

1 **The effect of sucrose addition on intake of a tropical grass hay by sheep**

2

3 A. de Vega^{A,B}, and Dennis P. Poppi^A

4 ^A Schools of Agriculture and Food Science and Veterinary Science, The University of Queensland.

5 Gatton 4343, Australia

6 ^B Corresponding author. Present address: Departamento de Producción Animal y Ciencia de los

7 Alimentos. Universidad de Zaragoza, Facultad de Veterinaria. Miguel Servet 177, 50013 Zaragoza,

8 Spain.

9 Running title: Intake as affected by sucrose addition

10

11 **Abstract.** Increasing levels of sucrose (15, 30, 45, and 60% of the total dry matter intake) were
12 added to a control diet of a tropical grass hay (pangola grass, *Digitaria eriantha*) to examine the
13 effect on dry matter intake (DMI) and digestible organic matter intake (DOMI). A pelleted lucerne
14 (*Medicago sativa*) diet provided a positive control diet with a higher metabolizable energy (ME)
15 intake. There were positive linear and quadratic effects of sucrose addition on DMI and DOMI,
16 reflecting increasing values from Control to 45% sucrose which then decreased for 60% sucrose.
17 Intake values at 45% sucrose were equivalent to 49% (DMI) and 60% (DOMI) of those shown by
18 animals fed on pelleted lucerne. At the same time there was a decrease in hay intake and neutral
19 detergent fibre (NDF) digestibility. Rumen concentration of butyric acid increased with sucrose
20 addition. Eating pattern changed with increasing sucrose inclusion in that meals extended over the
21 whole day rather than most food being consumed within 5 h as occurred with the high roughage
22 diet. Intake of sucrose was similar for all diets by 5 h, but by 24 h high sucrose diets had resulted in
23 a higher intake of sucrose. It was suggested that the underlying characteristics of the NDF present
24 in the diet regulate intake by posing a limit which can not be overridden.

25

1 Additional key words: intake regulation, water soluble carbohydrates, substitution, neutral
2 detergent fibre

3

4 **Introduction**

5 Intake by ruminants of forage-based diets is thought to be regulated by physical limitations
6 (Poppi *et al.* 1981), metabolic processes (Egan 1977, Poppi *et al.* 1994), or by some interaction
7 between these two processes (Egan 1977, Poppi *et al.* 1994). Gherardi and Black (1989) and
8 Weston (1996) clearly outlined the interaction of physical and metabolic mechanisms and proposed
9 that the interaction between net energy (NE) supply and demand affected the extent to which
10 ruminants were prepared to distend their rumen and hence digesta mass in the rumen and physical
11 limitation to intake. They showed a declining level of digesta mass in the rumen as NE supply
12 approached NE demand, which was consistent across a variety of diet types or infusion studies.
13 Physical limitations dominated in the low quality diet types whilst metabolic considerations became
14 more important for higher quality forages but there was an interaction between both major
15 mechanisms across the wide range of diet quality.

16

17 This experiment examined the effect of increasing the level of sucrose inclusion on the
18 intake of a tropical grass hay where the underlying neutral detergent fibre (NDF) fraction remained
19 constant but rate of release of nutrients from sucrose addition increased. The approach also has
20 application in examining the potential benefit of increasing the soluble carbohydrate level in
21 tropical grasses.

22

23 **Material and Methods**

24 The experiment was approved by the University of Queensland Animal Ethics Committee.

25 *Animals and diets*

1 taken on the first day of the measurement period, with the aid of an oesophageal tube, before (0 h)
2 and 4 h after feeding. The pH was measured immediately after obtaining the sample, which was
3 subsequently filtered through a metal mesh (1-mm pore size). From the filtrate, 10 mL were added
4 to 4-5 drops of concentrated HCl and stored frozen until their analysis for ammonia-N. A second
5 10 mL subsample was mixed with 1 mL of a 5% (v/v) solution of H₃PO₄, and kept frozen for
6 volatile fatty acid (VFA) analyses. Rate of intake was studied on the last day of the experiment by
7 weighing the refusals at 1, 2, 3, 4, 5 and 24 h after feeding. On the third day of the measurement
8 period, 50 mL of a Cr-EDTA solution (2.65 mg Cr/mL) and 10 g of Yb-labelled (4.72 mg Yb/g
9 DM) pangola particles (the fraction retained between screens of 0.5 and 1.18 mm after wet sieving
10 a sample previously ground through a 2-mm screen) were given to the animals through an
11 oesophageal tube, directly into the reticulo-rumen. Cr-EDTA was prepared by the method of
12 Downes and McDonald (1964) and Yb-labelled particles as described by de Vega and Poppi (1997).
13 Faecal samples were subsequently taken, directly from the rectum, at 3, 8, 15, 24, 32, 48, 56, 72,
14 80, 96, 104 and 120 h post dosing, oven dried at 80 °C for 48 h, ground through a 1 mm screen and
15 then analysed for marker concentration as outlined in de Vega and Poppi (1997). The slow
16 fractional outflow rate (k_1) of both particulate and liquid phases was calculated according to the
17 procedures described by Grovum and Williams (1973). Animals were weighed before and after the
18 digestibility trial.

