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# Effects of Herbage Allowance on Dry Matter Intake, Efficiency of Grazing, Milk Yield and Grazing Behaviour of Crossbred Holstein-Jersey Dairy Cows Grazing Alfalfa Pastures

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**Abstract**

The objectives of this study were to research the effects of herbage allowance (HA) on dry matter (DM) intake, milk yield, grazing efficiency and grazing behaviour of dairy cows grazing alfalfa (*Medicago sativa L.*) pastures, and to compare the estimates of herbage DM intake using either an external intake marker developed from a purified enriched lignin (LIPE®) or the animal performance method, the latter based on calculations of energy requirements.

Twenty six spring calving crossbred Holstein-Jersey dairy cows (84 days in milk) were balanced and assigned to either the low HA (Lo-HA; 10.0 kg DM/cow/day) or the high HA treatment (Hi-HA; 14.5 kg DM/cow/day). Cows were offered alfalfa (Medicago sativa L.) pastures at grazing, Sorghum (Sorghum bicolor) silage (3.7 kg DM/cow/day) and concentrates (5.6 kg DM/cow/day). Herbage DM intake was estimated using the sward cutting method, the animal performance method and an indigestible intake marker (LIPE®). Decreasing herbage allowance (HA) decreased herbage DM intake by 1.1 kg cow/day (P <0.05), increased the efficiency of grazing by 210 g/kg (P <0.01), increased silage DM intake by 0.3 kg cow/day (P <0.05), decreased total metabolisable energy intake by 12 MJ ME cow/day (P <0.05), but did not affect total DM intake (P >0.05). The cows on the Lo-HA treatment produced less milk (-4.1 kg cow/day (P <0.05) and grazed 26 min/day longer (P <0.05) than cows at the Hi-HA. Herbage DM intake measured with LIPE® was positively correlated with estimates of herbage DM intake from the animal performance method (R=0.65, P<0.01).

This study showed that cows at the Hi-HA treatment had greater herbage DM intake and milk yield, but had an efficiency of grazing 210 g/kg lower than cows at the Lo-HA in early lactation.

**Keywords:** Intake marker; LIPE; Holstein-Jersey; Milk composition; Early lactation.

## Introduction

In countries where the price of milk paid to farmers is low, such as Argentina [1], production costs need to be low, and this can be achieved by efficiently grazing pastures, the cheapest source of feed. In this situation, stocking rate (SR), which is closely related to herbage allowance, is a major factor determining the efficiency of the system [2,3].

Alfalfa (*Medicago sativa L.*) pasture is the main source of feed for dairy cows in Argentina [4], in combination with concentrates and conserved roughages. The efficiency of grazing of alfalfa pastures in Argentina is low, usually less than 650 g/kg DM [5]. The relationship between herbage allowance (HA) and herbage dry matter (DM) intake was studied for dairy cows grazing alfalfa pastures in Argentina [6-8], but in these experiments, herbage DM intake (HDMI) was estimated for a group of animals, from DM disappearance in the sward, using the sward cutting method, rather than for individual animals [9]. It is expected that, as HA decreases, herbage dry matter (DM) intake/cow and individual milk yield decreases, but grazing time and the efficiency of grazing increases [10].

The objectives of this study were i) to compare herbage and total DM intake per cow, milk yield, grazing efficiency and grazing behaviour of supplemented cows in early lactation, grazing alfalfa pastures at low HA (Lo-HA) or high HA (Hi-HA) and ii) to compare the estimates of individual herbage DM intake using either an external intake marker developed from a purified enriched lignin (LIPE®) [11] or the animal performance method, the latter based on calculations of energy requirements.

## Materials and Methods

### Experimental design

The experiment was carried out at Instituto Nacional de Tecnología Agropecuaria (INTA), Rafaela, Argentina, at latitude 31.11S, longitude 61.33W and altitude 99m. This study started on 19 September (Day 1) and finished on 26 September (Day 8) 2008. Twenty six crossbred Holstein-Jersey dairy cows were temporarily withdrawn from a 2 year farmlet experiment which explored the effects SR of 1.6 (low SR) and 2.6 cows/ha (high SR) on farm efficiency [12].

Thirteen cows from the high SR farmlet were allocated to the Lo-HA treatment (10.0 kg DM/cow/day) and 13 cows from the low SR farmlet were allocated to the Hi-HA treatment (14.5 kg DM/cow/day).

The present experiment was approved by Massey University Animal Ethics Committee: MUAET protocol 08/49.

