

# Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας

Τόμ. 73, Αρ. 2 (2022)



**Effects of spirulina (*Arthrospira platensis*) as feed additive of steers consuming high energy diets: growth performance and nutrient digestion**

*Alejandro Plascencia, Iván Guadalupe Martínez-Alvarez, Martín Montano, Alfredo Estrada-Angulo, Jesús David Urías-Estrada, Beatriz Isabel Castro-Pérez, Richard Avery Zinn*

doi: [10.12681/jhvms.26069](https://doi.org/10.12681/jhvms.26069)

Copyright © 2022, Alejandro Plascencia, Iván Guadalupe Martínez-Alvarez, Martín Montano, Alfredo Estrada-Angulo, Jesús David Urías-Estrada, Beatriz Isabel Castro-Pérez, Richard Avery Zinn



Άδεια χρήσης [Creative Commons Αναφορά-Μη Εμπορική Χρήση 4.0.](https://creativecommons.org/licenses/by-nc/4.0/)

## Βιβλιογραφική αναφορά:

Plascencia, A., Martínez-Alvarez, I. G., Montano, M., Estrada-Angulo, A., Urías-Estrada, J. D., Castro-Pérez, B. I., & Zinn, R. A. (2022). Effects of spirulina (*Arthrospira platensis*) as feed additive of steers consuming high energy diets: growth performance and nutrient digestion. *Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας*, 73(2), 4015–4022. <https://doi.org/10.12681/jhvms.26069>

## Effects of spirulina (*Arthrospira platensis*) as feed additive of steers consuming high energy diets: growth performance, carcass characteristics, morbidity, and nutrient digestion

A. Plascencia<sup>a</sup>, I.G. Martínez-Álvarez<sup>b</sup>, M. Montano<sup>b</sup>, A. Estrada-Angulo<sup>c</sup>,  
J.D. Urías-Estrada<sup>c</sup>, B.I. Castro-Pérez<sup>c</sup>, R.A. Zinn<sup>d</sup>

<sup>a</sup>Departamento de Ciencias Exactas y Naturales, Universidad Autónoma de Occidente, Unidad Guasave, Sinaloa, México

<sup>b</sup>Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja California, México

<sup>c</sup> Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa, México

<sup>d</sup>Department of Animal Science, University of California, El Centro 92243, USA

**ABSTRACT:** Two trials were performed in order to evaluate the effects of spirulina platensis (SP) supplemented (0, 0.5, and 1.0% of diet DM) in high-energy diet on nutrient digestion and growth-performance of feedlot cattle. The dose of each treatment was top dressed and mixed directly with the basal diet at time of feeding. In Trial 1, six cannulated Holstein steers ( $281 \pm 2$  kg) were used in a replicated  $3 \times 3$  Latin square experiment. Supplemental SP did not affect ruminal and total tract nutrient digestion. Ruminal microbial efficiency (quadratic component,  $P=0.06$ ), nitrogen efficiency (linear component,  $P=0.10$ ) and ruminal pH (linear,  $P=0.06$ ) tended to decrease with SP supplementation, without further effects on ruminal VFA or estimated methane production. In Trial 2, 108 medium-frame crossbred steers ( $182 \pm 1$  kg) were used in a 211-d study to evaluate growth performance. SP dietary supplementation did not affect feed intake, but linearly tended ( $P=0.09$ ) to decrease weight gain, gain efficiency, and estimated dietary net energy. Supplemental SP also tended ( $P=0.08$ ) to linearly increase steer morbidity. Effects of SP supplementation on carcass traits were not significant, but longissimus muscle area tended to linearly decrease ( $P=0.06$ ). It is concluded that low level ( $\leq 1\%$ ) of *Spirulina platensis* supplementation (powder form) has minimal effects on characteristic of digestion and may negatively impact on growth performance, feed efficiency, and morbidity of feedlot cattle fed a high energy finishing diet.

**Keywords:** spirulina, feedlot cattle, ruminal fermentation, digestion; performance.

*Corresponding Author:*

Alejandro Plascencia, Departamento de Ciencias Exactas y Naturales, Universidad Autónoma de Occidente, Unidad Guasave, Sinaloa, México  
E-mail address: alejandro.plascencia@uadeo.mx; aplas\_99@yahoo.com

Date of initial submission: 09-02-2021  
Date of acceptance: 03-04-2021

## INTRODUCTION

*Spirulina platensis* (SP, *Arthrospira spp.*) is a blue-green microalgae that has been defined as a functional food with nutritional qualities (FAO/WHO, 1991). As a foodstuff, it has been generally regarded as a good source of protein, vitamins, minerals, and fatty acids (Moor et al. 2016), and has a long history of use as a food for humans around the world. In livestock, SP has been used incorporated as a protein supplement at levels of 3 to 20% into the diets for ruminants and non-ruminant (Holman and Madau-Aduli, 2013). Since SP contains bioactive compounds including essential oils, phycocyanin, sterols, and sulphate polysaccharides, it may act as a nutraceutical, possessing anti-inflammatory, antiviral, antimicrobial, and anti-oxidant properties (Ramadan et al., 2008; Karkos et al., 2011). Low levels of SP supplementation (i.e. inclusion levels of  $\leq 1\%$ ) have been used commercially in fish and poultry production systems to enhance health and growth performance (Amer, 2016; Bonos et al., 2016; Naiel et al., 2021). Scarce data are available about the effects of SP as a feed additive in growing-finishing feedlot cattle. SP may have beneficial effects on ruminal fermentation, digestion, growth performance, and dietary energy utilization due its nutraceutical properties. Accordingly, the objective of the present study was to evaluate the effects of SP supplementation (0, 0.5, and 1.0% of diet DM) on characteristics of ruminal and total tract digestion and growth performance of steers that were fed a high-energy finishing diet.