19

20 *Chemical analysis*

21 Dry matter in forages, refusals, sucrose solutions and faeces was determined by drying at 70
22 °C for at least 48 hours, and OM by ashing at 550 °C for 6 hours. Samples of faeces, forages,
23 refusals and sucrose solutions were analysed for total N using the Leco CNS-2000 Combustion
24 Analyser (Leco Corporation). Ash-free NDF was measured on dried forages, refusals and faeces
25 (60 °C for 48 hours) as described by Van Soest *et al.* (1991) and using an Ankom 220 Fiber
26 Analyzer. Diets were analysed for water soluble carbohydrates as described by Thomas (1977).

1 Ammonia nitrogen was determined from 5 mL samples of ruminal fluid according to Panjaitan *et*
2 *al.* (2010). Determination of VFA was performed by gas-liquid chromatography following the
3 procedure described by Jouany (1982), with 0.5 mL of a 0.65% (w/v) solution of 4-methyl valerate
4 as internal standard, and a mixture of acetate, propionate, isobutyrate, butyrate, isovalerate and
5 valerate as external standard.

6

7 *Statistical analysis*

8 Intake, digestibility and rate of intake were analysed by means of one-way analysis of
9 variance. Two animals from treatment PL were removed from the intake and digestibility analysis
10 due to very low intakes, but one of them had recovered average intakes by the end of the
11 experiment, so it was included in the rate of intake analysis. A subsequent ANOVA was performed
12 excluding data from animals fed lucerne, and the sums of squares of significant factors were
13 partitioned in four polynomial orthogonal contrasts to test linear and quadratic responses to level of
14 sucrose in the diet. Ammonia and VFA concentrations in the rumen, and pH, were analysed as
15 repeated measures following the model

$$16 \quad y = \mu + D_i + A_{j(i)} + T_k + DT_{ik} + \epsilon_{l(ijk)}$$

17 where D_i (5 df) represents the diet effect, $A_{j(i)}$ (22 df) the animal effect within diet, T_k (1 df) the
18 sampling time effect, DT_{ik} (5 df) the interaction between diet and time and $\epsilon_{l(ijk)}$ (5 df) the
19 experimental error. Diet effect was compared to animals within diets whereas all other factors were
20 compared to the experimental error. The same two animals which were removed from the intake
21 and digestibility analysis were withdrawn. Orthogonal contrasts were performed separately for each
22 sampling time. Slow fractional rates (k_1) of both Yb-labelled particles and Cr-EDTA were analysed
23 as a split-plot, taking into account the animal effect (main plot), the marker type (subplot) and the
24 interaction between diet and marker. The same four animals from treatment PL used in the rate of
25 intake analysis were used here, and the orthogonal contrasts were done separately for each marker.
26 All calculations were performed with the SAS (version 8.01) statistical package, using the PROC

1 MIXED procedure and the *lsmeans* statement, and following the recommendations given by Littell
2 et al. (1998) (for repeated measures) and Kaps and Lamberson (2004). As there were only two
3 sampling times for rumen fermentation parameters (pH, and concentration of VFA and ammonia-
4 N), the compound symmetry structure of the variance was assumed (Littell et al., 1998). Contrasts
5 between mean values were tested using the Scheffe's test, recommended when there are missing
6 values (Kaps and Lamberson, 2004).