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## Experimental animals

Cow groups were balanced on the basis of days in milk (84 ± 14), lactation number (2.5 ± 0.64), proportion of Jersey genes (0.49 ± 0.16) with a range from 0.75 Holstein to 0.75 Jersey genes in individual cows, body weight (BW) at calving (455 ± 56 kg) and a feed conversion efficiency (FCE) index (1.13 ± 0.42 g milk fat plus crude protein (MFP) per kg DM). The FCE index was calculated from the previous lactation for each cow, as grams of MFP produced/kilogram of DM consumed [13]. On Day 1 of the experiment, body condition score averaged 2.5 ± 0.17 measured on a 5 point body condition score (BCS) scale [14].

**Animal management before the experiment [12]:** Calving occurred from 1 July to 30 September 2007 and from 3 July to 16 September 2008. Dry off (i.e., termination of milking) policy was based on management decision rules. Thus, cows were dried 60 days before their expected calving date, or when their daily milk fat plus crude protein production was less than 0.7 kg for two consecutive herd tests in autumn. In addition, cows with BCS less than 2.5 were dried after 9 April (i.e., average lactation length of 8 months).

## Feeding management

**Feeding management during the experiment:** Cows were kept in two different herds and each herd grazed a different paddock of alfalfa pastures. Electric fencing was used within paddocks to allocate a fresh strip of pasture for each herd after each milking (4:30 h and 15:00 h) to give target HA of 10.0 and 14.5 kg DM/cow/day (measured 5 cm above ground level) for the Lo-HA and Hi-HA, respectively. The target HA (kg DM offered/cow/day) was achieved on the basis of pre-grazing herbage mass (HM; kg DM/ha), daily grazing area (m2 of the pasture strip) and the number of cows.

At 9:00 h, cows were retrieved from pasture for LIPE® dosage and faecal sampling, in a specific place besides the milking shed. Pastures were located at 230 (Hi-HA) and 245 meters (Hi-HA) from the milking shed. After faecal sampling, cows were offered 3.74 ± 0.15 kg DM/cow/day of Sorghum silage (Sorghum bicolour) in individual boxes. Each box was placed within individual plots (3m x 5m), which were divided by electric fences, and located in a specific area, at 90 meters of the milking shed. Silage offered and rejected was weighted in individual bags, and silage intake was calculated from difference between silage offered and refused. Cows were removed from these plots after all cows in the treatment had stopped eating silage. Each cow received 2.8 ± 0.12 kg DM of concentrate twice daily at milking, individually through an automatic feeding system. Silage DM offered and rejected and concentrate DM offered to each cow were weighed from Day 4 to Day 8. Refusals of concentrates during milking were negligible, and were considered to be nil. Animals had free access to water the whole day, except the time around milking. Cow management was as follows: morning milking (4:00 h), grazing (after milking), faecal sampling (9:10 h), silage feeding in plots, grazing, afternoon milking (14:30 h), grazing (from finishing of afternoon milking until morning milking).

**Feeding management before the experiment [12]:** During the two-years farmlet experiment, cows grazed alfalfa pastures during the whole lactation and were individually offered concentrates twice daily through an automatic feeding system during milking. All cows were offered the same amount of concentrate per day. The concentrate ingredients were, on a proportional DM weight basis: wheat bran, 0.35; sorghum grain (high condensed tannins), 0.23; maize grain, 0.18; soya bean meal, 0.17; limestone flour, 0.03; mineral and vitamin mixture, 0.04. Annual intakes of concentrate were 1778 and 1780 kg DM/cow/year for the low and high SR treatments, respectively. Sorghum silage with a high condensed tannin concentration was offered from April to October. Silage intakes were 1130 and 965 kg DM/cow/year for the low and high SR treatments, respectively. Uniform management was applied to all cows in the farmlet experiment [12]. It was intended to optimise grazing rotation by grazing alfalfa pastures as closely as possible to their optimum growth stage, which is 10% bloom in spring–summer [15]. Mechanical cutting (topping) of pasture residuals to 5.0 cm was completed after every grazing to maintain pasture quality. A bloat preventative, ethoxylate alcohol and pluronic detergents (Bloker, Biotay, Argentina), was offered to cows fed with concentrate during milking at a rate of 80 g/t live weight.

