Animal Feed Science and Technology 225 (2017) 123–133

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Review article

The effect of euglena (*Euglena gracilis*) supplementation on nutrient intake, digestibility, nitrogen balance and rumen fermentation in sheep

Ashagrie Aemiro[a], Peter Kiiru[a], Shota Watanabe[c], Kengo Suzuki[c], Masaaki Hanada[b], Kazutaka Umetsu[a], Takehiro Nishida[a,*]

[a] *Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan*
[b] *Department of Life Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan*
[c] *Euglena Co., Ltd 4th Floor, Yokohama Leading Venture Plaza 1, 75-1 One-cho, Tsurumi-ku, Yokohama-shi, Kanagawa 230-0046, Japan*

ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form 16 January 2017
Accepted 21 January 2017

Keywords:
Euglena
Intake
Digestibility
Sheep
Protein

ABSTRACT

This *in vivo* study was conducted to evaluate the effect of supplementation with different rates of euglena (*Euglena gracilis*) on nutrient intake, digestibility, nitrogen balance and rumen fermentation. Four rumen cannulated Corriedale wethers sheep with an average body weight of 44.25 ± 3.86 kg were arranged in a 4 × 4 Latin square design and fed a basal diet of Guinea grass (*Panicum maximum*) hay and concentrate mixture at the maintenance level with four different rates of euglena (0, 50, 100 and 150 g/kg DM intake). The experiment was conducted over 80 days in four 20 day periods that consisted of 14 days of acclimatization, 5 days of measurement and 1 more day for rumen liquor sample collection. The data were subjected to polynomial regression analysis. Dry matter (DM), organic matter (OM), acid detergent fibre (ADF) and gross energy (GE) intake increased linearly ($P < 0.001$) and quadratically ($P = 0.002$) with increasing concentrations of euglena. Similarly, crude protein (CP) intake was increased linearly ($P < 0.001$). Dry matter, OM, NDF, ADF and GE digestibility were not affected by supplementation of euglena ($P > 0.11$) while apparent CP digestibility increased linearly ($P = 0.009$). As a result, protein retention (g/d) was increased linearly ($P < 0.001$) and quadratically ($P = 0.017$) with increasing concentrations of euglena. Ruminal NH_3-N concentration increased (linear, $P < 0.001$) while ruminal protozoa population reduced linear, quadratic and cubic ($P < 0.008$) with increasing doses of Euglena. Euglena supplementation at different concentration did not change ($P > 0.23$) the total volatile fatty acid (VFA) concentration and the molar proportions of acetate, propionate, butyrate and the acetate: propionate ratio. The finding of this study indicated that the addition of euglena increased nutrient intake without affecting total tract digestibility. It has been also demonstrated that addition of euglena at a dose of 150 g/kg DM improved CP retention by 31%, which may be associated with increased CP digestibility and efficiency of utilization. Thus, euglena supplementation up to 150 g/kg DM of the diet could be a possible option for substitution of protein and energy sources.

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; A, P, acetate to propionate ratio; CP, crude protein; DE, digestible energy; DM, dry matter; EE, ether extract; GE, gross energy; NDF, neutral detergent fibre; NH_3-N, ammonia-N; OM, organic matter; TVFAs, total volatile fatty acids; VFA, volatile fatty acid.

* Corresponding author.
E-mail address: nishtake@obihiro.ac.jp (T. Nishida).

http://dx.doi.org/10.1016/j.anifeedsci.2017.01.017
0377-8401/

Table 1
Chemical composition of experimental feeds and diets.

Items[1]	Diet ingredients (g/kg DM)			Levels of Euglena (g/kg DM)				SEM	Contrast[2]		
	Guinea grass	concentrate	Euglena	0	50	100	150		L	Q	C
DM (g/kg)	955	951	969	953	954	955	956	0.66	0.203	0.977	0.981
OM (g/kg DM)	915	928	964	921	922	923	925	0.60	0.058	0.897	0.900
Ash (g/kg DM)	84.7	71.7	35.9	79.5[a]	77.7[ab]	76.6[ab]	75.2[b]	0.24	0.001	0.749	0.749
CP (g/kg DM)	101	182	285	134	139	140	143	0.71	0.081	0.501	0.504
EE (g/kg DM)	21.1	36.3	132	27.2[b]	32[ab]	36.1[ab]	40.4[a]	0.77	0.001	0.91	0.916
GE (MJ/kg DM)	17.5	17.8	21.4	17.6	17.8	18.0	18.1	0.75	0.061	0.979	0.992
NDF (g/kg DM)	650	232	6.5	483	472	481	483	0.54	0.789	0.303	0.904
ADF (g/kg DM)	368	37.5	2.8	236	234	249	257	0.25	0.567	0.814	0.876
Lignin (g/kg DM)	20.3	7.4	0.8	15.2	14.8	15.1	15.2	0.37	0.886	0.771	0.827

