SQU Journal for Science, 2019, 24(2), 71-77 DOI:10.24200/squjs.vol24iss2pp71-77
Sultan Qaboos University

Nutritive Value of Spirulina as Livestock Feed in the Sultanate of Oman

Osman Mahgoub[1]*, Hafidh Al-Mahrouqi[2], Sadeq Al-Lawatia[1] and Rabea Al-Muqbali[1]

*[1]Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences; [2]Agricultural Experiment Station, P.O. Box 34, PC 123, Muscat, Al- Khoud, Sultan Qaboos University, Sultanate of Oman. *Email: osmanhgob@squ.edu.om.*

ABSTRACT: A study was carried out to evaluate the nutritional value of spirulina (*Arthrospira platensis*) to determine its potential use for feeding livestock in Oman. Spirulina was grown in wooden cubicles and harvested after 10 days. One batch of spirulina was dried by centrifugation (CS) and the other was dried in an oven without centrifugation (NCS). Samples were analyzed for dry matter (DM) and proximate chemical components. An *in vitro* assessment was carried out to measure gas production and *in vitro* DM degradability of spirulina. The DM was 56.1 and 57.1% in CS and NCS, respectively. The proximate composition for CS and NCS as a percentage of DM, respectively was: 60.8 and 62.5 for crude protein (CP); 0.97 and 1.05 for Ether extract (EE); 6.35 and 7.55 for ash. The CS and NCS contained: 0.25 and 0.37% DM Acid Detergent Fiber (ADF) and 1.03 and 1.92 % DM Neutral Detergent Fiber (NDF), respectively. The gross energy (cal/g DM) was 5730 and 5629 in CS and NCS, respectively. The CS produced more *in vitro* gas (73 and 71 ml/200mg DM) from 12 hr until the end of the experimental period (96 hr) compared to the NCS (51 and 48 ml/200mg DM), respectively. The CS had significantly higher metabolizable energy (ME) (approximately 12 MJ/kg DM) than NCS (about 9 MJ/kg DM). CS had significantly higher (81 and 79%) Organic Matter Digestibility (OMD) than NCS (61 and 58%). The CS had significantly higher Short Chain Fatty Acids (SCFA) (1.7 and 1.6 µmol) than NCS (1.2 and 1.1 µmol). It was concluded that spirulina is an excellent source of protein and can be used after drying as a potential animal feed. There was little effect of the method of drying of spirulina on its chemical composition or digestibility.

Keywords: Oman; Spirulina; Chemical composition; *In vitro* digestibility.

القيمة الغذائية لطحالب السبيرولينا كعلف للمواشي في سلطنة عمان

عثمان محجوب ، حافظ المحروقي ، صادق اللواتيا ، ربيع المقبالي

الملخص: أجريت دراسة لتقييم القيمة الغذائية للسبيرولينا (ارثروسبيرا بلاتينسيس) لتحديد مدى كفاءة استخدامها كعلف للماشية في عمان. لقد تم زراعة السبيرولينا في احواض خشبية وتم حصادها خلال عشرة ايّام. تم تجفيف السبيرولينا بطريقتين الاولى بالطرد المركزي والثانية بإستخدام فرن تجفيف. تم بعد ذلك تحليل العينات لحساب المواد الجافة، والبروتين، والكربوهيدرات، والدهون. كما تم مختبريا قياس كمية الغاز ومدى تحلل المواد الجافة بها. كمية المواد الجافة كانت ٥٦,١ و ٥٧,١٪ في المجففة بالطرد المركزي والفرن على التوالي . كما أعطت التحاليل النسب التالية للمواد الكيميائية للمجففه بالطرد المركزي والمجففة بالفرن على التوالي:- البروتين الخام ٦٠,٨ و٦٢,٨ ، مستخلص الإيثر ٠,٩٧ و١,٠٥ ، الرماد ٦,٣٥ و٧,٥٥٪ كذلك احتوت العينات على نسب ألياف ٠,٢٥ و٠,٣٧٪ (ADF) و نسب ألياف ١,٠٣ و١,٩٢٪ (NDF)على التوالي. إجمالي الطاقة (cal/g DM) كانت ٥٧٣٠ و ٥٦٢٩ على التوالي. العينات المحصودة بالطرد المركزي أنتجت كمية غاز مختبريا ٧٣ و٧١ مل/٢٠٠ ملغ ، من ١٢ ساعه وحتى نهاية فترة التجربة (٩٦ ساعه) مقارنة مع العينات المجففة بدون طرد مركزي والتي أنتجت كمية غاز ٥١ و٤٨ مل / ٢٠٠ ملغ . الطاقة الايضية للعينات المجففة بالطرد المركزي كانت اعلى من المجففة بالفرن (١٢ ميجاجول/كغ) (٩ ميجاجول/كغ) على التوالي وكذلك نسبة هضم المواد العضوية (٨١ و ٧٩٪) لعينات الطرد المركزي مقارنة ب(٦١ و ٥٨٪) للعينات المجففة بالفرن. كما كانت أيضا اعلى في كمية الأحماض الدهنية قصيرة السلسلة (١،٧ و ١،٦ ميكرومول) مقارنة ب(١،٢ و ١،١ ميكرومول) على التوالي. خلصت الدراسة الى اعتبار ان السبيرولينا مصدرا جيدا للبروتين لتضمينه في العليقة الحيوانيه مع وجود تأثير بسيط في طريقة التجفيف على التركيب الكيميائي والهضم.

