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RUMEN FERMENTATIVE PROPERTIES OF PREGNANT WEST AFRICAN DWARF DOES FED DIETS CONTAINING ALGAE BIOMASSOdunfa, O. A^{1*}DOI: <https://doi.org/10.54536/ajaset.v5i2.121>**ABSTRACT**

The study was conducted to investigate the effect of feeding algae biomass rich in polyunsaturated fatty acids at 0%, 1.5%, 3.0% and 4.5%, respectively, using Panicum maximum as basal feed, on rumen fermentative properties of pregnant West African Dwarf (WAD) does. A total of twenty (20) pregnant WAD does were grouped into four treatments containing five (5) animals per treatment and randomly allotted to the experimental diets. Rumen fluid were collected prior to mating and at the last trimester of pregnancy for determination of rumen fermentative properties. Data obtained were subjected to one way Analysis of Variance in a Completely Randomized Design. Inclusion of algae biomass up to 4.5% had no significant ($p>0.05$) effect on bacteria population in the rumen of the experimental does. The inclusion levels of algae biomass gave rise to the predominance of *Eischeria coli* (a gram negative bacterium) in the rumen of pregnant does fed the algae biomass diet. Results obtained showed a significant ($p<0.05$) decrease in the values obtained for ammonia nitrogen concentration in the rumen of the pregnant does when compared to the values recorded prior to mating. The total volatile fatty acid production and its molar proportion in the rumen fluid were not significantly ($p>0.05$) influenced by the inclusion of algae biomass.

Keywords: Algae biomass, polyunsaturated fatty acid, rumen, WAD does.

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INTRODUCTION

NRC (1981) recommends high plane of nutrition for pregnant goats from day 60 post-mating to term because the nutrient diversion to foetus and other associated tissues is extremely small before sixty days of gestation (Blanchart and Sauvant, 1974; Osuagwuh and Aire, 1990). West African Dwarf (WAD) goats were found to be very sensitive to nutritional stress during pregnancy especially between 90 to 120 days of gestation (Osuagwuh and Akpokodje, 1986; Osuagwuh and Aire, 1990). The rumen serves as the primary site for microbial fermentation of ingested feed components. Gerard and Frederique (2006) reported that the nature of feed given to ruminant to support productivity is one of the several abiotic factors that can alter the balance of rumen microbial population and their activities which may lead to either decrease in performance or increase the risk of health problems. The proportion of partial volatile fatty acid concentration in the rumen also depends largely on the type of feed consumed by the animals. The use of algae as feed materials for animals is more common than their use in the human diet (Christaki *et al.*, 2012). Algae have been found to be a valuable feed source for poultry, fish and pigs (Kotrbaček *et al.*, 2015). A large number of nutritional and toxicological evaluations showed that algae biomass could be used as a valuable feed supplement, which can successfully replace conventional sources of dietary fat such as soy and fish meal (Becker, 2007). New innovative algae feeding exist of late in livestock production systems which entails the feeding of algae biomass as the source of poly-unsaturated fatty acid (PUFA) (Adeleye, 2012). The ability to produce highly valuable molecules such as *n*-3 fatty acids (FA), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) makes it a considerable feedstuff for livestock (Spolaore *et al.*, 2006). Feeds or feed additives containing PUFA may change rumen fermentation, including decreasing methane production (Moate *et al.*, 2013). However, there is no comprehensive research on the use of algae in ruminant nutrition and their effect on rumen functions (Da Silva *et al.*, 2016). Hence the need for this study.

LITERATURE REVIEW

Algae: Algae are aquatic, photosynthetic microorganisms that appeared on earth about 3.5 billion years ago and are regarded as the first life form (Margulis, 1981). They are distinguished into multicellular organisms (macro algae) that can reach 60 m in length and single-celled organisms (micro algae or phytoplankton) with size from 0.2 to 2 mm (Harlin and Darley, 1988). They are reproduced by simple division one or two times per day. They have no roots, stems, flowers or leaves, so they grow much faster than terrestrial plants and

are characterized as the most productive plants in the world (Marshall, 2007). Although the micro algae biotechnology is similar to conventional agriculture, micro algae have many advantages over terrestrial plants. Although the micro algae biotechnology is similar to conventional agriculture, micro algae have many advantages over terrestrial plants. They have higher productivity than traditional crops and can be grown in climatic conditions and regions where other crops cannot. It has been estimated that more than 30,000 species of micro algae exist, but the chemical composition of only some hundreds of those has been studied and only a few are cultivated in significant (industrial) quantities (Gouveia *et al.*, 2008). Both marine and freshwater algae have been used in human nutrition, cosmetics, and pharmaceutical products and have the potential to be used as a supplement for livestock feeds (Paul and Tseng, 2012).

