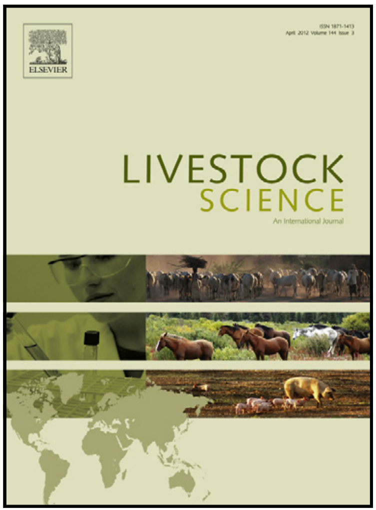

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Effect of cutting interval of Taiwan grass (*Pennisetum purpureum*) and partial substitution with duckweed (*Lemna* sp. and *Spirodela* sp.) on intake, digestibility and ruminal fermentation of Pelibuey lambs

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ABSTRACT

The effects of Taiwan grass (TW) cutting interval and partial substitution with duckweed on dry matter intake (DMI), *in vivo* DM digestibility (DMD), and digestibility of organic matter (OMD), crude protein (CPD), neutral (NDFD) and acid detergent fiber (ADFD), as well as on nitrogen balance, ruminal pH, production of volatile fatty acids (VFA) and ammonia nitrogen (NH_3-N), was evaluated. For each experimental period (P) of 30 (P1), 45

(P2), and 60 (P3) days of TW grass cutting interval, twelve Pelibuey lambs were randomly assigned to three groups of four lambs each in a completely random design. Average weights of lambs were 25.1 ± 1.5, 27.6 ± 1.9 and 28.6 ± 1.8 kg. Lambs were housed in individual metabolic cages, and assigned to three treatments (n=4): (T1) 100% TW, (T2) 80% TW+20% DW, and (T3) 70% TW+30% DW. Each experimental period lasted 16 days: 8 days for adaptation to the diet and 8 days for total feces collection and rumen liquor sampling; urine was collected the last two days. Data were analyzed with MIXED procedure and means compared with the Tukey test. Inclusion of DW decreased DMI ($P < 0.01$), whereas DMD, OMD, CPD, and NDFD increased ($P < 0.05$) as the age of the TW increased; besides, ADFD showed differences ($P < 0.05$) among treatments only in P_3. Nitrogen retention improved by DW ($P < 0.05$) in the three periods, and pH was affected by periods ($P > 0.05$) only in P3 ($P < 0.05$). In the three periods, 20 and 30% DW increased NH_3-N concentration and the acetate:propionate ratio ($P < 0.05$), but the VFA proportion did not change.

Key words: Sheep, duckweed, Taiwan grass, digestibility, ruminal variables.

1. Introduction

The efficiency of a ruminant production system based on forages depends on grass maturity, which determines their nutritional quality (Nelson and Moser, 1994); besides, maturity affects feed intake and digestibility as well as nutrient absorption efficiency (Da Silva et al., 2007). As forages mature, the cell wall increases and total and soluble nitrogen decrease (Merchen and Bourquin, 1994); therefore, protein content will be lower than the minimum required (6-8%) to supply enough ammonia for optimum ruminal microbial fermentation (Norton, 1994), thus decreasing dry matter intake and digestibility. To solve this problem supplements with commercial concentrates and non-conventional protein sources such as Gliricidia, Clitoria, and Mucuna (Juma et al., 2006), Guamuchil (Kahindi et al., 2007), and duckweed (Babayemi et al., 2006; Cheng and Stomp, 2009) have been used. Lemnoideae are aquatic plants with a great potential for supplying proteins with a high biological value for animals of economic interest such as ruminants (Damry and Nolan, 2009; Zetina-Córdoba et al., 2012), non-ruminants (Gutiérrez et al., 2001), birds (Akter et al., 2011; Witkowska et al., 2012) and fish (de Almeida et al., 2010). Taiwan grass (*Pennisetum purpureum* Schum) is an important forage species in tropical zones due to its large biomass production and if harvested at the right moment can supply a high amount of nutrients (Araya and Boschini, 2005), however it is affected by cutting interval (Kozloski et al., 2003). Therefore, the objective of this work was to evaluate the effect of duckweed (DW; an association of *Lemna* spp. and *Spirodela* sp.) as a suplement for Pelibuey lambs fed Taiwan grass (TW) at different stages after regrowth on voluntary intake, *in vivo* digestibility, nitrogen balance, concentration of ruminal nitrogen, volatile fatty acids (VFA), and ruminal pH.