## MATERIAL AND METHODS

All procedures involving animal care and management were in accordance with and approved by the University of California, Davis, Animal Use and Care Committee.

### Digestion trial (Trial 1)

Six Holstein steer calves ( $281 \pm 2.3$  kg) with "T" type tygon cannulas in the rumen and proximal duodenum (Zinn and Plascencia, 1993) were used in a replicated  $3 \times 3$  Latin square experiment to examine the effects of *Spirulina platensis* dietary supplementation on site and extent of nutrient digestion and ruminal fermentation of steers fed high energy diets. Animals were housed in individual pens ( $3.9 \text{ m}^2$ ) in an indoor facility, with a concrete floor covered by a neoprene carpet, automatic waterers and individual feed bunks. All steers were received *ad libitum* access to the corn-basal diet (Table 1) for 14 days before the

initiation of the trial. Upon initiation of the study, to avoid feed refusals during experimental period, intake of the basal diet of each steer was restricted to 90% of average *ad libitum* intake during the 14-d preliminary period (equivalent to 2.1% of average initial LW). Chromic oxide was used (3.0 g/kg of diet DM) as an indigestible marker to estimate nutrient flow and digestion. Chromic oxide was premixed with minor ingredients (urea and mineral supplements) before incorporation into complete mixed diets. Treatments consisted of a daily supplement (as feed basis) of 0, 32 or 64 g of *Spirulina platensis* (equivalent to supplementation level of 0, 0.5, and 1.0% of the basal diet, DM basis). The species of Spirulina used was *Arthrospira platensis* (Earthrise Nutritionals, Calipatria, CA, USA) which was supplemented in a dry powder form (93.8% DM). Its chemical composition is shown in Table 2. Spirulina was top-dressed on the basal diet at time of feeding. Diets were fed in equal proportions at 0800 and 2000 h daily. Experimental periods consisted of 14 days, with 10 days for dietary treatment adjustment and 4 days for collection (Trial lasted 42 days). During the collection period, duodenal and faecal samples were taken from all steers, twice daily as follows: d 1, 0750 and 1350 h; d 2, 0900 and 1500 h; d 3, 1050 and 1650 h; and d 4, 1200 and 1800 h. Individual samples consisted of approximately 500 mL of duodenal chyme and 200 g (wet basis) of faecal material. Samples from each steer and within each collection period were composited for analysis. During the final day of each collection period, ruminal samples were obtained from each steer at 4 h after feeding via the ruminal cannula. Ruminal pH was determined on fresh samples. Samples were then strained through four layers of cheese cloth. For VFA analysis, 2 mL of freshly prepared 25% (w/vol) meta-phosphoric acid was added to 8 mL of strained ruminal fluid, centrifuged ( $17,000 \times g$  for 10 min) and supernatant fluid stored at  $-20^{\circ}\text{C}$ . Upon completion of the trial, ruminal fluid was obtained from all steers and composited for isolation of ruminal bacteria via differential centrifugation (Bergen et al., 1968). The microbial isolate served as the purine:N reference for the estimation of microbial N contribution to chyme entering the small intestine (Zinn and Owens, 1986).

Dried ( $55^{\circ}\text{C}$ ) and ground (1 mm sieve) feed, duodenal and faecal samples were subject to the following analysis: dry matter (oven drying at  $105^{\circ}\text{C}$  until no further weight loss; method 930.15; AOAC, 2000); ash (method 942.05; AOAC, 2000), Kjeldahl N (method 984.13; AOAC, 2000); ADF (method 973.18;

AOAC, 2000); ammonia-N (method 941.04; AOAC, 2000); chromic oxide (Hill and Anderson, 1958) and starch (Zinn, 1990). Purines were determined on duodenal samples (Zinn and Owens, 1986), and VFA concentrations of ruminal fluid were assessed by gas chromatography (Zinn, 1988).

Total DM flow to duodenum and faecal DM excretion was estimated as follows: DM flow to the small intestine or faecal excretion, g/day = g Cr<sub>2</sub>O<sub>3</sub> intake/Cr<sub>2</sub>O<sub>3</sub> concentration of duodenal or faecal DM. Organic matter (OM) content of feed duodenal and faecal samples were estimated as DM concentration minus ash content. Microbial organic matter (MOM) and microbial nitrogen (MN) leaving the abomasum was calculated using purines as a microbial marker (Zinn and Owen, 1986). Organic matter fermented in the rumen (OMF) is considered equal to the OM intake minus the difference between the amount of total OM reaching the duodenum and the MOM reaching the duodenum. Feed N escape to the small intestine is considered equal to the total N leaving the abomasum minus ammonia-N and MN and, thus, includes any endogenous contributions. Ruminal microbial efficiency was estimated as duodenal MN, g·kg<sup>-1</sup> OM fermented in the rumen, and protein efficiency represent the duodenal nonammonia-N, g·g<sup>-1</sup> N intake. Methane production was calculated based on the theoretical fermentation balance for observed molar distribution of VFA and OM fermented in the rumen (Wolin, 1960).