Effect of algae on rumen fermentation: Previous studies have demonstrated that algae can affect rumen fermentation *in vitro* in whethers (Fievez *et al.*, 2007). Zhu *et al.* 2016 reported that algae infusion at 6.1g/d (10 g/kg DM) had no effect on rumen pH and total volatile fatty acid concentrations, whereas algae infusion at 18.3g/d (30 g/kg DM) increased rumen pH and decreased total volatile fatty acid concentration, which is in line with the general negative correlation between pH and total volatile fatty acid concentrations levels (Huhtanen and Kukkonen, 1995). High algae infusion (18.3g/d) induced an evident shift of the VFAs pattern, whereas no effect was observed with the low algae treatment (6.1g/d). The researcher further attributed the different responses to the amounts of algae supplemented and hence difference in the supply of long-chain polyunsaturated fatty acids (PUFA). Also, other researchers (Toral *et al.*, 2009; Toral *et al.*, 2010) did not observe shifts when dairy sheep were fed a diet containing similar amounts of eicosapentaenoic acid (EPA) + decosahexaenoic acid (DHA) as supplied through the low algae diet (approximately 2.0 g of DHA/kg DM). In contrast, in an earlier report (Boeckert *et al.*, 2008), supplementation of algae in the concentrate at a rate of 9.35 g/kg DM of the same algae species (*Schizochytrium*) modified rumen fermentation pattern in dairy cows. This might be related to difference between ruminant species with small ruminants eventually having a greater tolerance for dietary PUFAs and/or the mode of algae supplementation (ruminal infusion versus incorporation in the concentrate).

MATERIALS AND METHODS

Experimental site: The experiment was carried out at the Small Ruminant Unit of the Teaching and Research Farms, Federal University of Agriculture, Abeokuta. The University

is located on Lat. 7°5' N and Long. 3°11.2' E, Ogun State, Nigeria. It is located in the derived savannah zone of South Western Nigeria. Relative humidity ranged from 63-96% in the rainy season (late March to October) and 35-82% in the dry season (November to early March) with an average of 82%. The average annual rainfall was about 1100mm with peak rainfall occurring from July to September. The mean monthly ambient temperature ranged from 28°C in December to 36°C in February as recorded by the meteorological station of the Federal University of Agriculture, Abeokuta in the year 2016.

Experimental animals and their management: A total of twenty (20) West African Dwarf nulliparous does with weight ranging between 12 and 15kg, 12-18 months old purchased from villages in the neighborhood of Abeokuta were used for the experiment. The animals were quarantined in the experimental farm for 28 days in order to assess their health status and to ascertain their suitability for the experiment. The animals were given anti-stress (Vitalyte) at 1g/10kg body weight on arrival for five (5) days to prevent possible loss due to fatigue and stress during transportation. Experimental animals were de-wormed and treated against endo and ecto-parasites using Ivomec[®] injection (0.1ml/kg body weight) subcutaneously for three (3) days, oxytetracycline long acting injection (1.0ml/kg body weight) was also administered intramuscularly for three (3) days on those with diarrhea symptoms. They were also vaccinated against *pestes de petit ruminants (PPR)* using PPR vaccine. During this period, the animals were allowed to graze freely in a paddock sown with *Panicum maximum*. They were also fed concentrate supplement and water was supplied *ad-libitum*.

Mating and confirmation of pregnancy: After acclimatization, the estrus cycle of the does was synchronized by injecting 100µg of GnRH intramuscularly (Receptal[®]; Intervet International B.W., Boxer, Holland). After eleven days, animals received an injection of 2ml prostaglandin-F_{2α} (PGF_{2α}) and were introduced to four teasers in a paddock and estrus was closely monitored following synchronization. At the detection of estrus, the does were withdrawn from the paddock and introduced to bucks in the pen for mating. All does were tested for pregnancy 60 days following mating with the aid of a transabdominal ultrasonic scanning apparatus and this was carried out at the Veterinary teaching hospital of the Federal University of Agriculture, Abeokuta.