2. **Materials and Methods**

2.1 Animals and diets

Twelve Pelibuey male lambs were randomly assigned to three diets with Taiwan:duckweed rations of 100:0, 80:20 and 70:30. The study had three periods (3 cutting intervals), with the same Taiwan:duckweed rations. Average weights of lambs (n=4) were 25.1 ± 1.5, 27.6 ± 1.9 and 28.6 ± 1.8 kg, for each period. Lambs were housed in individual metabolic cages (1.2 x 15 m) and feed *ad libitum*, a las 08:00 y 16:00 h. DW was harvested daily at

14.00 h from a lagoon located in the aquaculture research unit of the Colegio de Postgraduados, Campus Veracruz. It was dried in a greenhouse and used the following day. In order to have the TW grass at the required harvesting time, 16 plots of 36 m^2 were established for each period (30, 45 and 60 days of regrowth) to get TW grass for 16 continuous days. TW grass was harvested at 7.00 h and chopped at a lenght of 3 cm, to be mixed latter on with DW. Because TW:DW relation was calculated in DM basis for the three treatments, every 48 h DM was determined in TW grass and DW, in order to adjust the amount of each as fed basis.

2.2. Sampling and chemical analysis

For each experimental period, lambs had an 8 days adaptation period to the diet and 8 days for total feces collection and rumen liquor sampling. Two days before the end of each period, total urine was collected (Valadares et al., 1997) and samples were stabilized with hydrochloric acid (50%) 50 ml L^{-1}. Rumen liquor samples were taken from the median ventral part of the rumen by esophageal probe 3 hours after feeding (07:00 h); pH was immediately measured with a portable pH meter (Orion™, model 3Star). The rumen liquor samples were stabilized with metaphosphoric acid (25%) at a 4:1 ratio, and ammonia nitrogen (NH_3-N) concentration was determined through absorbance in an ultraviolet light spectrophotometer (Varian™, model Cary-1-E) at 630 nm. From the stabilized solution, 1.0 mL was placed in a 13x100 mm glass tube containing 7.5 mL phenol and 7.5 mL sodium hypochlorite, shaken in a vortex mixer and incubated at 37 ºC for 30 minutes, and the reading was taken (McCullough, 1967). The determination of VFA was done according to Erwin et al. (1961) using a Perkin Elmer™ Claurus 500 chromatograph, with a FFAP Elite capillary column. Hydrogen was used as carrier gas with a 5.5 mL per minute flow. One µL sample was placed in an injector and detector temperature was 250 ºC per minute until reaching a temperature of 140 ºC, with a run time of 8 minutes (Kung and Hession, 1995). TW (30, 45, and 60 days of cutting interval), DW, and feces were analyzed for DM, organic matter (OM), crude protein (CP) according to AOAC (1990); neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. 1991). Feces and urine samples were analyzed for nitrogen (AOAC, 1990).

Individual DM intake (DMI) was determined as the difference between the diet supplied and rejected daily. *In vivo* digestibility was determined as the difference between the nutrient ingested and excreted and nitrogen balance was estimated as the difference between the ingested nitrogen and nitrogen excreted in feces and urine (Harris, 1970).

2.3. Statistical analysis

Data on digestibility, nitrogen balance, ruminal pH, VFA and NH_3-N were analyzed as repeated measures, completely randomized design (Steel and Torrie, 1997) using the Mixed procedure of SAS (2000). The model for the analysis included main effects of treatment, periods, and treatment*period interaction. Initial weight was considered as covariate, and least squares means were separated using adjust Tukey test. The model was the following:

$$\mathrm{Y}_{ijkl} = \mu + T_i + R_{j(i)} + P_k + (TP)_{ik} + \beta(X_{ijk} - \bar{X}_{i\cdot\cdot}) + E_{ijkl}$$

Where: Y_{ijkl} = response variable, μ= general mean, T_i = effect of the *i*th treatment , $R_{j(i)}$= effect of the jth replicate within the ith treatment, P_k = effect of *k*th period, $(TP)_{ik}$ = treatment*period interaction, β= regression coefficient, X_{ijk} =covariate, and E_{ijk} = random error.

3. Results

3.1. Chemical composition of the forage and experimental diets

The DM, NDF and ADF contents increased but OM and CP decreased as TW cutting interval increased, whereas the DW used in each period showed little variation in chemical composition. Including 20 (T2) and 30% (T3) DW decreased NDF and ADF content but increased CP (Table 1).

3.2. Dry matter intake

As shown in Table 2, DMI was different among treatments ($P < 0.01$) and periods ($P < 0.05$) but it was not affected by the treatment*period interaction ($P > 0.05$). In the three periods, TW dry matter intake was higher for the control (T1) as compared to treatments with 20 or 30% DW diets, with a greater decrease for 30% DW ($P < 0.01$). As the regrowth period increased DMI increased ($P < 0.01$), except at 60 days for 20% DW.