7

8 **Results**

9 Animals fed with PL showed the highest intakes and nitrogen digestibility coefficients
10 (P<0.0001) but the lowest DMD, OMD and NDF digestibility (NDFD) values (Table 2).
11 Differences between PL and Control were not significant for DM and OM digestibility, but they
12 were for NDFD. Both linear and quadratic components of the regressions of DM (P=0.024 and
13 0.009, respectively), ME (P<0.0001 and 0.006, respectively) and digestible OM intake (P=0.0004
14 and 0.006, respectively) on sucrose proportion in the diet were significant, reflecting increasing
15 values from Control to S45 and then much lower figures for S60. In the case of NDF intake, linear
16 (P<0.0001) and quadratic (P=0.019) components of the regression were significant too, but
17 increases occurred only from Control to S15 with a marked decrease from this to S60. Forage
18 intake decreased with sucrose proportion in the diet from S15 to S60, both linear (P<0.0001) and
19 quadratic (P=0.017) components of the regression being significant, whereas sucrose intake
20 increased from S15 to S45 and then remained unchanged with further addition to the diet up to 60%
21 of total DM. In this case also, linear (P<0.0001) and quadratic (P=0.008) components of the
22 regression were significant. For digestibility coefficients, only the linear component of the
23 regressions was significant, showing a constant increase with level of sucrose for digestibility of
24 DM, OM and N, and a constant decrease for NDFD.

25

(Table 2 here)

1 Both linear and quadratic components of the regression were also significant for cumulative
2 DM intake up to 3 h after feeding (Table 3), with the highest values at S15. Cumulative DM intake
3 at 4 and 5 h after feeding decreased linearly with sugar inclusion in the diet ($P < 0.0001$) but
4 followed a quadratic evolution for the 24 h period, with the highest value at S15, then a plateau and
5 finally a decrease at S60. S60 showed the lowest cumulative intake regardless of the time of
6 recording. As expected from Table 2, PL had the highest cumulative intake for the whole day
7 period. Cumulative sucrose intake (g/kg W) was similar for the first 5 h, with average values of
8 0.87, 0.82, 0.92 and 0.76 g/kg W.h for diets S15, S30, S45 and S60, respectively but very different
9 between diets for 24h. Cumulative NDF intake (g/kg W; Fig. 1) followed a pattern similar to that of
10 DM intake, with vastly different values across the range of sucrose addition.

11 (Figure 1 here)

12 (Table 3 here)

13 There were significant effects of diet type and sampling time (before and 4 h after feeding)
14 on rumen pH, ammonia concentration and total volatile fatty acid concentrations, and molar
15 proportions of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids (Table 4).
16 However, only diets means are presented as time effects were minor quantitatively except for
17 rumen ammonia which increased markedly after feeding from 39 to 87 mg $\text{NH}_3\text{N/L}$.

18 (Table 4 here)

19 The k_1 fractional outflow rates for passage of Cr-EDTA and Yb labelled particles were
20 affected by both diet type and marker ($P = 0.0001$; Table 2). As expected, fractional outflow rates of
21 Cr-EDTA were faster than those of Yb-labelled particles, whereas the higher values appeared in
22 animals fed lucerne. Both linear ($P = 0.024$) and quadratic ($P = 0.028$) components of the regression
23 on sucrose proportion were significant for Yb, although they were not for Cr ($P = 0.166$ and 0.600 ,
24 respectively).

25

26 Discussion

1 The lambs in this experiment exhibited a classical intake substitution effect of the basal hay
2 diet for inclusion of sucrose in the total mixed ration (TMR) with a substitution rate of 0.6 kg
3 forage DM/kg sucrose addition over the whole treatment range (Table 2). In summary, the data
4 indicate that with increasing level of sucrose inclusion there was a curvilinear increase and plateau
5 at around S15 in total DMI, a quadratic effect on DOMI with a peak around S45, a quadratic effect
6 on hay intake with a peak around S15 and thereafter the classical decline associated with
7 substitution with a similar effect on NDF intake, a linear increase in DMD and OMD but a marked
8 depression in NDFD from around S30. The values for DMI and DOMI were all much higher for the
9 pelleted lucerne diet but the DMD, OMD and NDFD were much lower. The data indicated similar
10 patterns to Gherardi and Black (1989) and Weston (1996) but the actual DMI and DOMI values
11 were lower. This suggests that the intake of the hay of the control treatment, as set by largely
12 physical mechanisms, given the digestibility and fractional outflow data, provided the maximum
13 level of NDF which could be processed through the rumen for this hay and controlled the intake
14 response to sucrose inclusion. It is suggested that if a different higher quality hay was used with a
15 higher intake of the hay control diet as a consequence of the physical NDF characteristics and
16 retention time of NDF setting the higher intake then the same pattern of substitution with addition
17 of sucrose would occur. Thus passage of small particles may not be the main limitation of retention
18 time as suggested by Poppi *et al.* (1981) and the rumen mat structure with its hindrance of particle
19 movement within the raft and its resistance to collapse under digestion (Poppi *et al.* 2001)
20 especially with C4 plants may provide the major control of intake with limited ability to markedly
21 increase intake by attempts to increase fluid passage from the rumen. Small increases in soluble
22 carbohydrate levels (up to approximately 15% units increase), such as could be achieved through
23 plant breeding, pasture management or supplement addition (e.g. molasses), appeared to increase
24 intake and DOMI of the basal diet but thereafter substitution occurred. For further significant
25 increases in intake the underlying structural and digestion characteristics of the NDF fraction have
26 to be changed. There was no evidence that the greater rate of release of nutrients through sucrose