Treatment effects on characteristics of digestion were analyzed as a replicated 3 × 3 Latin square design using the MIXED procedure according to SAS (2004). The fixed effect consisted of treatment, and random effects consisted of steer and period. The statistical model for the trial was as follows:

$Y_{ijk} = \mu + S_i + P_j + T_k + E_{ijk}$ , where: Y<sub>ijk</sub> is the response variable,  $\mu$  is the common experimental effect, S<sub>i</sub> is the steer effect, P<sub>j</sub> is the period effect, T<sub>k</sub> is the treatment effect and E<sub>ijk</sub> is the residual error. Least means squares and standard deviation are reported, and contrasts were considered significantly when the P value was  $\leq 0.05$ , and tendencies are considered when the P-value was  $> 0.05$  and  $\leq 0.10$ .

### Growth performance, health, and carcass characteristics (Trial 2)

One hundred and eight medium-frame crossbred steers (182±1.3 kg initial shrunk weight) were used

**Table 1.** Composition of basal diet used in experiments 1 and 2<sup>a</sup>

Ingredient	% of DM basis
Steam-flaked corn	40.00
Steam-flaked barley	35.00
Alfalfa hay	6.00
Sudan grass hay	6.00
Tallow	3.00
Cane molasses	6.50
Urea	0.90
Chromium oxide	0.50
Limestone	1.70
Trace mineral salt <sup>b</sup>	0.40
Nutrient composition, (DM basis) <sup>c</sup>	
Crude protein (%)	12.58
Starch (%)	44.75
ADF (%)	8.27
Calculated net energy (Mcal/kg) <sup>d</sup>	
Maintenance	2.11
Gain	1.46

<sup>a</sup> Chromic oxide (0.3%) was added as digesta marker in Exp 1.

<sup>b</sup> Trace mineral salt contained: CoSO<sub>4</sub>, 0.068%; CuSO<sub>4</sub>, 1.04%; FeSO<sub>4</sub>, 3.57%; ZnO, 1.24%; MnSO<sub>4</sub>, 1.07%, KI 0.052%; and NaCl, 92.96%.

<sup>c</sup> Dietary chemical composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.

<sup>d</sup> Based on tabular net energy (NE) values for individual feed ingredients (NRC, 2000).

**Table 2.** Characteristics of chemical composition of *Spirulina platensis*<sup>a</sup> used in experiments 1 and 2<sup>b</sup>

Item	
Dry matter	93.75
Composition, DM basis%	
Ash	8.5
Lipids	5.5
Carbohydrates	20.0
Crude protein	65.0
Amino acids	62.0
Calcium	1.0
Phosphorus	0.8
Trace minerals, mg/kg	
Chromium	2.5
Copper	12.0
Iron	1,500
Manganese	50.0
Selenium	1.0
Zinc	30.0
Pigments, %	
Phycocyanin	15.0
Chlorophyll	1.1
Carotenoids	0.4

<sup>a</sup> Product presentation as powder and provided by Earthrise Farms, Calipatria, CA, USA.

<sup>b</sup> Spirulina (SP) was supplemented daily at dose equivalent to 0, 0.5, and 1.0% SP/of offered basal diet DM and mixed directly with the basal diet at time of feeding.

in a 211-d trial to evaluate treatment effects on health and feedlot growth-performance. Upon arrival, steers were vaccinated against bovine rhinotracheitis-parainfluenza (Cattle Master Gold FP 5 L5, Zoetis, New York, NY), clostridials (Ultrabac-7, Zoetis, New York, NY), treated against internal and external parasites (Dectomax Injectable, Zoetis, New York, NY), subcutaneously injected with 1,500 IU vitamin E (as d-alpha-tocopherol) 500,000 IU vitamin A (as retinyl-palmitate) and 50,000 IU vitamin D3 (Vital E-A + D3, Stuart Products, Bedford, TX), and 2.4 g oxytetracycline (LA-200, Zoetis, New York, NY), branded, and ear-tagged, blocked by weight (6 blocks) and assigned within blocks to 18 pens (6 steers/pen). Pens were 50 m<sup>2</sup> with 26.7 m<sup>2</sup> of shade, and equipped with automatic waterers and fence-line feed bunks (4.3 m in length). Upon initiation of experiment, and then subsequently at 56-d intervals, steers were implanted with Synovex-S (Zoetis, Charles City, IA). Diet composition, source of SP were as in Trial 1 (Tables 1-2). Treatments consisted of a daily supplement (as feed basis) of 0, 36 or 72 g of *Spirulina platensis* (equivalent to supplementation level of 0, 0.5, and 1.0% of offered basal diet DM). The basal diet was prepared at weekly intervals and stored in plywood boxes located in front of each pen. Steers were allowed ad libitum access to dietary treatments. Fresh feed was provided twice daily (0600 and 1400 h). Spirulina were hand-weighed using a precision balance (Ohaus, mod AS612, Pine Brook, NJ, USA), and were premixed for 5 min with minor ingredients (urea, limestone and trace mineral salt) before incorporation into complete mixed diets. The final product was mixed with the rest of ingredients in a 1 m<sup>3</sup> capacity horizontal mixer (Davis, H.C. Davis Sons, manufacturers, Bonner Spring, KS, USA). To avoid contamination, the mixer was thoroughly cleaned between each treatment. To ensure the consumption of the planned dose, the total daily dose of SP of each treatment was provided in the morning feeding. Thus, the feed provided to all steers in afternoon was the basal diet without SP supplementation.