## Sward Measurements

**Herbage mass**: Herbage mass (>5 cm above ground level) was measured daily from Day 3 to Day 8. Pre-grazing herbage mass (HM) measurements were made from the next strip to be grazed and the post-grazing measurements were made from the same strip, immediately after the cows left the paddock. Herbage samples were collected with scissors from 12 randomly allocated quadrats of 50 cm × 50 cm. A pooled sample from each paddock of each treatment was made from mown herbage every fourth quadrats (1 $m^2$). Each of the resultant three pooled samples (12 quadrats ÷ 4) was immediately fresh weighed, sub-sampled (0.2 kg), dried at 60°C in a forced air oven until constant weight to determine DM content, ground to pass through a 1 mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and stored for analyses.

### Herbage DM intake based on sward cutting method

Herbage DM intake based on sward cutting method (HDMIc) was calculated to estimate group intake as follows:

HDMIc (kg DM cow/day) = (Pre-grazing HM – post-grazing HM) (daily grazing area / number of Cows).

Herbage growth between pre- and post-grazing samples was not accounted for because samples were within a 24 h period.

Herbage allowance and efficiency of grazing were calculated as follows:

**Herbage allowance (HA)-:** HA (kg DM cow/day) = Pre-grazing HM × daily grazing area / number of cows

Efficiency of grazing (EG) [16]

EG (at a single grazing, g/kg) = (HDMIc/cow) ÷ (HA/cow) × 1000

**Botanical composition:** The botanical composition of the herbage was determined once during the experimental period for each treatment. Twenty five samples of herbage of approximately 0.25 kg were collected from each paddock, randomly, to a cutting height of 5 cm above ground level with scissors before grazing. Fresh herbage was immediately separated into its botanical constituents (i.e., alfalfa, clovers, weed and grasses and dead matter), and each constituent was dried in a forced air oven at 60°C until constant weight to determine the botanical composition on a DM basis.

The botanical composition (g/DM) for the Hi-HA paddocks was: alfalfa 0.92 ± 0.074, clovers 0.04 ± 0.048, weeds and grasses 0.02 ± 0.45 and dead matter 0.02 ± 0.017 and for the Lo-HA paddocks: alfalfa 0.91 ± 0.103, clovers 0.03 ± 0.039, weeds and grasses 0.03 ± 0.077 and dead matter 0.03 ± 0.017.

**Chemical composition:** Twenty five herbage samples were collected by a hand plucking technique [17] in the morning (8:30 h) from Day 4 to Day 8, following close observation of cows to simulate grazing in each treatment. Samples of concentrate and silage were collected daily from Day 4 to Day 7. Sub-samples of 0.2 kg of alfalfa pasture, silage and concentrates were weighed and dried at 60ºC in a forced air oven until constant weight to determine DM content. Dry samples were ground using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) to pass through a 1 mm screen and analyzed for crude protein (CP) [18; method 976.05], neutral detergent fibre (NDF) [19], acid detergent fibre (ADF) [18; method 973.18], sulphuric acid lignin (lignin(sa)) [20], ether extract [18; method 920.39], ash [18; method 924.05] and in vitro DM digestibility (IVDMD) [21]. Heat-stable amylase and sodium sulfite were used for NDF determination, and NDF and ADF are expressed inclusive of residual ash. Chemical composition and DM digestibility of feeds are shown in (Table 1). Overall, herbage quality was similar in both treatments.

## LIPE® dosing, faeces sampling and analysis

The intake marker LIPE® was developed from a purified enriched lignin extracted from Eucalyptus grandis [11]. It is a modified hydroxyphenylpropane, that has proven to be a reliable estimator of faecal output for several animal species, including bovines [11,22,23]. The intake marker LIPE® is used to evaluate individual HDMI, based on total faecal output estimations and feed digestibility.

Cows were dosed once a day (from 9:10 h to 10:15 h) with a gelatin capsule containing 500 mg of LIPE® for 7 consecutive days, starting on Day 1 and finishing on Day 7. Animals were restrained in a headbail and capsules were inserted into the esophagus utilising a balling gun. To make sure that capsules were not regurgitated, animals were observed individually for three minutes after being dosed with the LIPE® capsule, and careful inspection of the yard in which the cows were kept was performed for 20 minutes after dosing.

Concentrations of LIPE® reach equilibrium in faeces approximately 48 hours after initial dosage. Therefore, faecal samples were taken from the rectum of each cow once a day (from 9:10 h) for five consecutive days, from Day 4 of LIPE® dosage until Day 8. All faecal samples (5 samples × 26 cows) were processed and analyzed individually. Faecal samples were weighed and dried immediately after sampling at 60ºC in a forced air oven until constant weight to determine DM content. Samples were ground to pass through a 1 mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) and stored for analysis. Dry matter was also determined using an oven at 105ºC until constant weight.

