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Spirulina (*Spirulina platensis*) algae supplementation increases microbial protein production and feed intake and decreases retention time of digesta in the rumen of cattle

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Abstract. Cattle consuming pastures low in protein have low liveweight gain due to low rumen degradable protein (RDP) supply and thus low microbial crude protein (MCP) production and efficiency of MCP production [EMCP, g MCP/kg digestible organic matter (DOM)]. Nitrogen supplements can increase MCP production and EMCP of cattle grazing low protein pastures. The objective of this study was to compare the effects of supplementation with a non-protein-N source (NPN), in this case urea and ammonium sulfate (US), with a single-cell algal protein source (*Spirulina platensis*), on intake, microbial protein supply and digestibility in cattle. Nine cannulated *Bos indicus* steers [initial liveweight 250.1 ± 10.86 (s.d.) kg] were fed Mitchell grass hay (*Astrebla* spp; 6.1 g N, 746 g NDF/kg DM) *ad libitum* and were supplied with increasing amounts of US (0, 6, 13, 19 and 33 g US DM/kg hay DM) or Spirulina 0, 0.5, 1.4, 2.5 and 6.1 g Spirulina DM/kg W.day in an incomplete Latin square design. The response of MCP production and EMCP to increasing amounts of the two supplements was different, with a greater response to Spirulina evident. The MCP production was predicted to peak at 140 and 568 g MCP/day (0.64 and 2.02 g MCP/kg W.day) for the US and Spirulina supplements, respectively. The highest measured EMCP were 92 and 166 g MCP/kg DOM for the US and Spirulina treatments at 170 and 290 g RDP/kg DOM, respectively, or a Spirulina intake of 5.7 g DM/kg W.day. Increasing RDP intake from US and Spirulina resulted in an increase in Mitchell grass hay intake and rumen NH_3-N concentration and reduced the retention time of liquid and particulate markers and digesta DM, NDF and lignin in the rumen with greater changes due to Spirulina. Total DM intake peaked at a Spirulina supplement level of 4.6 g Spirulina DM/kg W.day with a 2.3-fold higher DOM intake than Control steers. Rumen NH_3-N concentrations reached 128 and 264 mg NH_3-N/L for the US and Spirulina treatments with a significant increase in the concentration of branched-chain fatty acids for the Spirulina treatment. The minimum retention time of liquid (Cr-EDTA; 23 and 13 h) and particulate (Yb; 34 and 22 h) markers in the rumen were significantly lower for Spirulina compared with US and lower than unsupplemented animals at 24 and 34 h for Cr-EDTA and Yb, respectively. Spirulina could be provided safely at much higher N intakes than NPN supplements. The results suggest that, at an equivalent RDP supply, Spirulina provided greater increases than US in MCP production, EMCP and feed intake of *Bos indicus* cattle consuming low protein forage and could also be fed safely at higher levels of N intake.

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Introduction

Cattle consuming forages low in protein and digestibility are known to have low microbial crude protein (MCP) production and an associated low efficiency of MCP production (EMCP) primarily due to a deficit of rumen degradable protein (RDP) within the rumen (Poppi and McLennan 1995). Micro-algae are single-cell organisms, which may be high in protein, lipids and other nutrients (nucleic acids, vitamins, minerals), depending on the species and environment under which they are grown. They may increase MCP production within the rumen when fed to cattle. Increasing EMCP and hence protein supply and absorption should result in higher liveweight gain in cattle (Poppi and McLennan 1995). Feeding standards indicate that with the provision of non-protein nitrogen (NPN), EMCP will peak at ~130 g MCP/kg total digestible nutrients or digestible organic matter (DOM; NRC 1996; CSIRO 2007). By contrast, when true protein supplements are fed increases in EMCP to ~170 g MCP/kg DOM, typical of values achieved with temperate forages, have been reported (CSIRO 2007).

Algae have several features which could make them a valuable supplement for grazing livestock. They may provide a means of supplying degradable protein to cattle grazing in rangeland

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environments where forages are typically low in protein and digestibility for extended periods of the year. Some species are high in longer-chain fatty acids, in particular docosahexaenoic acid (DHA), and recently Pickard *et al*. (2008) demonstrated that supplementation with algae during pregnancy increased both plasma DHA concentration in the ewe and lamb activity at birth. The concentration of DHA has also been increased in the milk and rumen fluid of cows and sheep in response to algal supplementation (Franklin *et al*. 1999; Papadopoulos *et al*. 2002; Or-Rashid *et al*. 2008).

The objective of our experiment was to compare the effects of Spirulina algae (*Spirulina platensis*) and urea-sulfur (US) supplementation on intake, digestibility, retention time and MCP production of steers fed a low protein hay typical of that found in the rangelands of northern Australia.

Materials and methods

The experiment was conducted at the University of Queensland's Mt Cotton Research Farm, Queensland, Australia. All procedures were conducted in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Anon. 2004) and were reviewed and approved by the University of Queensland Animal Ethics Committee.

Animals

Nine rumen-cannulated (Bar Diamond; Parma, ID, USA) Brahman-Shorthorn crossbred steers were used. The steers were ~18 months of age and weighed 250.1 ± 10.86 (±s.d.) kg at the commencement of the experiment. All steers were provided with a cobalt pellet (300 g cobalt oxide/kg; Coopers Animal Health, Australia) and treated with moxidectin (Cydectin; Fort Dodge, Australia) to control internal and external parasites, before the commencement of the experiment. The steers had no previous exposure to algae.

Experimental design

Steers were randomly allocated to individual floor pens, metabolism crates and treatments (type and amount of supplement). They remained in the same floor pens and metabolism crates throughout the experiment but were allocated to different treatments for each run.

