The addition of external water to fresh grass does not affect dry matter intake, feeding behaviour and rumen characteristics in dairy cows

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Original article

The addition of external water to fresh grass does not affect dry matter intake, feeding behaviour and rumen characteristics in dairy cows

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Abstract — Fresh grass is often a fodder of high nutritive value for ruminants, but its intake is limited in spite of its high digestibility. The high water content of fresh grass could limit grass DM intake by cows. The effect of external water on intake, feeding behaviour and rumen fill was studied in dairy cows fed indoors with fresh perennial ryegrass offered ad libitum. Three treatments were compared: fresh grass as harvested (C: control); C with water added to the grass (SW: surface water) and C with perfusion of water in the rumen after each distribution (PW: perfused water). Grass was offered to cows at 14:00 h, 19:00 h on each day and 07:00 h the following morning. Free access was given to drinking water. In treatment SW, the DM content of the offered grass was reduced from 161 to 117 g·kg^{-1} on a fresh basis ($P < 0.02$) without changing the chemical composition of the grass on a DM basis. Compared to C and PW, the fresh matter intake and its rate of intake increased in SW (+ 36 and 39%, respectively), but the grass DM intake (16.4 kg DM·day^{-1}), the rate of DM intake (35.3 g DM·min^{-1}), the daily eating time (468 min) and rumination time (515 min) were not modified. Perfusion of water into the rumen did not affect intake or feeding behaviour. The weight of the rumen contents (150 kg) and their DM content (115 g·kg^{-1} fresh matter), rumen fluid osmolality, as well as proportions of free water and bound water and turnover rate were not modified by the treatments. In conclusion, for dairy cows fed on grass, important inputs of external water do not affect feeding behaviour, rumen fill nor grass DM intake.

dairy cows / fresh grass / surface water / intake / behaviour / rumen fill

Résumé — L'addition d'eau externe à l'herbe verte n'affecte pas la quantité de matière sèche ingérée, le comportement alimentaire ou les caractéristiques du rumen chez la vache laitière. L'herbe fraîche est un aliment de bonne valeur alimentaire pour les ruminants mais son ingestion par les vaches laitières reste limitée au regard de sa digestibilité. La forte teneur en eau de l'herbe fraîche pourrait limiter la quantité ingérée de MS d'herbe. L'effet de l'eau externe à la plante sur l'ingestion,

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le comportement alimentaire et l'encombrement ruminal a été étudié sur des vaches laitières nourries à l'auge avec du ray-grass anglais distribué ad libitum. Trois traitements ont été comparés : herbe verte témoin, fauchée une fois par jour (C) ; C plus de l'eau ajoutée sur l'herbe (SW) et C plus perfusion d'eau dans le rumen après chaque distribution (PW). La distribution d'herbe a été faite à 14:00 h, 19:00 h et 07:00 h le lendemain matin. L'eau de boisson était en accès libre. Dans le traitement SW, la teneur en MS de l'herbe offerte a été réduite de 161 à 117 $g \cdot kg^{-1}$ brut ($P < 0{,}02$) sans changer la composition chimique de l'herbe. Par rapport à C et PW, la quantité brute ingérée d'herbe et la vitesse d'ingestion en brut ont fortement augmenté (respectivement + 36 et 39 %), mais la quantité de MS d'herbe ingérée (16,4 $kg\ MS \cdot j^{-1}$), la vitesse d'ingestion de MS (35,3 $g\ MS \cdot min^{-1}$) et les durées journalières d'ingestion (468 min) et de rumination (515 min) n'ont pas été modifiées. Perfuser l'eau dans le rumen n'a affecté ni l'ingestion ni le comportement alimentaire. Le poids total du contenu du rumen (150 kg), la teneur en MS du contenu (115 $g \cdot kg^{-1}$), l'osmolarité du liquide ruminal, la proportion d'eau libre et d'eau liée ainsi que le taux de renouvellement des liquides n'ont pas été modifiés par les traitements. En conclusion, pour des vaches en production nourries à l'herbe, un apport même important d'eau externe ne constitue pas de limite à la prise alimentaire, ni à l'encombrement dans le rumen, et n'affecte pas la quantité de MS d'herbe ingérée.

vache laitière / herbe verte / eau externe / ingestion / comportement alimentaire / encombrement

1. INTRODUCTION

In the dairy cow, the intake of fresh grass is lower than the amount ingested in a total mixed ration and appears to be insufficient to allow a production close to the genetic potential of high-producing cows [2, 20, 37]. The digestibility of fresh grass, generally high, does not seem to limit intake [16]. However, other factors like plant structure or low dry matter content (DM) of grass are often quoted as factors that can limit intake [19]. In indoor feeding, a positive correlation between intake and DM content was observed in the case of sheep [18] and dairy cows [9, 36]. The variations in DM content of grass depend on internal water content and external water (rain or dew) unrelated to the physiological stage of the plant. This external water seems to reduce intake among cows fed indoors [36]. At pasture, Malossini et al. [23] also observed lower daily intakes in cows during periods of heavy rain. Similarly, in the more recent studies of Butris and Phillips [5] and Phillips et al. [28], the intake of grass DM by steers was reduced by the presence of surface water.