الكلمات المفتاحية: عمان، سبيرولينا، التركيب الكيميائي، هضم المواد العضوية مختبريا.

1. Introduction

Spirulina (*Arthrospira platensis*) as named by Stizenberger in 1852 is a prokaryotic cell-type blue green algae presently classified under the genus Arthrospira [1]. *Arthrospira platensis*'s cells are transparent and connected to one another forming a filament in non-heterocyte spiral form. The cell walls are easily digested in a moist environment or in the presence of certain enzymes. This is because the thick gelatinous cell walls are made of complex structure sugars. The cell walls have a diameter ranging between 1-12 mm and their length ranges between 3 to 20 mm [2].

Spirulina can grow in a variety of habitats like brackish water, hot springs, freshwater, desert, and seawater. Richmond [3] reported that spirulina can grow well in fresh water with an alkaline pH in hot springs and lakes. Nutrient uptake by microalgae such as spirulina does not depend only on the nutrients distributed within the growth medium but also on the physical factors affecting their growth such as salinity [4]; pH [5]; light intensity and temperature [6]. It also depends on biotic factors such as initial density, which is a major factor influencing growth. If the initial density is set up higher, the higher growth can be achieved and the more nutrient removal takes place [7]. *Spirulina platensis* has been considered a suitable natural antioxidant and immune-stimulant for humans and animals with few side effects [8-10].

Spirulina is edible and a highly nutritious potential feed resource for several animal species. It contains a high level of protein [9] and all essential amino acids, vitamins, minerals, carotenoids and fatty acids [11]. Although spirulina did not produce consistent positive effects on growth in chickens, with high levels resulting in declined growth, it can be used for vitamin and mineral supplementation and lowering egg cholesterol [11].

Spirulina can compose up to 20% ruminants' diets due to their ability to digest unprocessed algae especially as a suspension in water [11, 12]. It increases microbial protein production and by-pass protein by reducing protein rumen degradability [11]. Spirulina feeding improved body condition score and increased milk yield in dairy cows [13, 14]. Panjaitan *et al.* [15] found out that spirulina improved microbial crude protein production, efficiency of microbial crude protein and feed intake of cattle consuming low protein forage and that it could also be fed safely at higher levels of nitrogen intake.

Bezerra *et al.* [16] reported that spirulina feeding produced higher live weights and average daily gains in lambs. Holman *et al.* [11] also reported that dietary spirulina supplementation increased lamb live weight and improved body condition and conformation, although the difference in average daily gain was not statistically different. El-Sabagh [17] reported that spirulina supplementation improved final live body weight, daily live weight gain, feed intake and feed conversion ratio in lambs. They recommended that spirulina could be incorporated in fattening lamb's diets as an antioxidant, immune-stimulant and growth promoter feed additive. Shimkiene *et al.* [18] have shown that pregnant ewes receiving spirulina deliver up to 4% heavier lambs with subsequent higher weight gains compared to pregnant ewes receiving no spirulina.

The current study aimed to evaluate the nutritional value of spirulina species as a livestock feed. This was achieved by determining the chemical composition and *in vitro* digestibility of spirulina.