For calculation of growth performance, initial live weight (LW) is the off-truck arrival weight. Final live weight was reduced 4% to adjust for digestive tract fill. Average daily gain (ADG) was computed by subtracting the initial weight from the final adjusted weight and dividing the result by the number of days on feed. Gain to feed ratio (gain efficiency) was determined by dividing ADG by the daily dry matter intake (DMI). Health variables (morbidity, sick days, mortal-

ity, and liver abscess) were determined as described by Schunicht *et al.* (2003).

Hot carcass weights (HCW) were obtained at time of slaughter. After carcasses chilled for 48 h, the following traits were obtained: longissimus area (LM, cm<sup>2</sup>) by direct grid reading of the LM at the 12th rib; subcutaneous fat (cm) over the LM at the 12th rib taken at a location 3/4 the lateral length from the chine bone end (adjusted by eye for unusual fat distribution); kidney-pelvic-heart fat (KPH) as a percentage of HCW; marbling score (USDA 1997; using 3.0 as minimum slight, 4.0 as minimum small, 5.0 as minimum modest, 6.0 as minimum moderate, etc.), and estimated retail yield of boneless, closely trimmed retail cuts from the round, loin, rib and chuck (% of HCW; Murphrey *et al.*, 1960) = 52.56 - 1.95 × subcutaneous fat - 1.06 × KPH + 0.106 × LM area - 0.018 × HCW.

Treatment effects of on growth performance, estimated dietary NE, and carcassmeasures were analyzed as a randomized complete block design using pen means as the experimental unit, according to the following statistical model:  $Y_{ij} = \mu + B_i + T_j + E_{ij}$ , where  $\mu$  is the common experimental effect,  $B_i$  is block,  $T_j$  is treatment, and  $E_{ij}$  is residual error. Treatments effects were tested by means of orthogonal polynomials SAS (2004).Contrasts were considered significant when the *P*-value was  $\leq 0.05$ , and tendencies were identified when the *P*-value was  $> 0.05$  and  $\leq 0.10$ .

## RESULTS AND DISCUSSION

The chemical composition of *Spirulina platensis* used was provided by the Earthrise Farms, Calipatria, CA, USA. Composition of SP is shown in Table 2. The CP, fiber and lipid composition of the SP used in our experiment are within previously reported ranges (Babadzhanov *et al.*, 2004; Becker *et al.*, 2007). Likewise, concentrations of bioactives compounds are consistent with that reported by Moor *et al.* (2016). However, due to diverse growth media and production methods composition of microalgae *Arthrospira* may widely vary (Stokes, 2015).

### Digestion trial (Trial 1)

The influence of SP supplementation on characteristics of ruminal and total tract digestion (Trial 1) are shown in Table 3. There were no feed refusals during experiment. Intake of Spirulina was 0.11 and 0.23 g SP/kg LW for the 0.5 and 1.0% levels of supplementation, respectively. Supplemental SP did not affect ( $P > 0.10$ ) ruminal or total tract digestion of

**Table 3.** Influence of Spirulina platensis supplementation level on site and extent of digestion of cannulated Holstein steers (Exp. 1)