3.3. Nutrient digestibility

In P2 and P3 20 and 30% DW affected DMD and OMD ($P > 0.05$) but not in P1 (Table 2). As regrowth of TW increased DMD decreased ($P < 0.01$) with a higher digestibility in treatments with DW, but there was no effect of the treatment*period interaction. Period and treatment*period interaction did not change ($P > 0.05$) OMD. In the three periods, CPD increased with 20 and 30% DW in the diet ($P < 0.01$) and with the treatment*period interaction ($P < 0.05$); therefore, CPD is improved by DW but it is negatively affected by regrowth of TW. DW did not improve NDFD in P1, but it did so P2 and P3 ($P < 0.05$), whereas ADFD was increased ($P < 0.05$) by DW as compared to the control diet only in P3. Regrowth and treatment*period interaction did not affect ($P > 0.05$) NDFD nor ADFD (Table 2).

3.4. Effect on pH, NH_3-N, VFA, and acetate:propionate ratio

As shown in Table 3, ruminal pH ($P < 0.05$) was changed by 20% DW in P3 and also by regrowth ($P < 0.05$). The inclusion of 20 and 30% DW in the diet improved ($P < 0.01$) NH_3-N concentration in the three periods, decreasing ($P < 0.01$) with the regrowth and showing a higher concentration in diets containing DW. Both pH and NH_3-N concentration were not affected by the treatment*period interaction ($P > 0.05$).

Molar concentrations of acetate, propionate and butyrate showed no differences for diets with 0 or 30% DW, the highest amount of acetate was found with 20% DW, decreasing propionate ($P < 0.05$), and maintaining the level of butyrate, whereas the molar concentration of butyrate was not affected ($P > 0.05$) by the treatments (Table 3). Concentration of acetate increased ($P < 0.01$) due to regrowth, with a decrease in P3, and a similar response was observed for propionate concentration ($P < 0.05$). Treatments did not affect the acetate:propionate ratio ($P > 0.05$) in P1 and P3, and higher values ($P < 0.05$) were observed in P2 with 20% DW. The period did influence the acetate:propinate ratio ($P < 0.01$), but not by the treatment*period interaction($P > 0.05$).

3.5. Nitrogen balance

Diets with 20 and 30% DW increased ($P < 0.01$) nitrogen retention (NR) as compared to the control diet (0% DW), in P1, P2 and P3. A positive nitrogen balance was observed in all treatments and the three periods. The period influenced ($P < 0.01$) NR, decreasing with regrowth mainly in the control diet. The treatment*period interaction was also significant ($P < 0.05$).

4. Discussion

4.1. Nutritional value of the experimental diets and of duckweed

With the regrowth of TW there was an increase in DM, NDF, and ADF in T1, perhaps due to a decrease in the leaf:stem ratio (Minson, 1990), and a subsequent decrease in OM and CP. According to Merchen and Bourquin (1994), plant maturity affects the chemical composition and quality of forages with an increase in fiber and a decrease of total and soluble N. In this sense, CP content of TW in P3 was less than the 7.6 and 9.6 g kg^{-1} DM reported by Juma et al. (2006) and Kariuki et al. (2001), but similar to the 6.84 g kg^{-1} DM observed by Nyambati et al. (2003). The low CP content in the three periods may be attributed to the lack of fertilization of TW in this experiment, taking into account that CP is low in non-fertilized tropical grasses (Wuoters, 1987). The chemical composition of DW remained constant in the three experimental periods and CP content was 28%, lower than the 35.5% reported by Akter et al. (2011), but similar to the 29% found by Anderson *et al*. (2011). In DW, CP content is influenced by factors, such as species, insect presence and adherence of bacteria, ammonia concentration in the water and nutrient sources (Zetina et al., 2010); which would partially explain the variation in the reported values. In diets with DW, the increase in CP can be explained by the high content in the macrophyte, but the decrease in DM could be due to the greater humidity in the aquatic plant. Ash percentage could be the cause of the decrease of OM, while the lower NDF and ADF content in the diets could be explained by low percentages in the macrophyte. In P1, diets containing 20 and 30% DW showed a 2 and 4% NDF decrease, whereas in the control diet it decreased 5 and 7% in P2, and 7 and 10% in P3. ADF decreased in diets with 20 and 30% of DW compared to control diet (8 and 12%, 9 and 14%, 10 and 15%, in P_1, P_2 and P_3, respectively). Mertens (1997) mentions that NDF values below 25% favor problems of acidosis, laminitis, and abomasal displacement in dairy cows. The results of our study are congruent with those observed by Babayemi et al. (2006), who used diets based on *Panicum maximun* and varying DW (*Spirodela polyrhiza*) levels. Besides, the experimental diets except those with only TW, showed above the 8% minimum CP required for optimum ruminal function (Norton, 1994).