[a–d]Means within a row with different superscripts differ ($P < 0.05$).
[1] DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; GE: gross energy; NDF: neutral detergent fibre; ADF: acid detergent fibre.
[2] L: linear; Q: quadratic; C: cubic.

1. Introduction

Algae contain complex bioactive compounds and these are gaining importance in emerging technologies with nutritional and environmental applications (Dubois et al., 2013). Microalgae contain a large percentage of oil, with the remaining parts consisting of large quantities of proteins, carbohydrates, and other nutrients (Spolaore et al., 2006). This makes the post-oil extraction residue attractive for use as animal feed. The use of microalgae in addition to its nutritional importance, it is a simple and inexpensive method for carbon dioxide management, which is currently an important global issue (Poti et al., 2015). Our earlier *in vitro* study demonstrated that euglena (*Euglena gracilis*) is a rich source of amino and fatty acids, with the ability to affect protozoa population and therefore methane emission (Aemiro et al., 2016). The composition of euglena suggests that it can serve as a source of high-quality protein and energy. Studies on nutritional and toxicological evaluations demonstrated the suitability of microalgae biomass as a valuable feed supplement or substitute for conventional protein sources such as soybean meal, fish meal, and rice bran (Becker, 2007). Previous studies indicated that lipid supplementation in the diet of ruminants is the most promising approach to increase the energy density and product quality (Fiorentini et al., 2015). However, the performance response and supplemental lipid composition are complex and differ according to the specific diet (Grainger et al., 2010). It has been also reported that there was a reduction in DM intake with animals fed diets with supplemental fat (such as palm oil, linseed oil) compared with animals fed diets without fat (Fiorentini et al., 2014; Shingfield et al., 2010; Wanapat et al., 2011).

Limited *in vivo* studies are available on supplementation of microalgae in the ration of ruminants and the results are inconsistent. Enrichment in the polyunsaturated fatty acid was observed after supplementation of algae up to 94 g/d in the diet of ewe (Papadoulos et al., 2002); supplementation of 9.35 and 43 g/kg DM microalgae directly through the rumen fistula reduced DM intake by 10 and 45% compared to the control (Boeckaert et al., 2008); supplementation of microalgae to heifers at the dose of 50–150 g/d did not affect DM intake (Axman et al., 2015); inclusion of microalgae suspension (10% of their body weight) in the diet of calves did not improve CP and ME intake but crude fibre digestibility was improved (Chowdhury et al., 1995). Microalgae, despite its importance as a source of valuable nutrients for animals and management of environmental safety, its potential have not been fully exploited yet. Most of the previous studies with microalgae indicated that they contain a variable amount of CP, fibre and minerals depending on the production method (controlled environment, marine water or others). In addition to this the mode of feeding of microalgae was quite different (directly through the rumen cannula, Boeckaert et al., 2008; mixed with the ration, Stokes et al., 2015; or in suspension form, Chowdhury et al., 1995) which influenced the rumen fermentation process differently. It has been also observed that in some studies, specific microalgae were used in pure form (Aemiro et al., 2016) and in others in mixed or enriched form (Boeckaert et al., 2008). Because of these reasons, the results reported were inconsistent and incomparable. From our *in vitro* study (Aemiro et al., 2016), euglena supplementation up to 100 g/kg DM had shown better response in terms of rumen fermentation parameters, which was the basis to set the levels of euglena inclusion for our *in vivo* study and thus the objective of this work was to investigate the effect of increasing dose rates of Euglena in the diets of sheep on dry matter intake, *in vivo* digestibility, nitrogen balance and rumen fermentation.

2. Materials and methods

2.1. Euglena (Euglena gracilis)

Euglena, powder form with 100% purity, was obtained from Euglena Co. Ltd., Japan. The chemical compositions of euglena, Guinea grass hay and concentrate mixture are indicated in Table 1.