Item	SP supplementation, % diet DM basis <sup>a</sup>			SEM	Contrast P-value	
	0	0.5	1.0		Linear	Quadratic
Intake, g/d						
Basal diet (DM)	5,968	5,968	5,968			
Spirulina platensis (DM)	0	30	60			
Total dry matter intake	5,968	5,998	6,028	6.01	<0.01	0.77
Organic matter	5,652	5,685	5,713	5.31	<0.01	0.79
Acid detergent fiber	494	493	494	0.52	0.80	0.70
Starch	2,671	2,672	2,675	2.44	0.28	0.60
Nitrogen	120.2	123.2	126.1	0.13	<0.01	0.61
Flow to duodenum, g/d						
Organic matter	2,899	2,706	2,816	69	0.42	0.11
Acid detergent fiber	410	413	421	30	0.79	0.94
Starch	300	269	301	20	0.97	0.25
Total N	145	139	141	4.1	0.45	0.44
N-NH <sub>3</sub>	5.05	4.76	4.83	0.15	0.34	0.39
Non-ammonia N	140	134	136	4.1	0.48	0.46
Microbial N	82.1	80.5	82.7	2.86	0.89	0.60
Dietary N	57.9	53.5	57.7	4.86	0.50	0.74
Ruminal digestion, %						
Organic matter	63.23	66.62	65.18	1.36	0.48	0.18
Acid detergent fiber	17.00	16.22	14.77	6.46	0.66	0.86
Starch	88.76	89.93	88.74	0.81	0.89	0.21
Dietary N	51.83	56.57	57.73	3.83	0.40	0.87
Microbial Efficiency <sup>b</sup>	22.97	21.25	22.20	0.53	0.46	0.06
Protein efficiency <sup>c</sup>	1.11	1.09	1.08	0.02	0.10	0.46
Postruminal digestion %						
Organic matter	66.12	64.03	66.51	1.85	0.51	0.79
Acid detergent fiber	26.34	25.18	30.64	4.50	0.49	0.50
Starch	96.16	94.80	95.81	0.43	0.80	0.16
Nitrogen	80.10	79.16	78.56	0.62	0.12	0.82
Faecal excretion, g/d						
Dry matter	1,158	1,147	1,113	22.8	0.20	0.69
Organic matter	982	972	943	25.7	0.31	0.76
Acid detergent fiber	302	309	292	11.8	0.57	0.44
Starch	11.5	14.0	12.6	1.2	0.52	0.21
Nitrogen	28.9	29.1	29.8	0.65	0.32	0.75
Total tract digestion, %						
Dry matter	80.56	80.87	81.53	0.35	0.15	0.70
Organic matter	82.62	82.90	83.49	0.43	0.31	0.80
Acid detergent fiber	38.86	37.23	40.89	2.35	0.71	0.51
Starch	99.56	99.47	99.52	0.18	0.27	0.50
Nitrogen	75.95	76.37	76.37	0.50	0.83	0.62

<sup>a</sup> Spirulina platensis (DM = 94.4%) was including astop dressed at time of feeding. SP was provided by Earthrise Farms, Calipatria, CA, USA.

<sup>b</sup> Microbial efficiency estimated as duodenal MN, g kg<sup>-1</sup> OM truly fermented in the rumen.

<sup>c</sup> N efficiency estimated as duodenal non-ammonia-N, g g<sup>-1</sup> N intake.

OM, starch, ADF, and N. Based on incubation studies (Wild *et al.*, 2018), estimates of ruminally degradable protein and intestinal digestibility of SP average 50 and 73%, respectively. However, estimates of ruminal degradation of microalgae crude protein have been variable, ranging within 41 and 100% (Panjaitan *et al.*, 2015; Tayyabet *et al.*, 2016). These differences may be due in part to differences in microalgae species (Wild

*et al.*, 2018). Lack of an effect of SP supplementation on apparent ruminal and total tract N digestion in our trial is largely a reflection of the low dietary inclusion levels.

Ruminal microbial efficiency (duodenal MN, g /kg of OM truly fermented in the rumen) and nitrogen efficiency (duodenal non-ammonia-N, g/g of N intake) tended ( $P \leq 0.10$ ) to decrease with SP supplementation.

**Table 4.** Influence of *Spirulina platensis* supplementation on ruminal pH, VFA molar proportions and estimated methane production<sup>a</sup>

Item	SP supplementation, % diet DM basis <sup>b</sup>			Contrast P-value		
	0	0.5	1.0	SEM	Linear	Quadratic
pH	5.61	5.40	5.37	0.07	0.06	0.33
Total VFA, Mmol/L	113	129	129	6.21	0.11	0.34
Ruminal VFA, mol/100 mol						
Acetate	48.11	47.08	49.85	1.64	0.47	0.37
Propionate	40.55	42.66	37.07	2.01	0.26	0.16
Butyrate	11.34	10.26	13.08	1.20	0.34	0.22
Acetate:propionate ratio	1.19	1.10	1.34	0.13	0.26	0.25
Estimated methane <sup>c</sup>	0.35	0.33	0.39	0.025	0.32	0.17

<sup>a</sup> Average of the samples taken at 4 and 8-h post-feeding.

<sup>b</sup> *Spirulina platensis* (DM = 94.4%) was including astrop dressed at time of feeding. SP was provided by Earthrise Farms, Calipatria, CA, USA.

<sup>c</sup> Methane, mol/mol of glucose equivalent fermented (Wolin, 1960).

Effects of SP on microbial efficiency are inconsistent. In some instances SP inclusion have been no effects on microbial efficiency when compared in forage-based diets, but when compared in concentrate-based diets SP inclusion decreased microbial efficiency (Lodge-Iveyet *et al.*, 2014). In the other hand, Panjaitan *et al.* (2014) observed that in steers fed poor quality forage (low in N), consumption of SP (290 g/steer/d) increased ruminal microbial protein production. However, this effect may have been due to increased (33 g) daily N intake with SP supplementation, rather than a nutraceutical activity originated from SP. The basis for these inconsistencies are uncertain, but may be associated, among others, with potential inhibitory effects of SP on some ruminal bacteria (Finamore *et al.*, 2015).