Two mg of faecal sample and 300 mg of powdered Potassium bromide (KBr) were ground in an agate mortar and homogenised for 20 minutes. Pressed pellets were then obtained after 1 minute at constant pressure. Concentration of LIPE® in faeces was analysed by infrared spectroscopy using an infrared spectrometer VARIAN 800 FT-IR (Varian BV, Middelburg, and The Netherlands). Standard calibration curves for LIPE® were prepared by measuring the absorbance of five different concentrations of LIPE® in faeces to produce a regression equation relating concentration to absorbance, which had an R2 higher than 0.95. This equation allowed calculations of LIPE® concentration by measuring absorbance in faecal samples.

## Animal measurements

**Milk production:** Milk yields (kg/cow) were recorded daily whit individual milk meters at the two successive morning and evening milkings during the five days in which faecal samples were collected (Day 4 to Day 8). Milk samples (50 mL/cow/day) were also collected at these milkings. A weighted compound sample (five-days samples per cow) was made and analysed for fat, crude protein and lactose (g/kg of milk) by infrared methods (Milkoskan Model 4000, Foss Electric, DK-3400, Hillørod, Denmark).

**Total faecal output:** Concentrations of LIPE® reach equilibrium in faeces approximately 48 hours after initial dosage. Therefore, from Day 3, i.e., 72 hours after initial dosage, faecal concentrations of LIPE® were assumed constant and faecal output calculations were made by applying the following equation:

$$\text{Total faecal output (g DM/day)} = \frac{\text{(Dosed intake marker (g/day)}}{\text{(Marker concentration in faeces (g/g DM)}}$$

The recovery of a marker, which is the quantity of the marker obtained from the total collection of faeces expressed as a proportion of that consumed, is an important indication of its efficacy. The estimated total faecal output should be multiplied by the rate of recovery of the marker.

In experiments using LIPE®, faecal recovery rates were 102.6 g/100g for pigs, 95.9 g/100g for sheep [11,22,23], and 104.7 g/100g for beef cattle [23]. These recovery rates did not differ from 100g/100g/ (P >0.05). Therefore, a 100g/100g recovery rate was assumed for LIPE® in the calculation of total faecal outputs in the present study.

**Herbage DM intake calculated from faecal output and feed digestibility (HDMIm)**

Individual HDMI was determined from the total faecal output estimated for each cow and the group IVDMD value for consumed herbage. To obtain the faecal output from herbage (FOH), the faecal production associated with concentrate and silage was discounted from the total faecal output estimated with LIPE® and the remaining faecal material was attributed to the herbage intake [24]. The relative contributions of concentrates and silage to the total faecal output were estimated from the estimated intake of each feed for each cow and the IVDMD of each feed. The faecal output from herbage (FOH) was then calculated as follows:

$$\text{FOH} = \text{TFO} - (\text{C.DMI} \times (1 - \text{C.IVDMD}) + \text{S.DMI} \times (1 - \text{S.IVDMD}))$$

Where: TFO is the total faecal output estimated with LIPE®, C.DMI is the concentrate DM intake and C.IVDMD is the IVDMD of the concentrate, S.DMI is the silage DM intake and S.IVDMD is the IVDMD of the silage. Finally, herbage DM intake was calculated as follows:

$$\text{HDMIm (kg DM/cow/day)} = \text{FOH}/\ (1 - \text{H.IVDMD})$$

Where: H.IVDMD is the IVDMD of herbage.

**Herbage DM intake calculated from the animal performance method (HDMIa):** Herbage DM intake/cow was also calculated with the animal performance method (HDMIa), as the sum of metabolisable energy (ME) requirements for milk production, maintenance, pregnancy and BW change [25], minus ME consumed as supplements, i.e., silage and concentrates, divided by the estimated ME concentration of herbage.

**Body weight and body condition score:** The BW was recorded electronically, on two consecutive days immediately before and after the experimental period, using a portable weighing scale (Basculas Magris, HA 2000, Argentina). Body condition score was recorded on the same days, by two people simultaneously, using the 5 points BCS scale (1 =thin to 5 =fat) [14].