4.2. Dry matter intake

Oldman and Alderman (1980) report that *ad libitum* intake frequently increases due to a higher CP level in the diet, which does not coincide with the observations of our study since DMI decreased as CP levels in the diet increased. This could be explained by the fact that DW was fertilized with sheep manure, affecting taste and smell and decreasing intake. On the other hand, the high moisture content in fresh forages is often mentioned as a factor regulating intake (Forbes, 1995). In this sense, Babayemi et al. (2006) report a decrease in DMI when including 40% DW in diets based on *P. maximum*, and attribute it to the moisture content in the diet, caused by DW inclusion. Zetina-Córdoba et al. (2012) showed that the supplementation of DW to sheep did not affect DMI of *P. purpureum* hay, mainly because DW was not given *ad libitum*. This, however, does not coincide with the findings of Kahindi et al. (2007), who observed an increase in DMI in diets with *P. purpureum* supplemented with guamuchil (*Pithecellobium dulce*), which like DW increases CP in the diet; thus taste, moisture, and volume of the diets could have been determining factors in DMI. Another explanation could be the presence of antinutritional factors such as trypsin inhibitors, phytic acid, cyanide, calcium oxalate, and tannins (Bairagi et al., 2002; Kalita et al., 2007); however, no negative effects have been reported on the intake or health caused by these antinutritional factors, from DW feeding in sheep.

4.3. In vivo digestibility

The DMD results obtained in P2 and P3 when substituting 0, 20, and 30% TW for DW are similar to those observed by Babayemi et al. (2006), who reported an increase of 55% in the control diet, up to 61 and 60% when substituting 0, 20, and 40% *P. maximun* for DW, respectively. On the other hand, Juma et al. (2006) point out that including *Gliricidia sepium* and *Mucuna pruriens* in diets based on *P. purpureum* improved DMD by 57.9% in the control to 60.3 and 60.8%. Kahindi et al. (2007) report an increase of 57.45 to 62.29% when including 15% *Pithecellobium* in diets with *P. purpureum*. It is known that a high DMI increases passage rate and reduces digestibility of the feed (Fox et al., 2004). When increasing DMI of the diet with only TW (T1) in the three periods, DMD could have decreased, but the inclusion of DW increased CP in the diet above the 8% minimum required to supply enough ammonia for optimum microbial function (Norton, 1994); therefore, it is probable that N availability for ruminal bacteria increased, improving digestibility at the same time. Huque et al. (1996) and Nguyen (1997) reported a high DMD of DW (67-91%), which might explain the high digestibility of diets with DW. The increase of OMD due to DW as the regrowth of TW increased could be because of the low DMI with regard to the control diet. A longer retention of DM in the rumen has a positive effect on the digestion rate (Tolera and Sundstol, 2000). According to Huque et al. (1996), there is an OMD of 66.9% for DW and the inclusion of this macrophyte in the diet can improve digestibility. The results of our study are consistent with those observed by Kahindi et al. (2007), Aregheore (2006), and Kariuki et al. (2001), who found an increase in OMD when supplementing diets based on *P. purpureum* with guamuchil (*Pithecellobium dulce*), copra cake (*Cocos nucifera*) and potato foliage (*Ipomea batatus*), respectively.

The inclusion of DW in the diet increased CPD and since Huque et al. (1996) found a CPD of 80, 86, and 93% in different DW species and Khan et al. (2002) point out a high CPD of DW, this result would not be a bypass protein source, as suggested by Damry and Nolan (2002). Kozloski et al. (2003) report that NDFD and ADFD of TW hay at 30, 40, 50 and 60 days regrowth, as well as 70 and 90 days regrowth (Kozloski et al., 2005) were not different, which does not coincide with our results that as the regrowth age increased in TW there was a significant decrease in NDFD (62.49, 60.73, 57.27%). However, partial substitution of TW for DW increased NDFD, which could explain DW being responsible of an increased digestibility of NDF and ADF. Another factor that apparently influenced the results was DM retention in the rumen, since DMI decreased in diets with DW. It is worth considering that a greater retention time in the rumen increased digestibility; likewise, the lower NDF and ADF content in the diets with DW could partly explain our results.