1 addition could markedly increase fractional outflow rate of Cr-EDTA but rate of passage of Yb
2 labelled small particles increased.

3 The plateau levels of DMI and DOMI which were reached at the first level of sucrose
4 inclusion (15%) were 44 and 50%, respectively, of the intake and DOMI achieved by those animals
5 consuming pelleted lucerne. They were also 40% of the DOMI achieved by lambs grazing high
6 quality white clover or lucerne (Cruickshank *et al.* 1992). The animals were clearly not regulating
7 intake to achieve a high NE intake. There was a quadratic relationship between sucrose % and hay
8 intake (Table 2) of:

$$\begin{aligned} \text{Hay intake (g DM/kg W.day)} &= 17.96 + 0.0176 \% \text{ Sucrose} - 0.0028 \% \text{ Sucrose}^2 \\ & \quad (n = 20; r^2 = 0.74) \end{aligned}$$

11 This agrees in principle with similar curves proposed by Weston (1996). However, DOMI
12 reached a quadratic peak around the 45% sucrose level but this level was not as high as could be
13 achieved by other diet types as evidenced by the results from pelleted lucerne and an estimated NE
14 intake compared to NE requirement for high growth rate. Thus intake was not limited by NE supply
15 meeting requirement. NDFD declined from around 30% sucrose inclusion onwards which suggests
16 that sucrose was depressing the digestion of NDF in the rumen and so the decline in hay intake was
17 limited by a physical mechanism (Table 2).

18 This aspect was studied by examining the rate of passage of NDF by reference to Yb-
19 labelled particles (Table 2). The fractional outflow rate of these small particles appeared to increase
20 with level of inclusion of sucrose and this would reduce retention time not increase retention time
21 of NDF in the rumen. This increase in fractional outflow rate might be related to the greater release
22 of nutrients and increased rumen osmolarity (Beever *et al.* 1980-81) but there was no linear or
23 quadratic effect of sucrose level on fractional outflow rate of CrEDTA within the fluid medium
24 which would carry particles out of the rumen. However conditions within the rumen depressed
25 NDFD (Table 2) suggesting less time for digestion of NDF within the rumen (consistent with the
26 marker data) or a depression in rate of NDF digestion under the rumen conditions established by a

1 high sucrose load (Huhtanen and Khalili, 1992). The major difference between a study of this type
2 and one where NDF level is decreased by choice of forage, e.g. temperate forages, is the
3 characteristics of the underlying NDF fraction. When DMI and DOMI of the control and sucrose
4 inclusion diets are compared to those of the pelleted lucerne (Table 2), temperate grass or legume
5 diets (Cruickshank *et al.* 1992) the values are always much lower despite comparisons at similar
6 NDF levels. Pelleted lucerne had a NDF value (41%) similar to S45 (37%) whilst temperate
7 legumes had a NDF content of 19% and temperate grasses 39% (Cruickshank *et al.* 1992). The
8 values for DMI and DOMI for temperate legumes were 35 and 30, and for temperate grasses 27 and
9 22 g/kgW.day, respectively, with NDFD of 74 and 82%, respectively (Cruickshank *et al.* 1992).
10 This suggests that the digestion and passage characteristics of the underlying NDF fraction drive
11 the level of intake rather than the level of NDF *per se*. A tropical grass cannot be manipulated to
12 have the same level of intake as a temperate grass or legume simply by varying the level of non-
13 structural carbohydrates without tackling the characteristics which determine retention time of NDF
14 in the rumen, *viz* rate of particle breakdown, potential digestibility of NDF, rate of NDF digestion
15 and rate of passage of small particles. These are all much lower for tropical forages compared to
16 temperate forages (Poppi *et al.* 1981). De Vega and Poppi (1997) also showed that the rumen
17 conditions as set by the diet type had most influence on fractional outflow rate of small particles
18 labelled with a marker rather than the dietary source of the small particles which further emphasises
19 that the conditions established in the rumen by the underlying NDF characteristics of the diet drive
20 intake. Thus a base level of intake is set by the NDF characteristics of the hay around which
21 nutrient addition will modify it but the intake and retention time of low quality hay NDF will never
22 reach the values for high quality hay NDF even with nutrient addition.