A regression polynomial of third order was fitted to actual BW measurements for each cow during lactation (15 measurements, 11 obtained every three weeks during lactation and four performed during experiment) as follows:

$$y_t = b_0 + b_1 t + b_2 t_2 + b_3 t_3$$

where yt represents BW at day t of lactation, while $b_0$, $b_1$, $b_2$ and $b_3$ are the regression coefficients of the polynomial equation. These parameters were estimated for each cow using the REG procedure of SAS [26]. Predicted BW for each day of lactation, for each cow, were then obtained from the polynomial equation. Daily BW changes during the experimental period were calculated as the difference between the predicted values from the polynomial equation across two consecutive days.

**Grazing behavior:** Visual observation of the 13 animals in each treatment was carried out from sunrise (6:30 h) to sunset (19:20 h) at intervals of 10 minutes, from Day 5 to Day 8. The following activities were recorded: grazing, walking, standing, standing ruminating, lying, lying ruminating, eating silage, other (faecal sampling and milking). Cows drinking were record as standing, cows grazing while lying were recorded as lying, cows ruminating at the same time as walking were recorded as walking. The activity observed was regarded as being effective over a 10 minutes period. Observations of behavior were made by two observers simultaneously, each located so as to view one of the herds.

### Statistical analyses

All data were analysed using SAS [26]. Animal variables were analysed considering cow as the experimental unit. Milk yield, milk fat content, milk crude protein content, HDMIm, HDMIa, total DM intake and grazing behaviour variables were averaged per cow for the experimental period (Day 4 to Day 8) and analysed using the MIXED procedure. The linear model included the fixed effects of HA and parity and proportion of Jersey genes and days in milk as covariables. Mean differences between treatment effects were declared significant at a probability <0.05.

Pre-grazing HM, HA and HDMIc were evaluated using the MIXED procedure. The linear model included the fixed effects of HA and the random effect of day of sampling and the interaction of HA x day of sampling, which was used as the error term to derive the F value for testing the significance of treatment effect.

The accuracy of HDMIm estimations, i.e., from LIPE® method, was tested against the estimates of HDMIa, i.e., animal performance method, using the mean square prediction error (MSPE) defined as [27]:

$$MSPE = \frac{1}{n}\sum_{i=1}^{n}(A_i - P_i)^2$$

where A is the HDMI calculated from ME requirements, P is the HDMI estimated using LIPE® and n is the number of pairs of values of A and P being compared. The fitness of the model was evaluated by the relative prediction error (RPE) defined as the ratio between the positive root square of the MSPE and the mean of the actual intake values (A). It was suggested [27] that a RPE value lower than 10% is an indication of satisfactory prediction, between 10% and 20 % indicates relatively good or acceptable predictions, and greater than 20% indicates poor predictions.

The concordance correlation coefficient (CCC) was also calculated to test the agreement between methods [28]. It is calculated as: CCC = $\rho \times C_b$, with ρ the Pearson correlation coefficient and $C_b$ the bias correction factor. The Pearson correlation coefficient reflects precision, i.e., degree to which the predicted against actual values cluster about the regression line. The bias correction factor reflects accuracy, i.e., degree to which the regression line adheres to the 45° line through the origin. A scale [29] has been used here to describe the degree of concordance, with: 0.21–0.40 being "Fair"; 0.41–0.60 being "Moderate"; 0.61–0.80 being "Substantial"; and 0.81–1.00 being "Almost perfect".

## Results

### Weather

From Day 4 to Day 8, when all measurements were taken, mean air temperature was 17.7 ± 2.54°C, minimum air temperature was 9.2 ± 3.34°C, maximum air temperature was 26.3 ± 2.12°C and no rainfall was recorded.

### Feed intake and efficiency of grazing

Pre-grazing HM was similar for both HA treatments (Table 2). Herbage DM intake was higher for Hi-HA than Lo-HA (P <0.05) as estimated by the LIPE® method and the animal performance method, but no differences were detected between treatments when intake was calculated from pre- and post-grazing HM measurements (Table 2).

Cows at Lo-HA had higher silage DM intake (P <0.05), which partially compensated for their lower HDMI, and therefore, total DM intake was similar for both treatments (Table 2). Total ME intake was higher (P <0.05) for Hi-HA cows and EG was 210 g/kg higher (P <0.05) for cows at Lo-HA than for cows at Hi-HA (Table 2).