*Spirulina* supplementation tended ( $P=0.06$ ) to decrease ruminal pH (Table 4). Panjaitan *et al.* (2015) likewise observed a lowering of ruminal pH with SP supplementation (0.50 to 6 g /kg LW) of a forage-based diet. Similarly, Lamminen *et al.* (2019) registered lower ruminal pH in dairy cows fed with a diet contained a 57:43 forage:concentrate ratio supplemented with 2.6% of SP. The basis for this is not certain. In both experiments (Panjaitan *et al.*, 2015; Lamminen *et al.* 2019), the total VFA numerically increased (3.8 and 6.7%) in SP diets. In this experiment supplemental SP numerically increased 12.4% total ruminal VFA, then, this effect on VFA by SP supplementation could affect ruminal pH decreasing it. Increased ruminal propionate and decreased methane production have been observed in vitro when substrates were supplemented with Chlorella, a high-oil microalgae (Wild *et al.*, 2019).

### Growth performance, health, and carcass characteristics (Trial 2)

Daily SP consumption averaged 0, 0.11 and 0.22 g SP/ kg LW (consistent with that in Experiment 1). The influence of SP supplementation on parameters associated with growth performance and health are shown in Table 5. *Spirulina* supplementation did not affect dry matter intake. However, SP tended (linear effect  $P=0.09$ ) to decrease daily weight gain and gain efficiency. At high levels of supplementation (i.e. > 5% of diet) supplemental SP enhanced ADG and/or feed intake (Stokes, 2015; Norman *et al.*, 2018). These responses at high levels of supplementation of SP are possible associated with changes in diet composition (increased dietary concentration of protein and fat; Moor *et al.*, 2016). Bezerra *et al.* (2010) observed that inclusion of SP in milk replacer (intake of SP equivalent to 0.55 g SP/kg LW) did not affect lamb growth performance during the first 60 days of life. El-Sabagh *et al.* (2014) observed that supplementation (powder form) of finishing lambs with 0.10 g SP/kg LW enhanced 35-d ADG, DMI and gain efficiency. In feedlot cattle, supplementation with low levels (0.02% of DM intake) of a high-oil algae biomass (liquid form) enhanced 90-d dry matter intake, and daily weight gain of finishing feedlot steers (Gutierrez *et al.*, 2016). The divergence in responses to supplemental SP may be due delivery method (dry vs liquid form) and chemical composition of *Spirulina* (lipid concentration and bioactive compounds) (Capelli and Cysewski, 2010; Holman and Madal-Aduli, 2013).

In contrast with the expectation that supplemental SP might stimulate immunity (Capelli and Cysewski, 2010), supplemental SP tended to increase (linear component,  $P<0.08$ ) morbidity, without effect on mortality. The low dosage level ( $\leq 1\%$  of diet DM) may not have been high enough to produce an effective protective effect (Zotte *et al.*, 2013). However, the reasons why supplemental SP tended to increase morbidity in

**Table 5. Influence of *Spirulina platensis* on health and growth-performance of feedlot cattle feeding during 211 days (Trial 2)**

Item	SP supplementation, % diet DM basis			SEM	Contrast P value	
	0	0.5	1.0		L	Q
Shrunk body weight, kg <sup>a</sup>						
Initial	181.4	183.4	182.8			
28-d	219.4	219.8	220.8	2.77	0.74	0.93
Final	493.5	486.8	476.8	7.08	0.13	0.85
Daily gain, kg						
28-d	1.360	1.300	1.358	0.096	0.98	0.63
28-211-d	1.497	1.459	1.399	0.031	0.05	0.77
1-211-d	1.479	1.438	1.393	0.033	0.09	0.97
Dry matter intake, kg/d						
28-d	3.94	4.03	4.00	0.216	0.82	0.83
28-211-d	7.28	7.20	7.25	0.181	0.89	0.80
1-211-d	6.84	6.78	6.81	0.168	0.91	0.84
Gain to feed, kg/kg						
28-d	0.343	0.322	0.338	0.009	0.71	0.12
28-211-d	0.206	0.203	0.193	0.004	0.07	0.49
1-211-d	0.217	0.213	0.205	0.004	0.06	0.66
Health, %						
Morbidity	27.76	38.89	49.99	8.04	0.08	0.99
Sick days	3.9	4.0	4.0	1.0	0.88	0.96
Mortality	0	5.55	8.33	4.24	0.20	0.80
Liver abscess	2.77	0	2.77	2.37	0.99	0.36

<sup>a</sup>Initial and final full body weights were shrunk 4% to account for digestive tract fill.