2. Materials and Methods

2.1 Growing spirulina

The experiment was conducted in an open area in the Agricultural Experimental Station (AES) of Sultan Qaboos University, Muscat, Oman by culturing spirulina in wooden tanks (400 × 400 cm by 15 cm deep) lined with clear polythene sheets (Figure 1A). Kosaric medium used as a control was prepared [19] with minor modification using commercial fertilizer (g L^{-1}): 5.0 $NaHCO_3$, 0.25 NaCl, 0.1 $CaCl_2$, 0.2 $MgSO_4.7H_2O$, 0.221 Urea, 0.07 H_3PO_4, 0.242 KOH, 0.02 $FeSO_4.7H_2O$ and 0.5 mlL^{-1} of trace metals solution composed of the following (g L^{-1}): 2.86 H_3BO_4, 1.81 $MnCl_2.4H_2O$, 0.22 $ZnSO_4.7H_2O$, 0.08 $CuSO_4.5H_2O$, 0.01 MoO_3, and 0.01 $COCl_2.6H_2O$ (Sukumaran *et al.*, 2014). Urea was added to the culture medium using fed-batch methods (pulse fertilization) [20]. The fed batch methods were invented to feed the culture with from smaller to increasingly larger amounts. At the premature period, the fed batch method is known to introduce and adapt the culture slowly to the specific nutrient. Without this step, the culture could collapse due to medium stress. Spirulina culture was added after the chemicals had been added to the water. The spirulina was harvested after 10 days when it had reached its highest density. The spirulina was harvested by the filtration method by using nylon cloth (Figure 1B). The cells on the cloth were rinsed several times in tap water to remove traces of growth medium prior to drying.

2.2 *In vitro* Gas production

Rumen liquor was collected into a pre-warmed thermo flask at 39 °C from a fistulated goat before it was offered the morning feed and strained using a cheese cloth. The incubation procedure was carried out as reported by Menke and Steingass [24] using 120 ml calibrated transparent glass syringes fitted with silicon tubes. The 200 mg (n=3) samples were carefully dropped into syringes and thereafter, 30 ml inoculums containing strained rumen liquor and buffer. The buffer (g/l) containing 9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_3$ $7H_2O$ + 16 $CaCl_2$. $2H_2O$ (1:4 v/v) was dispensed under continuous flushing with CO_2. Incubation was carried out at 39±1°C and the volume of the gas production was measured at 3, 6, 9, 12, 15, 18, 21, 24, 48, 72, and 96 hr. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample against the incubation time and from the graph. Metabolizable energy (ME) and Organic Matter Digestibility (OMD) were calculated according to the methods of Menke and Steingass [24] as: ME (MJ/kg DM) = 2.20 + 0.136*GV + 0.057*CP + 0.0029*CF; OMD (%)= 14.88 + 0.889*GV + 0.45*CP + 0.651*CF. The Short Chain Fatty Acids (µmol) was estimated according to the equation of Getachew *et al.* [25] as: 0.0239*GV -0.0601.

2.3 Statistical analysis

Data obtained on *in vivo* digestibility and *in vitro* gas production parameters were subjected to one-way analysis of variance using the analysis of variation model in SPSS [26] PC package. Multiple comparison tests used Tukey's multiple-range test on SPSS [26].

3. Results and Discussion

3.1 Spirulina cultivation

The current experiment proved that spirulina could be cultivated very well under Oman's weather conditions. Spirulina grew well and reached a high density after 10 days. The open system used in the current study appeared to be suitable for growing spirulina under local conditions. The requirements for growing spirulina are within reach and the procedures may be adopted by Omani farmers. Therefore, spirulina offers an excellent potential high protein source for feeding livestock in Oman. It will be an excellent product under saline conditions. This is of significant national importance as large areas of Omani land and water resources are becoming progressively salinized especially in the Al Batinah plains due to low rainfall and sea water seepage.

3.2 Chemical composition of dried spirulina

The proximate chemical analyses of the CS and NCS are given in Table 1 with the contents expressed as a % of DM. The DM for both spirulina species ranged between 56 and 57%. The high moisture contents in spirulina should be taken into consideration during storage and processing. Drying would be an excellent option of processing to reduce moisture for better storage and incorporation of livestock feeds.

