Sweet forages and organic milk: Quite a sustainable combination!?!

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Executive summary

Forages are the cornerstone of all sustainable milk production systems. However, recent studies suggest that growing cattle prefer forages prepared with alfalfa harvested at sunset (between 19:00 and 21:00) rather than at sunrise (between 06:00 and 08:00) (Burns et al., 2005), although there is no difference in the digestibility of the forage. The purpose of harvesting alfalfa at sunset is to benefit from the plant's ability to accumulate non-fibrous carbohydrates (NFC) during the day (Lechtenberg et al., 1971). These studies, however, have not been successful in determining the minimum number of hours of sunlight required to produce an optimal increase in NFC. Furthermore, very little is known on how exactly this process works. The primary objective of this study was to determine the effect of the duration of sunlight on sugar, starch and NFC accumulation in alfalfa. The second objective was to determine just how the levels of soluble carbohydrates, starch and NFC affect rumen fermentation by measuring in vitro gas production.

Materials and methods

Plant material

Twenty alfalfa seedlings (AC Caribou) were grown under controlled conditions with temperatures ranging from 17°C at night to 22°C during the day and a photoperiod of 16 hours. Four seedlings were harvested during early bloom, i.e., after either 0, 2, 4, 8 or 12 hours of sunlight, then dried immediately at 55°C and grinded into 1 mm pieces.

Preparation of medium and samples

The growing medium was prepared according to the method described by Longland et al. (1995). All 20 forage samples (1 g) were weighed and inserted into 160-ml bottles. Four additional bottles containing no forages were used as controls. Total soluble sugar content was evaluated by adding up individual sugar concentrations, and total non fibrous carbohydrates were estimated by adding the starch concentration to total soluble sugar content.

Inoculation

Two litres of ruminal digesta were drawn from a cow that had been equipped with a ruminal cannula and fed grass silage ad libitum. This sample was mixed thoroughly then sent to the lab in a preheated, airtight container. The ruminal digesta was then filtered through cheesecloth into a beaker saturated with CO_2. The bottles containing the forage samples suspended in an anaerobic environment were inoculated with 10 ml of filtered digesta using a syringe. The bottles were then connected to a gauge in order to measure gas pressure in the top portion of the bottles and bring this pressure back to 0. The bottles were then placed in an incubator equipped with an agitator plate and set at a stable temperature of 39°C. Measurements were taken of gas pressure and volume on a regular basis during 142 hours in order to estimate the rate of fermentation using the model proposed by France et al. (1993).

Sampling

Gas pressure and volume were recorded at 0, 3, 6, 9, 12, 16, 20, 24, 30, 36, 48, 60, 72, 96, 120 and 142 hours post-inoculation. At the end of the experimental period, the liquid fraction was separated from the solid fraction (undigested forages + attached bacteria) by vacuum filtration through pre-weighed crucibles (100 µm porosity 1; Gallenkamp; Fisher Scientific UK, Loughborough, Leicestershire). This residue was freeze-dried to determine the quantity of residual dry matter. One ml of liquid was collected and acidified with 100 µl of HCl (2M) in order to assay ammonia nitrogen (NH_3-N), and another 1-ml sample of liquid was taken and acidified with 2 drops of orthophosphoric acid to determine the concentration of VFA.

Chemical and statistical analyses

Non-fibrous carbohydrate (soluble sugars and starch) concentrations were determined after extracting dry forage samples (0.1 g) in a blend of methanol/chloroform/water at 65°C, as in the method described by Castonguay et al. (1995). An aliquot of the water fraction was evaporated to dryness then resuspended in a blend of water and EDTA for analysis of soluble sugars (glucose, fructose, raffinose, sucrose and pinitol) by high-performance liquid chromatography (Waters Scientific, Milford, USA). The pellet containing non soluble residues was rinsed with methanol, and the starch present was resuspended by gelatinization at 100°C in an alkali environment, then hydrolyzed using amyloglucosidase (Sigma Chemical Co. St-Louis, USA). Starch levels were assayed based on glucose equivalents after a colorimetric reaction with p-hydroxybenzoïc acid hydrazide (PAHBAH) and absorbance reading at 414 nm by spectrophotometer. VFA concentrations in the supernatant were determined by gas chromography with a Chrompack CP 9002 (CP-Sil 5CB column 10 m x 0.25 mm ID; Chrompack UK) using the method described by Zhu et al. (1996). Ammonia nitrogen was determined by enzymatic method using glutamate dehydrogenase on a discrete analzyer (FP-901M Chemistry Analyzer, Labsystems, Oy, Helsinki, Finland; Test kit No. 66-50, Sigma-Aldrich Co. Ltd., Poole, Dorset).