**Table 6. Influence of *Spirulina platensis* supplementation level on carcass characteristics (Trial 2)**

Item	SP supplementation, % diet DM basis			SEM	Contrast P value	
	0	0.5	1.0		L	Q
Hot carcass weight, kg	320.8	316.5	309.9	4.606	0.13	0.84
Dressing percentage	65.37	64.92	64.71	0.335	0.20	0.77
Longissimus muscle area, cm <sup>2</sup>	86.85	85.21	81.25	1.799	0.06	0.61
Fat thickness	1.25	1.45	1.18	0.131	0.68	0.18
Kidney-pelvic-heart fat, %	1.80	1.83	1.91	0.165	0.66	0.89
Marbling score	3.60	3.71	3.63	0.112	0.86	0.51
Retail Yield <sup>a</sup>	53.90	53.79	53.44	0.191	0.12	0.61
Yield grade	1.32	1.37	1.52	0.082	0.12	0.61

<sup>a</sup> Estimated retail yield of boneless, closely trimmed retail cuts from the round, loin, rib and chuck (% of HCW; Murphrey et al., 1960) = 52.56 - 1.95 × subcutaneous fat - 1.06 × KPH + 0.106 × LM area - 0.018 × HCW.

this experiment are far away to be clear. The main causes of morbidity observed during the experimental period were associated with respiratory and gastrointestinal disorders. The present knowledge of animal response to dietary *Spirulina* supplementation is relatively scanty and largely unknown. Furthermore, to our knowledge, data about morbidity in cattle that have been received SP are not available in scientific literature. Due to the nature of the experiment and level of SP used more research is needed about of the effects of supplemental SP on the frequency and proportion of diseases on feedlot. Anyway, morbidity is often inversely associated with growth performance of feedlot cattle (Irsiket et al., 2006; Erickson et al., 2011), consistent with the apparent negative effect of SP supplementation on daily weight gain observed in

the present study.

The influence of SP supplementation on carcass characteristics (Trial 2) are shown in Table 6. Longissimus muscle area tended to decrease (linear effect,  $P=0.06$ ) with increasing SP. Erickson et al. (2011) reported a negative association between morbidity and LM area. However, in the present study this treatment effect may have been partially due to SP effects on final carcass weight (Owens and Gardner, 2000).

## CONCLUSION

It is concluded that low-level ( $\leq 1\%$ ) *Spirulina platensis* supplementation (powder form) has minimal effects on characteristic of digestion and may negatively impact on growth performance, feed effi-

ciency, and morbidity of feedlot cattle fed a high energy finishing diet.

## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors

## REFERENCES

- AOAC (2000) Official methods of analysis (17th ed) Assoc. Off. Anal. Chem. Gaithersburg, MD.
- Amer SA (2016) Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (*Oreochromis niloticus*). *Benha Vet. Med J* 30:1-10.
- Babadzhanyan S, Abdusamatova N, Yusupova FM, Faizullaeva N, Mekhlyumyan LG, Malikova M (2004) Chemical composition of *Spirulina platensis* cultivated in Uzbekistan. *Chem Nat Compd* 40: 276-279.
- Becker EW (2007) Micro-algae as a source of protein. *Biotech Adv.* 25:207-210.
- Bergen WG, Purser DB, Cline JH (1968) Effect of ration on the nutritive quality of rumen microbial protein. *J AnimSci* 27:1497-1501.
- Bezerra LR, Silva AMA, Azevedo SA, Mendes RS, Mangueira JM, Gomes AK (2010) Performance of Santa Ines lambs submitted to the use of artificial milk enriched with *Spirulina platensis*. *CiêncAnim Bras* 11: 258-263.
- Bonos E, E. Kasapidou E, Kargopoulos A, Karampampas A, Christaki E, Florou-Paneri P, Nikolakakis I (2016) Spirulina as a functional ingredient in broiler chicken diets. *South Afr J AnimSci* 46:94-102.
- Capelli B, Cysewski GR (2010) Potential health benefits of Spirulina microalgae. *Nutrafoods* 9:19-26.
- EL-Sabagh MR, Eldaim MA, Mahboub DH, Abdel-Daim M (2014) Effects of spirulina platensis algae on growth performance, antioxidative status and blood metabolites in fattening lambs. *J AgricSci* 6:92-98.
- Erickson GE, Bremer VR, Klopstein TJ, Smith DR, Hanford KJ, Peterson RE, Burciaga-Robles LO, Faulkner DB, Krehbiel CR (2011) Relationship between morbidity and performance in feedlot cattle. *Nebraska Beef Cattle Rpt* 87-89.
- FAO/WHO (1991) Protein Quality Evaluation: Report of the Joint FAO/WHO Expert Consultation, FAO. Food and Nutrition Paper 51. Rome: FAO.
- Finamore A, Palmery M, Bensehaila S, Peluso I (2017) Antioxidant, immunomodulating, and microbial-modulating activities of the sustainable and ecofriendly *Spirulina*. *Oxid Med Cell Longev Art ID* 3247528.
- Gutierrez BH, Alvarez E, Arrizon AA, Carrasco R, Salinas-Chavira J, Zinn RA (2016) Influence of high-oil algae biomass as a feed intake and growth-performance enhancer in feedlot cattle during period of high ambient temperature. *J ApplAnim Res* 44:118-120.
- Hill FN, Anderson DL (1958) Comparison of metabolizable energy and productive determinations with growing chicks. *J Nutr* 64:587-603.
- Holman BWB, Malau-Aduli EO (2013) Spirulina as a livestock supplement and animal feed. *Anim Phys Anim Nutr* 97: 615-623.
- Irsik M, Langemeier M, Schroeder T, Spire M, Roder JD (2006) Estimating the effects of animal health on the performance of feedlot cattle. *BovPract* 40:65-74.
- Karkos PD, Leong SC, Karkos CD, Sivaji N, Assimakopoulos DA (2011) Spirulina in clinical practice: Evidence-based human applications. *Complement Alter Med* 1-4.
- Lamminen M, Halmemies-Beauchet-Filleau A, Kokkonen T, Vanhatalo A, Jaakkola S (2019) The effect of partial substitution of rapeseed meal and faba beans by *Spirulina platensis* microalgae on milk production, nitrogen utilization, and amino acid metabolism of lactating dairy cows. *J Dairy Sci* 102:7102-7117.
- Lodge I, Tracey LN, Salazar A (2014) RUMINANT NUTRITION SYMPOSIUM: The utility of lipid extracted algae as a protein source in forage or starch-based ruminants diets. *J AnimSci* 92:1331-1342.
- Moor VJ, Pieme CA, Biapa PC, Matip ME, Moukette B, Nzufu F, Nannack P, Ngogang J (2016) Chemical composition of *Spirulina platensis* of Nomayos-Yaounde (Cameroon). *Annals Food SciTechnol* 17:524-528.
- Murphy CE, Hallett DK, Tyler WE, Pierce JC (1960) Estimating yields of retail cuts from beef carcasses. In: *Proceedings of American Society of Animal Production*, Chicago, IL, USA: pp. 1-12.
- Naiel MAE, Alagawany M, Patra AK, El-Kholy AI, Amer MS, El-Hack MEA (2021) Beneficial impacts and health benefits of macroalgae phenolic molecules on fish production. *Aquaculture* 534:Art736186.
- Norman MM, Carlson ZE, Hilscher FH, Erickson GE, Brodersen BW, Loy JD, Wilson JW, Rabe C, Watson AK (2018) Evaluation of the safety of an algae biomass as an ingredient for finishing cattle. *Prof AnimSci* 34:618-630.
- NRC. 2000. Nutrient Requirements of Beef Cattle. Updated (7<sup>th</sup> Rev. ed.) National Academy Press, Washington, DC, USA.
- Owens FN, Gardner BA (2000) A review of the impact of feedlot management and nutrition on carcass measurements of feedlot cattle. *J AnimSci* 77 (Suppl E):1-18.
- Panjaitan, T, Quigley SP, McLennan SR, Swain AJ, Poppi DP (2015) Spirulina (*Spirulina platensis*) algae supplementation increases microbial protein production and feed intake and decreases retention time of digesta in the rumen of cattle. *Anim Prod Sci* 55:535-543.
- Ramadan MF, Asker MMS, Ibrahim ZK (2008) Functional bioactive compounds and biological activities of *Spirulina platensis* lipids. *Czech J Food Sci* 26:211-222.
- SAS (2004). Statistical Analysis Software. Version 90 SAS Institute Inc. Cary, North Carolina, USA.
- Stokes RS (2015) Evaluation of algae meal as a novel feedstuff for ruminants. Graduate Theses and Dissertations. 14909. <https://lib.dr.iastate.edu/etd/14909>. Iowa State University, USA.
- Schunicht OC, Booker CW, Jim GK, Guichon PT, Wildman BK, Hill BW (2003) Comparison of a multivalent viral vaccine program versus a univalent viral vaccine program on animal health, feedlot performance, and carcass characteristics of feedlot calves. *Can Vet J* 44:43-50.
- Tayyab U, Novoa-Garrido M, Roleda MY, Lind V, Weisbjerg MR (2016) Ruminal and intestinal protein degradability of various seaweed species measured in situ in dairy cows. *Anim Feed Sci Technol* 213:44-54.
- USDA (1997) United States Standards for Grading of Carcass Beef. Washington (DC): Agricultural Marketing Service, United States Dep. Agric.
- Wild KJ, Steingab H, Rodehutscord M (2019) Variability of *in vitro* ruminal fermentation and nutritional value of cell-disrupted and nondisrupted microalgae for ruminants. *Bioenergy* 11: 345-359.
- Wolin MJ (1960) A theoretical rumen fermentation balance. *J Dairy Sci* 43:1452-1459.
- Zinn RA (1988) Comparative feeding value of supplemental fat in finishing diets for feedlot steers supplemented with and without monensin. *J Anim Sci*. 66:213-227.
- Zinn RA (1990) Influence of steaming time on site of digestion of flaked corn in steers. *J AnimSci* 68:776-781.
- Zinn RA, Plascencia A (1993) Interaction of whole cottonseed and supplemental fat on digestive function in cattle. *J AnimSci* 71:11-17.
- Zinn RA, Barreras A, Owens FN, Plascencia A (2008) Performance by feedlot steers and heifers: ADG, mature weight, DMI and dietary energetics. *J AnimSci* 86:1-10.
- Zinn RA, Owens FN (1986) A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can J AnimSci* 66:157-166.
- Zotte AD, Sartori A, Bohatir P, Rémignon H, Ricci R (2013) Effect of dietary supplementation of *Spirulina (Arthrospira platensis)* and Thyme (*Thymus vulgaris*) on growth performance, apparent digestibility and health status of companion dwarf rabbits. *Liv Sci* 152: 182-191.