Experimental diets preparation: A concentrate diet with the following ingredients: whole maize, groundnut cake, wheat offal, palm kernel cake, bone meal and salt with 0, 1.5, 3.0, and 4.5% algae biomass per Kg dry matter (DM) was offered to the experimental animals. Table 1 shows the proximate composition and calculated analysis of the experimental diets.

Table 1: Ingredient composition of the experimental diets

Ingredients	0PAB	1.5PAB	3.0PAB	4.5PAB
Maize (%)	15.0	15.0	15.0	15.0
Wheat offal (%)	44.0	44.0	44.0	44.0
Palm kernel cake (%)	35.0	33.5	32.0	30.5
Groundnut cake (%)	3.0	3.0	3.0	3.0
Bone meal (%)	2.0	2.0	2.0	2.0
Salt (%)	1.0	1.0	1.0	1.0
Algae biomass (%)	-	1.5	3.0	4.5
Total (%)	100	100	100	100

0PAB - 0 percent algae biomass, 1.5PAB – 1.5 percent algae biomass, 3.0PAB- 3.0 percent algae biomass and 4.5PAB- 4.5 percent algae biomass

Experimental design

The twenty (20) experimental animals were grouped into four treatments containing five (5) animals per treatment with each animal being a replicate on its own. Each group was balanced for weight and allotted to one of the four experimental diets containing 0%, 1.5%, 3.0% and 4.5% algae biomass respectively, in a completely randomized design.

Dietary treatment of experimental goats: The experimental goats were housed and fed individually throughout the period of the experiment. They were fed *Panicum maximum* as basal diet at 4% of their body weight in the morning and the experimental concentrate diets was fed at 3% of their body weight in the afternoon. Water was supplied *ad-libitum*

Data collection: Rumen fluids were collected prior to mating and at the last trimester of pregnancy for total bacteria count, rumen ammonia nitrogen and volatile fatty acids determination. Rumen fluids were collected from each animal prior to feeding and four hours after feeding through the oesophagus by the use of a suction tube. 10ml of rumen fluid was taken and filtered through four layers of cheese cloth into sample bottles to determine the rumen nitrogen and volatile fatty acids.

Laboratory analysis of rumen fluid

Determination of rumen microbial type and population: A portion of the rumen fluid was mixed with formalin solution in normal saline water for total direct count. Total counts of bacteria were enumerated microscopically using a Hausser counting chamber (Boeco). Each bacteria count was repeated twice and the average was recorded.

Determination of the volatile fatty acids (VFA) concentration: Samples of the filtrate was acidified with 1ml of 5% (v/v) tetraoxosulphate (vi) acid solution and stored at -20°C in an air tight bottle for subsequent determination of VFA concentration by steam distillation process

using Markham micro-distillation apparatus (Markham, 1942) as modified and described by Wei *et al.* (2001). Individual VFAs (propionic, acetic and butyric acid) was determined using chromatography (Mebrahtu and Tenaye, 1997).

Determination of rumen ammonia nitrogen: 5mls of the liquor was siphoned into a 30ml corked test tube containing 20ml of sodium acetate buffer at pH 8.2. The mixture was thoroughly shaken to ensure complete trapping of ammonia nitrogen. The mixture was filtered through a Whatman No 42 filter paper into 30ml test tube. The absorbance of sample as well as working standards was read on a 21D spectrophotometer at wavelength of 630nm. (AOAC 2000).

Proximate and chemical analysis of experimental diets: The chemical composition of the experimental diets was carried out to determine the percentage moisture, ash, fat, crude fibre fraction, crude protein and carbohydrate. The moisture content was determined by drying in an oven at 70°C to constant weight. Ash by incineration in a muffle furnace at 550°C for 48 hours, Protein was determined by nitrogen determination using kjeldahl method and conversion of nitrogen to protein by the factor of 6.25. Fat determination was by Bligh dyer technique. Fibre fraction such as neutral detergent fibre (NDF), acid detergent fibre (ADF) was analyzed according to Van Soest (1994). Percentage carbohydrate was calculated using the formular: $100 - (\text{percentage of ash} + \text{percentage of moisture} + \text{percentage of fat} + \text{percentage of protein})$ (A.O.A.C, 2000).