The control of herbage intake due to the action of water could involve mechanisms that may be related to behavioural aspects of feeding or regulating factors of digestive origin. Because the water ingested with the forage dilutes its dry matter and energy content, it could reduce the daily DM intake by decreasing the eating rate [19].

However, contrasted conclusions have been given by different authors since Butris and Phillips [5] found that water external to the plant affected the daily eating time without decreasing the eating rate. The large amounts of water eaten with fresh grass could also contribute to the rumen fill but this hypothesis remains a matter of debate [6].

The aim of this trial was to establish the role of external water as a factor limiting herbage intake in the lactating dairy cow and to establish the mechanisms involved. In order to dissociate the potential effects of water on rumen fill from the DM dilution during feeding, water was added either to grass or in the rumen by perfusion at the time of the main meals. The hypothesis tested here is that water added to fresh grass generates both a behavioural limit and filling of the rumen, whereas the perfusion of water during meals induces only a potentially filling effect.

2. MATERIALS AND METHODS

2.1. Treatments and experimental design

The effects of external water on grass intake were studied by comparing three treatments: fresh grass as harvested (C: control), C with water added to the grass (SW: surface water) and C with perfusion of water in the rumen (PW: perfused water).

The control treatment C consisted of perennial ryegrass offered indoors ad libitum three times per day at 07:00 h, 14:00 h and 19:00 h. Treatment SW consisted of sprinkling the ryegrass control with water, using an amount representing 33% of the fresh weight of the offered grass. This proportion is the maximum quantity of water that the grass can retain on its surface without leading to water losses in the trough. In practice, the addition of water was carried out just before the three distribution times by sprinkling crates containing 15 kg of grass with 5 kg of water using a fine jet while mixing regularly.

Treatment PW corresponds to the treatment C plus a perfusion of 30 L of water per day in the rumen. This amount corresponds to the expected quantity of surface water supplied in treatment SW and was estimated from the quantity of grass voluntarily ingested by the cows in the week prior to the beginning of the trial and the proportion of water retained by the grass during sprinkling. The perfusion of water started at the beginning of each distribution time, the quantities of perfused water being proportional to the size of the three main meals, that is to say 8.7, 11.4 and 9.9 litres in the meals at 07:00 h, 14:00 h and 19:00 h, respectively. The perfusion was performed with a peristaltic pump using a flow rate of 5 litres per hour.

The trial was conducted according to a 3 × 3 Latin-square experimental design with four cows, with two of them following the same succession of treatments. Each of the three periods lasted ten days, with three days for adaptation to the treatments and seven days for measurements. The trial began on 17 May 2000 and finished on 16 June 2000. A pre-experimental period of two weeks was carried out indoors for accustoming to the diet and adjusting the grass quantities to be offered.

2.2. Animals and feeding

Four multiparous Holstein dairy cows were used. They were fitted with a large rumen canula (internal diameter: 123 mm) and kept in individual stalls. They were milked twice daily at 06:30 h and 17:00 h. During the reference period three weeks before the beginning of the trial, the cows were on average in their 13th week of lactation, with a milk production of 34 kg per day and a live weight of 662 kg. The diet was made up of fresh perennial ryegrass distributed three times per day ad libitum (minimum of 10% refusals). One paddock was used for accustoming to the diet, and one paddock was used for each experimental period. Each paddock was prepared by mowing to obtain 30 days of regrowth in the middle of each experimental period and immediately afterwards received a fertilisation of 60 kg $N \cdot ha^{-1}$. During the experiment, the grass was cut once a day in the morning around 10 h using a flail harvester, then stored in a cold room at 4 °C until the feeding times. The first meal was offered at 14:00 h, the second at 19:00 h and the third on the following day in the morning at 07:00 h. Refusals were removed each day before the meal at 14:00 h. Drinking water and a salt block were freely accessible for each cow.

2.3. Measurements

The individual intakes were measured each day by weighing the quantities of grass offered and refused. Samples of offered and refused grass were taken each day in order to determine the DM contents. For offered grass, a 700-g sample was taken before each meal on treatments C and SW. For grass refusals, a representative 700-g sample was taken each day for each cow during the weighing of the refusals. The DM content of grass was determined by the drying samples in an oven for 48 hours at 80 °C.