| Chemical composition | Concentrate | Sorghum silage | Pasture (High HA) | Pasture (Low HA) |
|---|---|---|---|---|
| DM (g/kg) DM composition (g/kg) | 906 ± 9.2 | 363 ± 263 | 217 ± 7.2 | 228 ± 3.8 |
| CP | 154 ± 5.7 | 95 ± 3.8 | 242 ± 9.5 | 233 ± 10.7 |
| NDF | 241 ± 4.1 | 576 ± 4.8 | 432 ± 19.3 | 441 ± 13.7 |
| ADF | 95 ± 3.9 | 316 ± 0.7 | 182 ± 10.7 | 199 ± 9.6 |
| Lignin(sa) | 27 ± 4.9 | 65 ± 2.7 | 52 ± 8.2 | 52 ± 3.9 |
| Ether extract | 58 ± 3.0 | 38 ± 2.9 | 28 ± 5.4 | 29 ± 2.2 |
| Ash | 73 ± 2.8 | 133 ± 1.5 | 119 ± 11.1 | 121 ± 10.6 |
| IVDMD (g/kg) | 840 ±68.2 | 510 ± 28.5 | 82 ± 29.3 | 803 ± 21.6 |

CP: Crude Protein; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; lignin(sa): sulphuric acid lignin; IVDMD: in vitro Dry Matter Digestibility.

**Table 1:** Chemical composition and DM digestibility of the concentrates, silage and hand-plucked samples [16] of alfalfa based pasture (mean ± SD).

| | Herbage allowance | | | |
|---|---|---|---|---|
| | Hi-HA | Lo-HA | SEM | P value |
| Pre-grazing herbage mass (kg DM/ha) | 1743 | 1608 | 105.6 | 0.32 |
| Herbage allowance (HA, kg DM/cow/day) | 14.3 | 10.2 | 0.73 | 0.01 |
| Herbage intake[z] | | | | |
| Sward cutting method(HDMIc), kg DM/cow/day | 9.6 | 8.9 | 0.74 | 0.43 |
| Animal performance (HDMIa), kg DM/cow/day | 11 | 9.7 | 0.42 | 0.05 |
| LIPE® method (HDMIm), kg DM/cow/day | 10.8 | 9.7 | 0.37 | 0.05 |
| Efficiency of grazingy (EG, g/kg) | 666 | 872 | 337 | 0.01 |
| Silage consumed (kg DM /cow/day) | 3.1 | 3.4 | 0.08 | 0.05 |
| Concentrates consumed (kg DM) | 5.6 | 5.6 | - | - |
| Total intake (kg DM /cow/day) | 19.5 | 18.7 | 0.33 | 0.11 |
| Total ME intake (MJ ME/cow/day) | 227 | 215 | 4.2 | 0.03 |

zHerbage DM intake (HDMI). yCalculated using the sward cutting method (herbage DM intake/herbage allowance × 1000; g/kg DM) [15].

**Table 2:** Pre-grazing herbage mass, herbage allowance (HA), herbage DM intake estimated with LIPE®, animal performance and sward cutting methods, metabolisable energy (ME) intake, efficiency of grazing (EG) and supplements intake at the high and low herbage allowance treatments.

| | Herbage allowance | | SEM | P value |
|---|---|---|---|---|
| | **Hi-HA** | **Lo-HA** | | |
| Days in milk | 83 | 85 | 1.8 | 0.42 |
| Milk yield (kg/cow/day) | 33.2 | 29.1 | 1.84 | 0.02 |
| Fat yield (kg/cow/day) | 1.26 | 1.11 | 0.034 | 0.01 |
| Crude protein yield (kg/cow/day) | 1.13 | 1.00 | 0.032 | 0.01 |
| Milk composition (%) | | | | |
| Fat | 3.86 | 3.83 | 0.154 | 0.92 |
| Crude protein | 3.43 | 3.46 | 0.639 | 0.70 |

**Table 3:** Milk yield cow and milk composition at the high and low herbage allowance treatments.

| Time (min/day) | Herbage allowance | | SEM | P value |
|---|---|---|---|---|
| | **Hi-HA** | **Lo-HA** | | |
| Grazing | 258 | 284 | 7.2 | 0.04 |
| Standing and ruminating | 16 | 20 | 3.4 | 0.44 |
| Lying and ruminating | 23 | 14 | 3.5 | 0.10 |
| Standing[z] | 109 | 91 | 7.9 | 0.13 |
| Lying[y] | 112 | 58 | 7.7 | 0.01 |
| Walking[x] | 23 | 40 | 1.7 | 0.01 |
| Eating silage[w] | 125 | 157 | - | - |
| Other[w,v] | 103 | 105 | - | - |

[z]Cows drinking were record as standing.
[y]Cows grazing while lying were recorded as lying.
[x]Cows ruminating at the same time as walking were recorded as walking.
[w]All the cows in each treatment spent the same time in this activity (no individual measurements available).
[v]Other: Milking and faecal extraction.