Statistical analysis: All data collected were subjected to one-way Analysis of Variance (ANOVA) in a Completely Randomized Design as contained in Statistical Package for Social Sciences (SPSS, 2010). Significantly different means were separated using new Duncan Multiple Range Test within the same package.

RESULTS AND DISCUSSION

Table 2 shows the proximate composition of the experimental basal diet (*Panicum maximum*) and test ingredient (algae biomass). The nutrient composition per 100g for *Panicum maximum* is as follows: Dry matter 97.33%, crude protein 11.40%, crude fibre 62.00%, fat 6.00%, ash 5.33% and nitrogen free extract 15.72%. For algae biomass, the values of 90.24%, 15.77%, 6.72%, 30.82%, 10.91 and 26.02 were recorded for dry matter, crude protein, crude fibre, fat, ash and nitrogen free extract respectively.

The proximate composition of experimental diets is as shown in Table 3. The diet containing 4.5PAB had the highest values in the dry matter content, crude protein, crude fibre, ether extract, ash, calcium, phosphorus, neutral detergent fibre and acid detergent fibre. These

values were closely followed by the diet containing 3.0PAB and 1.5PAB while the control diet had the least values for all the nutrients considered.

Table 2: Proximate composition of the experimental basal diet and test ingredient

Nutrient parameters	<i>Panicum maximum</i>	Algae biomass
Dry matter (%)	97.33	90.24
Crude protein (%)	11.40	15.77
Crude fibre (%)	62.00	6.72
Fat (ether extract) (%)	6.00	30.82
Ash content (%)	5.33	10.91
Nitrogen free extract (%)	15.72	26.02

Table 3: Proximate and chemical composition of experimental concentrate diets

Parameter	Control	1.5PAB	3.0PAB	4.5PAB
Dry matter (%)	84.80	85.40	87.50	89.62
Crude protein (%)	13.72	13.96	14.10	14.25
Crude fibre (%)	7.40	7.62	7.85	8.10
Ether extract (%)	6.25	6.48	6.50	7.21
Ash (%)	12.0	12.47	13.50	13.80
Calcium (%)	1.20	1.30	1.42	1.48
Phosphorus (%)	0.68	0.74	0.78	0.96
Neutral detergent fibre (%)	48.85	56.30	58.58	62.57
Acid Detergent fibre (%)	40.25	43.58	48.65	51.35

***PAB - Percent algae biomass**

The relatively higher dry matter observed in the experimental concentrate diet can be attributed to the fact that they were prepared from mixture of dry concentrate and the algae biomass ingredient which were high in dry matter. Higher dry matter is of very significant importance as less moisture will mean more nutrients. The crude protein range from 13.72-14.25% recorded in the experimental feedstuff was higher than the critical level of 8% for ruminants as required by rumen microorganisms to support optimum activity (Gatenby, 2002) and also comparable to 14-18% crude protein recommended for pregnant does (NRC, 1981). This implies that the experimental diets provided adequate nitrogen required by rumen microorganisms to maximally digest the component of dietary fibre leading to the production of volatile fatty acids (Trevakis *et al.*, 2001). The crude fibre increased with inclusion level of algae biomass across the treatments. The crude fibre range of the experimental feed was lower than 24.66% reported by Ukanwoko (2007). The fibre content in diets has been found useful in predicting feed intake as it measures the total fibre components of the feed (Van Soest, 1994).

Table 4: Total bacteria count in the rumen of pregnant WAD does fed varying levels of algae biomass

Parameters	0PAB	1.5PAB	3.0PAB	4.5PAB
Total bacteria count (10³cfu/ml)				
Pre-feeding	29.70 ± 0.35 ^a	20.80 ± 4.03 ^b	17.55 ± 0.92 ^b	18.98 ± 2.07 ^b
Post-feeding	22.00 ± 2.42	26.1 ± 3.94	26.75 ± 8.56	21.48 ± 4.85

^{a,b} Mean values with different superscripts within rows differed (p<0.05) significantly. PAB - Percent algae biomass.

