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Saltbush (*Atriplex nummularia* L.) reduces efficiency of rumen fermentation in sheep

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Abstract. In a preliminary experiment, *in vitro* methane production in rumen fluid from sheep fed old man saltbush (*Atriplex nummularia*) was approximately four times higher than methane production in rumen fluid from sheep fed straw or a mixed oaten-hay and lupin ration. To understand this result further we tested the hypotheses that *in vivo* methane production would be higher in sheep fed saltbush compared to sheep fed a traditional forage, and that the increase in methane production would be due to the high level of salt in saltbush. Forty merino wethers (average weight 43 kg) with no previous experience on salty diets were allocated to one of four treatment diets; control pellet of wheaten chaff, barley, oats and lupins (NS), control pellet + 10% salt (MS), control pellet + 20% salt (HS), or saltbush leaves containing approximately 16% salt (SB). The salt in all diets was approximately 2NaCl:1KCl, and sheep had free access to fresh drinking water at all times. Measurements were taken after sheep had been fed the diets for a minimum of two weeks, and included methane production over a 22-hour period, the number of methane-producing archaea in the rumen, and volatile fatty acid production. Sheep fed saltbush produced more methane (l/22h/kg DOMI), had higher numbers of methane-producing archaea and a higher ratio of acetate to propionate in the rumen. There was no difference in these measurements between the NS, MS and HS treatments. We conclude that the reduction in efficiency of rumen fermentation is not related to the salt content of the forage, but may explain why animal production from saltbush pastures is poor.

Keywords. Saltbush – Salt – Methane – Rumen efficiency.

L'*Atriplex nummularia* réduit l'efficacité de la fermentation dans le rumen du mouton

Résumé. Dans une expérience préliminaire, nous avons montré que la production *in vitro* de méthane dans le rumen de moutons ayant consommé l'*Atriplex nummularia* était approximativement quatre fois plus élevée que celle de moutons ayant consommé de la paille ou une ration mixte de foin d'avoine et de lupin. Nous avons donc supposé que la production *in vivo* de méthane est plus importante dans le rumen des moutons recevant l'*Atriplex* que dans celui de moutons alimentés avec un fourrage classique, et que cette augmentation de la production de méthane serait due à une concentration élevée de sel dans l'*Atriplex*. Quarante moutons ont reçu un des quatre régimes suivants ; le régime "témoin" (NS), le témoin + 10% de sel (MS), le témoin + 20% de sel (HS), ou l'*Atriplex* contenant approximativement 16% de sel (SB). Les mesures ont porté sur la production de méthane pendant 22 heures, le nombre d'archaea-méthanogènes dans le rumen, et la production d'acides gras volatils. Les moutons ayant consommé l'*Atriplex* ont produit plus de méthane (l/22h/kg DOMI), ont eu un nombre plus élevé d'archaea productrices de méthane, et un rapport acétate/propionate plus élevé dans le rumen. Il n'y a eu aucune différence dans ces mesures entre les traitements NS, MS et HS. Nous concluons que la réduction de l'efficacité de la fermentation dans le rumen n'est pas liée à la teneur en sel du fourrage, mais plutôt au fait que l'*Atriplex* est peu valorisé par les animaux.

Mots-clés. *Atriplex* – Sel – Méthane – Efficacité du rumen.

I – Introduction

Saltbush (*Atriplex*) species are highly valued by pastoralists and considered to be among the most productive natural pastures in the Australian rangelands (Lefroy, 2002). They are also being

planted in agricultural areas, where they have become an important tool in the revegetation and rehabilitation of salt-affected land. Compared to the dried annual herbage available in summer and autumn, species such as oldman saltbush (*A. nummularia*) have a moderate nutritive value, yet sheep struggle to eat and digest enough saltbush to maintain weight (Casson *et al.*, 1996; Morcombe *et al.*, 1996).