Figure 1. Growing (A) and collecting (B) spirulina in wooden frames at the Agricultural Experiment Station in Sultan Qaboos University.

3.3 Chemical analyses of spirulina

The spirulina samples were dried in two ways: by centrifugation at 7,500 rpm (CS) and without centrifugation in (NCS). The dried spirulina was analyzed according to the standard methods of the AOAC [21]. Dry matter was determined by drying in an oven for 24 h at 80 ºC (Method 934.01). Crude protein (CP) was determined using a Foss Tecator Kjeltec 2300 Nitrogen/Protein Analyzer (Method 976.05). Lipids (Essential fatty acids and Photosynthetic pigments) and Ether extract (EE) were determined by Soxhlet extraction of the dry sample, using petroleum ether (Method 920.39). Ash was determined by ashing samples in a muffle furnace at 500 ºC for 24 hr (Method 942.05). Acid detergent fiber (ADF) was determined using cetyltrimethyl ammonium bromide (CTAB) and 1N H_2SO_4 as described by Roberston and Van Soest [22]. Neutral detergent fiber (NDF) was determined using sodium sulphite and sodium lauryl sulphate as described by Van Soest *et al*. [23]. ADF was expressed with ash whereas NDF was expressed without ash. Gross energy (GE) was measured using a bomb calorimeter.

Table 1. Chemical composition of spirulina dried in two different ways.

Spirulina	DM (%)	CP (%DM)	EE (%DM)	Ash (%DM)	ADF (%DM)	NDF (%DM)	Gross Energy (cal/g)
Centrifuged Spirulina (CS)	56.08	60.84 ± 0.25	0.97 ± 0.06	6.35 ± 0.03	0.25 ± 0.30	1.03 ± 0.15	5730
Non-centrifuged Spirulina (NCS)	57.06	62.48 ± 1.25	1.05 ± 0.00	7.55 ± 0.06	0.37 ± 0.36	1.92 ± 0.18	5629

The CP content was 61% for CS and 62% for NCS. This value falls within the range of 60-70% of CP reported by Holman and Malau-Aduli [11]. This CP content in spirulina is excellent compared to other plant sources such as SBM (44%) and animal sources such as fish meal (60%). Spirulina is more edible compared to fish meal and contains all essential amino acids, vitamins, minerals, carotenoids, fatty acids [11]. It is readily digestible by ruminants and it increases microbial protein production and by-pass protein by reducing protein rumen degradability [27, 11]. Moreover, spirulina protein contains a balanced composition of amino acids with concentrations of essential amino acids such as methionine and tryptophan similar to that of casein, depending on the culture used [27].

The lipid (EE) contents in CS and NCS were 0.97 and 1.05%. This level is very low compared to the 4-16% DM reported by Habib *et al.* [27] and Holman and Malau-Aduli [11]. The reason behind this low lipid content compared with reports in the literature was explained by Vonshak *et al*. [4], in that salt-adapted cells had a modified biochemical composition with a reduced chlorophyll content, and increased carbohydrate content which might affect lipid levels. However, low fat levels are recommended for ruminant rations as high fat levels negatively influence rumen fermentation.

The ash content was 6.4 and 7.6%, for CS and NCS, respectively. Habib *et al.* [27] and Holman and Malau-Aduli [11] reported a comparable range of 3-11% DM. Spirulina's ash was reported to contain all the essential minerals (about 7% of total weight) depending on the media. It should be noted that high ash content in non-conventional feeds poses a problem for using them in high proportions in mixed pelleted feeds.

CS and NCS had an ADF content of 0.25 and 0.37% DM and an NDF content of 1.03 and 1.92% DM in the CS and NCS, respectively (Table 1). A special value of spirulina is that it is readily digested due to its low fiber content or absence of cellulose in its cell walls [27]. About 85% of its protein is digested or assimilated after 18 hours [28]. This explains the low level of energy in spirulina. The gross energy (cal/g DM) in the current study was 5730 and 5629 for CS and NCS, respectively. Holman and Malau-Aduli [11] reported a gross energy of 1504 kj/100 g. This implies that spirulina should be used mainly as a protein supplement to a high fiber diet for ruminants due to its extremely low fiber content.