The effect of varying levels of algae biomass in diets of WAD does on total bacteria counts is as shown in Table 4. There was a significant (P<0.05) difference in the bacteria count obtained pre-feeding among treatment groups with the highest value of 29.70x10³cfu/ml obtained in the control group while pregnant does on the algae biomass diets had the lowest values (20.80 x10³cfu/ml, 17.55 x10³cfu/ml and 18.98 x10³cfu/ml). At post feeding, the highest value of 26.75x10³cfu/ml was observed in pregnant does on 3.0PAB and the least value of 22.00x10³cfu/ml was observed in pregnant does fed the control diet. Although, the values obtained did not differ statistically, the total bacteria count in the rumen of does fed the algae biomass diets increased numerically when compared to the values obtained pre-feeding while the control group had a reduction in total bacteria count after feeding. Bacteria population in the rumen at a given time largely determine the extent and rate of fiber digestion (Khampa *et al.*, 2006). Diet is one of the main factors influencing the rumen microbial population and specifically the milieu of substrate derived from microbial fermentation of ingested feed (Carberry *et al.*, 2012). The results obtained in this study indicated that the population of bacteria in the rumen of the experimental does were not affected by the dietary inclusion level of the algae biomass. Jenkins (1993) and Ueda *et al.* (2003) observed that diets with high forage to concentrate ratios may reduce the effects of polyunsaturated fatty acids (PUFA) on ruminal fermentation because the high fiber concentration promotes ideal conditions for the rapid growth of the microorganisms responsible for the hydrolysis and bio-hydrogenation of PUFA. This may be the reason for the increase in total bacteria count in the rumen of does fed the algae biomass diet since the experimental does were fed diets with high forage to concentrate ratios.

Table 5 shows the bacteria species isolated from the rumen of pregnant WAD goats fed varying levels of algae biomass. The bacteria species isolated were *Staphylococcus spp.*, *Eischeria Coli*, *Streptococcus spp.*, *Bacillus spp.* and *Pseudomonas spp.* *Streptococcus spp.*

and *Bacillus spp.* were found in all treatment groups. *Eischeria Coli* was absent in the control diet but present in the rumen of does fed the algae biomass diet.

Table 5: Bacteria species isolated from the rumen of pregnant WAD does fed varying levels of algae biomass

0PAB	1.5PAB	3.0PAB	4.5PAB
<i>Staphylococcus spp.</i>	<i>Staphylococcus spp.</i>	-	<i>Staphylococcus spp.</i>
-	<i>Eischeria Coli</i>	<i>Eischeria Coli</i>	<i>Eischeria Coli</i>
<i>Streptococcus spp.</i>	<i>Streptococcus spp.</i>	<i>Streptococcus spp.</i>	<i>Streptococcus spp.</i>
<i>Bacillus spp.</i>	<i>Bacillus spp.</i>	<i>Bacillus spp.</i>	<i>Bacillus spp.</i>
<i>Pseudomonas spp.</i>	<i>Pseudomonas spp.</i>	<i>Pseudomonas spp.</i>	-

Streptococcus spp. and *Bacillus spp.* which are gram positive bacteria were found in all the treatment groups. Similar result was obtained by Valinote *et al.* (2005) who evaluated cottonseed and the calcium salts of fatty acids as sources of fat for beef cattle. Findlay (1998) also listed *Streptococcus spp.* and *Bacillus spp.* as part of the predominant cellulolytic bacteria in the rumen. The predominance of the genus *Eischeria coli* in the rumen fluid of does on the algae biomass diet is consistent with the observations of other authors who investigated microbial populations in cattle under different feeding conditions such as diets rich in sugar cane (Franzolin and Franzolin, 2000), diets with or without addition of fat (Towne *et al.*, 1990) and the addition of ionophores to diets rich in forage or concentrate (Guan *et al.*, 2006). *Escherichia coli* ranks among the most common causative agents of bacterial infections in several ruminant animals with symptoms including bloody diarrhea, diarrhea leading to dehydration, prolonged diarrhea and weight loss. (Brown *et al.*, 1997; Dean-Nystrom *et al.*, 1999). During the course of this study, some of the above symptoms were observed confirming the effect of the gram negative bacteria on the experimental does.

