



ELSEVIER

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

APPLIED ANIMAL
BEHAVIOUR
SCIENCE

Applied Animal Behaviour Science 94 (2005) 245–254

www.elsevier.com/locate/applanim

Effect of added sugar on preference and intake by sheep of hay cut in the morning versus the afternoon

E.A. Burritt^{a,*}, H.F. Mayland^b, F.D. Provenza^a,
R.L. Miller^c, J.C. Burns^d

^a *Department of Forest, Range and Wildlife Sciences, Utah State University, Logan, UT 84322-5230, USA*

^b *USDA Agricultural Research Service, Northwest Irrigation and Soils Research Laboratory,
Kimberly, ID 83341-5076, USA*

^c *Agricultural Systems and Technology and Education Department,
Utah State University Logan, UT 84322-2300, USA*

^d *USDA–Agricultural Research Service, Crop and Animal Science Departments, North Carolina State
University, Raleigh, NC 27695-7620, USA*

Accepted 7 March 2005

Available online 22 April 2005

Abstract

Ruminants prefer hay cut in the afternoon to hay cut in the morning, presumably because hay cut in the afternoon contains higher concentrations of non-structural carbohydrates than hay cut in the morning. We determined if adding sugars (glucose and sucrose) to ground hay would account for differences in preference and affect intake of hay. Alfalfa hay cut either in the afternoon (PM) or the following morning (AM) was used in the trials. Glucose and sucrose were added to AM hay (AMS) to make its sugar content similar to PM hay. During the first trial, lambs received a choice of either: (1) AM and PM hay; (2) AMS and PM hay; or (3) AMS and AM hay. Lambs preferred PM to AMS or AM hay. We also studied how increasing the concentration of added sugars affected preference for hay. Lambs received a choice of AM hay and AM hay with either 1, 2, 3, or 4% added sugar. On the first day of the trial, lambs ate similar amounts of each hay type, regardless of the amount of sugar added. By the end of the trial, lambs preferred hay with 2, 3, or 4% added sugar compared with AM hay without added sugar. During the intake trial, lambs ate similar amounts of AM, AM hay with added starch and sugar (AMSS), and PM hay. After the intake trial, a final preference trial determined that prolonged exposure

* Corresponding author. Tel.: +1 435 797 3576; fax: +1 435 797 3796.
E-mail address: bethb@cc.usu.edu (E.A. Burritt).

to AMSS hay increased preference for AMSS hay compared with AM hay. Our results indicate that preference for PM hay is likely related to increased levels of sugars, such as glucose and sucrose, and that lambs learn about the post-ingestive benefits of exogenous sugars added to hay.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Sheep; Intake; Preference; Non-structural carbohydrates; Sugars

1. Introduction

Hay cut in the afternoon contains higher quantities of total non-structural carbohydrates (TNC) than hay cut in the morning. Though differences in TNC between morning and afternoon cut hay are small (generally less than 2%), numerous studies have demonstrated that ruminants (Fisher et al., 1999, 2002) and horses (MacKay et al., 2003) prefer hay cut in the afternoon to hay cut in the morning. Preference for hay cut in the afternoon may be due to the additional energy provided by these slight increases in TNC. Ruminants may reap additional benefits from TNC beyond the extra energy they receive because soluble carbohydrates, especially sugars, increase microbial protein synthesis from inorganic nitrogen in the rumen (Chamberlain et al., 1985).

Sheep form strong preferences for foods and flavors based on feedback from nutrients, such as energy and protein (Provenza, 1995; Villalba and Provenza, 1996, 1997a,b). Even small quantities of nutrients can condition preferences. For example sheep formed preferences for flavored straw when gavaged with a starch and water solution that provided only 2.5% of their daily energy requirement (Villalba and Provenza, 1997b).

To date, evidence supporting the hypothesis that higher levels of TNC in hay cut in the afternoon accounts for the increase in preference is purely correlative. We investigated whether adding small amounts of sugar and starch would cause sheep to increase their preference for hay cut in the morning. In addition, we determined if lambs would ingest more hay if it were cut in the afternoon rather than the morning or if it contained a larger proportion of sugars and starch.

2. Materials and methods

2.1. *Animals and housing*

Feeding trials were conducted at Utah State University's Green Canyon Ecology Center in Logan, UT, USA. We used 5-month-old white-faced lambs (commercial crossbreeds) in all trials. Lambs were weaned at 60 days and reared on alfalfa pellets, grass hay and barley. They were placed in individual pens prior to the onset of the trials.