23 Weston (1996) further proposed that the signals controlling the meal pattern might be
24 invoked to maintain the internal homeostasis but that the average NE intake over several days may
25 manipulate these to achieve a NE intake as close as possible to the NE required. By varying sucrose
26 content, the rate of digestion and release of nutrients presumably increased with sucrose content.

1 The animals appeared to regulate intake within the short term of 5h to achieve a reasonably
2 constant sucrose intake (Fig. 1) but vastly different NDF intake.

3 Changes in VFA pattern and rumen ammonia reflected the supply of sucrose and had no
4 association with intake of NDF. The higher butyric acid with sucrose fermentation has been found
5 previously (McLennan *et al.* 1998). pH values were within normal ranges.

6 It may be concluded that addition of sucrose increased total DM intake and DOMI but not
7 as much as expected because the underlying NDF characteristics of the hay posed a physical upper
8 limit which could not be overridden. At very high levels of sucrose addition some metabolic
9 interaction appeared to further limit intake.

10

11 **Acknowledgements**

12 A. de Vega was in receipt of a fellowship under the OECD Co-operative Research
13 Programme: Biological Resource Management for Sustainable Agricultural Systems. The skilled
14 technical assistance of M. Nielsen, T. Hodby, A. Gibbon and J. Hales is gratefully acknowledged.

15

16 **References**

17 Beaver DE, Black JL, Faichney GJ (1980-81) Simulation of the effects of rumen function on the
18 flow of nutrients from the stomach of sheep, part 2-assessment of computer predictions.
19 *Agricultural Systems* **6**, 221-241.

20 Cruickshank GJ, Poppi DP, Sykes AR (1992) The intake, digestion and protein degradation of
21 grazed herbage by early-weaned lambs. *British Journal of Nutrition* **68**, 349-364.

22 de Vega A, Poppi DP (1997) Extent of digestion and rumen conditions as factors affecting passage
23 of liquid and digesta particles in sheep. *Journal of Agricultural Science, Cambridge* **128**, 207-
24 215.

25 Downes AM, McDonald IW (1964) The chromium-51 complex of ethylenediaminetetraacetic acid
26 as a soluble rumen marker. *British Journal of Nutrition* **18**, 153-162.

- 1 Egan AR (1977) Nutritional status and intake regulation in sheep. VIII. Relationships between the
2 voluntary intake of herbage by sheep and the protein/energy ratio in the digestive products.
3 *Australian Journal of Agricultural Research* **28**, 907-915.
- 4 Gherardi SG, Black JL (1989) Influence of post-ruminal supply of nutrients on rumen digesta load
5 and voluntary intake of a roughage by sheep. *British Journal of Nutrition* **62**, 589-599.
- 6 Grovum WL, Williams VJ (1973) Rate of passage of digesta in sheep. 4. Passage of marker through
7 the alimentary tract and the biological relevance of rate constants derived from the changes
8 in concentration of marker in faeces. *British Journal of Nutrition* **30**, 313-329.
- 9 Huhtanen P, Khalili H (1992) The effect of sucrose supplements on particle-associated
10 carboxymethylcellulase (EC 3.2.1.8) activities in cattle given grass-silage-based diets. *British*
11 *Journal of Nutrition* **67**, 245-255.
- 12 Jouany JP (1982) Volatile fatty acid and alcohol determination in digestive contents, silage juices,
13 bacterial cultures and anaerobic fermentor contents. *Science des Aliments* **2**, 131-144.
- 14 Kaps, M., Lamberson, W., 2004. Biostatistics for Animal Science. CABI Publishing, Wallingford,
15 UK.
- 16 Littell, R.C., Henry, P.R., Ammerman, C.B., 1998. Statistical analysis of repeated measures data
17 using SAS procedures. *J. Animal Science* **76**, 1216–1231.
- 18 McLennan SR, Leng RA, Nolan JV (1998) Whole cottonseed supplements for cattle given a
19 molasses-based diet. *Animal Production in Australia* **22**, 141-144.
- 20 Panjaitan, T. Quigley, SP, McLennan, SR, and Poppi, DP (2010). Effect of concentration of *Spirulina*
21 (*Spirulina platensis*) algae in the drinking water on water intake by cattle and the proportion of
22 algae bypassing the rumen. *Animal Production Science* **50**, 405-409.
- 23 Poppi DP, Gill M, France J (1994) Quantification of theories of intake regulation in growing
24 ruminants. *Journal of Theoretical Biology* **167**, 129-145.