In a preliminary experiment (Mayberry, unpublished data) *in vitro* methane production in rumen fluid from sheep fed saltbush was approximately four times higher than methane production in rumen fluid from sheep fed barley straw or a mixed oaten hay and lupin ration. This increase in methane production corresponded to an increase in the salt content of the feed and the salinity of the rumen fluid. Methane is considered to be a major inefficiency in ruminant production (Pelchen and Peters, 1998; Ulyatt *et al.*, 2002), and production of extra methane by sheep grazing saltbush could contribute to poor animal performance.

To understand this result further we tested the hypotheses that: (i) *in vivo* methane production would be higher in sheep fed saltbush compared to sheep fed a traditional forage; and (ii) that the increase in methane production would be due to the high level of salt in the feed.

II – Materials and methods

Forty 18-month-old merino wethers with no prior exposure to salty diets were individually penned in an animal house for 13 weeks. For the first six weeks all animals were fed a control pellet (NS) of wheaten chaff (52.5%), barley (20%), oats (20%), lupins (5%), Siromin (White *et al.*, 1992) (1%) and binders (met lime and gypsum, 1.5%) at maintenance to allow an assessment of natural variation in methane production between animals. Animals were then allocated to one of four treatment diets based on measured methane production, with the average methane production for each group being between 24.6 and 26.5 litres of methane over a 22 hour period per kg of digestible organic matter intake (l/22h/kg DOMI). Average liveweight of each group varied between 43.1 and 44.2 kg. Four sheep had to be removed during the experiment due to poor appetite, so there were only nine animals in each treatment group.

The four diets offered to the animals (Table 1) were the control pellet (NS), control + 10% salt pellet (MS), control + 20% salt pellet (HS) and old man saltbush (*Atriplex nummularia*) containing approximately 16% salt (SB). The proportion of salts added to all diets was approximately 2NaCl:1KCl. Animals were gradually introduced to the new diets over two weeks, before being fed 100% of the treatment diets for the remaining five weeks of the experiment. The animals were offered a maintenance ration, but did not consume it all on the MS, HS and SB diets. All sheep had free access to fresh drinking water at all times during the experiment. Components of the diets were analysed as described by Thomas *et al.* (2007).

Table 1. Chemical composition of diets

% of dry matter	Diet			
	NS	MS	HS	SB
Na	0.21	3.04	6.11	5.44
K	0.84	2.01	3.50	2.38
Cl	0.56	5.71	11.3	7.83
Ash	5.40	18.3	27.9	23.6
DOMD (<i>in vivo</i>)	66.0	62.8	57.6	47.6
NDF	53.4	52.1	41.9	36.3
ADF	21.5	20.5	16.9	19.9

Methane production was measured using four open system methane chambers (Klein and Wright, 2006) after sheep had been fed the treatment diets for a minimum of two weeks. Because there were only four chambers, measurements took place over nine days, with one sheep from each treatment group included in each day's measurements. The sheep were offered their daily ration and immediately enclosed in the chambers at around 09:00 h. They remained in the chambers for approximately 23 hours, before being removed at around 08:00 h the following morning. Methane measurements were corrected for differences in temperature, humidity, air pressure, feed intake and duration of measurements between chambers and animals.

When the animals were removed from the chambers they were returned to their pens in the animal house and fed their normal daily ration. A sample of rumen fluid was taken from each animal on the day after they had been in the methane chambers, three hours after feeding using a stomach tube attached to a vacuum pump. Analysis of the rumen fluid included electrical conductivity (used as an indication of rumen salinity), volatile fatty acid (VFA) concentration and numbers of methane-producing archaea. The numbers of methane-producing archaea were measured using the real-time PCR method of Christopherson *et al.* (unpublished), which targets the 16SrRNA gene.

During the final week of the experiment, five sheep from each treatment group were fitted with faecal harnesses and total feed intake and faecal output were measured for six days. Approximately 10% of the faeces from each sheep and a sub sample of each diet were dried, ground and ashed to determine the average organic matter digestibility.

A one-way analysis of variance with Tukey's pairwise comparisons was used to determine the effects of diet on methane production, populations of methane-producing archaea, VFA and rumen electrical conductivity. Analyses were conducted using the Genstat statistical package (GenStat, 2005).