An incomplete Latin square design was used with 9 steers, 9 diets (2 supplement types) and 3 experimental runs. The basal diet was native grass, hereafter Mitchell grass (*Astrebla* spp.) hay [6.1 g N and 746 g ash-free neutral detergent fibre (NDF)/kg DM]. The two supplements were US [9 parts (w/w, as-fed) urea (Incitec Pivot Ltd, Australia] mixed with 1 part ammonium sulfate (AMSUL; Hifert Ltd, Australia) and Spirulina (Life Stream International, New Zealand). Each run included 1 Control steer fed Mitchell grass hay alone and 1 replicate (steer) of each supplement type at 4 levels of intake, that is, 6, 13, 19 and 33 g US DM/kg hay DM and 0.5, 1.4, 2.5 and 6.1 g Spirulina DM/kg liveweight (W).day. The intention was to supply equivalent and increasing amounts (g) of RDP/kg DOM for both supplement types, i.e. 90, 130, 170 and 210 g for US (US90, US130, US170 and US210, respectively), and 90, 130, 170 and 290 g for Spirulina (S90, S130, S170 and S290, respectively). The US was not supplied at the comparable highest amount relative to Spirulina due to potential risks of urea toxicity.

The duration of each experimental run was 30 days, including the first 13 days in floor pens and the remaining 17 in metabolism crates. Each run comprised, sequentially, a 14-day preliminary feeding period, 7-day collection period when total faecal and urinary output were measured, 2 days for rumen sampling and measurement of retention times, 1 day for rumen emptying and a further 6 days for other procedures not reported here.

Feeds and feeding procedures

Mitchell grass hay, so named because it was obtained from the Mitchell grassland community of the Barkly Tableland, Northern Territory, Australia, was actually a mixture of rangeland grasses comprising ~35% Mitchell grass and 55% Flinders grass (*Iseilema* spp.) plus a mixture of other native grasses. The hay was chopped to less than 10 cm in length before feeding. *Ad libitum* feed intake for each steer was established during the first 13 days of each experimental run while the steers were in floor pens and intake was thereafter fixed at 90% of this value. Steers were fed once each day at 0830 hours in floor pens and then continuously in equal hourly increments using an automatic feeder (Minson and Cowper 1977) in the metabolism crates.

Spirulina was mixed with water to make a 25% (w/w) Spirulina mixture and administered through the cannula immediately before feeding hay each day during the preliminary feeding period and twice daily at 0830 hours (30%) and 1430 hours (70%) during the 7-day collection period. When Spirulina was provided, water intake via the cannula was equilibrated for all steers by adding sufficient volume equal to that provided with the S290 treatment. Thus ~10 L of water total was administered via the cannula of each steer daily. During the measurement of retention time (2 days following the collection period), the Spirulina was administered in equal amounts at hourly intervals over 24 h before rumen emptying.

The US supplement was mixed with water to make a 25% (w/w) US solution, which was sprayed onto, and mixed thoroughly with, the hay. Water application to the hay was equilibrated for all steers at the level provided for the US210 treatment to attain similar moisture content in the hay offered. The steers had access to fresh drinking water at all times.

Measurements and sample collection

Samples of hay offered were collected daily and bulked for each run and the chemical composition and DM proportion of leaf and stem determined. Feed intake and faecal output were measured in metabolism crates over the 7-day collection period. Daily urine output of each steer was collected into containers (with 10% H_2SO_4 to maintain total daily urine pH below 3), weighed, mixed thoroughly and a subsample collected, bulked and frozen over the 7-day collection period.

Rumen fluid was sampled via the cannula of each steer after the completion of the collection period and before rumen emptying for the measurement of rumen pH and the concentrations of NH_3-N and volatile fatty acid (VFA) at 0900, 1000, 1200, 1400, 1500, 1700 and 1900 hours. Samples

for VFA analysis were bulked across sampling times within steers.

Retention time was measured by complete emptying of the rumen and also by marker dilution. Retention time of the fluid using chromium-EDTA (Cr-EDTA) and particle matter using ytterbium chloride [$YbCl_3.6H_2O$ (YbCl)], markers was determined by administration of marker on the final day of each faecal collection period. A single dose of Cr-EDTA solution was administered (~154 mg Cr/100 kg W) across 4 different sites in the rumen of each steer, via the rumen cannula. Similarly, YbCl (~1000 mg Yb/100 kg W) was injected into 4 different sites in the rumen. Duplicate rumen fluid (~10 mL) and digesta (~100 g) samples were collected from several sites in the rumen before dosing (0 h) and 1, 3, 5, 8, 10, 24, 32 and 48 h after dosing and stored at −20°C. The Ln concentration of Cr (in fluid) and Yb (in digesta) was regressed against time and the slope (k) provided the fractional outflow rate while retention time of markers was calculated as 1/k.

Retention time of DM, NDF and lignin using the rumen emptying procedure was measured 48 h after each faecal collection period. The rumen of each steer was completely emptied and total contents weighed ~30 min after the last hourly feed. After thorough mixing, triplicate homogenous subsamples of total rumen contents (~1000 g) were dried to a constant weight at 65°C, ground (1 mm) and stored for NDF and lignin analysis. Retention time (h) was calculated by dividing the total rumen contents of DM, NDF or lignin (kg) by the average hourly intake rate (kg/h), according to Minson (1966).