2.2. *Foods*

Alfalfa hay was harvested at the Northwest Irrigation and Soils Research Laboratory in Kimberly, ID, USA, at sundown on June 16 (PM) and at sunup on June 17 (AM). Hay was field-dried and baled prior to shipment to Logan, UT, USA, for preference and intake trials.

Table 1
Non-structural carbohydrate composition of AM and PM hays^a

Carbohydrate	PM (%)	AM (%)
Monosaccharides	1.73	1.29
Disaccharides	3.43	3.11
Short-chain polysaccharides	1.25	1.25
Starch	0.80	0.50
Total non-structural carbohydrates	7.21	6.15

^a Data expressed on a dry matter basis.

After baling, core samples were taken from five bales from each cutting (AM and PM). Core samples were ground and mixed together and analyzed for monosaccharides and disaccharides by an adaptation (Fisher and Burns, 1987) of the method described by Smith (1969). Starch and short-chained polysaccharide composition were estimated using near-infrared reflectance (NIR). Non-structural carbohydrate compositions of AM and PM hays are listed in Table 1. The amounts of sugars and starch added to the AM hay varied with the trial and are described below. Prior to the onset of the trials, hay was ground in a tub grinder. Ground hays were offered ad libitum in separate food boxes and the position of the food boxes (left or right) was switched each day. All intake data are expressed on an as-fed basis.

2.3. Trial 1

The objective of Trial 1 was to determine if adding sugars (glucose and sucrose) to make the concentration of sugar in AM and PM hay equal would account for the preference for PM over AM hay by livestock. During Trial 1, sugars were added to the hay but not starch because Fisher et al. (1999) reported that cattle, sheep and goats preferred tall fescue hay cut in the evening to hay cut in the morning; but in their study, the hays differed in sugar but not starch content. Based on the data reported in Table 1, we added 0.45% glucose (Sigma Chemical Co.) and 0.35% sucrose (C & H Sugar) to the AM hay to make AM hay plus sugars (AMS) equal to the sugar content of PM hay.

Prior to Trial 1, lambs were offered hay they would receive during Trial 1 to familiarize lambs with the hays. For 9 h/day, lambs received 1200 g of one hay, the following day they received 1200 g of the alternate hay.

Lambs were blocked by weight and randomly assigned to one of three treatments ($n = 10$ /treatment). Each morning from 08:00 to 10:30, lambs received a choice of either (1) AM and PM, (2) AM and AMS, or (3) PM and AMS hay. At the end of each trial, food remaining in the boxes was weighed and intake for each lamb was calculated. Lambs then received alfalfa pellets ad libitum for 1 h/day and food boxes were removed until the following morning. The trial lasted 3 days.

Analyses of variance for this and all other trials were conducted using SAS Proc Mixed. In Trial 1, data were analyzed using an analysis of variance for repeated measures. Food was the main effect. Day ($n = 3$) was the repeated measure. Pairs of foods (AM versus PM; AM versus AMS; AMS versus PM) were analyzed separately.

2.4. Trial 2

The objective of Trial 2 was to determine if lambs preferred hay with 1, 2, 3, or 4% added sugar versus hay without added sugar. Lambs from Trial 1 were re-randomized and assigned to one of four groups ($n = 8/\text{group}$) that received a choice between AM hay and either AM hay plus 1% (AM1), 2% (AM2), 3% (AM3), or 4% (AM4) added sugar (50:50 glucose:sucrose). Lambs received a choice of two hays for 2.5 h each morning for 5 days. At the end of each daily trial, food remaining in the boxes was weighed and intake calculated. Lambs then received alfalfa pellets *ad libitum* for 1 h and food boxes were removed until the following morning. After 5 days, lambs received 1000 g of either AM hay or AM hay with added sugars on alternate days so that lambs received each hay as their only food for 2 days. Most lambs consumed all of their hay in 24 h. Lambs received hay with the same concentration of sugars as they received during the first 5 days of the trial. Lambs were then given a choice between AM hay and AM hay plus sugars for 2.5 h each morning for an additional 2 days.

Food preferences were compared using an analysis of variance for repeated measures design. Food was the main effect, and day ($n = 7$) was the repeated measure. Pairs of foods (AM versus AM1; AM versus AM2; AM versus AM3; AM versus AM4) were analyzed separately.