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4.4. Ruminal pH and VFA concentration

The values of ruminal pH in all diets were between 6.5 and 7.0, a range considered as optimum for cellulolytic bacteria (Erdman, 1988), digestion of cellulose (Mould and Orskov, 1983), and VFA absorption (Dijkstra *et al*., 1993). Besides, Mouriño et al. (2001)

mention that a pH lower than 6.2 can negatively affect growth and activity of cellulolytic bacteria, given the decrease in bicarbonate availability. The lowest pH value found in this study was 6.5, which is higher to the minimum value that could affect rumen function (Erdman, 1988). The buffer capacity of ruminal liquid is higher with fiber-rich diets, resulting in higher pH (Feng et al., 1993). The results obtained in our experiment coincide with those reported by Muinga et al. (1995), Abdulrazak et al. (1996), and Kariuki et al. (2001) who mentioned that pH levels were not affected by TW supplementation with *Gliricidia sepium, Leucaena leucocephala*, *Desmonium intortum* or *Ipomea batatus,* respectively. VFA are the main energy source for ruminants, contributing to 80% of their daily energy requirements, therefore a change in the proportion of acetate:propionate might affect animal production (Biazon et al., 2012). The molar proportion of acetate decreases when fiber intake decreases (□rskov and McDonald, 1979). Substituting TW for DW in P1 and P3 did not modify the acetate:propionate ratio. In P2, the acetate:propionate proportion was different for T1 and T2. The high ratio of acetate and low ratio of propionate are consistent with the results obtained by Muinga et al. (1995), when supplementing TW with *Leucaena leucocephala*.

4.5. Ammonia nitrogen (NH_3-N) concentration

The increment in NH_3-N concentration in the three periods due to DW could be consequence of the CP content of this plant; according to Huque et al. (1996) and Khan et al. (2002), CP of DW shows high rumen degradability. According to Forbes and France (1993), the amount of NH_3-N in the ruminal liquor is related to protein degradation and a lower digestibility decreases ammonia liberation; thus, microbial protein synthesis is limited. Kariuki et al. (2001) point out that reducing protein in the diet decreases ruminal fermentation, since less NH_3-N is available for microbial synthesis. The minimum for low-quality tropical grasses is 150 mg L^{-1} (Preston and Leng, 1987), or over 200 mg L^{-1} might be necessary with low-quality forages (Dixon, 1987). The NH_3-N values obtained in the diets are within the ranges recommended by Preston and Leng (1987), with the exception of T_1 in P_3, which can be explained by the decrease of CP in the diet. In our study the values in P3 coincide with that reported by Kariuki et al. (2001), who found that NH_3-N concentration improved up to 130-214 mg L^{-1} and 139-235 mg $^{-1}$, when using diets based on TW and 10, 20 or, 30% of *Desmodium intortum* and *Ipomacea batatus* vines.

4.6. Nitrogen balance

Including DW improved NR, which decreased as the age of TW regrowth increased. The results are lower than those obtained with goats by Babayemi et al. (2006), who reported increases in NR in diets based on *Panicum maximum* when including 20 and 40% DW, reaching 83.7% with 20% substitution. In our study there was a variation of 75.8 to 78.9% for 20 and 30% TW and only 59.4 to 65.9% of the control diet; the difference with Babayemi et al. (2006) could be due to greater N losses in feces and urine, as well as to the different ruminal conditions between sheep and goats. However, in complete diets, Eisemann et al. (2005) report that using N from DW is less efficient than soy pastes.

5. Conclusions

The results obtained in this study suggest that the inclusion of 20 and 30% DW in diets based on TW at 30, 45, and 60 days cutting interval decreased DMI, but improved DMD, OMD, CPD, NDFD, ADFD, NR, and NH_3N concentration, showing minimal changes in the acetate:propionate ratio and ruminal pH.

Conflict of interest statement

On behalf of all coauthors, I wish to confirm that there is no conflict of interest.

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References

Abdulrazak, S.A., Muinga, R.W., Thorpe, W., Ørskov, E.R., 1996. The effects of supplementation with *Gliricidia sepium* or *Leucaena leucocephala* forage on intake, digestion and live-weight gains of *Bos Taurus* x *Bos Indicus* steers offered napier grass. Anim. Sci. 63,381-388.

Akter, M., Chowdhury, S.D., Akter, Y., Khatun, M.A., 2011. Effect of duckweed (*Lemna minor*) meal in the diet of laying hen and their performance. Bangladesh Res. Pub. J. 5(3), 252-261.

Anderson, K.E., Loeman, Z., Stomp, A.M., Chang, J., 2011. Duckweed as a feed ingredient in laying hen diets and its effect on egg production and composition. Int. J. Poult. Sci. 10(1), 4-7.

AOAC, 1990. Official methods of analysis. In: Horwitz Willian (Ed.), Association of Official Analytical Chemists, 15th ed. Arlintog.

