

Effect of the concentration of *Spirulina* (*Spirulina platensis*) algae in the drinking water on water intake by cattle and the proportion of algae bypassing the rumen

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Abstract. *Spirulina*, a freshwater microalgae, has previously been shown to increase the efficiency of microbial protein production in cattle fed hay with a low crude protein content. The present study was carried out to determine the effect of increasing the concentration of *Spirulina* in the drinking water on the intake of water and the amount of water containing *Spirulina* bypassing the rumen of cattle. Five rumen-cannulated steers were given a fixed amount of pangola grass hay (14 g DM/kg W.day⁻¹) and water containing 0, 1, 2, 2.7 and 3.5% (w/w) *Spirulina* in an incomplete Latin square design. Water intake by the control steers (0% *Spirulina*) was 29.7 and 49.3 g/kg W for the first drinking event after it was made available and over 24 h, respectively. For steers receiving the algae, intake of water plus *Spirulina* increased linearly ($P < 0.01$) from 42.7 to 60.2 g/kg W during the first drinking event, as the concentration of *Spirulina* in the drinking water increased, but over 24 h was not affected by *Spirulina* concentration and averaged 74.4 g/kg W. The bypass of water through the rumen, as determined using chromium-EDTA as a marker, averaged $20.5 \pm 1.2\%$ and was not affected by the concentration of *Spirulina* in the drinking water. Increasing inclusion of *Spirulina* was associated with a decrease in rumen pH, an increase in urea concentration in blood serum, and an increase in ammonia-N concentration, propionate and branched-chain fatty acids, and a decrease in butyrate proportions in rumen fluid. *Spirulina* inclusion in the drinking water increased water intake and may provide a potential safe and inexpensive alternative to urea for extensively grazed ruminants.

Introduction

Spirulina (*Spirulina platensis*) feeding has been shown to increase the efficiency of microbial crude protein production in the rumen of cattle consuming forages of low crude protein (CP) content (Panjaitan 2010). Microalgal sources, such as *Spirulina*, grown in algal ponds and in the drinking water of cattle, may provide a source of N for cattle managed under extensive rangeland conditions. Intensive monogastric and ruminant production systems routinely administer nutrients to animals via the drinking water. The advantage is that all animals obtain the supplement in proportion to their water intake, in contrast to feeding nutrients in blocks or loose licks where supplement intake is more variable (Dixon *et al.* 2003). Under extensive grazing conditions in northern Australia, urea is often fed in the drinking water as a N source for cattle. However, one disadvantage with this approach is the risk of urea toxicity (Entwistle and Jephcott 2005). The provision of some of the N as algae within the drinking water or in a pond system would reduce the risk of toxicity from urea and, by virtue of the degradable protein, lipid, minerals and vitamin present in the algae, potentially enhance the efficiency of microbial CP production above that from the non-protein N source alone (Panjaitan 2010).

Cafe and Poppi (1994) reported that ~19% of imbibed water bypassed the rumen. *Spirulina* is rich in microelements, such as K, Na, Ca, Mg, Fe, Zn, Cu and Mn (Durand-Chastel 1980).

Spirulina contains Na (7.5 g/kg DM) and Cu (2.7 mg/kg DM), in addition to other minerals (Campanella *et al.* 1998), which may influence the closure of the oesophageal groove at high contents (Ruckebusch 1988) and increase the amount of water, and hence *Spirulina*, which bypasses the rumen. This experiment investigated the effect of the concentration of *Spirulina* in the drinking water of cattle on water intake and on the proportion of imbibed water that bypassed the rumen.

Materials and methods

All procedures were reviewed and approved by the University of Queensland Animal Ethics Committee.

Animals and experimental design

Five rumen-cannulated (Bar Diamond, Parma, ID, USA) commercial *Bos indicus* crossbred steers were used in the experiment. The steers were ~21 months of age and 294 ± 20 (\pm s.e.m.) kg liveweight at the start of the pre-experimental period. They had never had access to *Spirulina* under experimental conditions before the experiment.