Chemical analyses

Dry matter content of feeds, feed refusals and faeces was determined by drying samples to a constant weight at 65°C in a forced fan oven. Organic matter content was determined by combusting samples in an electric muffle furnace (Carbolite, England) at 550°C for ~4.5 h. The Ankom fibre analyser (ANKOM 220, USA), incorporating the use of α-amylase, was used to measure the concentration of ash-free NDF, ash-free acid detergent fibre (ADF) and lignin based on the method of Van Soest *et al*. (1991). Total N content was measured using the Leco system (LECO FP-428, USA) and CP content calculated (N × 6.25). Crude lipid content of the Spirulina was determined using a chloroform/methanol extraction procedure as described by Hara and Radin (1978).

Chromium in rumen fluid was analysed by first centrifuging samples at 2500*g* for 5 min, removing the supernatant and then further centrifuging at 2500*g* for 7 min before direct analysis on an Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES, USA). Ytterbium was analysed in dried, ground whole digesta by the method of de Vega and Poppi (1997). A background matrix was prepared for standards and all samples were analysed by ICP-AES. Ammonia-N concentration in rumen fluid was measured by distillation using a Buchi 321 distillation unit (Buchi Scientific Apparatus Flawil, Switzerland) with saturated sodium tetraborate (>260 g/L) used to adjust pH. The distillate was titrated (TritaLab 840 radiometer, France) with 0.01 M HCl to calculate total N concentration. Rumen fluid samples for VFA were prepared by immediately placing an 8-mL sample into 2 mL of a 20% solution of metaphosphoric acid containing an internal standard (50 μM 4-methyl n-valeric acid), which were stored at −20°C. After thawing, centrifugation and filtration (0.45-μm membrane filter), concentrations of VFA and branched-chain fatty acids (BCFA) were determined by gas chromatography (GC-17 A, Shimadzu, Japan) using a polar capillary column, automatic injector and flame ionisation detector. Serum urea-N concentration was determined on an Olympus AU 400 automated clinical analyser, using a commercially available kit (Thermo Electron, Australia). Total S in Mitchell grass hay was measured on a Vista Pro ICP-OES instrument (Varian, USA) following an acid digest (5 : 1, nitric : perchloric acid mixture).

The acidified urine samples were thawed, mixed and diluted (1 : 20) in stock buffer containing 10% ammonium phosphate ($NH_4H_2PO_4$) solution and allopurinol (100 μM), as an external standard. Prior to analysis for purine derivatives, diluted urine samples were filtered through a 300-mg C-18 Sep-Pak cartridge. Solutions of known concentrations of allantoin, uric acid, xanthine and hypoxanthine were used to produce standard values, as described by Balcells *et al*. (1992). Purine derivatives (i.e. allantoin, uric acid, xanthine and hypoxanthine) in urine samples were determined by HPLC (Agilent 1100 Series, USA). Separation and quantification were achieved by using a Bondaclone C-18 reversed-phase column (300 mm × 3.9 mm i.d.; Phenomenex; USA). Sample injection volume was 20 μL with the detector set at 205 nm. Data were analysed using Agilent 'Chemstation' software. Estimation of MCP production was based on the method of Chen and Gomes (1995) with a value of 0.190 mmol/kg $W^{0.75}$.day used for the endogenous purine derivative excretion for *Bos indicus* cattle (Bowen *et al*. 2006).

Statistical analyses

For each variable the data were initially summarised by fitting a general linear mixed model, with run, supplement type, supplement level and interaction between type and level as fixed effects and steer as a random effect. Graphs of the residuals were used to check for outliers and the assumption of homogeneity of variance and minimal skewness. A sequence of general linear mixed models was then fitted to determine an appropriate low order polynomial model to describe the responses to intake of supplement or RDP (g/kg DOM) for each variable. For curve fitting all RDP values had the mean RDP for Control subtracted from them so that the origin for RDP corresponded to no supplement. Initially, quadratic models for the response to RDP for each supplement type were compared with linear responses, and then the responses between types were compared, based on either linear or quadratic response curves depending on the result of the initial test. Where differences between types were not significant, a common function was fitted. Finally, if the slope for a linear response was not significant the term was removed; i.e. a constant response was used in the model. The resulting model was illustrated by plotting the fitted curves with means from the initial summary included on the plot. The residual standard deviation (RSD) was determined and 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 RDP terms. Where the final model had separate curves for US and Spirulina supplementation, the

no-supplement responses were included in the RSD and R^2 calculations for both supplement types. Because of high variability in the EMCP data at high Spirulina intakes, data were transformed by the natural logarithm (Ln) before analysis, to stabilise the variance.

The pattern of change in rumen NH_3-N over time of measurement within a day (0, 1, 3, 5, 6 and 10 h after feeding) was estimated by fitting a general linear mixed model with run, supplement type, supplement level, type by level interaction and time as fixed effects and steer and steer by run as random effects.

Statistical significance was determined at $P < 0.05$ in all cases. All analyses were done using the statistical package GENSTAT 2007 (GENSTAT for Windows, 10th edition, VSN International Ltd, Hemel Hempstead, UK).

Results

The Mitchell grass hay contained 887 g OM, 6.1 g N, 3.4 g S, 746 g NDF, 481 g ADF and 65 g lignin/kg DM while the Spirulina contained 912 g OM, 114.3 g N, 35 g NDF, 18 g ADF and 100 g crude lipid/kg DM. The hay comprised 52% leaf. The estimated values for RDP/DOM (g RDP/kg DOM), determined from the measured values of feed CP content and OM digestibility, were 62 for the Control (Mitchell grass hay) and 109, 138, 175 and 245 as US supply increased and 116, 155, 199 and 358 as Spirulina supply increased with treatment.