DM digestibility was calculated as the difference between the quantity of DM in the sample placed in the bottle and the quantity of DM retrieved after residue centrifugation. Gas production data pertaining to the fermentation of each type of forage were corrected to take into account the gas produced by rumen microbes (blanks) and introduced in the model described by France et al. (1993). The equation used was:

$$Y = A \{1- e^{[-b (t-T) - c (\sqrt{t} - \sqrt{T})]}\}$$

Where Y represents cumulated gas production (ml); A represents the asymptote (maximal production); T represents the latency period (time before the beginning of gas production); b (h^{-1}) and c ($h^{-0.5}$) represent production rates determined by the model. These computed parameters, as well as the results obtained from the assays performed on effluents (VFA and ammonia nitrogen) were subjected to statistical analysis.

Experimental design
This experience was conducted using a fully randomized experimental design with two factors (time of harvest and incubation period) and four replications.

Results
Starch and NFC concentrations increased with the increase in the duration of sunlight before harvesting (see Table 1), except for the 8-hour period. However, the duration of sunlight had no effect on sucrose, fructose, pinitol or total sugar concentrations, but did cause a linear decrease in raffinose concentration ($P_{lin} < 0.0001$). The increases in starch (248%) and NFC (55%) concentrations observed in this study after 12 hours of sunlight were substantially larger than previously reported (20% for starch and 28% for NFC; Burns et al., 2005). This can probably be attributed to environmental factors since our study was conducted in controlled conditions whereas Burns et al. (2005) conducted their research in the field. The decrease in NFC concentrations after 8 hours, compared to the decrease observed after 4 hours of sunlight, is the result of the drop in starch levels. However, starch concentrations did increase markedly after 12 hours of sunlight, which in turn produced an increase in NFC levels. As far as we know, this decrease, after 8 hours, has never been reported before in the literature and remains difficult to explain.

Table 1: Chemical composition (% DM) of alfalfa harvested after 0, 2, 4, 8 and 12 hours of sunlight

	Duration of sunlight (hours)					SEM	*P*		
	0	2	4	8	12		L	Q	C
NFC[1]	11.9	13.7	14.0	12.9	18.5	0.92	0.0002	0.0986	0.0154
Total sugar[2]	7.7	8.1	8.5	8.5	8.0	0.45	0.5450	0.1832	0.9540
Sucrose	4.8	5.1	5.6	5.6	5.1	0.34	0.4059	0.1003	0.9394
Fructose	0.1	0.1	0.1	0.1	0.1	0.01	0.6780	0.6339	0.6559
Raffinose	0.3	0.2	0.3	0.2	0.1	0.02	<0.0001	0.2931	0.2400
Starch	4.2	5.6	5.6	4.5	10.4	0.70	<0.0001	0.0057	0.0029
Pinitol	2.5	2.7	2.5	2.6	2.7	0.15	0.5101	0.9169	0.6525

[1]NFC: Non-fibrous carbohydrates = Total sugar + Starch
[2]Total sugar: Sucrose + Fructose + Raffinose + Pinitol

Figure1 illustrates in vitro gas production curves, whereas Table 2 outlines computation of mathematical parameters as in the model described by France et al. (1993). These parameters indicate that the duration of sunlight had no effect on the latency period or on the quantity of DM lost in the rumen. However, the duration of sunlight had a cubic effect on the half-life ($P_{cub} = 0.01$), which is the time needed to produce half of the total volume of gas, as well as on the maximum gas production ($P_{cub} = 0.02$) within the rumen based on the curve's asymptote. Gas production within the rumen varied in the same fashion as the accumulation of starch and NFC. This suggests that an increase in the metabolic activity of bacteria within the rumen is associated with higher NFC concentrations. Lee et al. (2002) have reported similar observations after comparing perennial rye grass cultivars containing different levels of soluble sugar.