- 1 Poppi DP, Minson DJ, Ternouth JH (1981) Studies of cattle and sheep eating leaf and stem
2 fractions of grasses. II Factors controlling the retention of feed in the reticulo-rumen. *Australian*
3 *Journal of Agricultural Research* **32**, 109-121.
- 4 Poppi, DP, Ellis, WC, Matis, JH and Lascano, CE (2001). Marker concentration patterns of
5 labelled leaf and stem particles in the rumen of cattle grazing bermuda grass (*Cynodon dactylon*)
6 analysed by reference to a raft model. *British Journal of Nutrition* **85**, 553-564.
- 7 Standing Committee on Agriculture. Ruminants Subcommittee (1990) 'Feeding standards for
8 Australian livestock. Ruminants.' (CSIRO Publishing: Melbourne)
- 9 Thomas TA (1977) An automated procedure for the determination of soluble carbohydrates in
10 herbage. *Journal of the Science of Food and Agriculture* **28**, 639-642.
- 11 Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber,
12 and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583-
13 3597.
- 14 Weston RH (1996) Some aspects of constraint to forage consumption by ruminants. *Australian*
15 *Journal of Agricultural Research* **47**, 175-197.

16

17

1 **Table 1. Composition of the final mixtures given to the animals. A 4% (on dry matter basis) of**
 2 **mineral + vitamin premix was also included in Control and S15 to S60 diets (for its**
 3 **composition see the Materials and Methods section)**

	Control	S15	S30	S45	S60	PL
4 Mineral + vitamin premix %	4	4	4	4	4	0
5 Sucrose %	0	15	30	45	60	0
6 Urea %	0.6	1.1	1.6	2.0	2.5	0
7 Dry matter %	66.3	67.3	68.2	69.2	70.3	90.1
8 Organic matter %	89.8	90.7	91.6	92.8	93.8	89.5
9 Neutral detergent fibre %	70.5	58.7	48.9	36.9	26.3	40.8
10 Crude protein %	8.3	9.0	9.4	10.1	11.1	20.8
11 g CP/kg DOM	151	148	150	147	159	391
12 Water soluble carbohydrates %	4.4	18.7	33.1	47.4	61.8	6.2
13 Estimated M/D	7.9	9.1	9.5	10.6	10.8	7.8

15 Control: pangola hay plus urea solution; S15: pangola hay plus 15% of total dry matter as sucrose
 16 plus urea; S30: pangola hay plus 30% of total dry matter as sucrose plus urea; S45: pangola hay
 17 plus 45% of total dry matter as sucrose plus urea; S60: pangola hay plus 60% of total dry matter as
 18 sucrose plus urea; PL: pelleted lucerne)

19 CP/DOM (g CP/kg DOM) values from Table 1 and 2.

20 M/D: MJ of metabolizable energy per kg DM estimated from $M/D = 0.18 \text{ DOMD}\% - 1.8$ (SCA
 21 1990; $\text{DOMD}\% = \text{OM}\% \times \text{OMD}\% / 100$; values of OMD are given in Table 2)

22

1 **Table 2. Dry matter intake (DMI), neutral detergent fibre intake (NDFI) digestible organic**
2 **matter intake (DOMI) (g/(kg W.day)), forage intake (Forage), sucrose intake (Sucrose) (g**
3 **DM/(kg W.day)) and estimated metabolizable energy intake (MEI; kJ/(kg W.day)), and**
4 **digestibility coefficients (%) of dry matter (DMD), organic matter (OMD), nitrogen (ND) and**
5 **neutral detergent fibre (NDFD) of the diets used in the experiment and measured fractional**
6 **outflow rate (h⁻¹) of Cr-EDTA and Yb labelled particles from the rumen.** Within rows, values
7 followed by the same letters are not significantly different at P=0.05. * Probability of the linear and
8 quadratic contrasts between the variables and the level of sucrose in the diet. PL was excluded from
9 this analysis.