Effect of supplement intake on microbial protein production, intake and digestibility

Microbial protein production increased quadratically in response to increasing US or Spirulina intake (Table 1), with peak values of 0.64 and 2.02 g/kg W.day (~140 and 568 g/day), respectively. The Ln EMCP increased linearly over a narrow range of DM intakes of US and quadratically with Spirulina (Table 1). In the latter case, EMCP peaked at 166 g MCP/kg DOM (back-transformed value) when Spirulina intake was 5.7 g/kg W.day. Both hay and total DM intakes increased quadratically with increasing US and Spirulina intake, with no difference between supplement responses (Table 1), and predicted peak values were 22 and 26 g/kg W.day with supplement intakes of 3.7 and 4.6 g DM/kg W.day, respectively. Organic matter digestibility increased linearly with increasing intake of US or Spirulina (Table 1). There was a quadratic increase in DOM intake with both supplement types (Table 1) with peak intakes of 7.6 and 12.1 g/kg W.day when intakes of US and Spirulina were 0.4 and 4.9 g/kg W.day, respectively.

Effect of RDP supply on microbial protein production, intake and digestibility

The MCP production and EMCP for Control steers were 66 g/day (0.27 g/kg W.day) and 54 g MCP/kg DOM, respectively. Microbial protein production increased quadratically in response to increasing RDP supply, relative to DOM intake, from both Spirulina and US supplements, with the response much greater to Spirulina (Fig. 1; Table 2). The Ln EMCP increased quadratically with increasing RDP supply from Spirulina and linearly from US, with higher responses being from the Spirulina treatment (Fig. 1; Table 2).

Mean hay intake of the Control steers was 12.8 g DM/kg W.day. Hay and total DM intake increased quadratically with increasing RDP supply, with the response in both cases being higher for Spirulina than US (Fig. 2; Table 2). Hay intake peaked at 18.5 and 24.5 g DM/kg W.day when RDP supply was 200 and 300 g/kg DOM for the US and Spirulina treatments, respectively. The digestibility of DM, OM and NDF for Control steers was 43.5, 46.4 and 52.7%, respectively, and the total DOM intake was 5.3 g/kg W.day. There was a linear increase in OM digestibility in response to increasing RDP supply from both US and Spirulina,

Table 1. Effect of urea-ammonium sulfate (US) and Spirulina intake (X; g DM/kg W.day) on microbial crude protein (MCP) production and the efficiency of MCP production (EMCP), on hay and total DM intake, digestibility of organic matter (OMD) and total digestible organic matter intake (DOMI) of steers fed Mitchell grass hay alone or supplemented with US or Spirulina

Where there was no significant difference between response relationships for the two supplement types a combined regression equation is given and the degree of fit of different supplement treatments to that combined equation is given separately. R^2, proportion of variability about mean response for supplement group (including Controls) accounted for by fitted curve. RSD, residual standard deviation about fitted curve for supplement group (including Controls). *P*, significance probability for highest order coefficient in equation

Y	Supplement	Equation	R^2	RSD	*P*
MCP production (g/kg W.day)	US	$Y = 0.314 + 1.655\ X - 2.106\ X^2$	0.81	0.078	<0.05
	Spirulina	$Y = 0.314 + 0.436\ X - 0.0279\ X^2$	0.99	0.043	<0.001
Ln EMCP (g MCP/kg DOM)	US	$Y = 4.149 + 0.716\ X$	0.38	0.219	<0.01
	Spirulina	$Y = 4.149 + 0.337\ X - 0.0296\ X^2$	0.92	0.138	<0.05
Hay DM intake (g/kg W.day)	Combined	$Y = 14.68 + 3.890\ X - 0.5270\ X^2$			<0.001
	US		0.34	1.74	
	Spirulina		0.89	1.19	
Total DM intake (g/kg W.day)	Combined	$Y = 14.68 + 4.890\ X - 0.5270\ X^2$			<0.001
	US		0.43	1.74	
	Spirulina		0.95	1.19	
OMD (%)	Combined	$Y = 46.51 + 1.1054\ X$			<0.001
	US		0.14	1.54	
	Spirulina		0.77	1.59	
DOMI (g/kg W.day)	US	$Y = 5.237 + 12.498\ X - 16.7051\ X^2$	0.81	0.51	<0.001
	Spirulina	$Y = 5.237 + 2.802\ X - 0.2847\ X^2$	0.99	0.33	<0.001