2.5. Trial 3

The objective of Trial 3 was to determine if lambs consumed more hay if it contained a higher concentration of sugars and starch. Prior to the onset on Trial 3, lambs were offered AM hay *ad libitum* continuously for 2 days and daily intake was recorded. Lambs from Trial 2 were blocked by intake and previous experience and re-randomized and divided among three treatments ($n = 10/\text{treatment}$). During the trial, lambs received either (1) AM hay, (2) PM hay, or (3) AM hay plus 0.45% glucose, 0.35% sucrose and 0.30% corn starch (AMSS). Starch was added to the AM hay because the AM and PM hays differed in their starch contents (Table 1). Each morning at 08:00, food boxes were removed and refusals were weighed. Food boxes were refilled and returned to lambs by 1000. Hays were offered *ad libitum*. The trial lasted 7 days.

The statistical design for Trial 3 was a repeated measures analysis of variance. Food (AM, AMSS, PM) was the main effect. Day ($n = 7$) was the repeated measure.

2.6. Trial 4

The objective of Trial 4 was to determine the preference for AM, PM and AMSS by all lambs ($n = 30$). For 2 h each morning, lambs received a choice between AM and PM hay for 2 days, lambs then received a choice between AM and AMSS the following 2 days, and finally they received a choice of AMSS and PM hay for 2 days.

The statistical design for the analysis of variance for Trial 4 was a split-plot with repeated measures. History (consumed AM, AMSS, or PM during Trial 3) was the main effect; food was nested within history. Only experience with the food eaten during Trial 3 (history) was considered because prolonged exposure (7 days) to AMSS hay may have

enabled lambs to discriminate between AMSS and AM. Day ($n = 2$) was the repeated measure. Pairs of foods (AM versus PM; AM versus AMS; AMS versus PM) were analyzed separately.

2.7. Trial 5

The objective of Trial 5 was to determine if lambs naive to the alfalfa hays used in the above trials would prefer PM or AM hay. Twenty-six-month-old crossbred lambs reared on barley, alfalfa pellets and grass pastures were fasted overnight. The following morning lambs were offered a choice of 500 g each of AM and PM hay in separate boxes for 2 h. Refusals were weighed after 2 h and intake calculated. Data were analyzed using a *t*-test to determine differences between food intake means (AM versus PM).

3. Results

3.1. Trial 1

Lambs consumed more PM hay than AM hay (205 g versus 134 g, $P = 0.03$) and they consumed more PM hay than AMS hay (224 g versus 145 g, $P = 0.02$). They consumed similar amounts of AMS hay and AM hay (225 g versus 198 g, $P = 0.21$). Total hay intake by lambs increased during each of the 3 days, regardless of which pairs of hay lambs received, showing that the lambs were adjusting similarly to these novel hays (Table 2).

3.2. Trial 2

In Trial 2, lambs receiving AM and AM1 ate similar amounts of hay (205 g versus 211 g, $P = 0.66$) during the 7-day trial (initial 5-day trial plus 2-day follow-up trial). Although lambs receiving AM and AM2 ate similar amounts of hay on day 1 of the trial, overall they ate more AM2 than AM hay (248 g versus 190 g, $P = 0.01$; Fig. 1). Lambs preferred AM3 to AM hay (277 g versus 168 g, $P = 0.002$). They consumed similar amounts of each hay on days 1 and 2 of the trial but consumed more of the hay with sugars on days 3–7 of the trial (Fig. 2). The same pattern occurred for lambs receiving AM4 and AM hay (Fig. 3). Lambs preferred AM4 hay to AM hay (246 g versus 162 g, $P = 0.0014$).

Table 2
Total hay intake of lambs when offered a choice of hays in Trial 1^a

Hay ^b	Day 1 (g)	Day 2 (g)	Day 3 (g)	S.E.	<i>P</i> -value
AM and PM	282	356	379	24.4	0.001
AM and AMS	354	442	474	38.8	0.001
AMS and PM	318	381	407	29.8	0.001

^a Data expressed on an as-fed basis.

^b AM = AM hay; PM = PM hay; AMS = AM hay + .045% glucose + 0.35% sucrose.