Araya, M.M, Boschini, C.F., 2005. Producción de forraje y calidad nutricional de variedades de P*ennisetum purpureum* en la Meseta Central de Costa Rica. Agronomía Mesoamericana. 16(1), 37-43.

Aregheore, E.M., 2006. Utilization of concentrate supplements containing varying levels of copra cake (*Cocos nucifera*) by growing goats fed a basal diet of *Pennisetum purpureum*. Small Rumin. Res. 64, 87-93.

Babayemi, O.J., Basmikole, M.A., Omojola, A.B., 2006. Evaluation of the nutritive value and free choice intake of two aquatic weeds (*Nephrolepis biserrata* y *Spirodela polyrrhiza*) by West African dwarf goats. Trop. Subtrop. Agroecosyst. 6, 15-21.

Bairagi, A, Ghosh, K.S., Sen, S.K. Ray, A.K., 2002. Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita* Ham.) fingerlings after fermentation with a fish intestinal bacterium. Biores. Technol. 85, 17-24.

Biazon, F.A., Frota, M.J.M., Ferraz, F.C.L., Silva, A.M., Morenz, D.A., Moscat de Faria, B., da Silva C.L., Moreira C.E.F., 2012. Bakery waste in sheep diets: intake, digestibility, nitrogen balance and ruminal parameters[1]. R. Bras. Zootec. 41(1), 147-153.

Cheng, J.J., Stomp, M.A., 2009. Growing duckweed to recover nutrients from wastewater and for production of fuel ethanol and animal feed. Clean. 37(1), 17-26.

Da Silva, M.J., Bonnecarrére, S.L., Kozloski, G.V., Dorneles, L., Mauricio, T.L., Vizzotto, R.M., Cadorin, J.R., 2007. Dwarf elephant grass hay (*Pennisetum purpureum* Schum. cv Mott) digestion by sheep at different levels of intake. Ciência Rural. 37(2), 482-487.

Damry J., Nolan, J.V., 2002. Degradation of duckweed protein in the rumen of sheep. Anim. Produc. Aust. 24, 45-48.

Damry J., Nolan, J.V., 2009. Nitrogen dynamics in the rumen and abomasun of sheep determined with 15N-labelled ammonia or 15Nlabelled duckweed. J. Indon. Trop. Agric. 31, 1-7.

de Almeida, T.F., Rubens, L.F., Roubach, R., Jungles, M.K., Machado, F.D., Moreira, M.A., 2010. Use of domestic effluent through duckweeds and red tilapia farming in integrated system. PANAMJAS, 5(1), 1-10.

Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M., Tamminga, S., 1993. Absorption of volatile acids from the rumen of lactating dairy cows as influenced by volatile acid concentration, pH and rumen liquid volume. Brit. J. Nutr. 69, 385-396.

Dixon, R.M., 1987. Maximising the rate of fibre digestion in the rumen. In Dixon R.M. (Ed.). Ruminant feeding systems utilising fibrous agriculture residues. IDP., Camberra, Australia. p. 46-49.

Eissemann J., Huntington, G., Poore, M., 2005. Digestibility and N flux in steers feed diets with differing sources of supplemental protein. J. Dairy Sci. 88(Sup.1), 320-321.

Erdman, R.A., 1988. Dietary buffering requirements of the lactating cow: a review. J. Dairy Sci. 71, 3246-3266.

Erwin, E.S., Marco, G.J., Emery, E.M., 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44, 1768-1776.

Feng, P., Hoover, W.H., Miller, T.K., Blauwie, R., 1993. Interaction of fibre and nonstructural carbohydrates on lactation and ruminal function. J. Dairy Sci. 76, 1324-1333.

Forbes, J.M., 1995. Ruminant gastrointestinal tract. In: Forbes, J.M. (Ed.), Voluntary Food Intake and Diet Selection in Farm Animals. CAB International, Wallingford, p. 59-80.

Forbes, J.M., France, J., 1993. Quantitative aspects of ruminal digestion and metabolism. CAB International. Oxon, UK, p. 123-130.

Fox, D.G., Tedeschi, L.O., Tylutki, T.P., Russell, J.B., Van Amburgh, M.E., Overton, T.R., 2004. The Cornell net carbohydrate and protein system model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112, 29–78.

Gutiérrez, K., Sanginés, L., Pérez, G.P., Martínez, L, 2001. Estudios de la planta acuática Lemna gibba en la alimentación de cerdos. Rev. Cub. Cienc. Agric. 35(4), 367-372.

Harris, L. E., 1970. Nutrition research techniques for domestic and wild animals, Vol. I. Logan, UTAH, USA.