The chemical composition of the offered and refused grass was determined on samples that were frozen and then freeze-dried. For the offered grass, samples of 100 g were taken from day 4 to 10 of each period at each meal on treatments C and SW. These samples were frozen at –30 °C and then grouped together by period and grass type. For grass refusals, from day 4 to day 10 of each period, representative subsamples of 150 g were taken for each cow during the weighing of refusals, frozen at –30 °C, and then grouped together by cow and by period.

The quantity of water drunk by each cow was measured automatically each day. According to the treatments, the quantities of surface water added to the grass or perfused in the rumen were weighed and recorded every day.

The feeding behaviour was studied from day 4 to day 10 in each period by simultaneous and continuous recording of the number of jaw movements and the weight of the trough [4]. These measurements thus provided the daily eating and rumination times. The average eating rate was calculated as the ratio between the daily DM intake and the eating time.

Milk production of the cows was recorded at each milking. The protein and fat contents of the milk were determined for each milking from Monday to Friday throughout the trial by infrared spectrophotometry (Milkoscan, Foss Electric, Hillerood, Denmark).

Two ruminal emptyings per cow and period (days 8 and 10) were carried out at 06:15 h and 23:00 h to determine weight and composition of rumen contents as well as turnover rate of the rumen liquids (CrEDTA).

At each emptying, the contents of the reticulo-rumen were removed via the canula, the remaining liquid being sucked out at the end of the emptying. The whole contents were weighed and then homogenised before taking a representative sample (5% w/w). After collection of the samples the rumen contents were placed back into the rumen immediately in the reverse order of the emptying. Several subsamples were taken from the initial sample of the rumen contents.

DM content was determined by placing a 500-g subsample in a drying oven (80 °C, 48 h). Chemical composition of the rumen contents was analysed from a 700-g subsample that was frozen at –30 °C and then freeze-dried. The proportion of free and bound water in the contents was obtained on 2 subsamples of 500 g each. Each sample was placed in a nylon bag of 100 μm pore size and compressed at 4 bars. The extracted filtrate was weighed and regarded as representing the free water. Filtrates from the two subsamples were then pooled, homogenised and frozen at –30 °C until determination of CrEDTA. The residues of the two subsamples were also pooled together, weighed as fresh and then dried in an oven at 80 °C for 48 h to determine the quantity of bound water. Free and bound water were expressed as a percentage of the total fresh weight of the rumen contents.

Turnover rate of the rumen liquid contents was determined by adding 1.5 L of a solution of CrEDTA (1200 mg CrEDTA·L^{-1}) in the rumen, either on day 8 and day 9 at 18:00 h. The turnover rate of the liquids was estimated from the decrease in the quantity (Q) of CrEDTA present in the rumen between two emptyings, carried out 5 h (Q1, emptying in the evening) and 12 h 15 min (Q2, emptying in the morning) after the addition of the CrEDTA-solution. The quantity of

CrEDTA present in the rumen was calculated by multiplying the total quantity present in the rumen by the content of CrEDTA of the filtrate obtained after compressing, by assuming a homogeneous diffusion of CrEDTA throughout the rumen liquids. The turnover rate of the liquids (K) was then calculated by the following formula, assuming that all the CrEDTA of the first dose disappears after 24 h by the time the second dose was added:

$$K = (\mathrm{Ln}\ Q2 - \mathrm{Ln}\ Q1) / 7.25.$$

The ruminal fermentation was characterised on day 6 of each period. The pH, the VFA concentration and composition, osmolality as well as the ammonia concentration of the ruminal fluid were measured 0, 2, 3:30 and 5 h after each meal, i.e. at 7:00, 9:00, 10:30, 12:00, 14:00, 16:00, 17:30, 19:00, 21:00, 22:30 and 00:00 h. At each sampling, 50 mL of the rumen liquid were drawn out of the ventral sac, the pH was immediately measured, and then the samples were filtered through six layers of cheesecloth. Eight mL of filtrate were frozen at –20 °C with a preservative solution (0.8 mL $HgCl_2$ 1% p/v in H_3PO_4 5% v/v) and stored until VFA and osmolality analysis. Four mL of filtrate with 4 mL of preservative solution (NaCl 20%) were frozen at –20 °C and stored until analysis of ammonia concentration. The average values by day were calculated from the arithmetic means of the 11 samples.