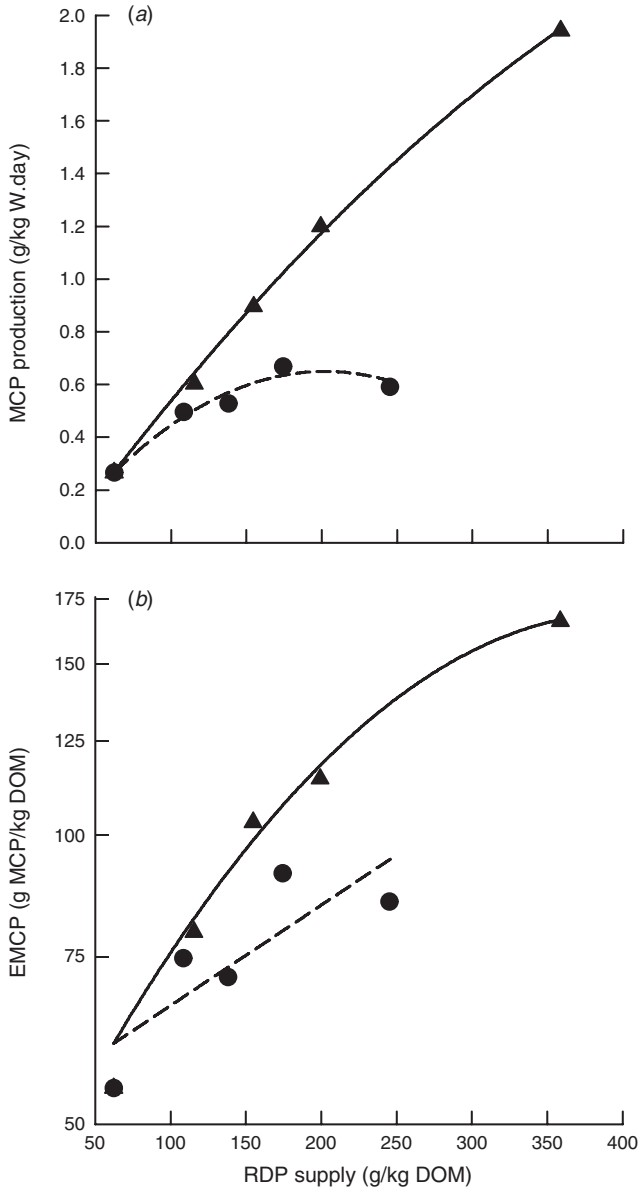

Fig. 1. Effect of rumen degradable protein (RDP) supply [g/kg digestible organic matter (DOM)] on (*a*) microbial crude protein (MCP) production and (*b*) the efficiency of MCP production (EMCP) for steers given Mitchell grass hay alone or supplemented with urea-ammonium sulfate (US; ●; dashed line) or Spirulina (▲; solid line). Points shown on the graph are treatment means for steers across runs. Values for EMCP are treatment means (geometric means) back-transformed after the analysis of variance of Ln-transformed data. The equations describing the response relationships are shown in Table 2.

the rate of increase being greater with the algae source (Table 2). Total DOM intake increased quadratically with increasing intake of both supplements but the response was greater for Spirulina compared with US (Fig. 2; Table 2).

Effect of RDP supply on retention time of markers and of DM, NDF and lignin in the rumen

The retention time of Cr-EDTA and Yb in the rumen of steers consuming Mitchell grass alone was 24.0 and 33.9 h, respectively. Retention time of Cr-EDTA declined quadratically with increasing RDP supply from US and Spirulina with the effect being greater for the Spirulina treatment (Fig. 3; Table 2). The minimum retention time of Cr-EDTA was predicted to occur at 164 g RDP/kg DOM for US (23 h) and at 326 g RDP/kg DOM for Spirulina (13 h). Retention time of Yb was not significantly affected by increasing RDP supply from US but decreased linearly when Spirulina was fed and was 22 h at the highest rate of inclusion (358 g RDP/kg DOM or 6.1 g Spirulina DM/kg W.day; Fig. 3; Table 2). The retention times of DM, NDF and lignin in the rumen of steers consuming Mitchell grass hay alone, as determined by gravimetric measurement, were 44.2, 44.5 and 79.2 h, respectively, and all declined quadratically with increasing RDP supply (Table 2). The lowest retention times of DM, NDF and lignin for the US supplement were 32 and 33 and 56 h and were predicted to occur at 187, 179 and 192 g RDP/kg DOM while the corresponding values for the Spirulina were 24, 29 and 45 h at 303, 273 and 268 g RDP/kg DOM, respectively.

Effect of RDP supply on pH, ammonia concentration and the concentration and proportion of VFA in the rumen fluid

Mean rumen fluid pH was 6.9 in steers fed Mitchell grass hay alone. There was a small linear decline in rumen pH as RDP supply from US and Spirulina increased with no significant difference between the supplement types (Table 2). Data for ammonia concentration was Ln transformed due to the high variability at high RDP intakes. The Control steers had a very low concentration of NH_3-N in the rumen of 5.0 mg/L but there was a quadratic increase as RDP supply from both supplements increased (Table 2). Rumen NH_3-N concentrations peaked at 128 and 264 mg/L for the US and Spirulina treatments when the RDP concentrations were 223 and 296 g/kg DOM, respectively.

Total VFA concentration in the rumen of Control steers averaged 65 mM. Increasing RDP supply from the supplements resulted in a linear increase in total rumen VFA concentration, which was not significantly different between supplements (Table 2). The molar percentages in total VFA of acetate, propionate and butyrate averaged 77.3, 13.5 and 7.7 for unsupplemented Controls. There was a linear decrease in the molar percentage of acetate from 77 to 72 and a linear increase in that of BCFA from 1.2 to 4.2 across the range of Spirulina intakes but neither was affected by RDP supply from US (Table 2). The molar percentage of propionate in total VFA increased marginally from 13.5 to 14.6 in response to increasing intakes of Spirulina and US, with no significant difference between the supplements (Table 2), but supplements did not affect butyrate proportion in total VFA.

Discussion

Spirulina algae is a novel feed source which provides a range of nutrients including nucleic acids, vitamins, minerals, fatty acids and amino acids for the rumen microbes. It can be included in the drinking water and is thus a potential N supplement for ruminants grazing under extensive production systems (Panjaitan *et al.* 2010). The present experiment appears to be the first experiment to use Spirulina algae as a source of RDP for ruminants consuming low protein tropical pastures. It was

Table 2. Effect of rumen degradable protein (RDP) supply [X; g/kg digestible organic matter (DOM).day] on microbial crude protein (MCP) production and the efficiency of MCP production (EMCP), on hay and total dry matter (DM) intake, digestibility of organic matter (OMD) and total digestible organic matter intake (DOMI), the retention time of Cr-EDTA, Yb, DM, neutral detergent fibre (NDF) and lignin, and the concentration of volatile fatty acids (VFA) and molar percentages in total VFA of individual VFA and branched-chain fatty acids (BCFA) in rumen fluid, of steers fed Mitchell grass hay alone or supplemented with urea-ammonium sulfate (US) or Spirulina