Huque, K.S., Chowdhury, S.A., Kibria, S.S., 1996. Study on the potentiality of duckweeds as a feed for cattle. Asian-Aust. J. Anim. Sci. 9(2), 133-134.

Juma, H.K., Abdulrazak, S.A., Muinga, R.W., Ambula, M.K., 2006. Evaluation of Clitoria, Gliricidia and Mucuna as nitrogen supplements to Napier grass basal diet in relation to the performance of lactating Jersey cows. Livest. Sci.103, 23-29.

Kahindi, R.K., Abdulrazak, S.A., Muinga, R.W., 2007. Effect of supplementing Napier grass (*Pennisetum purpureum*) with Madras thorn (*Pithecellobium dulce*) on intake, digestibility and live weight gains of growing goats. Small Rumin. Res. 69, 83-87.

Kalita, P., Mukhopadhyay., P.K. Mukherjee, A.K., 2007. Evaluation of the nutritional quality of four unexplored aquatic weeds from northeast India for formulation of cost-effective fish feeds. Food Chem. 103, 204-209.

Kariuki, J.N., Tamminga, S., Gachuiri, C.K., Gitau, G.K., Muia, J.M.K., 2001. Intake and rumen degradation in cattle fed napier grass (*Pennisetum purpureum*), supplemented with various levels of *Desmodium intortum* and *Ipomoea batatus* vines. South African J. Anim. Sci. 31(3), 149-157.

Khan, M. J., Steingass, H., Drochner, W., 2002. Evaluation of some aquatic plants from Bangladesh through mineral composition, *in vitro* gas production and *in situ* degradation measurements. Asian-Aust. J. Anim. Sci. 15(4), 537-542.

Kozloski, G.V., Perottoni, J., Ciocca, M.L.S., Rocha, J.B.T., Raiser, A.G., Sanchez, L., 2003. Potential nutritional assessment of dwarf elephant grass (*Pennisetum purpureum* Schum. cv. Mott) by chemical composition, digestion and net portal flux of oxygen in cattle. Anim. Feed Sci. Technol. 104, 29-40.

Kozloski, G.V., Perottoni, J., Sanchez, L., 2005. Influence of regrowth age on the nutritive value of dwarf elephant grass hay (*Pennisetum purpureum* Schum. cv. Mott) consumed by lambs. Anim. Feed Sci. Technol. 119, 1-11.

Kung, L., O. Hession., 1995. Preventing *in vitro* lactate accumulation in ruminal fermentation by innoculation with *Megasphaera elsdenii*. J. Animal Sci. 73, 250-256.

McCullough, H., 1967. Determination of ammonia in whole blood by a direct colorimetric method, Clin. Chim. Acta. 17, 297-301.

Merchen, N.R., Bourquin, L.D., 1994. Processes of digestion and factors influencing digestion of forage-based diets by ruminants. In: Fahey Jr., G.C. (Ed.), Forage Quality, Evaluation and Utilization. ASA, CSSA, SSSA, Madison, WI, USA, p. 564-612.

Mertens, D.R., 1997. Creating a system for meeting the fiber requeriments of dairy cows. J. Dairy Sci. 80, 1463-1481.

Minson, D.J., 1990. Forages in Ruminant Nutrition. Academic Press, London. p. 483.

Mould, F.L., Ørskov, E.R., 1983. Manipulation of rumen fluid pH and its influence on cellulolysis, *in sacco* dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Anim. Feed Sci. Technol. 10, 1-14.

Mouriño, F.L., Akkarawongsa, R.A., Weiner, P.J., 2001. Initial pH as a determinant of cellulose digestion rate by mixed ruminal microorganisms *in vitro*. J. Dairy Sci. 84, 848-859.

Muinga, R.W., Thorpe, W., Topps, J.H. 1995., The effects of supplementation of *Leucaena leucocephala* and maize brain on voluntary intake, digestibility, live-weight change and milk yield of *Bos Indicus x Bos Taurus dairy* cow and rumen fermentation in steer offered *Pennisetum purpureum ad libitum* in the semi-humid tropics. Anim. Sci. 60, 13-23.

Nelson, C.J., Moser, L.E., 1994. Plant factors affecting forage quality. In: Fahey Jr., G.C. (Ed.), Forage Quality, Evaluation and Utilization. ASA, CSSA, SSSA, Madison, WI, USA, p. 115–154.

Nguyen, V.T., 1997. A study of feed degradability and rumen environment of swamp buffaloes. Livest. Res. Rural Develop. 9(3), 1- 4.

Norton, B.W., 1994. Antinutritive and toxic factors in forage tree legumes. In: Tropical Tree Legumes in Tropical Agriculture. CAB International, Wallingford, UK, p. 202–215.