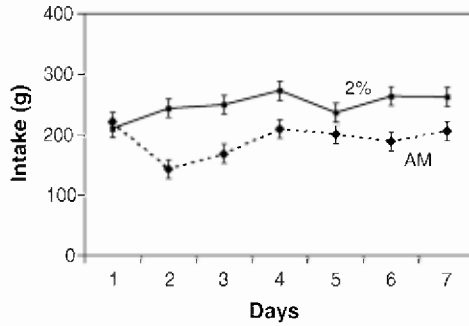


Fig. 1. Intake (g) by lambs of AM hay and AM hay plus 2% added sugar (1% glucose and 1% sucrose). Intake is expressed on an as-fed basis.

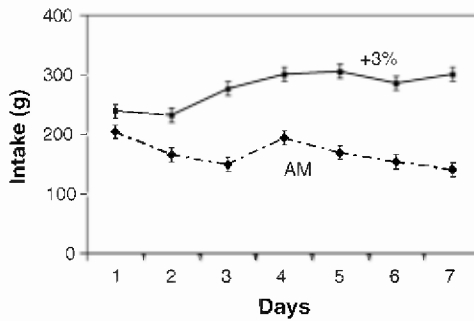


Fig. 2. Intake (g) by lambs of AM hay and AM hay plus 3% added sugar (1.5% glucose and 1.5% sucrose). Intake is expressed on an as-fed basis. Least significant difference 0.05 = 47.

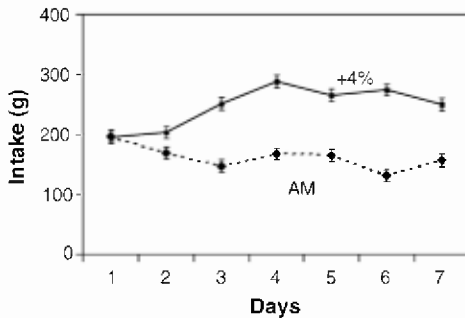


Fig. 3. Intake (g) by lambs of AM hay vs. AM hay plus 4% added sugar (2% glucose and 2% sucrose). Intake is expressed on an as-fed basis. Least significant difference 0.05 = 41.

3.3. Trial 3

Lambs ingested similar amounts of hay, regardless of treatment (AM = 1845 g, AMSS = 1762 g and PM = 1747; $P = 0.76$).

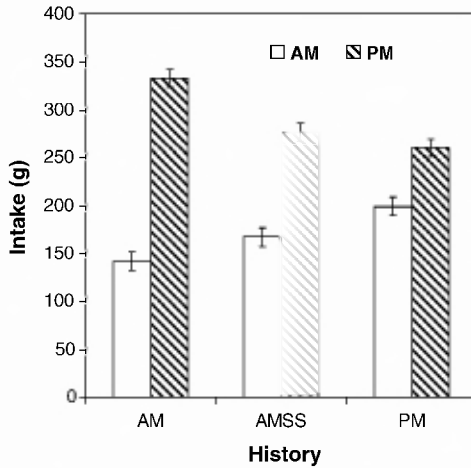


Fig. 4. Intake (g) of AM and PM hay by lambs with different dietary histories. Intake is expressed on an as-fed basis. Least significant difference 0.05 = 37.

3.4. Trial 4

During Trial 4, lambs preferred PM (290 g) to AM (169 g; $P < 0.0001$), AMSS (242 g) to AM (196 g; $P < 0.0001$) and PM (237) to AMSS (202 g; $P = 0.029$). The history by food interaction was significant. Lambs preferred PM to AM, regardless of the food they consumed during the intake study, but lambs that ate AM during the intake study showed a stronger preference for PM than lambs that received either AMS or PM hay during the intake trial (Fig. 4). Lambs preferred AMSS to AM hay if they ate AM or AMSS during the

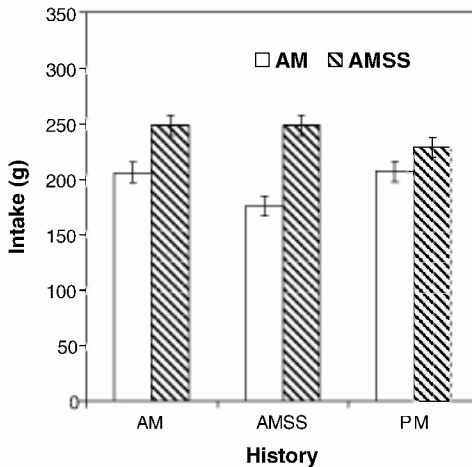


Fig. 5. Intake (g) of AM and AM hay plus 0.45% glucose, 0.35% sucrose and 0.30% corn-starch (AMSS) by lambs with different dietary histories. Intake is expressed on an as-fed basis. Least significant difference 0.05 = 36 and 0.01 = 48.