Where there was no significant difference between response relationships for the two supplement types a combined regression equation is given and the degree of fit of different supplement treatments to that combined equation is given separately. R^2, proportion of variability about mean response for supplement group (including Controls) accounted for by fitted curve. RSD, residual standard deviation about fitted curve for supplement group (including Controls). *P*, significance probability for highest order coefficient in equation

Y	Supplement	Equation	R^2	RSD	*P*
MCP production (g/kg W.day)	US	$Y = -0.150 + 0.0079\ X - 0.000020\ X^2$	0.78	0.082	<0.01
	Spirulina	$Y = -0.217 + 0.0081\ X - 0.000006\ X^2$	0.99	0.064	<0.05
Ln EMCP (g MCP/kg DOM)	US	$Y = 3.964 + 0.0024\ X$	0.42	0.220	<0.01
	Spirulina	$Y = 3.702 + 0.0071\ X - 0.00012\ X^2$	0.93	0.128	<0.05
Hay DM intake (g/kg W.day)	US	$Y = 6.507 + 0.1178\ X - 0.00032\ X^2$	0.91	0.705	<0.01
	Spirulina	$Y = 6.135 + 0.1181\ X - 0.00023\ X^2$	0.97	0.738	<0.01
Total DM intake (g/kg W.day)	US	$Y = 6.054 + 0.1127\ X - 0.00032\ X^2$	0.92	0.751	<0.01
	Spirulina	$Y = 5.081 + 0.1313\ X - 0.00021\ X^2$	0.99	0.767	<0.01
OMD (%)	US	$Y = 44.8 + 0.0141\ X$	0.38	1.40	<0.05
	Spirulina	$Y = 44.1 + 0.0254\ X$	0.78	1.61	<0.01
DOMI (g/kg W.day)	US	$Y = 1.972 + 0.0566\ X - 0.00014\ X^2$	0.81	0.519	<0.01
	Spirulina	$Y = 1.537 + 0.0600\ X - 0.000087\ X^2$	0.98	0.479	<0.01
Cr-EDTA RT (h)	US	$Y = 27.70 - 0.0842\ X + 0.00026\ X^2$	0.32	1.616	<0.05
	Spirulina	$Y = 27.75 - 0.0764\ X + 0.00012\ X^2$	0.87	1.310	<0.01
Yb RT	US	No relationship			
	Spirulina	$Y = 35.106 - 0.0354\ X$	0.46	4.149	<0.01
DM retention time (h)	US	$Y = 60.54 - 0.3013\ X + 0.00081\ X^2$	0.80	2.94	<0.01
	Spirulina	$Y = 57.21 - 0.2202\ X + 0.00036\ X^2$	0.86	3.36	<0.01
NDF retention time (h)	US	$Y = 61.22 - 0.3139\ X + 0.00087\ X^2$	0.82	2.64	<0.01
	Spirulina	$Y = 55.89 - 0.1961\ X + 0.00036\ X^2$	0.70	4.02	<0.01
Lignin retention time (h)	US	$Y = 108.05 - 0.5408\ X + 0.00141\ X^2$	0.77	6.55	<0.05
	Spirulina	$Y = 103.84 - 0.4360\ X + 0.00081\ X^2$	0.82	6.15	<0.01
Rumen fluid pH	Combined	$Y = 6.814 - 0.00077\ X$			<0.05
	US		0.13	0.12	
	Spirulina		0.30	0.13	
Ln rumen ammonia-N concentration (mg/L)	US	$Y = 1.061 + 0.0471\ X - 0.00015\ X^2$	0.95	0.37	<0.01
	Spirulina	$Y = 1.061 + 0.0386\ X - 0.00008\ X^2$	0.82	6.15	<0.01
VFA concentration (mM)	Combined	$Y = 65.463 + 0.0973\ X$			<0.01
	US		0.11	8.97	
	Spirulina		0.60	9.75	
Acetate molar percentage	US	No relationship			
	Spirulina	$Y = 78.64 - 0.0169\ X$	0.89	0.75	<0.01
Propionate molar percentage	Combined	$Y = 13.49 + 0.0031\ X$			<0.05
	US		0.12	0.65	
	Spirulina		0.36	0.48	
Branched-chain fatty acids percentage	US	No relationship			
	Spirulina	$Y = 0.084 + 0.0115\ X$	0.94	0.32	<0.01

carried out to compare the effects of Spirulina with US, a supplement traditionally fed as a N source in northern Australia, on MCP production, EMCP and intake in steers fed a low quality tropical pasture hay. The results demonstrated that, at the same rate of supply of RDP, Spirulina increased MCP production and the EMCP above that for US. These effects on MCP were associated with increases in intake, digestibility and rumen NH_3-N and a decrease in digesta retention time in the rumen.

In the experiment reported here, supplementation with Spirulina resulted in similar response patterns, namely increases in intake, digestibility and MCP production, to those observed previously with various protein meals (McLennan 1997). In our study, DOM intake peaked at 12 g/kg W.day with a moderate inclusion rate of Spirulina of 4.9 g DM/kg W.day. The primary mode of action appears to be through increases in MCP production and EMCP, which would increase the protein : energy (P : E) ratio of absorbed substrates with consequential increases in intake of the low quality forage (Egan 1977). Higher Spirulina intakes were associated with increases in rumen NH_3-N concentration and markedly lower retention time of digesta within the rumen. Thus it appears this combination of higher rumen NH_3-N and increased rumen dilution rate has resulted in a considerably enhanced MCP