3.4 *In vitro* digestion of spirulina

The gas volume produced by spirulina over 96 hr is given in Figure 2. The centrifuged spirulina produced a total in vitro gas of 39.7 ml whereas the non-centrifuged spirulina produced a total of 31.2 ml (Figure 2). The difference between the two types started to be significant from 12 hr and continued until the end of the experimental period at 96 h. This total gas production is very low compared to that of forages. For instance, Kamalak *et al.* [29] reported much higher gas volumes (40-80 ml at 96 hr) for wheat straw, barley straw, alfalfa hay and maize silage. Gas production by fermentation largely arises from the carbohydrate fraction of forage. This is because ash and fat produce no gas and protein produces very little [30].

Drying spirulina by centrifugation resulted in higher gas production indicating that spirulina dried by this method is more digestible. This may be explained by its cell walls being easily digested in a moist environment or in the presence of certain enzymes. This is because of the lack of cellulose in its thick gelatinous cell walls which are made of complex structure sugars. The cell walls have a diameter ranging between 1-12 mm and their length ranges between 3 and 20 mm [2].

The CS in the current study had significantly higher ME (9.13 MJ/kg DM) than the NCS (8.25 MJ/kg DM) (Table 2). High energy levels in spirulina indicate its excellent potential as a livestock feed. Tobias Marino *et al.* [31] reported that ME levels followed the same pattern of gas production which is similar to the findings of the current study.

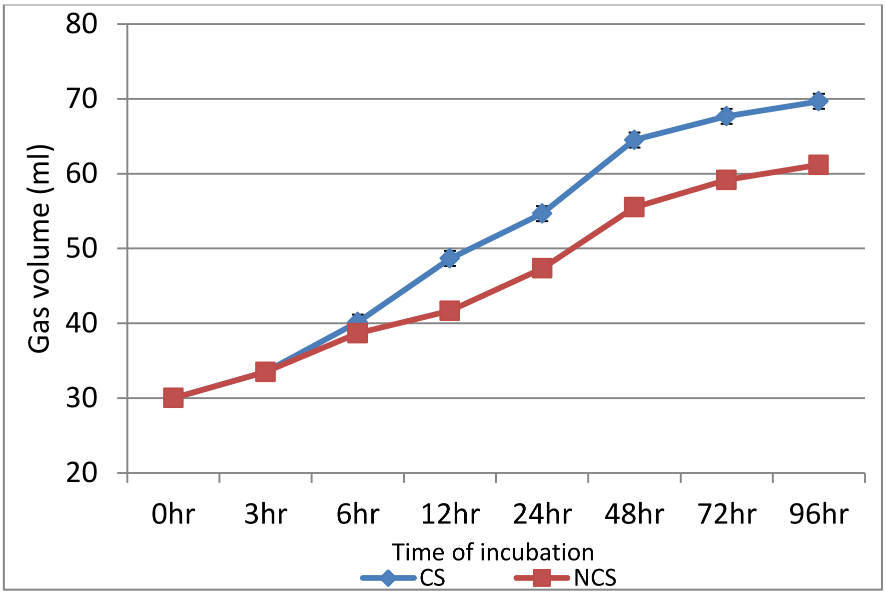

Figure 2. *In vitro* gas production pattern from spirulina dried by CS or NCS.

The CS had higher ODM (67.86%) than NCS (61.37%) and ME (9.1 and 8.25 MJ/kg) (Table 2). Calculations indicated that the CS had higher short chain fatty acids (SCFA) than NCS (0.59 and 0.41 µmol), respectively (Table 2). This adds to the value of spirulina as a potential livestock feed if it has been properly processed.

Table 2. Gas production parameters of spirulina dried by centrifugation (CS) and non-centrifuged spirulina (NCS).

Parameter	CS		NCS	
	Mean	SD	Mean	SD
Total gas (ml)	39.7	1.65	31.2	1.65
ME (MJ/kg)	9.13	0.275	8.25	0.079
ODM (%)	67.86	1.796	61.34	1.119
SCFA (µmol)	0.59	0.048	0.41	0.030

ME (Metabolizable energy) = 2.20 + 0.136*GV + 0.057*CP + 0.0029*CF (Menke & Steingass, 1988)
OMD (Organic Matter Digestibility) = 14.88 + 0.889*GV + 0.45*CP + 0.651*CF (Menke & Steingass, 1988)
SCFA (Short Chain Fatty Acids) = 0.0239*GV -0.0601 (Getachew *et al.*, 1999)
Means on the same row with similar denoting letters do not differ significantly (P>0.05)

Conclusion

In general, the findings of the current study supported published reports that, based on chemical composition and digestibility, spirulina proves to be an excellent non-conventional feedstuff especially as a protein source. Drying of spirulina by centrifugation improved its quality.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The technical help of Animal Science students Hussein Al-Madhani and Waleed Al-Madhani during the experimental period is acknowledged. This study was supported by a Sultan Qaboos University internal grant No. IG/AGR/ANVS/15/01.