**Table 4:** Means of time (min/day) spent at grazing, standing, lying, ruminating, walking, eating silage and at other activities for cows at the two herbage allowances, based on visual observations during 12.9 h of daylight.

| Reference | | Sward cutting | Animal performance |
|---|---|---|---|
| [38] | Intake marker[z] | 0.09 | 0.06 |
| | Animal performance | 0.57*** | |
| [39] | Intake marker[y] | 0.40** | 0.50*** |
| | Sward cutting | | 0.43** |
| Present study | Intake marker[x] | | 0.65** |

*$P$<0.05; **$P$<0.01; ***$P$<0.001.
[z]*P*ulse-dosed marker (chromium-mordant fiber).
[y]n-alkane.
[x]LIPE®.

**Table 5:** Correlation coefficients of the relationships between the sward cutting method, the intake marker methods and the animal performance method in three studies.

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## Milk production and milk composition

Milk yield was higher (P <0.05) for cows at the Hi-HA than for cows at the Lo-HA, but percentages of milk fat and milk crude protein did not differ between treatments (Table 3). This resulted in increased milk crude protein yield by (+0.13 kg/cow/day) and milk fat yield (+0.15 kg cow/day) for Hi-HA compared to Lo-HA (P <0.01).

## Grazing behavior

Daily times at pasture were 9.1 hours for Hi-HA and 8.6 hours for Lo-HA cows, because Hi-HA cows spent less time eating silage, the recorded grazing times of 4.3 h and 4.7 h represented 47% and 55% of daily time at pasture for Hi-HA and Lo-HA, respectively (Table 4).

Cows at the Hi-HA, spent less time grazing (P <0.05) and walking (P <0.01), more time lying (P <0.01) than cows at the Lo-HA (Table 4). The time spent standing and ruminating did not differ between treatments (Table 4).

## Comparison of LIPE® versus animal performance estimates of herbage DM intake

Figure 1 show the relationship between HDMIm estimated with the LIPE® method and HDMIa estimated with the animal performance method, using the average of 5 days/cow in both cases. The relationship between the animal performance method and the LIPE® method was significant (P<0.01), with a correlation coefficient of 0.65, a CCC of 0.57 and a RPE of 16%.

# Discussion

The aim of this study was to evaluate the effects of HA on individual HDMI, milk yield, grazing efficiency and grazing behaviour of supplemented dairy cows grazing alfalfa based pastures in early lactation, as well as to compare estimates of HDMI using either the animal performance method or the LIPE® method, both estimating individual HDMI .

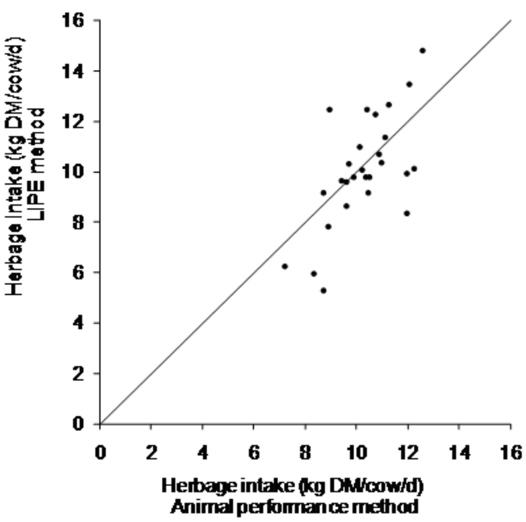


**Figure 1:** Relationship between herbage DM intake (HDMI) estimated from the LIPE® method and the animal performance method. One data point plotted per cow, averaged over the 5-day period. The relationship was significant (P<0.01), with a correlation coefficient of 0.65, a CCC of 0.57 and a relative prediction error of 16%. Line X=Y.

## Feed intake and efficiency of grazing

The HDMI was higher for cows in the Hi-HA treatment than for cows in the Lo-HA treatment when the LIPE® method and the animal performance method were used; however, no differences between treatments were detected with the sward cutting method. This highlights the importance of using dietary markers to investigate the relationship between HA and HDMI for cows grazing alfalfa based pastures, because the intake marker provides information of HDMI for each cow, giving more statistical power to detect differences than methods that measure HDMI for a group of cows, i.e., sward cutting method. However, this does not necessarily mean that the intake marker method is more accurate than other methods to estimated HDMI.