Table 6: Rumen fermentative products of dry WAD does

Parameters	0PAB	1.5PAB	3.0PAB	4.5PAB
PH				
Pre-feeding	7.39 ± 0.96	7.54 ± 0.90	6.91 ± 0.19	7.37 ± 0.15
Post-feeding	6.38 ± 0.04	6.57 ± 0.48	6.45 ± 0.36	6.67 ± 0.22
Ammonia nitrogen (ml/100ml)				
Pre-feeding	3.28 ± 0.62 ^b	4.71 ± 0.61 ^a	3.75 ± 0.34 ^b	2.82 ± 0.28 ^b
Post-feeding	3.64 ± 0.58 ^b	5.16 ± 0.66 ^a	4.20 ± 0.22 ^b	3.40 ± 0.52 ^b
Total volatile fatty acid (ml/100ml)				
Pre-feeding	1.146 ± 0.17	1.270 ± 0.41	1.176 ± 0.16	1.176 ± 0.42
Post-feeding	1.236 ± 0.08	1.140 ± 0.33	1.266 ± 0.20	1.536 ± 0.22

Acetate (ml/100ml)				
Pre-feeding	0.764 ± 0.11	0.851 ± 0.27	0.784 ± 0.10	0.784 ± 0.29
Post-feeding	0.840 ± 0.05	0.760 ± 0.22	0.844 ± 0.13	1.024 ± 0.15
Butyrate (ml/100ml)				
Pre-feeding	0.076 ± 0.11	0.084 ± 0.28	0.078 ± 0.11	0.078 ± 0.03
Post-feeding	0.084 ± 0.01	0.076 ± 0.02	0.084 ± 0.01	0.103 ± 0.03
Propionate (ml/100ml)				
Pre-feeding	0.255 ± 0.04	0.280 ± 0.09	0.261 ± 0.34	0.261 ± 0.93
Post-feeding	0.280 ± 0.18	0.253 ± 0.07	0.281 ± 0.44	0.341 ± 0.05
Acetate: Propionate ratio				
Pre-feeding	3.00 ± 0.00	3.05 ± 0.08	3.00 ± 0.00	3.00 ± 0.00
Post-feeding	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00

^{a,b} : Mean values with different superscripts within rows differed (P<0.05) significantly.

PAB- percent algae biomass.

Table 7: Rumen fermentative products of pregnant WAD does fed diet containing varying levels of algae biomass at the last trimester of pregnancy

Parameters	0PAB	1.5PAB	3.0PAB	4.5PAB
PH				
Pre-feeding	7.10 ± 0.35	7.10 ± 0.22	6.95 ± 0.21	7.15 ± 0.31
Post-feeding	7.27 ± 0.23	7.10 ± 0.26	7.25 ± 0.35	7.30 ± 0.22
Ammonia nitrogen (ml/100ml)				
Pre-feeding	0.183 ± 0.40	0.165 ± 0.40	0.150 ± 0.85	0.183 ± 0.06
Post-feeding	0.173 ± 0.46 ^{ab}	0.145 ± 0.03 ^b	0.235 ± 0.01 ^a	0.200 ± 0.05 ^{ab}
Total volatile fatty acid(ml/100ml)				
Pre-feeding	0.530 ± 0.08	0.383 ± 0.05	0.450 ± 0.13	0.488 ± 0.08
Post-feeding	0.407 ± 0.04	0.352 ± 0.05	0.440 ± 0.01	0.438 ± 0.08
Acetate (ml/100ml)				
Pre-feeding	0.333 ± 0.46	0.255 ± 0.40	0.300 ± 0.85	0.322 ± 0.52
Post-feeding	0.273 ± 0.29	0.232 ± 0.31	0.295 ± 0.07	0.290 ± 0.05
Butyrate (ml/100ml)				
Pre-feeding	0.037 ± 0.01	0.028 ± 0.01	0.030 ± 0.01	0.035 ± 0.01
Post-feeding	0.027 ± 0.01	0.025 ± 0.01	0.028 ± 0.00	0.030 ± 0.01
Propionate (ml/100ml)				
Pre-feeding	0.151 ± 0.08	0.085 ± 0.01	0.100 ± 0.03	0.110 ± 0.01
Post-feeding	0.093 ± 0.09	0.078 ± 0.01	0.100 ± 0.00	0.093 ± 0.02