The experiment was conducted between July and September 2007 at the University of Queensland, Mt. Cotton Research Farm, Australia. The steers were randomly allocated to five *Spirulina* treatments and five pens at the start of the pre-experimental period and remained in the same pens throughout. The experimental

design was a 5 by 3 incomplete Latin square, with five treatments (i.e. five Spirulina concentrations) and one replicate (steer) per treatment, represented in each of three runs. Each run consisted, sequentially, of a 7–9 day preliminary period; 5 days to measure food and water intake, 1 day to measure water bypass and 1 day to test the steer's preference for water containing Spirulina.

Immediately before the main experiment, feed and water intake of the steers were monitored for 20 days (pre-experimental period). Steers were offered a low-quality pangola grass (*Digitaria eriantha*) hay *ad libitum* and drinking water was available *ad libitum*. Mean daily feed intake was 14.7 g DM/kg W.day⁻¹ (4.2 kg DM/day) and mean daily water intake was 55 g/kg W.day⁻¹ (16.2 kg/day) during the pre-experimental period.

Diets and feeding management, and preparation and offering of Spirulina treatments

Pangola grass hay was chopped to less than 10 cm in length before feeding. Throughout each of the three runs, steers were offered a fixed amount of hay (14 g DM/kg W.day⁻¹). They were offered hay at 0930 h each day and allowed to eat until 1130 h, defined as the 'first eating activity', as determined in the pre-experimental monitoring period to be the average duration of the initial feeding event before the first water drinking event. The feed residue at 1130 h was collected, weighed and then returned to the feed trough and was available until feed residues were weighed and removed the following day.

Water containing the allocated amount of Spirulina was given to the steers at 1130 h each day. The Spirulina treatments were prepared by adding increasing amounts of Spirulina (Phytofoods Australia, Labrador, Qld, Australia) to a fixed amount of water (16.2 kg) in a 50-L bucket (Table 1). Suspension and even distribution of the Spirulina in the drinking water was maintained by constant circulation with a portable aerator pump. The steers were allowed to drink the water containing Spirulina voluntarily until they returned to consume the hay, defined as the 'first drinking activity'. The length of time of the 'first drinking activity' and the quantity of water containing Spirulina consumed were recorded before the remaining water containing Spirulina was returned to the animals. At 1430 h, any remaining water containing Spirulina, or water only for control steers, was removed, weighed and discarded and fresh water was offered *ad libitum* until 0800 h the following morning, when water residues were collected and weighed.

Table 1. Concentration of Spirulina in the drinking water offered and quantity of Spirulina imbibed by steers offered a fixed amount of pangola grass hay

Spirulina concentration (% of water; w/w)	Amount of Spirulina imbibed	
	(g DM/day)	(g DM/kg W.day ⁻¹)
0	0	0
1.0	148	0.5
2.0	289	1.0
2.7	406	1.3
3.5	528	1.7

Sampling procedures and measurements

Samples of hay offered were collected daily and bulked within a run for chemical analysis. Feed DM intake was recorded daily over the 5-day measurement period. Intake of water containing Spirulina was measured by weighing the amount of water containing Spirulina offered and remaining each day. Intake of untreated water was also determined daily. Water bypassing the rumen was measured by the method of Cafe and Poppi (1994). Briefly, following the 5-day measurement period, steers were deprived of water for 16 h during which time hay was available. This was chosen to simulate the episodic drinking events of cattle grazing in extensive areas. After 16 h without water, chromium-EDTA (Cr-EDTA)-labelled water (400 mg Cr-EDTA in 4 kg water) containing the allocated treatment levels of Spirulina was offered to steers 2 h after feed was offered in the morning. Labelled water was withdrawn when the steers stopped drinking (~10 min) and the rumen was emptied to determine water and Cr content in rumen contents. Intake of Cr-EDTA-labelled water was measured by weighing that offered and refused. Bypass of water from the rumen was estimated by determining the difference between Cr intake from the labelled water intake and the amount of Cr remaining in the rumen.

Rumen pool size was measured after emptying the rumen following a single drinking event where steers had access to Cr-EDTA-labelled water. Rumen digesta was removed manually by hand through the fistula and any liquid was removed by a vacuum pump. Total rumen contents were weighed, mixed thoroughly and subsampled. Triplicate subsamples (~500 g) were collected and DM content determined by drying to a constant weight at 65°C. From the rumen fluid collected, pH was recorded immediately and subsamples were taken for determination of the concentrations of Cr, NH₃-N and volatile fatty acids (VFA). Rumen fluid samples were stored at -20°C. The rumen contents were removed from and returned to the rumen within ~30 min.