2.4. Chemical analyses

The chemical compositions of the offered and refused grasses as well as the rumen contents were determined on freeze-dried samples ground in a mill through a 0.8 mm screen. Cell-wall constituents (NDF and ADF) were analysed on a Fibersac instrument (Ankom, US) according to the method initially described by van Soest et al. [35]. Total nitrogen was determined by the Dumas method [1] and ash by calcination at 550 °C for 5 h [1]. Non structural carbohydrates (NSC) were determined by the method of Luff-Schoorl [1] and the cellulase digestibility according to the procedure described by Aufrère and Michalet-Doreau [3]. The proportion of soluble nitrogen in the grass was analysed by the Kjeldahl method after extraction in water and precipitation of proteins with tungstic acid according to the method described by Licitra et al. [22]. The methods used for analysing ammonia and VFA in the rumen contents were as described previously by Peyraud et al. [27]. The ruminal fluid osmolality was determined with an osmometer (Roebling, Berlin) using the freezing point depression procedure, and the values were corrected from the osmolality of the preservative solution. Cr-EDTA was determined by atomic absorption spectrophotometry (Varian, AA-20) with a nitrous oxide-acetylene flame [24].

2.5. Statistical analyses

Grass intake on a fresh and DM basis, quantity of water drunk, feeding behaviour parameters, and milk yield and composition were averaged from day 4 to 10. The whole set of animal variables was then analysed according to the GLM procedure of SAS [30], taking into account the effects of the cow, period and treatment. The evolution of intake in the experimental periods was treated on a day-by-day basis according to the same analytical model. The “supply” effect of adding water (C vs. SW + PW) and the “site” effect of adding water (SW vs. PW) were analysed by orthogonal contrasts.

3. RESULTS

Sprinkling grass in the SW treatment led to a reduction in the DM content of the offered grass from 161 to 117 $g \cdot kg^{-1}$ ($P < 0.02$, Tab. I). For a fixed amount of

Table I. Effect of adding surface water on the chemical composition of offered grass.

Grass component	C	SW	Syx	Prob
DM	161	117	7.8	0.017
OM	871	878	107.8	0.642
CP	153	152	4.1	0.942
NDF	468	476	20.1	0.656
ADF	254	253	9.3	0.953
NSC	147	151	5.7	0.454
Soluble protein	29.7	27.3	0.67	0.044
Cel. Dig. (% DM)	75.7	77.0	1.63	0.427

C: control grass, SW: grass with surface water, NSC: non structural carbohydrates, Cel. Dig.: pepsin-cellulase digestibility. Chemical composition is given in g·kg DM^{-1} except DM with values in g·kg fresh.

Table II. Effect of adding surface water or ruminal water perfusion on the chemical composition of refused grass.

Grass component	C	SW	PW	Syx	Contrast	
					Supply	Site
DM	161	107	161	0.5	0.002	0.001
OM	836	842	824	7.5	0.763	0.187
CP	153	151	141	0.4	0.051	0.026
NDF	488	486	495	1.7	0.786	0.544
ADF	266	270	275	0.8	0.244	0.390
NSC	102	108	111	0.6	0.105	0.491
Cel. Dig. (% DM)	68.2	70.6	68.6	3.25	0.537	0.452

C: control grass, SW: grass with surface water, PW: grass with perfused water, NSC: non structural carbohydrates, Cel. Dig.: pepsin-cellulase digestibility. Chemical composition is given in g·kg DM^{-1} except DM with values in g·kg fresh; contrast "supply" compares C vs.W+PW; contrast "site" compares SW vs. PW.

DM to be ingested, this reduction corresponds to an increase of 37% in the total fresh weight of grass fed. The chemical composition of offered grass was not modified by adding water (Tab. I). The DM content of the refused grass was the same as the offered grass irrespective of the treatment (Tab. II). The proportion of grass refused (17.3% of that offered) and the chemical composition of the refusal did not show any significant differences among the treatments. The refused grass was on average less digestible (–7 digestibility units) and with lower NSC content (–42 g·kg^{-1} DM) than the offered grass.

Compared to the control treatment, a significant increase in fresh intake with the SW treatment was observed from the first day in each period, leading to an equivalent amount of DM intake among treatments (Fig. 1) (+37 kg, i.e. 36% higher) but did not vary when water was perfused directly into