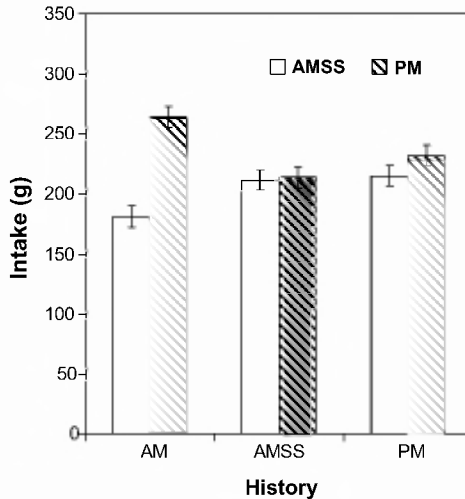


Fig. 6. Intake (g) of AM hay plus 0.45% glucose, 0.35% sucrose and 0.30% corn starch (AMSS) and PM hay by lambs with different dietary histories. Intake is expressed on an as-fed basis. Least significant difference 0.05 = 34.

intake trial, but showed equal preference for both hays if they ate PM during the intake trial (Fig. 5). Lambs preferred PM to AMSS if they consumed AM during the intake trial, but did not prefer PM over AMSS if they ate AMSS or PM during the intake trial (Fig. 6).

3.5. Trial 5

Lambs naive to the experimental hays consumed 106 g of AM hay and 224 g of PM hay during the 2 h trial ($P = 0.0004$).

4. Discussion

In Trial 1, lambs did not prefer AMS to AM even though the sugar content of AMS was similar to PM. Thus, sweetness may not be the only flavor difference between AM and PM hay. Just as adding sugar to slightly green strawberries does not make them taste like strawberries ripened on the plant, adding sugar to AM probably does not make AMS taste like PM. Ruminants probably use additional flavor cues to discriminate AM from PM.

In Trial 2, adding sugar up to 4% did not produce an immediate increase in intake of AM. Lambs increased intake of hays containing 2, 3, or 4% sugar only after consuming the hay for a day or two, indicating they learned about the beneficial effects of added sugar. Lambs receiving hay with 1% added sugar did not prefer one hay over the other. However, lambs may be able to learn to discriminate between AM and AM with 1% added sugar-starch, but only after a longer period of exposure to AMS as indicated by the observation that lambs receiving AMSS during the intake study came to prefer AMSS to AM after 7-day exposure to AMSS (Trial 4).

Intake of hays by lambs was not affected by the concentration of soluble sugars and starches in hays. Burns et al. (2005) found that goats and cattle, but not sheep, ate slightly more PM than AM hay during an 18-day feeding trial. A variety of factors affect total intake of foods. Ruminants do not increase food intake based solely on the energy content of the diet, rather they tend to consume a relatively constant level of energy provided the diet contains adequate energy and is not limited by fill. Provenza et al. (1996) found that sheep consumed more of a poor quality diet compared with a higher quality diet because a smaller quantity of the higher quality diet was required to meet their energy needs. The higher concentration of sugars or other flavor cues may have been great enough to impact preference but not intake.

In Trial 4, the significant history by day interaction suggests that preferences for AM, AMSS and PM hay depended on which food they ate during the previous intake trial (Trial 3). Lambs preferred the PM- to AM-cut hay, regardless of the food they ate during the intake trial, but lambs that ate AM during the intake trial had the highest preference for PM, probably because they were forced to eat a lower quality food than the other groups (Fig. 4). Lambs that consume diets even slightly inadequate in nutrients have stronger preferences for alternative diets (Early and Provenza, 1998). Lambs that ate AMSS during the intake trial preferred AMSS to AM (Fig. 5). This is in contrast to results in Trials 1 and 2 where lambs did not discriminate between AMS and AM. Prolonged exposure to AMSS, the addition of starch or both may have caused lambs to discriminate AMSS from AM. Lambs that ate AM hay during the intake trial also preferred AMSS to AM hay. Lambs forced to eat the same food for several days seek foods with different flavors (Early and Provenza, 1998). The addition of sugar likely gave the hays slightly different flavors. When lambs were allowed to choose between AMSS and PM, only lambs that had consumed AM hay during the intake trial preferred PM to AM (Fig. 6). Lambs that received AMSS during the intake study may have learned after prolonged exposure to AMSS that AMSS and PM were similar in nutrient content.