Whole blood (10 mL) was obtained from the jugular vein of each steer and dispensed into a glass tube, stored at room temperature overnight before centrifugation at 870g for 15 min. An aliquot of serum was stored at -20°C for later analysis.

Maximum and minimum ambient temperature and average humidity over the previous 24 h period were recorded at the same time each day.

The preference of steers for water containing Spirulina compared with water alone was determined by offering 7 kg of water containing 2% Spirulina alongside 7 kg of water alone, in identical buckets, to the steers at 1130 h. The position of the buckets was randomly allocated for each steer and between runs. Time of first drink and intake for the first drinking activity, as well as cumulative intake after 10, 30 and 120 min was determined.

Chemical analysis

Dry matter content was determined by drying samples to a constant weight at 65°C in a fan forced oven. Organic matter (OM) content was determined by combusting samples in an electric muffle furnace (Carbolite, Hope Valley, UK) at 550°C for ~4.5 h. Ash-free neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were measured using an Ankom fibre analyser (ANKOM 220; Ankom Technology, New York, NY, USA) (Van Soest *et al.* 1991). Total N

content was measured using the Leco system (LECO FP-428; LECO Australia, Castle Hill, NSW, Australia). Crude lipid content of the Spirulina was determined using a chloroform:methanol extraction procedure as described by Hara and Radin (1978). Chromium was analysed in rumen fluid samples by inductively coupled plasma optical emission spectrometry (Varian Vista-PRO Radial; Varian Australia, Melbourne, Vic., Australia) after the background matrix was prepared for standards and samples. Ammonia-N ($\text{NH}_3\text{-N}$) concentration in rumen fluid was measured by distillation using a Buchi 321 distillation unit (Buchi; Labortechnik AG, Flawil, Switzerland) with saturated sodium tetraborate (>260 g/L) used to adjust the pH. The distillate was titrated (Titralab 840; Radiometer Analytical SAS, Lyon, France) with 0.01 M HCl to calculate total N content. The concentrations of VFA and branch chain fatty acids (BCFA) were measured in rumen fluid diluted 1 : 4 in 20% metaphosphoric acid containing an internal standard (50 μM 4-methyl *n*-valeric acid) by gas chromatography (GC-17A; Shimadzu, Tokyo, Japan). Serum urea concentration was determined enzymatically with an automated analyser (Olympus AU400; Olympus, Mount Waverly, Vic., Australia) and a blood urea-N kit (BUN Reagent; Thermo Electron, Noble Park, Vic., Australia).

Statistical analyses

For each variable, the data were initially summarised by fitting a general linear mixed model, with run and supplement level as fixed effects and steer as a random effect. A sequence of general linear mixed models was then fitted to determine an appropriate low order polynomial model to describe the responses to Spirulina concentration (%) for each variable. All models included run as a fixed effect and steer as a random effect, and all concentration values had the mean concentration for control subtracted from them. Initially, quadratic models for the response to Spirulina concentration were compared with linear responses, based on either linear or quadratic response curves depending on the result of the initial test. An approximate R^2 for the model was calculated from the reduction in the sum of squares of residuals from the corresponding model with no concentration terms. The statistical significance of preference for water containing Spirulina or water

alone was tested using the sign test, with the preference outcome considered positive if more water containing Spirulina was drunk than water alone in the interval being considered. All analyses were carried out using the statistical package GENSTAT for Windows (GENSTAT 2007, VSN International, Oxford, UK).

Results

The chemical composition (g/kg DM) of the pangola grass hay included OM: 936; N: 5.5; NDF: 735; ADF: 396 and lignin: 38. The chemical composition (g/kg DM) of the Spirulina included OM: 907; N: 115; and EE: 98.

The average daily minimum and maximum temperatures during the experiment were 12°C and 25°C, respectively, with an average relative humidity of 59%.