Table 2: Ruminal gas production kinetics for alfalfa harvested after 0, 2, 4, 8 and 12 hours of sunlight

	Duration of sunlight (hours)					SEM	*P*		
	0	2	4	8	12		L	Q	C
Half-life (h)	10.09	10.61	10.56	10.24	11.32	0.21	0.004	0.21	0.01
Latency period (h)	1.75	1.75	1.78	1.87	1.70	0.05	0.981	0.08	0.15
Asymptote(ml)	231.5	236.3	236.9	230.7	248.9	3.46	0.009	0.09	0.02
% decomposed	57.76	57.53	56.20	57.54	58.42	0.67	0.302	0.09	0.57

Average ammonia nitrogen and VFA concentrations are presented in Table 3. The increase in starch and NFC concentrations had no effect on ammonia nitrogen, acetate or n-butyrate concentrations, or on total NFC levels present in the ruminal fluid. However, the duration of sunlight caused a linear increase ($P_{lin} = 0.01$) in propionate concentrations and a decrease ($P_{cub} < 0.04$) in branched-chain VFA (isobutyrate, isovalerate, valerate). This usually indicates a drop in the catabolism of branch-chain amino acids. The linear increase in propionic acid concentrations caused a quadratic increase ($P_{quad} = 0.02$) in the propionate/(acetate + butyrate) [P/(A+B)] ratio, which indicates glucogenic, rather than lipogenic, ruminal fermentation, a more energy-efficient process.

Table 3: Ammonia nitrogen and volatile fatty acids concentrations in ruminal fluid after in vitro incubation of alfalfa harvested after 0, 2, 4, 8 and 12 hours of sunlight

	Duration of sunlight (hours)					SEM	*P*		
	0	2	4	8	12		L	Q	C
	mmol/L								
Ammonia N	5.2	5.1	5.1	5.2	4.9	1.72	0.10	0.42	0.17
Acetate	40.2	37.2	38.5	41.7	44.7	2.96	0.08	0.35	0.47
n-Butyrate	7.1	7.3	7.5	7.4	8.4	0.55	0.10	0.53	0.48
Propionate	11.8	11.6	12.8	13.6	14.6	1.00	0.01	0.96	0.81
Isobutyrate	1.5	1.1	1.1	1.1	1.0	0.08	0.001	0.07	0.01
Isovalerate	2.3	2.0	2.0	2.0	1.7	0.10	0.001	0.79	0.04
Valerate	1.6	1.2	1.3	1.4	1.2	0.09	0.01	0.42	0.009
Total	64.5	60.5	63.2	67.2	71.5	4.54	0.09	0.45	0.55
P/(A+B) ratio	0.25	0.26	0.28	0.28	0.27	0.01	0.01	0.02	0.63
	Mol/100 Mol								
Acetate	62.3	61.4	61.0	62.0	62.5	0.36	0.16	0.009	0.07
N-Butyrate	11.0	12.1	11.8	10.9	11.7	0.36	0.93	0.88	0.006
Propionate	18.3	19.2	20.3	20.3	20.3	0.44	0.002	0.03	0.40
Isobutyrate	2.3	1.8	1.8	1.7	1.4	0.13	0.0001	0.26	0.06
Isovalerate	3.6	3.4	3.1	3.1	2.4	0.17	<0.0001	0.52	0.19
Valerate	2.5	2.0	2.1	2.1	1.6	0.13	0.0002	0.91	0.02