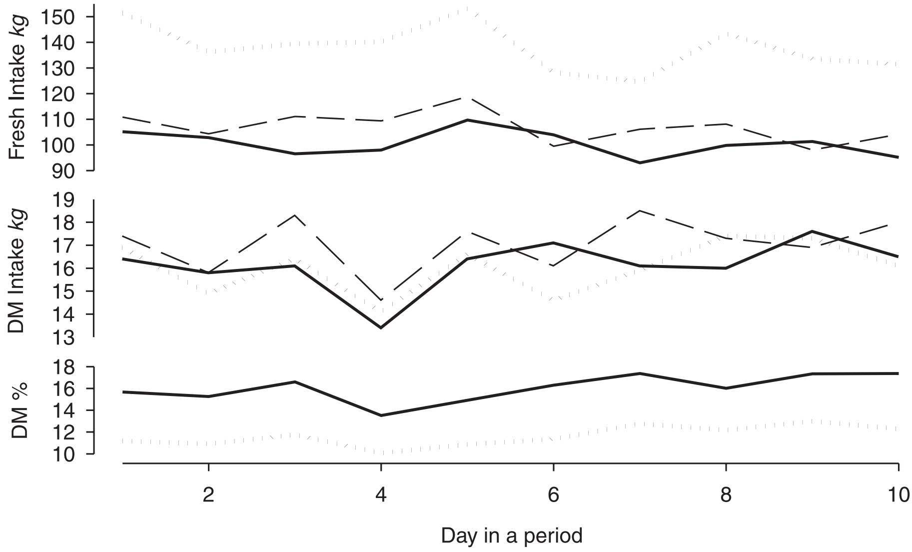

Figure 1. Effect of adding surface water or ruminal water perfusion on the day-to-day variation of fresh matter intake, dry matter intake and grass DM content from day 1 to day 10 of a mean period. (treatments C —, PW – – and SW ııııı).

the rumen. On the contrary, DM intake showed no variation among the treatments (Tab. III, Fig. 2).

The quantity of water drunk was lower for the SW and PW treatments compared to C (–26 L, $P < 0.002$), but without differences according to the method of water addition ($P = 0.08$).

Finally, the total water intake (through grass, drinking water and added water), normalised to the kg of DM intake, did not vary among the treatments (Tab. III).

Table III. Effects of adding surface water or ruminal water perfusion on intake of fresh grass, dry matter grass and water.

Parameter	C	SW	PW	Syx	Contrast	
					Supply	Site
Fresh intake (kg)	100.4	136.2	106.2	5.29	0.005	0.003
DM intake (kg)	16.2	16.0	17.0	1.07	0.631	0.271
Refused (%)	17.7	18.5	15.7	2.59	0.758	0.214
Total water intake						
By drinking (L)	37.6	8.2	15.2	4.12	0.002	0.080
Total input [a] (L)	121.9	128.4	134.4	4.8	0.036	0.162
L per kg DM Intake	7.61	8.02	7.94	0.423	0.233	0.797

C: control grass, SW: grass with surface water, PW: grass with perfused water; contrast "supply" compares C vs. SW+PW, contrast "site" compares SW vs. PW.
[a] Total input is calculated from the water contents of forage and water used in the treatments.

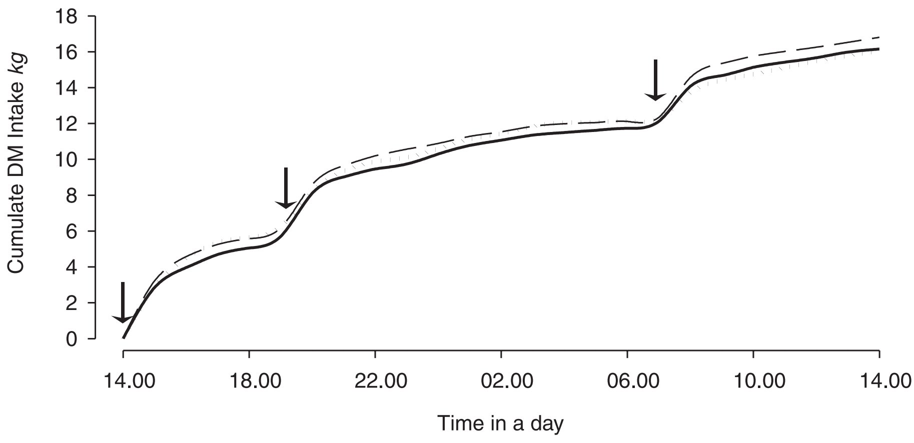

Figure 2. Effect of adding surface water or ruminal water perfusion on cumulated grass DM intake during the day (treatments C —, PW – – and SW ııı). Arrows indicate the distribution time.

No significant differences were observed among the treatments in the number of meals, the rate of DM intake, DM intake per meal or the daily eating, rumination and chewing times (Tab. IV). The average intake of fresh grass per meal was higher with the SW treatment (+3.8 kg). Compared to the control treatment, the rate of fresh matter intake increased strongly when water was added to the grass (+84 $g \cdot min^{-1}$, i.e. 39% higher), but showed no variation when water was added directly into the rumen. The total weight of the rumen contents was significantly higher in the evening than in the morning (164 and 137 kg fresh basis, respectively; $P < 0.001$). No significant

Table IV. The effect of adding surface water or ruminal water perfusion on feeding behaviour.