Intake of water and Spirulina

In the absence of Spirulina, the water intake and the time spent drinking during the 'first drinking activity' were 29.7 g/kg W and 1.29 min, respectively, while for the intake of water plus Spirulina (hereafter referred to as treated water) both measurements increased linearly with increasing concentration of Spirulina in the drinking water (Table 2). During this activity, intake of treated water increased from 42.7 to 60.2 g/kg W and the duration increased from 2.12 to 4.25 min as the concentration of Spirulina in the drinking water increased from 1 to 3.5%. Intake of water plus Spirulina over a 24 h period was not affected by Spirulina concentration and averaged 74 g/kg W for the Spirulina treatments, compared with 49.2 g/kg W for the control untreated water.

Water bypassing the rumen

The steers consumed almost all Cr-EDTA-labelled water containing different amounts of Spirulina within 6 min of offering, after they were deprived of water for 16 h. The mean intake of the labelled water containing Spirulina was 3.8 ± 0.1 kg/steer. The amount of labelled water bypassing the rumen was not affected by Spirulina concentration, with $20.5 \pm 1.2\%$ of labelled water containing Spirulina bypassing the rumen. There was no effect of Spirulina concentration in the

Table 2. Relationships between the concentration of Spirulina in the drinking water and the intake of water plus Spirulina, and the pH, concentration of ammonia-N ($\text{NH}_3\text{-N}$) and molar proportions of propionate, butyrate and branched chain fatty acids (BCFA) in the rumen fluid and the concentration of urea in the serum of steers fed a fixed level of pangola grass hay
x, 0–3.5% Spirulina w/w. RSD, residual standard deviation

Parameter	Equation	R^2	RSD	P-value
<i>Water containing Spirulina intake</i>				
First drinking activity (g/kg W)	$y = 35.7 + 7.01x$	0.41	9.71	0.009
Duration of first drinking activity (min)	$y = 1.26 + 0.86x$	0.55	1.16	0.012
<i>Rumen parameters and serum urea</i>				
Rumen pH	$y = 7.15 - 0.07x$	0.14	0.14	0.023
Ln $\text{NH}_3\text{-N}^A$ (mg/L)	$y = 3.38 + 1.23x - 0.22x^2$	0.89	0.27	0.003
Propionate molar proportion (mmol/mol)	$y = 157.1 + 26.8x - 6.7x^2$	0.46	1.43	0.025
Butyrate molar proportion (mmol/mol)	$y = 144.2 - 9.1x$	0.61	1.55	0.029
BCFA molar proportion (mmol/mol)	$y = 10.5 + 0.9x$	0.75	0.09	0.001
Serum urea (mM)	$y = 0.72 + 0.84x$	0.95	1.99	<0.001

^ANatural logarithm of $\text{NH}_3\text{-N}$ concentration.

drinking water on total wet (52.1 ± 2.6 kg) or dry (6.3 ± 0.1 kg) weight of digesta, or weight of water (45.8 ± 2.5 kg) in the rumen.

Rumen parameters

Rumen pH declined gradually (7.2–6.9) but linearly with increasing Spirulina concentration in the drinking water (Table 2). A quadratic increase in Ln rumen $\text{NH}_3\text{-N}$ concentration was observed with increasing Spirulina concentration in the drinking water and peaked at ~ 160 mg $\text{NH}_3\text{-N/L}$ (backtransformed from Ln 5.1) when Spirulina concentration was 2.7%. There was a linear increase in serum urea from 0.7 to 3.7 mM with increasing Spirulina concentration. The mean total VFA concentration in the rumen fluid of steers was 85 ± 2.3 mM, which was not affected by increasing Spirulina concentration. With increasing Spirulina concentration in the drinking water, there was a quadratic change in the molar proportion of propionate in total VFA (Table 2), increasing from a low of 157 for the control to 184 mmol/mol at 2% Spirulina inclusion in the drinking water. Correspondingly, there was a linear decrease in butyrate proportion from 144 (control) to 112 mmol/mol (3.5% Spirulina concentration) and a linear increase in the proportion of BCFA from 10.5 to 13.7 mmol/mol over the same range. The molar proportion of acetate in total VFA was not affected by the concentration of Spirulina in the drinking water (679 ± 7 mmol/mol).