	Control	S15	S30	S45	S60	PL	P*	
							Linear	Quadratic
12 DMI	18.9 a	25.5 b	24.2 b	28.3 b	23.6 ab	57.7 c	0.0241	0.0089
13 Forage	17.4 cd	19.0 d	15.1 bc	12.9 b	9.3 a	57.7 e	<0.0001	0.017
14 Sucrose	0.0 a	5.8 b	8.5 c	14.5 d	13.4 d	0.0 a	<0.0001	0.008
15 NDFI	13.1 cd	14.3 d	11.4 bc	9.7 b	7.0 a	23.6 e	<0.0001	0.019
16 DOMI	10.2 a	15.5 bc	15.2 b	18.6 c	15.9 bc	30.9 d	0.0004	0.006
17 MEI	150 a	232 b	230 b	300 c	255 bc	451 d	<0.0001	0.006
18 DMD	60.0 a	66.4 b	67.5 b	73.0 c	73.2 c	58.3 a	<0.0001	0.144
19 OMD	60.3 a	67.0 b	68.4 b	74.0 c	74.6 c	59.4 a	<0.0001	0.125
20 ND	40.1 a	49.6 b	49.3 b	62.9 c	62.3 c	70.0 c	<0.0001	0.601
21 NDFD	69.5 d	67.9 d	61.6 c	60.4 bc	54.2 b	44.2 a	<0.0001	0.587
22 Cr-EDTA	0.039 ^a	0.044ab	0.041ab	0.048b	0.045abc	0.062c	0.166	0.600
23 Yb particles	0.024 ^a	0.029ab	0.031b	0.034ab	0.030ab	0.053c	0.024	0.028

24
25 Hay and Sucrose intake were calculated assuming all NDF in refusals were from Pangola. Sucrose
26 intake was corrected for urea contents in the solutions. Premix was assumed to be completely
27 consumed with the hay.

1 DMI and Forage intake are not the same for Control because DMI includes forage, urea solution
2 and premix.

3 Average liveweights of the groups were 30.1, 28.8, 29.0, 27.1, 27.1 and 34.8 kg for Control, S15,
4 S30, S45, S60 and PL, respectively.

5

1 **Table 3. Cumulative DM intake (g/kg W), at different recording intervals, on the last day of**
 2 **the experiment.** Within rows, values followed by the same letters are not significantly different at
 3 P=0.05. * Probability of the linear and quadratic contrasts between the variables and the level of
 4 sucrose in the diet. PL was excluded from this analysis.

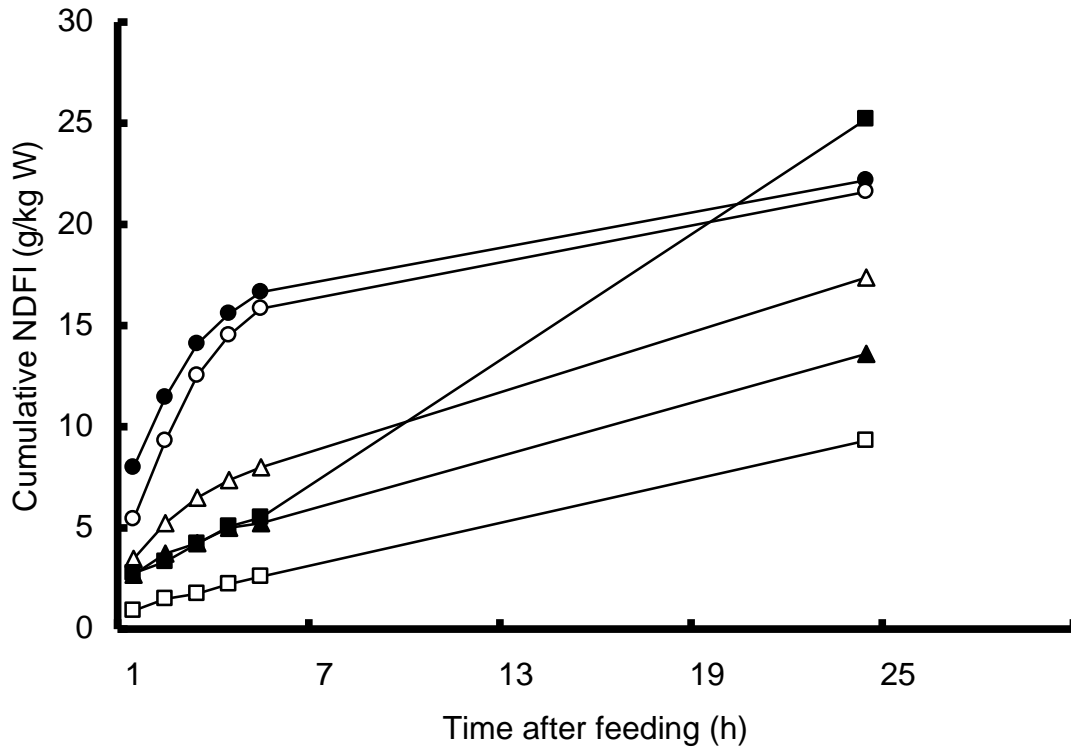
	Control	S15	S30	S45	S60	PL	P*		
							Linear	Quadratic	
5	<hr/>								
6	Cumulative DM intake								
7									
8	0-1 h	7.9 b	14.2 c	7.5 b	7.6 b	3.1 a	6.8 b	<0.0001	0.0002
9	0-2 h	13.5 bc	20.3 c	11.2 b	10.6 b	5.1 a	9.1 ab	<0.0001	0.0042
10	0-3 h	18.1 c	25.0 d	13.9 bc	12.0 bc	5.9 a	11.2 ab	<0.0001	0.0458
11	0-4 h	21.0 c	27.7 d	15.7 bc	14.2 b	7.5 a	13.1 ab	<0.0001	0.0650
12	0-5 h	22.9 c	29.6 d	17.1 bc	14.9 ab	8.7 a	14.0 ab	<0.0001	0.0780
13	0-24 h	31.2 a	39.4 b	37.0 ab	39.2 b	31.1 a	60.8 c	0.9530	0.0022
14	<hr/>								