Parameter	C	SW	PW	Syx	Contrast	
					Supply	Site
Number of meals	13.3	12.4	13.2	0.64	0.248	0.181
Grass intake by meal (kg)						
Fresh	7.85	11.80	8.10	0.736	0.012	0.004
Dry matter	1.25	1.45	1.35	0.101	0.080	0.247
Eating rate ($g \cdot min^{-1}$)						
Fresh matter	214	300	225	19.99	0.020	0.008
Dry matter	34.6	35.2	36.1	2.91	0.591	0.695
Eating time (min)	471	455	479	36.0	0.869	0.418
Rumination time (min)	483	543	520	40.5	0.133	0.490
Chewing time (min)	955	998	1000	48.2	0.220	0.969

C: control grass, SW: grass with surface water, PW: grass with perfused water; contrast "supply" compares C vs. SW+PW, contrast "site" compares SW vs. PW.

differences were observed between the morning and evening emptyings regarding DM content, proportions of free and bound water, as well as the chemical composition of the rumen contents. Thus, the average data are presented in Table V. The weight of the rumen contents, on a fresh or dry matter basis, the proportions of free and bound water in the same way as the chemical composition and turnover rate of the rumen liquids showed no significant differences among the treatments.

Table V. Effect of adding surface water or ruminal water perfusion on rumen content characteristics.

Parameter	C	SW	PW	Syx	Contrast	
					Supply	Site
Fresh weight (kg)	152.7	147.4	151.0	8.28	0.549	0.584
DM content ($g \cdot kg^{-1}$)	114	117	115	6.1	0.675	0.724
DM weight (kg)	17.4	17.2	17.4	0.90	0.898	0.713
CP ($g \cdot kg\ DM^{-1}$)	23.0	22.6	23.0	0.86	0.749	0.567
NDF ($g \cdot kg\ DM^{-1}$)	56.0	56.5	57.7	1.34	0.257	0.279
ADF ($g \cdot kg\ DM^{-1}$)	27.9	27.5	28.3	0.88	0.955	0.250
Free water (%)	52.7	50.9	52.9	2.38	0.658	0.301
Bound water (%)	35.8	37.4	35.6	2.12	0.658	0.301
Liquid turnover rate ($\% \cdot h^{-1}$)	10.66	12.20	12.42	2.29	0.319	0.899

Values are the average of two emptying times; C: control grass, SW: grass with surface water, PW: grass with perfused water; contrast "supply" compares C vs. SW+PW, contrast "site" compares SW vs. PW.

Table VI. Effect of adding surface water or ruminal water perfusion on ruminal fermentation patterns by treatment.

Parameter	C	SW	PW	Syx	Contrast	
					Supply	Site
pH	6.17	6.24	6.15	0.115	0.744	0.357
NH_3 ($mg \cdot L^{-1}$)	108.1	88.5	91.2	10.32	0.049	0.726
VFA (mM)	112.9	109.5	113.9	5.33	0.734	0.321
C_2 (%)	67.2	66.5	66.6	0.63	0.220	0.827
C_3 (%)	20.0	20.9	20.5	0.41	0.063	0.333
C_4 (%)	9.4	9.4	9.6	0.32	0.725	0.530
C_5+C_6 (%)	1.0	1.0	1.0	0.07	0.944	0.758
Iso acids	2.3	2.1	2.2	0.12	0.104	0.465
Osmolality	249.3	237.9	249.1	8.78	0.529	0.332

Osmolality values are given in $mOsmol \cdot kg^{-1}$; C: control grass, SW: grass with surface water, PW: grass with perfused water; contrast "supply" compares C vs. SW+PW, contrast "site" compares SW vs. PW.

Table VII. Effect of adding surface water or ruminal water perfusion on milk production.

Parameter	C	SW	PW	Syx	Contrast	
					Supply	Site
Milk yield ($kg \cdot day^{-1}$)	23.0	24.4	24.9	1.17	0.088	0.593
Protein content ($g \cdot kg^{-1}$)	28.0	28.4	28.1	0.21	0.232	0.182
Fat content ($g \cdot kg^{-1}$)	39.3	38.4	37.9	0.85	0.094	0.479
Protein production ($g \cdot d^{-1}$)	646	693	702	35.8	0.085	0.747
Fat production ($g \cdot d^{-1}$)	908	939	944	32.1	0.170	0.827
4% fat corrected milk ($kg \cdot d^{-1}$)	22.8	23.9	24.1	0.918	0.114	0.699

C: control grass, SW: grass with surface water, PW: grass with perfused water; contrast "supply" compares C vs. SW+PW, contrast "site" compares SW vs. PW.