III – Results and discussion

The amount of methane produced by sheep fed saltbush (SB) was significantly higher ($P > 0.05$) than the amount of methane produced by sheep fed the NS, MS and HS diets (Table 2), supporting our first hypothesis. This indicates an increase in the amount of ingested energy lost from the rumen, and a decrease in the efficiency of rumen fermentation. There was no difference in methane production between the NS, MS and HS diets despite an increase in the salinity of the rumen fluid. Thus, the high levels of methane produced by sheep fed saltbush are not caused by the high concentration of salt in the feed and our second hypothesis is rejected. However, it is worth noting that the addition of salt to the control diet (MS and HS) did not suppress methane production, despite the increase in rumen salinity, rate of passage and dilution of feed particles associated with high salt diets (Hemsley *et al.*, 1975).

Table 2. Effect of diet and rumen salinity on products of rumen fermentation

	Diet			
	NS	MS	HS	SB
Electrical conductivity (mS/cm)	11.4 ^a	15.7 ^{ab}	18.3 ^b	15.3 ^{ab}
Methane production (l/22h/kg DOMI)	31.6 ^a	34.4 ^a	30.9 ^a	44.8 ^b
Methane producing archaea (10 ⁹ /ml of rumen fluid)	3.68 ^a	1.88 ^a	1.70 ^a	55.3 ^b
Total VFA concentration (mmol/l)	84.0 ^a	71.3 ^{ab}	50.6 ^b	55.8 ^{ab}
Proportion acetate (%)	64.1 ^a	65.2 ^a	65.2 ^a	77.1 ^b
Proportion propionate (%)	18.9 ^{ab}	22.0 ^{bc}	23.5 ^c	15.5 ^a

^{a,b,c}Values with the same letter in the same row are not significantly different ($P > 0.05$).

The increase in methane production is also unrelated to the digestibility or fibre content of the feed (Table 1). This is contrary to the results of Pelchen and Peters (1998), who linked high methane production with high digestibility and fibre concentration. The most likely explanation for the high level of methane production is the presence of many secondary compounds in the leaves of saltbush.

The high methane production by sheep fed saltbush was accompanied by a significant increase in the numbers of methane-producing archaea in the rumen (Table 2). The increase in methane-producing archaea was much greater than the increase in methane production, suggesting that although there are many more methane-producing archaea in the rumen of sheep fed saltbush, they are not all more active.

Across all three salty diets (MS, HS and SB) there was a decrease in the total concentration of VFA in the rumen (Table 2), though this was only significant for the HS treatment. This result appears to be a direct result of the increased salt concentration of the feed and salinity of the rumen fluid, and is consistent with previous research by Hemsley *et al.* (1975) and de Waal *et al.* (1989). Rather than reflecting a decrease in the production of VFA, this result is probably due to the dilution of VFA by increased water intake (Masters *et al.*, 2005). Although there may not be less VFA produced, absorption of VFA would be reduced by the increased rate of passage, and more energy would be lost from the rumen.

The relative proportions of acetate and propionate in the rumen of sheep fed saltbush also reflects inefficiencies in rumen fermentation (Table 2). There was a significant increase in the ratio of acetate to propionate in the rumen fluid of sheep fed saltbush compared to sheep fed the NS, MS and HS diets. This occurred through a significant increase in the proportion of acetate and a small decrease in the proportion of propionate in the rumen. The MS and HS diets had no effect on the proportion of acetate, and slightly increased the proportion of propionate, but there was no significant decrease in the ratio of acetate to propionate in the rumen.

IV – Conclusions

The results from this experiment demonstrate that consumption of old man saltbush reduces the efficiency of rumen fermentation in sheep. This was illustrated by an increase in the amount of methane produced (through increased populations of methane-producing archaea), a decrease in the total concentration of VFA, and an increase in the proportion of acetate to propionate in the rumen. The reduction in rumen efficiency is clearly not due to the high concentration of salt in saltbush, but may instead be due to the effects of secondary plant compounds on rumen microbial populations. The associated loss of ingested energy may help to explain poor animal production from saltbush pastures, and we are currently investigating the use of an energy supplement to improve rumen fermentation.

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