Acetate :propionate ratio

Pre-feeding	2.514 ± 0.88	2.998 ± 0.14	3.000 ± 0.00	2.920 ± 0.22
Post-feeding	2.930 ± 1.45	3.013 ± 0.15	2.950 ± 0.07	2.990 ± 0.11

^{a,ab,b} Mean values with different superscripts within rows differed ($p < 0.05$) significantly.

PAB- percent algae biomass

Table 6 and 7 shows the rumen fermentative properties of dry WAD does and pregnant WAD does respectively. Rumen pH is the best indicator that reflects the ruminal health. The mean values of pH observed during pregnancy were not significantly affected by the dietary inclusion of algae biomass. According to Sung *et al.* (2007), the pH level is one of the most important factors in the rumen environment because cellulolytic bacteria are very sensitive to pH change. The values obtained in this study were comparable to the range of 6.0-7.0 reported by Muia *et al.* (2000) which was considered optimal for the activity of rumen microbial population for their growth and VFA absorption. Zhu *et al.* (2016) reported a non-significant increase in rumen pH and a decreased total volatile fatty acid concentration with algae inclusion at 30 g/kg DM which is in line with the negative correlation between pH and total volatile fatty acid concentrations levels (Huhtanen and Kukkonen, 1995). According to Firkins *et al.* (2006), the proportion of VFA produced in the rumen depends largely on the composition of diets consumed by the ruminant in particular the fractions contained in the feed. The non-significant results obtained for total volatile fatty acid (TVFA) concentration and its molar fractions is in agreement with previous studies (Bateman and Jenkins, 1998; Harvatine and Allen, 2006) who reported that the use of polyunsaturated fatty acid has a minor or insignificant effect on ruminal parameters. Toral *et al.* (2009) reported that TVFA concentration was not affected at any specific time post-feeding and that only mean values are lowered with oil supplementation. In contrast, in an earlier report (Boeckaert *et al.*, 2008), supplementation of algae in concentrate at a rate of 9.35 g/kg DM of an algae specie (*Schizochytrium*) influenced TVFA and its fractions in dairy cows. The observed differences can be related to difference between ruminant species with small ruminants having a greater tolerance for dietary PUFAs and also the higher inclusion levels used in this study.

The concentration of rumen ammonia nitrogen ($\text{NH}_3\text{-N}$) has been used as a qualitative reference to detect the microbial activity in fibrous carbohydrate in the rumen (Detmann *et al.*, 2009). The values obtained for ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$) in this study were significantly influenced by the inclusion levels of algae biomass. There was a significant reduction in the post-feeding values recorded in the pregnant does compared to values obtained prior to mating. Juliana *et al.* (2013) attributed the observed decrease in the

ammonia concentration to an increase in the use of ammonia nitrogen by cellulolytic bacteria in the rumen. It could also be attributed to the increased needs of proteins for the foetus development during pregnancy (Castillo *et al.*, 1997). Loor *et al.* (2002) also reported that ruminal ammonia nitrogen was decreased in response to fat supplementation. The values obtained during gestation in this study were within the range of 0.1-2.0mM required to optimize digestion of feed for rumen microorganism as reported by Leng (1990). This implies that the level of NH₃-N obtained in all dietary treatments were sufficient for optimum rumen fermentation and microbial growth.

CONCLUSION

From the results obtained in this study, the following conclusions can be deduced; inclusion of algae biomass up to 4.5% had no significant effect on bacteria population in the rumen of the experimental does. The inclusion levels of algae biomass gave rise to the predominance of *Eischeria coli* (a gram negative bacteria) in the rumen of pregnant does fed algae biomass diet. Inclusion of algae biomass up to 4.5% did not significantly affect rumen pH, volatile fatty acid concentration and its molar proportions. During gestation, the inclusion levels of algae biomass significantly affected the concentration of rumen ammonia nitrogen produced with the highest value recorded in does fed 3.0% algae biomass.

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