The pH, concentration and composition of VFA and osmolality in the rumen liquid did not vary among the treatments (Tab. VI). The ammonia content was higher with treatment C (+18 $mg \cdot L^{-1}$, $P < 0.05$) than for the two other treatments.

Milk yield, fat and protein contents of milk, and daily fat and protein yields were not modified by the treatments (Tab. VII).

4. DISCUSSION

The aim of this trial was to quantify the effect of adding external water on the fresh herbage intake of dairy cows and to determine the principal mechanisms involved. In our study, adding water did not modify the chemical composition of the grass, and probably the mineral content was not affected because no loss of water during sprinkling grass or at the trough was observed. Hence, in contrast with some previous studies [18, 25], the effect of the water content of the forage on intake was not confused with the effect of the other forage nutrients involved in the regulation of intake.

Under the indoor conditions of the present trial, the intake of grass DM by dairy cows fed ad libitum remained unaffected when water was either added to the offered forage or perfused into the rumen. This result is analysed and discussed here with regards to the experimental conditions, then compared to the adaptation of the cows to the supply of external water in terms of feeding behaviour and rumen fill.

4.1. External water and intake

The supply of external water did not affect the total DM intake, therefore the grass fresh matter intake increased in proportion to its reduction in DM content. On the contrary, several authors have mentioned a decreased grass DM intake in the presence of water external to the plant. Vérité and Journet [36] observed a slight but significant reduction (5%) of DM intake in dairy cows fed indoors with grass wet by dew (12.8 kg DM) compared to grass without dew (13.5 kg DM). In the trials of Butris and Phillips [5] and Phillips et al. [28], the daily grass intake of steers was significantly reduced by 2.0 and 1.3 kg DM (–22 and –14%, respectively) due to an external supply of water. In these two trials, the grass was immersed completely in tanks filled with water and then drained for 5 min before being offered to the cows. On the contrary, the addition of water onto conserved forage does not modify the DM intake [29].

In our trial, the experimental conditions were favourable to study the effect of external water on intake. The DM content of control grass (16%) was quite low and any further reduction in DM content would be in the range generally quoted as limiting for intake [18, 36]. Moreover, the difference of DM content between the control and wet grass represents a 37% dilution of the fresh matter. This dilution is close to the maximum value possible to get in the field since the amount of water added was all that the grass could retain. Only a complete soaking of the grass could lead to even greater dilutions [5, 28]. However, such experimental conditions do not exist in practice. Moreover, a high level of roughage intake is probably necessary to study the intake regulation due to large amounts of water. The intake level of the cows in our trial (16.4 kg DM, which is 2.5% of the live weight and 2.8 times the maintenance requirements) was higher than that reported in the trials of Vérité and Journet [36], Butris and Phillips [5] and Phillips et al. [28].

4.2. External water and feeding behaviour

From a behavioural point of view, daily intake can be regarded as the product of eating rate and time. Thus, any factor limiting eating rate and/or eating time will represent a potential limitation on the daily grass intake [14, 15, 26].

Under the conditions of our experiment, the presence of surface water in no way constituted a limit to the feeding behaviour. Indeed, the rate of DM intake did not vary with the supply of surface water, so the cows did not compensate by longer daily eating times. Such a compensation was generally observed in the case of *physical constraints* on feed intake as seen with low sward heights in continuous grazing [26]. The cows were able to increase their rate of intake in the same proportion to the falling content of DM, thus maintaining the rate of DM intake. If the animals judged wet grass less palatable than dry grass (lower motivation), then a reduction in the intake rate and/or eating time would have been observed [5, 33].

The influence of the DM content of grass on the intake rate and eating time is seldom described in the literature. With steers fed with fresh grass indoors, Butris and Phillips [5] observed an important reduction of daily eating time with no modification of DM intake rate in the control grass compared to the soaked grass. This decrease of eating time was perhaps related to the weak motivation for eating of the low requirement animals that were used. Sheep appear more sensitive to surface water than cattle, and seem to reduce their intake rate significantly when the grass is too wet [19].

In our trial, the regulation of eating rate was thus carried out in an overall way on a dry matter and not on a fresh matter basis. Two hypotheses may be proposed to explain this result. Firstly, the external water did not cause a variation in the structure of the forage offered. In particular, it did not increase the apparent volume to be ingested since surface water filled the free spaces between the grass blades. Our results thus suggest that, indoors, feeding is controlled by the prehension of the grass blades more than by the weight of the fresh matter to be ingested.