14
 15
 16
 17
 18
 19
 20
 21
 22
 23
 24
 25

Table 4. Mean of before (0 h) and 4 h after feeding of rumen pH, ammonia concentration (NH₃N; mg/L) and total volatile fatty acid concentrations (VFA; mmol/L), and molar proportions (%) of acetic (Ac), propionic (Prop), butyric (But), iso-butyric (Ibut), valeric (Val) and iso-valeric (Ival) acids. Within rows, values followed by the same lower case letters are not significantly different at P=0.05. RSD: Residual standard deviation of the mean for comparisons between diets. Individual time values not presented.

		Diet (D)						Significance			
		C	S15	S30	S45	S60	PL	RSD	D	Time (T)	D x T
7	pH Mean	7.21bc	7.10b	6.92b	6.92b	6.56a	7.57c	0.366	0.0006	<0.0001	0.7125
8	NH ₃ N Mean	43.2	88.1	86.2	52.1	29.4	78.4	50.36	0.0778	0.0015	0.0187
9	VFA Mean	48.2	51.3	45.0	46.6	55.1	69.8	16.14	0.0873	0.0092	0.3283
10	Ac Mean	78.7c	71.5b	64.6a	61.6a	63.4a	72.4b	5.11	<0.0001	0.0003	0.0061
11	Prop Mean	15.7a	19.2abc	21.0bc	17.0ab	23.7c	20.6abc	5.07	0.0250	0.1568	0.9103
12	But Mean	3.8a	7.8ab	12.7c	18.3d	10.8bc	5.4a	4.65	<0.0001	<0.0001	0.0007
13	Ibu Mean	0.9b	0.6a	0.6a	0.5a	0.4a	0.6 ^a	0.21	0.0015	0.0007	0.2911
14	Val Mean	0.3a	0.3ab	1.0ab	2.2c	1.0b	0.7ab	0.78	0.0003	0.2435	0.1926
15	Ival Mean	0.7	0.5	0.3	0.4	0.7	0.4	0.55	0.5355	0.0395	0.1723

1



2

3

Fig. 1.

4

5

6

7

8

9

10

11

12

13

14

15

16

17

1 **Fig. 1.** Cumulative neutral detergent fibre intake (NDFI, g/kg W) of the different diets used in the
2 experiment (○, Control: pangola hay; ●, S15: pangola hay plus 15% sucrose; △, S30: pangola hay
3 plus 30% sucrose; ▲, S45: pangola hay plus 45% sucrose; □, S60: pangola hay plus 60% sucrose;
4 ■, PL: pelleted lucerne) measured at times 1, 2, 3, 4, 5 and 24 h after feeding..

5