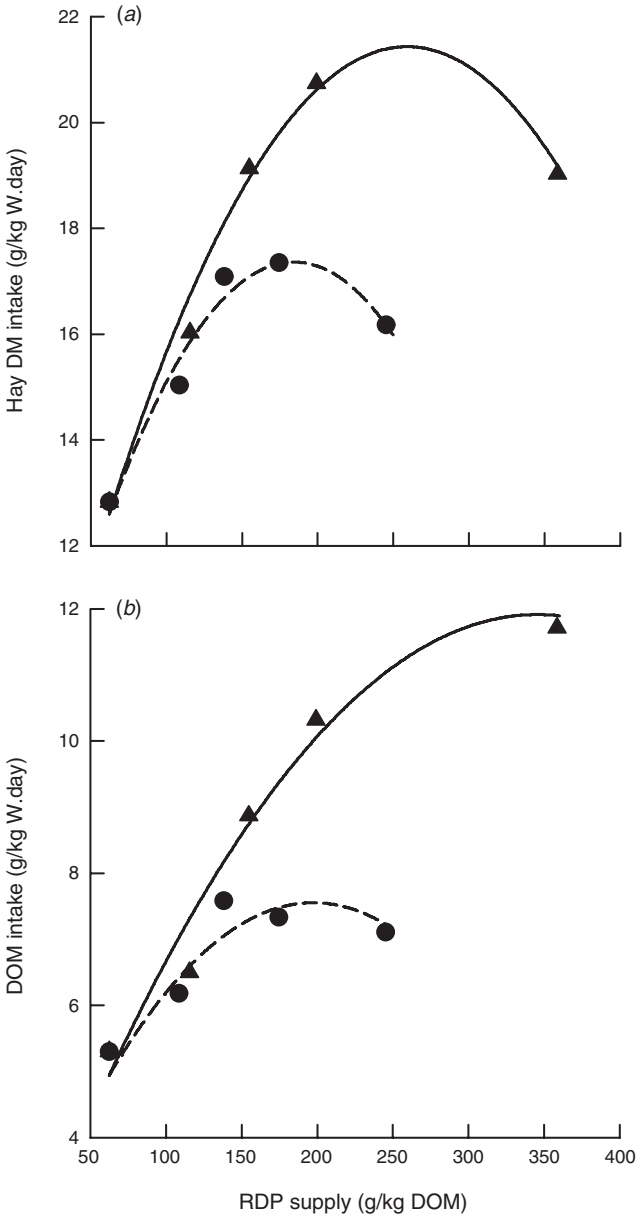

Fig. 2. Effect of rumen degradable protein (RDP) supply [g/kg digestible organic matter (DOM)] on the intake of (*a*) hay DM and (*b*) total DOM for steers given Mitchell grass hay alone or supplemented with urea-ammonium sulfate (US; ●; dashed line) or Spirulina (▲; solid line). Points shown on the graph are treatment means for steers across runs. The equations describing the response relationships are shown in Table 2.

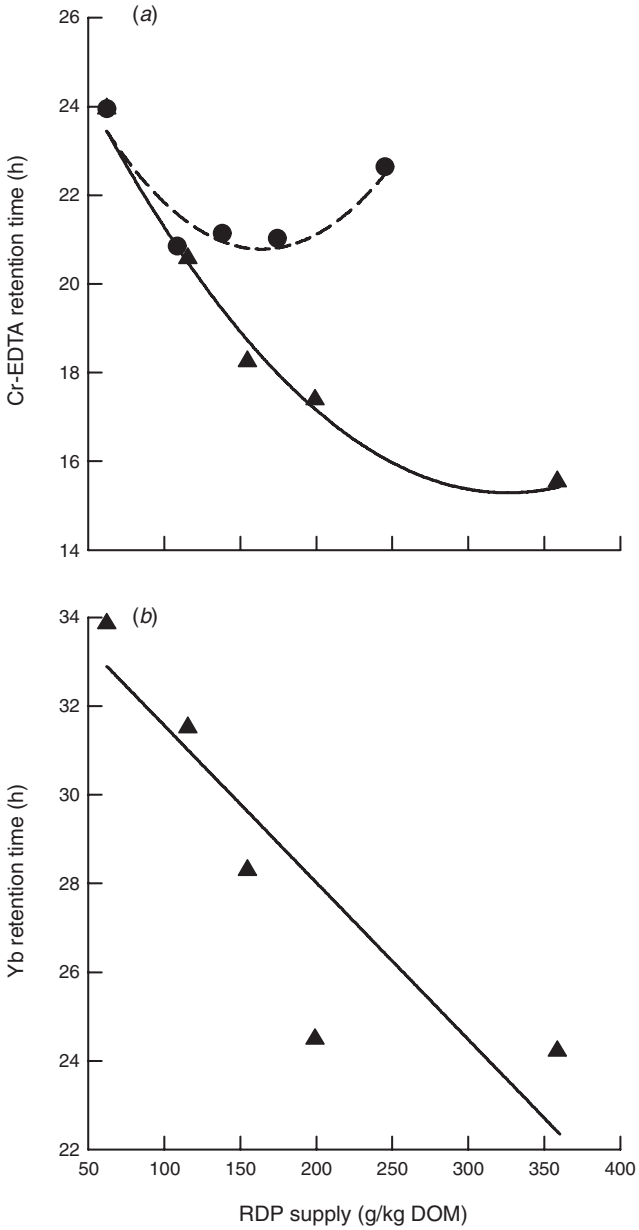

Fig. 3. Effect of rumen degradable protein (RDP) supply [g/kg digestible organic matter (DOM)] on the retention time of (*a*) Cr-EDTA and (*b*) Yb for steers given Mitchell grass hay alone or supplemented with urea-ammonium sulfate (US; ●; dashed line) or Spirulina (▲; solid line). Points shown on the graph are treatment means for steers across runs. The equations describing the response relationships are shown in Table 2.

production, EMCP (and hence higher P : E) and intake at Spirulina intakes below 7 g/kg W.day.

