fat percentage and milk fatty acid composition were measured in each of the 32 goats. The measurements were performed during the 4^{th}, 8^{th} and 12^{th} weeks of the experiment, and the mean value of these three measures was taken for each goat. Moreover, 3 goats from each group were placed in metabolism crates where digestibility measurements were performed during the three experimental weeks, and urinary nitrogen excretion measured.

Table 1. Composition of compound feeds

% of ingredients	Concentrate rich in starch	Concentrate rich in cellwalls
Barley	30	
Wheat	20	
Wheat bran	15	
Oats	10	
Sugar beet pulp		30
Soyabean hulls		20
Citrus pulp		10
Lupin		10
Soyabean meal	10	10
Peanut meal	5	
Maizegerm meal		10
Molasses	6	6
Mineral and vitamin mixture	4	4

Results

Assuming that the additive method is valid, it is possible to calculate for each compound feed its theoretical *in vitro* production of ammonia and volatile fatty acids after 6 hours of incubation. Since the respective percentages of forage and concentrate ingested have been measured in each goat, it was also possible to calculate theoretical *in vitro* concentrations of fermentation end-products for each diet. Estimations of ammonia intakes (expressed per kg of metabolic weight, MW or $W^{0.75}$) were calculated by multiplying the *in vitro* ammonia concentrations by individual dry matter intakes.

Theoretical *in vitro* ammonia concentrations differed significantly between groups, and were higher with lucerne hay than with maize silage diets. Within each forage group, theoretical *in vitro* ammonia concentrations were also higher with the concentrate rich in cellwalls than with the concentrate rich in starch (Table 2). Plasma uraemia of goats fed with maize silage (MS and MC) was lower than that of

those receiving lucerne hay (LS and LC), but within a forage group there was no significant difference between C and S concentrates (Table 2) and the tendency was the opposite to that from theoretical *in vitro* ammonia concentrations. Moreover, for the 32 goats, there was a significant correlation between uraemia and theoretical *in vitro* ammonia concentrations:

Plasma uraemia (g/l) = 7.50 x Theoretical ammonia concentration (g NH_3/l)

(r = 0.74, n = 32, RSD = 0.101)

Table 2. Influence of diets on theoretical ammonia concentrations and plasma uraemia (32 goats)

	Diets				Significance
	MS	MC	LS	LC	
Theoretical ammonia concentration (g/l)	0.0671^a	0.0756^b	0.0980^c	0.1063^d	P<0.001
Plasma uraemia (g/l)	0.567^a	0.524^a	0.775^b	0.749^b	P<0.001

M: maize silage; S: starch; C: cellwalls; L: lucerne hay
a,b,c,d: Means not bearing the same superscript letters within rows are significantly different (P<0.05)

For the 12 goats in the digestibility experiment (Table 3), urinary nitrogen excretion was more closely related to theoretical ammonia intake (r = 0.86, n = 12) than to theoretical ammonia concentrations (r = 0.82), and poorly related to uraemia (r = 0.53).

Urinary nitrogen excretion (g/kg MW) = 3.85 x Theoretical ammonia intake (g NH_3/kg MW)

(r = 0.86, n = 12, RSD = 0.199)

Urinary nitrogen excretion (g/kg MW) = 2.00 x Uraemia (g/l)

(r = 0.53, n = 12, RSD = 0.346)

Table 3. Influence of diets on theoretical ammonia and nitrogen metabolism (12 goats)

	Diets				Significance
	MS	MC	LS	LC	
Ammonia concentration (g/l)	0.0700^a	0.0763^a	0.0997^b	0.1075^b	P<0.001
Ammonia intake (g/kg MW)	0.255^a	0.259^a	0.382^a	0.451^a	P<0.057
Uraemia (g/l)	0.607^{ab}	0.472^b	0.801^a	0.718^{ab}	P<0.046
Urinary nitrogen excretion (g/kg MW)	1.07^a	0.94^a	1.59^b	1.72^b	P<0.002