References

1. Castenholz, R.W. Subsection III, Order Osciilatoriales. In: J. T. Stanley, M. P. Bryant, N. Pfenning and J.G. Holt (Eds.). *Bergey's Manual of Systematic Bacteriology*, 1989, **3**,1771-1775.
2. Salleh, B. *Pengenalan alam tumbuhan*. *Dewan Bahasa dan Pustaka, Kuala Lumpur*, 1987, 47-59.
3. Richmond, A. Spirulina. In: Micro-algal Biotechnology. Borowitzka, M. A. and Borowitzka, L. Y. (Eds.), p 85-101. Cambridge, Cambridge Univ. Press. U.K., 1988.
4. Vonshak, A., Kancharaksa, N., Bunnag, B. and Tanticharoen, M. Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. *Journal of Applied Phycology*, 1996), **8(2)**, 119-124.
5. Azov, Y. and Shelef, G. The effect of pH on the performance of the high-rate oxidation ponds. *Water Science Technology*, 1987, **19(12)**, 381-383.
6. Talbot, P. and De la Noue, J. Tertiary treatment of wastewater with *Phormidium bohneri* (Schmidle) under various light and temperature conditions. *Water Research*, 1993, **27(1)**, 153-159.
7. Lau, P.S., Tam, N.F.Y. and Wang, Y.S. Effect of algal density on nutrient removal from primary settled wastewater. *Environmental Pollution*, 1995, **89**, 56-66.
8. Abdel-Daim, M.M., Abuzead, S.M.M. and Halawa, S.M. Protective Role of *Spirulina platensis* against Acute Deltamethrin-Induced Toxicity in Rats. *PLoS ONE,* **8(9)**, e72991, 2013, http://dx.doi.org/10.1371/journal.pone.0072991.
9. Belay, A. The potential application of Spirulina (Arthrospira) as a nutritional and therapeutic supplement in health management, Review. *Journal of the American Nutraceutical Association*, 2002, 5, 27-48.
10. Khan. Z., Bhadouria, P. and Bisen, P.S. Nutritional and therapeutic potential of Spirulina. *Current Pharmaceutical Biotechnology*, 2005, **6**, 373-379. http://dx.doi.org/10.2174/138920105774370607.
11. Holman, B.W.B. and Malau-Aduli, A.E.O. Spirulina as a livestock supplement and animal feed. *Journal of Animal Physiology and Animal Nutrition*, 2013, **97(4)**, 615-623.
12. Panjaitan, T., Quigley, S.P., McLennan, S.R. and Poppi, D.P. 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. *Animal Production Science*, 2010, **50**, 405-409. http://dx.doi.org/10.1071/AN09194.
13. Kulpys, J., Paulauskas, E., Pilipavicius, V. and Stankevicius, R. Influence of cyanobacteria *Arthrospira* (*Spirulina*) *platensis* biomass additive towards the body condition of lactation cows and biochemical milk indexes. *Agronomy Research*, 2009, **7**, 823-835.
14. Simkus, A., Oberauskas, V., Laugalis, J., Zelvyte, R., Monkeviciene, I., Sedervicius, A., Simkiene, A. and Pauliukas, K. The effect of weed *Spirulina Platensis* on the milk production in cows. *Veterinarija ir Zootechnika*, 2007, **38**, 60.
15. Panjaitan, T., Quigley, S.P., McLennan, S.R., Swain A.J. and Poppi, D.P. Spirulina (*Spirulina platensis*) algae supplementation increases microbial protein production and feed intake and decreases retention time of digesta in the rumen of cattle. *Animal Production Science*, 2014, 55(4) 535-543.
16. Bezerra, L.R., Silva, A.M.A., Azevedo, S.A., Mendes,R.S., Mangueira, J.M. and Gomes, A.K.A. Performance of Santa Ineˆs lambs submitted to the use of artificial milk enriched with *Spirulina platensis*. *Cieˆncia Animal Brasileira*, 2010, **11**, 258-263.
17. EL-Sabagh, M.R., Abd Eldaim, M.A., Mahboub, D.H. and Abdel-Daim. M. Effects of *Spirulina Platensis* algae on growth performance, antioxidative status and blood metabolites in fattening lambs. *Journal of Agricultural Science*, 2014, **6(3)**, ISSN 1916-9752 (Print) ISSN 1916-9760 (Online).
18. Shimkiene, A., Bartkevichiute, Z., Chernauskiene, J., Shimkus, A., Chernauskas, A., Ostapchuk, A. and Nevitov, M. The influence of *Spirulina platensis* and concentrates on lambs' growth. *Zhivotnov'dni Nauki* , 2010, **47**, 9-14.
19. Tompkins, J., De Ville, M.M., Day, J.G. and Turner, M.F. Culture collection of algae and protozoa. Catalogue of Strains, (6th Edition). CCAP, Ambleside. 208 pp. 1995. [accessed Jun 25 2018].
20. Danesi, E.D.G., Rangel-Yagui, C.O., de Carvalho J.C.M. and Sato, S. An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by *Spirulina platensis*. *Biomass and Bioenergy*, 2002, **23**, 261-269.
21. The Official Methods of Analysis. Association of Official Analytical Chemists (AOAC.), 15thEdn. Washington, DC, USA. 2000.
22. Roberston, J.B. and Van Soest, PJ. The detergent system of analysis. In: James, W.P.T., Theander, O. (Eds.). The Analysis of Dietary Fiber in Food. Marcel Dekker, NewYork,USA,1981, 123-158.