The main supplementation requirement for animals consuming forages of low protein content is a supply of N in the rumen. Provision of N and S through mixes such as the US employed here is a commonly used, practical method of supplementing grazing cattle in northern Australia (Winks *et al*. 1970; McLennan *et al*. 1991). Previous research has shown that provision of N in the form of non-protein-N sources will increase EMCP to the lower end of the range of values reported in the various feeding standards, that is ~130 g MCP/kg DOM (e.g. CSIRO 2007) but that the upper values in the range, i.e. ~170 g MCP/kg DOM will only be attained with increases in intake associated with higher rumen dilution rate (AFRC 1993) or when a proportion of the N comes from degradable true protein (NRC 1996). Algae supplies a range of substrates including peptides, amino acids, nucleic acids and minerals known to be associated with higher EMCP (Dijkstra *et al*. 1998; Baker and Dijkstra 1999) but also vitamins. Our comparison of US and Spirulina, on the basis of RDP/DOM

supply from both supplement types, showed that feeding the algae resulted in greater MCP production, EMCP and DOMI in cattle than did the US supplement. In our study, the highest EMCP of steers supplemented with US was ~95 g MCP/kg DOM, well below the minimum value cited from the feeding standards (CSIRO 2007). In contrast, Spirulina supplementation increased EMCP to the higher end of the range indicated in the feeding standard range (170 g MCP/kg DOM; CSIRO 2007).

The mechanisms responsible for these effects with Spirulina compared with US are not clear. An obvious possibility relates to the mix of nutrients supplied to the rumen microbes of cattle by Spirulina, including the higher concentration of BCFA and the lower retention time of digesta within the rumen with consequences for increased intake of forage and total DOM. The retention time of digesta in the rumen has an important role in determining MCP production and EMCP (Dijkstra *et al*. 2002; Firkins *et al*. 2007). Increasing dilution rate of rumen fluid and digesta increases MCP supply to the host animal and also increases EMCP (Owens and Goetsch 1988; AFRC 1993; Firkins *et al*. 2007). This is associated with a reduced maintenance energy requirement of bacteria through reduced recycling within the rumen (Owens and Goetsch 1988; Baker and Dijkstra 1999). Owens and Goetsch (1988) estimated that the ATP used for maintenance decreased from 65 to 32% of the total ATP when digesta retention time decreased from 50 to 17 h. The results reported here indicate that retention time of water, digesta, DM, NDF and lignin decreased with increasing RDP intake from both supplements with the decline in retention times greater with Spirulina. Van Soest (1994) suggested that a reduction in retention time of water in the rumen by ~50% (from 25 to 12.5 h) could increase EMCP by ~35% (from 130 to 200 g MCP/kg DOM). In our experiment, increasing RDP intake from Spirulina was associated with a decrease in water retention time, as indicated by Cr-EDTA dilution, by 38% from 24 to 15 h compared with that for unsupplemented hay.

For an equivalent RDP supply of 250 g/kg DOM, Spirulina was associated with an approximate 2-fold increase in EMCP compared with the Control (133 vs 64 g MCP/kg DOM), with the US value intermediate at ~90 g MCP/kg DOM. Thus for a 300-kg steer, this would result in an extra 360 g MCP/day from Spirulina supplementation compared with an extra 105 g MCP/day from US. This increase would have a significant impact on the nutrient status and liveweight gain of the animal. Mbongo *et al*. (1994) reported that for Belmont Red steers of similar liveweight to those used in the present study an extra 150 g/day of intestinal protein supplied in the form of formaldehyde-treated casein resulted in an increase in liveweight gain of 250 g/day above that of unsupplemented steers grazing a setaria (*Setaria sphacelata*)-dominant pasture with 13% CP content.

The intake response to the Spirulina supplement was greater than that to the US supplement. The Spirulina supplement markedly increased hay intake presumably by increasing the rate of passage of material through the rumen. An intake substitution effect occurred when Spirulina intake exceeded 3.7 g DM/kg W.day, as denoted by peak hay intake. This is similar to the results of Marsetyo (2004) who found that intake of forages of low CP content was stimulated with intakes of protein meals up to ~5 g DM/kg W.day, and declined thereafter. In the present study, very high intakes of Spirulina were not used so it was not possible to establish a substitution rate. In fact, total DM intake plateaued when Spirulina intake was ~4.6 g DM/kg W.day, not much less than the highest inclusion rate of 6.1 g DM/kg W.day. At this plateau level, total DOMI was more than 2.3-fold higher than that for Control steers. This represents a large increase in DOMI and thus ME intake and, combined with the increased metabolisable protein supply described above, would be expected to result in a considerable increase in liveweight gain by cattle consuming low quality native grass pasture.

Values for rumen pH, rumen ammonia and VFA proportions were all within the range for a forage of this type with or without N supplements. The concentration of BCFA increased with Spirulina supplementation most likely reflecting the greater extent of branch chain amino acid degradation.

Spirulina was supplemented via the rumen cannula in this experiment. Panjaitan *et al*. (2010) demonstrated that it could be supplied in drinking water but to supply the amounts determined in this experiment for a high response in the animal will require a higher concentration than natural algal blooms can achieve. Thus the algae will need to be supplied in pre-mixed drinking troughs or through the commonly used loose licks, blocks or molasses based supplementation strategies.

In conclusion, our results have demonstrated that Spirulina, and presumably other algae, has the potential to be used as a novel protein source for cattle grazing low quality tropical grasses. Protein sources based on algae can provide production increases necessary for a stimulus in growth rate of the animal through their effect on increasing protein supply to the animal and ultimately on increasing intake of the forage under grazing conditions.

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