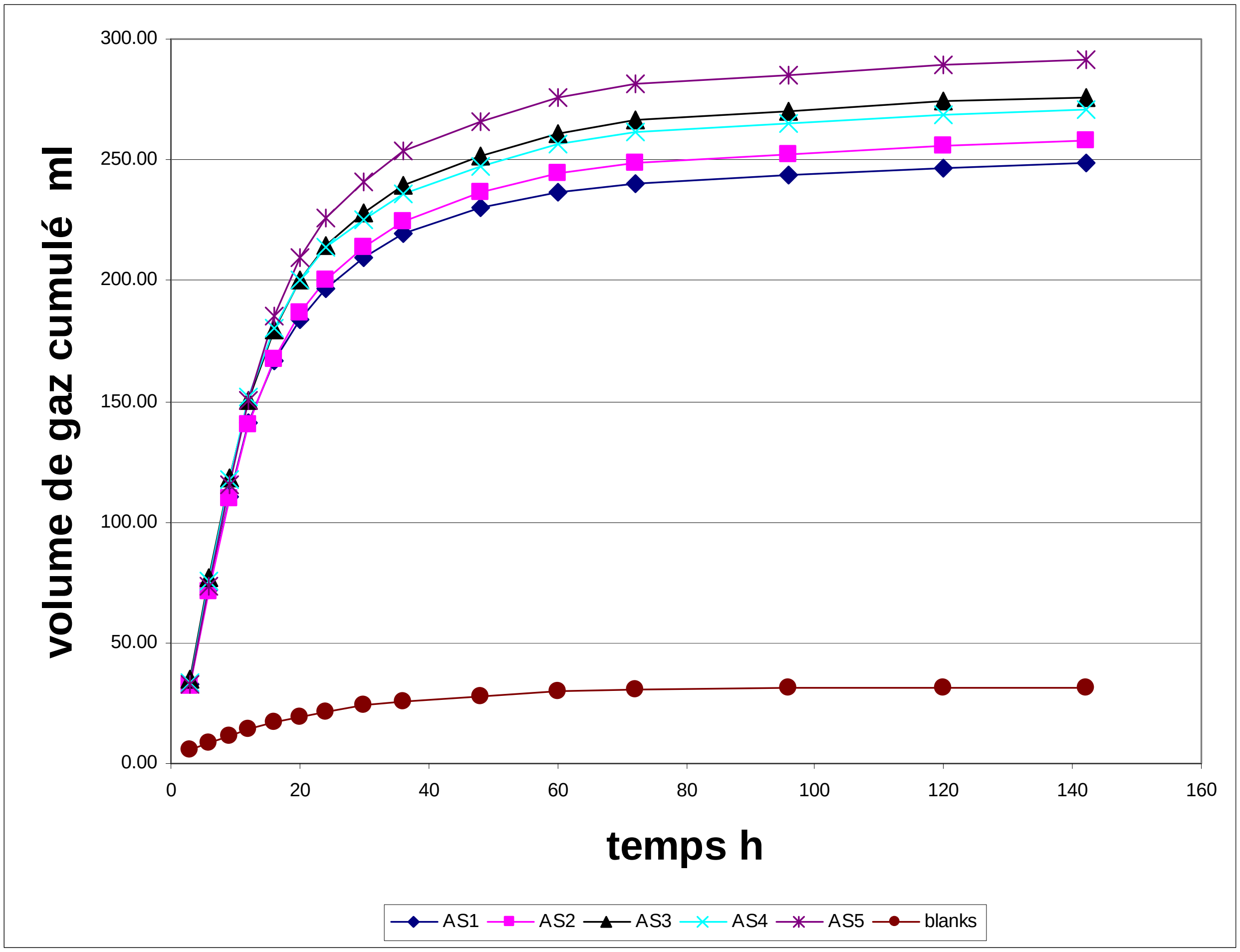

Figure 1: Accumulated gas production in artificial rumen as a function of duration of sunlight before mowing alfalfa: AS1 = 0 h; AS2 = 2 h; AS3 = 4 h; AS4 = 8 h; AS5 = 12 h and blanks = control bottles containing no forages (n = 4).

Conclusion

An increase in the duration of sunlight before mowing alfalfa plants promotes the accumulation of starch and NFC in alfalfa. Also, this increase in starch and NFC concentrations has a positive effect on ruminal fermentation of alfalfa (greater gas production and glucogenic ruminal fermentation) despite the absence of effect on total sugar concentrations.

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