M: maize silage; S: starch; C: cellwalls; L: lucerne hay
a,b: Means not bearing the same superscript letters within rows are significantly different (P<0.05)

Assuming that the additive method is also valid for C fluxes, it is possible to calculate theoretical C fluxes, after 6 hours of incubation, available for *de novo* milk synthesis from VFA by multiplying dry matter intake (expressed per kg of MW) by *in vitro* C_2 and C_4 VFA production per g of dry matter. The expression in moles of C implies that a mole of C_4 is twice as efficient as a mole of C_2. With the knowledge of the milk yield, the milk fat percentage and the percentage of the different milk fat fatty acids, it is possible to calculate the output of short and medium chain fatty acids (C_4 to C_{14}) in the milk, as well as C_{16} expressed in g/kg MW. Mean values are given in Table 4 for each group.

Mean C fluxes were higher with hay (LS and LC) than with silage diets (MS and MC), but variations were higher for hay than for silage diets. *De novo* synthesis of short and medium chain fatty acids (C_4 to C_{14}) was highly correlated to theoretical C input from VFA (r = 0.69). Assuming that about half of C_{16} is also *de novo* synthesized, the total theoretical output of fatty acids was highly correlated to the theoretical C input which was calculated from VFA (r = 0.72).

Table 4. Influence of diets on theoretical C intake and short and medium chain milk fatty acid output (32 goats)

	Diets				Significance
	MS	MC	LS	LC	
C available from VFA (moles C/kg MW)	0.659[a]	0.660[a]	0.805[b]	0.809[b]	P<0.011
C_4 to C_{14} milk output (g/kg MW)	1.061[a]	1.095[a]	1.178[a]	1.223[a]	P<0.771
C_{16} milk output (g/kg MW)	1.301[a]	1.294[a]	1.538[a]	1.577[a]	P<0.384

M: maize silage; S: starch; C: cellwalls; L: lucerne hay
a,b: Means not bearing the same superscript letters within rows are significantly different (P<0.05)

Discussion

It is well known that uraemia is highly correlated to rumen ammonia concentrations, and thus crude protein intake and fermentescibility (Preston *et al.,* 1965). The *in vitro* method tested in this study seems to be of interest to mimic rumen fermentation, since *in vitro* ammonia concentrations are positively correlated to uraemia.

Urinary nitrogen losses are highly correlated to *in vitro* theoretical intake, which may be of practical use if this relationship is confirmed using more data involving more diets. It seems logical that losses are more closely correlated to ammonia intake, than to ammonia concentrations, since the quantity of ingested feeds has an influence on excretion (McIntyre, 1970). The poorer relationship between uraemia and urinary nitrogen losses may be explained by the fact that they do not have the same biological significance, even if they could be correlated (Giger *et al.,* 1986). Uraemia is a balance between the rate of urea entry mainly related to the ammonia level in the rumen, and the rate of urea elimination through the kidneys which depends upon urea entry (Harmeyer and Martens, 1980).

Data about C fluxes are of particular interest, since the volatile fatty acid profile and production might explain milk fatty acid synthesis. If the relationship is confirmed with more diets, this may be of particular interest for the conception of new feeding systems with the possibility of discriminating between volatile fatty acids available for milk production from those which are more useful in fattening.

Conclusion

This short-term *in vitro* method could be of great interest for dairy husbandry since theoretical *in vitro* ammonia concentrations may be a good predictor of uraemia, since calculated ammonia intake may predict urinary nitrogen excretion, and since *de novo* milk synthesis may be predicted from the *in vitro* fatty acid profile and production. All these aspects are very important in ruminant feeding and even if *in vivo* absorption or transit time cannot be directly taken into account in this short-term *in vitro* method, they could be included in a model containing *in vitro* parameters.

The relationships between *in vitro* and *in vivo* data which were studied in this paper highlight the usefulness of some *in vitro* methods to predict *in vivo* data.

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