Additionally, surface water would not represent for the animal a fresh matter to be ingested, because it could be swallowed independently and more quickly than the remainder of the bolus. Jarrige et al. [17] mentioned that, during regurgitation in the rumination phase, ruminants are able to sort the digestive contents in their oral cavity into a fibrous fraction and a more liquid fraction. The latter is swallowed immediately before chewing the selected fraction of the bolus.

4.3. External water and rumen characteristics

The hypothesis tested was that the addition of water during the main meals could create a constraint on the rumen fill. Indeed, it is known that additional rumen load changes its storage capacity as well as the transit rates, with a possible modification of the total DM intake according to the nature of the diet [8] and the type of the animal [11].

Our results showed that rumen fill was not increased by either the perfusion of 30 L of water into the rumen during the main meals or the addition of water to the grass. Neither of these treatments modified the structure of the rumen contents. The immediate increase of fresh matter intake from the first day of supplying external water showed that the fill effect of adding water was never observed (Fig. 1). This confirmed the results of Campling and Balch [6] who already showed that the perfusion of 45 L of water into the rumen during the main meals does not affect the intake of cows fed with hay. Such a result can be explained by the strong reduction in the quantity of water drunk when water is added either by perfusion or by sprinkling the grass. Indeed, to cover their requirements in water, the cows can modify the quantity they drink to maintain total water intake per kg of DM [6, 21].

The absence of a fill effect can also be explained considering that the quantity of external water added does not contribute significantly to the total amount of water entering or leaving the rumen. In fact, the daily production of saliva, estimated at 18 L per kg DM ingested [10], would reach 250 to 300 L per day, thus largely exceeding the water supplied by grass (80–100 L) and the volume of water drunk or added (40 L). Moreover, it is rather unlikely that the daily production of saliva varied much among the treatments since the daily chewing times were equal in all treatments. From the observed turnover rates of the liquids, the total quantity of liquids passing through the rumen would be close to 400 L per day whatever the treatment, this means more than 10 times the amount of water added. Such values are frequently observed in producing cows fed grass (Peyraud et al., unpublished data).

Ruminal osmolality could be a short term regulating factor of feeding behaviour [7]. Both the average values of ruminal fluid osmolality and the values at the end of each feeding period were not affected by the treatments. Osmolality regulation was observed on free water access, even with important loading of VFA [12]. On the contrary, osmolality was low, perhaps due to the large quantity of ruminal water in this experiment. Osmolality at the end of each feeding period was always lower than the reported values affecting the regulation of meal size [7].

Finally, rumen fluid composition was not affected by the treatments. No differences in VFA, and NH3 concentration and pH values were observed. This result allows to suppose that the cow's rumen environment is strongly equalised in spite of external water addition.

The fact that the composition of the rumen contents (DM content and the proportion of free water and water bound to particles) was not modified by the supply of water could suggest that the structure of the ruminal contents was effectively controlled. Van Soest [34] mentioned that, for a given diet, the osmotic pressure in the rumen is controlled by salivation and by the exchange of water through the wall of the rumen. In the same way, the proportion of water retained by the rumen contents would not vary for a given type of fodder [13, 31].

Whether offering wet grass or perfusing water into the rumen, the amount of water added was lower than the water requirements since the cows continued drinking. Because the quantity of water drunk seems strongly controlled by the DM intake, it cannot be ruled out that the herbage DM intake would fall if the external supply of water was higher than the water requirements of the cows. However, this case must be rare in practice since cows fed on herbage commonly drink 20–30 L of water for an intake of 15–16 kg DM [32]. Overall, 30 L of water is thus probably the maximum quantity that a cow can be led to consume with wet grass. Actually, this figure is rather low compared with water fluxes in the rumen and is of the same order of magnitude as the water need for a cow.

5. CONCLUSIONS

Under the experimental conditions in this trial, an important supply of external water did not modify the grass DM intake of lactating dairy cows. The total water intake was determined by the quantity of DM ingested.

The surface water on the grass did not constitute a factor affecting feeding behaviour since neither the rate of DM intake nor the eating time showed any modification. The added water into the rumen did not increase the rumen fill, the amount of water drunk being proportionally reduced.

With fresh grass offered ad libitum, the cows are able to manage their voluntary intake based on DM whatever the amount of the external water. In practice, surface water constitutes a water input that is balanced by the amount of water drunk. However, it is possible that the internal water of the grass has a greater influence on DM intake due to the existence of more marked effects on the studied mechanisms.

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