Lambs naive to the hays also preferred PM to AM hay. These data may cause some to conclude that sheep innately discriminate between forages based on the concentration of sugar or some other cue. However, this study does not support the notion that sheep innately prefer sweet. Lambs in Trial 2 receiving hay with 3 or 4% sugar did not immediately prefer hay with added sugar but preferred the hay with added sugar after eating the hay for a day. In addition, Grovum and Chapman (1988) concluded that a preference for sweet is not innate in ruminants and that sucrose is relatively unpalatable when the flavor of sucrose is separated from its post-ingestive consequences. Lambs used in Trial 5 were naive to the hays used in this study but were familiar with alfalfa pellets. Experience with alfalfa pellets may have enabled lambs to discriminate between different qualities of alfalfa based on level of sweetness or other flavor cues.

5. Conclusions

Lambs in this study preferred hay cut in the evening to hay cut in the morning but adding sugar to hay did not account for this preference until lambs learned about the benefits of added sugar. This study and others on AM–PM hay emphasizes that ruminants prefer

forages based on small changes in plant chemistry that are difficult to detect in the lab. Thus, it is important that animal preference is determined with feeding trials and not to rely on forage chemical composition to predict forage preference.

Acknowledgements

This research was supported by the Utah Agricultural Experiment Station, Utah State University, Logan, Utah, and USDA-ARS, Kimberly, Idaho, and Raleigh, North Carolina.

References

- Burns, J.C., Mayland, H.F., Fisher, D.S., 2005. Dry matter intake and digestion of alfalfa harvested at sunset and sunrise. *J. Anim. Sci.* 83, 250–262.
- Chamberlain, D.G., Thomas, P.C., Wilson, W., Newbold, C.J., MacDonald, J.C., 1985. The effect of carbohydrate supplements on ruminal concentrations of ammonia in animals given diets of grass silage. *J. Agric. Sci.* 104, 331–340.
- Early, D.M., Provenza, F.D., 1998. Food flavor and nutritional characteristics alter dynamics of food preference in lambs. *J. Anim. Sci.* 76, 728–734.
- Fisher, D.S., Burns, J.C., 1987. Quality of summer annual forages. I. Sample preparation methods and chemical characterization of forage types and cultivars. *Agron. J.* 79, 236–242.
- Fisher, D.S., Mayland, H.F., Burns, J.C., 1999. Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. *J. Anim. Sci.* 77, 762–768.
- Fisher, D.S., Mayland, H.F., Burns, J.C., 2002. Variation in ruminant preference for alfalfa hays cut at sunup and sundown. *Crop Sci.* 42, 231–237.
- Grovum, W.L., Chapman, H.W., 1988. Factors affect the voluntary intake of food by sheep. 4. The effects of additives representing the primary tastes of sham intake by oesophageal-fistulated sheep. *Br. J. Nutr.* 59, 63–72.
- MacKay, L.C., Mayland, H.F., MacKay, W.P., 2003. Horse preference for alfalfa-grass hay harvested in the afternoon or morning. *Proc. West. Sec. Am. Soc. Anim. Sci.* 54, published on CD-ROM. Not paginated.
- Provenza, F.D., 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *J. Range Manage.* 48, 2–17.
- Provenza, F.D., Scott, C.B., Phy, T.S., Lynch, J.J., 1996. Intake by sheep of foods varying in flavours and nutrients. *J. Anim. Sci.* 74, 2355–2361.
- Smith, D., 1969. Removing and analyzing non-structural carbohydrates from plant tissue. *Res. Rep.* 41. College of Agric. and Life Sci., Univ. Wisconsin, Madison.
- Villalba, J.J., Provenza, F.D., 1996. Preference for wheat straw by lambs conditioned with intraruminal infusions of sodium propionate. *J. Anim. Sci.* 74, 2362–2368.
- Villalba, J.J., Provenza, F.D., 1997a. Preference for flavoured foods by lambs conditioned with intraruminal administration of nitrogen. *Br. J. Nutr.* 78, 545–561.
- Villalba, J.J., Provenza, F.D., 1997b. Preference for wheat straw by lambs conditioned with intraruminal infusions of starch. *Br. J. Nutr.* 77, 287–297.