Preference test

All steers, regardless of previous treatment, preferred water containing Spirulina compared with untreated water 10 min after offering ($P < 0.001$). Steers offered 0, 1.0, 2.0, 2.7 and 3.5% Spirulina as their treatment before the preference test, consumed 100, 99, 92, 99 and 99%, respectively, of the 2% Spirulina solution, within 10 min of commencing the preference test. In contrast, no untreated water was consumed within the first 10 min of the test. Only one steer, which received 2% Spirulina concentration before the preference test, did not consume the full amount of 2% Spirulina and commenced consumption of untreated water before the completion of the 2% Spirulina solution, between 10 and 20 min of commencement of the preference test. Some steers consumed water alone immediately on completion of drinking the treated water, but no consistent pattern in subsequent water intake was observed.

Discussion

This experiment demonstrated that drinking water containing Spirulina was highly acceptable by cattle and had no deleterious effects on total water intake over a 24 h period. Steers strongly preferred water that contained Spirulina to water alone when given a choice between both. Approximately 20% of imbibed water bypassed the rumen and this was not influenced by Spirulina concentration in the drinking water.

Total daily water intake (treated and untreated water) was increased in animals receiving the Spirulina treatment due to an increased preference for Spirulina in the water. There are several animal and environmental factors that may affect water intake including bodyweight, physiological state, DM intake, Na intake

and ambient temperature and humidity (Beede 1991; Murphy 1992). However, it is commonly accepted that water quality also influences its intake. Several studies have indicated that bad-tasting water and odour limit water intake (Willms *et al.* 2002; Frank *et al.* 2004; Lardner *et al.* 2005). Total daily intake of water plus water containing Spirulina by steers receiving the Spirulina treatments was greater than that of control steers, perhaps as a function of increased DM intake due to increasing Spirulina DM in the drinking water and the high palatability of water containing Spirulina. Water intake was not depressed but stimulated with the inclusion of Spirulina over short (2 h) or longer (24 h) periods. The preference test reinforced that when steers were offered Spirulina in the drinking water and fed pangola grass hay deficient in CP, a preference to consume Spirulina treated water over untreated water was demonstrated. Spirulina contains Na (Durand-Chastel 1980), for which ruminants have a recognised appetite (Denton 1982) and this may be one explanation for the preference for water containing Spirulina. Alternatively, the steers may have preferentially selected water containing Spirulina to address a nutrient deficiency (e.g. CP) of the pangola grass hay.

The amount of water bypassing the rumen is influenced by several factors, such as the duration of water deprivation, extent of rumen fill and availability of minerals such as Na and Cu (Ruckebusch 1988). The Na and Cu content of Spirulina was not determined in the present study, but the results indicate that increasing Spirulina concentration in the drinking water did not change the amount of water bypassing the rumen, suggesting that the Spirulina was not included at a sufficient concentration, or did not contain high enough mineral content, to influence the closure of the oesophageal groove. This amount of water bypassing the rumen is in agreement with Cafe and Poppi (1994), where 19% of labelled water bypassed the rumen. More importantly, this suggests that $\sim 20\%$ of Spirulina fed in this way may also escape rumen degradation and thus be available for digestion and absorption directly from the abomasum. This has important implications for N supply to the animal, indicating that this protein source may provide a source of both rumen degradable and undegraded protein to the animal. The increasing ammonia concentrations in the rumen with increasing Spirulina intake indicate that there is still appreciable degradation of the protein within the rumen for microbial utilisation.

The intake of Spirulina in the drinking water by cattle will also provide an additional source of fermentable organic matter to the animals. In the current experiment, total VFA proportion was unaffected by Spirulina concentration in the drinking water, however, there was a small increase in the molar proportion of propionate and BCFA, and a small decrease in butyrate. These changes in the molar proportion of VFA are biologically small but may provide substrates that promote microbial protein production within the rumen and enhance the growth of the animal.

In conclusion, it appears that algal sources, such as Spirulina, can be successfully delivered to animals in their drinking water, up to 3.5% Spirulina w/w, and that this may provide a potential method to supply a rich and safe source of protein to animals. It is conceivable that algae grown on property could be delivered in the drinking water as a safe and inexpensive alternative to urea. A comparison of efficacy for animal production with other traditional protein sources is warranted.

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