23. Van Soest, PJ., Roberston, J.B. and Lewis, B.A. Methods for dietary fibre NDF and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Sci*ence,1991,**74**, 3583-3597.
24. Menke, K.H. and Steingass, H. Estimation of the energetic feed value from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development*, 1988, **28**, 7-55.
25. Getachew, G., Makkar, H.P.S. and Becker, K. Stoichiometric relationship between short chain fat acids and *in vitro* gas production in presence and polyethyleneglycol for tannin containing browses. European Federation of Animal Science (EAAP) Satellite Symposium, 1999.
26. *Statistical Packages for Social Sciences* (SPSS). Version 16, SPSS Inc., Illinois, USA. 2007.
27. Habib M.A.B., Parvin, M., Huntington, T.C. and Hasan M.R. A Review on Culture, Production and Use of *Spirulina* as Food for Humans and Feeds for Domestic Animals and Fish. FAO Fisheries and Aquaculture Circular. No. 1034 FAO, Rome, 2008, pp. 33.
28. Sasson, A. Micro Biotechnologies: Recent Developments and Prospects for Developing Countries. BIOTEC Publication 1/2542, 1997, pp. 11-31. Place de Fontenoy, Paris. France. United Nations Educational, Scientific and Cultural Organization (UNESCO).
29. Kamalak, A., Canbolat, O., Gurbuz Y. and Ozay, O. Comparison of *in vitro* gas production technique with *in situ* nylon bag technique to estimate dry matter degradation. *Czech Journal of Animal Science*, 2005, 50(2) 60-67.
30. Robinson, P.H. and Getachew. G. University of California CE: A Practical Gas Production Technique to Determine the Nutritive Value of Forages: The UC Davis Approach, 2000 (Available from: https://www.researchgate.net/publication/265997062_UC_CE_A_Practical_Gas_Production_Technique_to_Determine_the_Nutritive_Value_of_Forages_The_UC_Davis_Approach [accessed Jun 06 2018].
31. Tobias Marino, C., Hector, B., Mazza Rodrigues, P.H., Oliveira Borgatti, L.M., Marques Meyer, P., Alves da Silva, E.J. and Ørskov, E.R. Characterization of vegetables and fruits potential as ruminant feed by *in vitro* gas production technique. *Livestock Research for Rural Development*, 2010, *22, Article #168.* Last accessed 18.06.2017.from http://www.lrrd.org/lrrd22/9/mari22168.htm

Received 25 June 2018
Accepted 3 January 2019