The EG was 210 g/kg higher (P <0.05) for cows at Lo-HA than for cows at Hi-HA. This indicates that cows grazing alfalfa pastures can partially compensate for the lower HA by increasing the EG, as suggested previously [7]; however, this compensation is only possible within a range of HA, which depends on the possibility of the animal to extend grazing time and increase biting rate. These results also suggest that high EG of 870 g/kg can be obtained at Lo-HA, even when cows were offered moderate to high amounts of supplements, which can be the case in dairy systems with high SR and moderate to high levels of supplementation.

## Milk production and milk composition

Cows at the Hi-HA treatment produced 4.1 kg milk more per cow/day than 006E cows at Lo-HA (Table 2). Estimates of ME consumed according to requirements [25] indicate that cows at the Hi-HA treatment consumed 12 MJ ME/cow/day more than cows at Lo-HA (Table 2). The rest of the extra energy required to produce the extra milk (4.1 kg milk x 5.1 MJ ME/kg milk = 20.9 MJ ME/cow/day) in the Hi-HA treatment must have been provided from body lipid mobilisation. However, it could also be explained by experimental errors in estimations of intake and feeds quality.

Milk fat and milk crude protein percentages were not affected by HA, in agreement with previous short term studies of cows grazing ryegrass based pastures at different allowances in early lactation [30], cows grazing a mix of grasses in mid lactation [31] and cows grazing alfalfa based pastures in mid lactation [8].

## Grazing behavior

Grazing conditions such as HA or sward height, which partly determines herbage availability and herbage intake rate, can affect the time spent grazing [32,33]. Sheep and cattle increase grazing time as HA declines, in an effort to meet their intake requirements [34].

Grazing time increased at the higher of two SR for steers grazing alfalfa pastures [35] and steers grazing tall fescue [36]. Consistent with these studies, grazing time increased by 26 minutes/day as HA decreased from 14.3 to 10.3 kg DM/cow/day in the present study (Table 4).

It was reported [37] that virtually no grazing occurred between 21:00 h and milking time the next morning, and that rumination and resting mainly occurred at night for dairy cows. This possibly explains the short time that cows spent ruminating during the day time in our study. A further explanation is that ruminating time was not recorded while cows were eating silage, at milking or at faecal sampling in our study. Even though our study reports only daylight grazing behavior in the paddock, this is the first study reporting grazing behavior of dairy cows on alfalfa based pastures.

## Comparison of LIPE® versus animal performance estimates of herbage DM intake

Estimates of HDMI from the animal performance method were compared with estimates from an intake marker (chromium-mordanted fiber) and the sward cutting method for dairy cows grazing at two SR [38]. They reported a positive correlation between the animal performance method and the sward cutting method, but no significant correlation between the animal performance method and the pulse-dose marker and between the pulse-marker and the sward cutting method (Table 5). These authors attributed the lack of empirical relationships between the marker and the other two methods to possible dosing errors, the frequent disturbance of animals, numerous laboratory analyses, and the complexity of modelling marker flow and calculating the parameters.

Estimates of HDMI from the animal performance method were compared with an intake marker method (n-alkane) and with the sward cutting method (using individual animals) for grazing dairy cows [39]. In their study, positive and significant correlations were found between all three methods (Table 5).

In our study, there was a positive correlation (R= 0.65, P<0.01) between the animal performance method and the intake marker (LIPE®) method. The accuracy of the prediction of LIPE® against the animal performance method is considered relatively good, given that the RPE is between 10% and 20% [27] and is considered moderate according to the CCC of 0.57 [29]. The sward cutting method could not be compared to the other two methods, since the former was used to record HDMI of a group of cows rather than intake of individual cows.

## Conclusions

Cows at the Hi-HA treatment had greater HDMI and milk yield, but had lower EG than cows at Lo-HA. This study showed that alfalfa pasture can be utilised at grazing with high efficiency by restricting HA, with cows offered supplements in early lactation.

Herbage DM intake differed between treatments when using both the LIPE® method and the animal performance methods, which are based on estimates of individual intakes. However, when using the sward cutting method, based on herd average intake, HDMI did not differ between treatments. This highlights the importance of using dietary markers to investigate the relationship between HA and HDMI for cows grazing alfalfa based pastures.

Estimates of HDMI measured with LIPE® were close to animal performance estimates. However, further research is needed to explore the suitability of LIPE® to predict HDMI for dairy cows at grazing. More research is also required to investigate the relationship between HA and individual HDMI, exploring a wider range of herbage allowances for dairy cows grazing alfalfa based pastures.

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