Nyambati E.M., Sollemberger, L.E., Kunkle, W.E., 2003. Feed intake and lactation performance of dairy cows offered napiergrass supplemented with legume hay. Livest. Prod. Sci. 83, 179-189.

Oldham, J.D., Alderman, G., 1980. Recent advances in understanding protein-energy interrelationships in intermediary metabolism in ruminants. In: Protein and energy supply of high producing milk and meat. Pergamon Press. Oxford. p. 728.

Ørskov, E.R., McDonald, I., 1979. Utilización de volatile fatty acids for maintenance and for energy retention. In Energy metabolism. Ed. by L.E. Mount Butterworths, London. p. 147-150.

Preston T.R., Leng, R.A., 1987. Matching ruminant production system with available resources in the tropic and sub-tropics. Penambul Books, Armidale p. 245.

SAS, 2000. Language Guide for Personal Computer, Version 8.1. SAS Institute Inc. Cary. NC, USA.

Steel, R.G.D., Torrie, J.H., Dickey, D.A. 1997. Principles and procedures of statistics. McGraw Hill, Inc. p 666.

Tolera, A., Sundstol, I.F., 2000. Supplementation of graded levels of *Desmodium intortum* hay to sheep feeding on maize stover harvested at three stages of maturity. 2. Rumen fermentation and nitrogen metabolism. Anim. Feed Sci. Technol. 85, 215-229.

Valadares, R.F.D., Goncalves. L.C., Rodríguez, N.M., Barbosa, S.I., de Campos, V.S.F. 1997. Metodología de coleta de urina em vacas utilizando sondas de Folley. R. Bras. Zootec. 26(6), 1279-1282.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and nonstach polysacharides in relation to animal nutrition. Symposium: carbohydrate methodology, metabolism and nutritional implications in dairy cattle. J. Dairy Sci. 74, 3583-3597.

Witkowska, Z., Saeid, A., Chojnacka, K., Dobrzanski., Gorecki, H., Michalak, I., Korczynski, M., Opalinski, S., 2012. New biological dietary feed supplement for laying hens with microelements based on duckweed (*Lemna minor*). Am. J. Agric. Biol. Sci. 7(4), 482-493.

Wouters, A.P., 1987. Dry Matter Yield and Quality of Napier Grass on Farm Level (1983–1986) Research Report. Ministry of Livestock Development, Nairobi, Kenya.

Zetina, C.P., Ortega, C.M.E., Ortega, J.E., Reta, M.J.L., Sánchez-Torres, M.T.E., Haro, H.J.G., 2010. Utilización de la lenteja de agua (Lemnaceae) en la producción de tilapia (*Oreochromis* spp). Arch. Zootec. 59, 133-155.

Zetina-Córdoba, P., Ortega-Cerrilla, M.E., Sánchez Torres-Esqueda, M.T., Herrera-Haro, J.G., Ortega-Jiménez, E., Reta-Mendiola, J.L., Vilaboa-Arroniz, J., 2012. Reproductive Response of Ewes Fed with Taiwan Grass Hay (*Pennisetum purpureum* Schum.) Supplemented with Duckweed (*Lemna* sp. and *Spirodela* sp.). Asian-Aust. J. Anim. Sci. 25(8), 1117-1123.

Table 1. Chemical composition of diets fed to sheep with Taiwan grass with or without duckweed

	PERIOD 1 (PI)					PERIOD 2 (P2)					PERIOD 3 (P3)				
	30 days^{1}					45 days^{1}					60 days^{1}				
	T_1	T_2	T_3	SEM	DW	T_1	T_2	T_3	SEM	DW	T_1	T_2	T_3	SEM	DW
DM (%)	15.50^{a}	15.10^{ab}	14.90^{c}	0.14	13.50	17.00^{a}	16.40^{ab}	16.10^{c}	0.15	12.95	21.50^{a}	20.00^{b}	19.25^{c}	0.17	13.44
Analysis on DM basis															
OM (%)	92.01^{a}	90.08^{a}	89.12^{a}	1.30	82.40	90.10^{a}	88.56^{a}	87.79^{a}	1.21	84.94	89.10^{a}	87.76^{a}	87.09^{a}	1.05	82.59
CP (%)	7.50^{c}	11.68^{b}	13.77^{a}	0.21	28.40	6.50^{c}	10.56^{b}	12.59^{a}	0.25	28.03	6.20^{c}	10.06^{b}	11.99^{a}	0.16	28.25
NDF (%)	54.10^{a}	52.78^{b}	52.12^{c}	0.12	47.50	61.33^{a}	58.56^{b}	57.18^{c}	0.19	48.15	71.77^{a}	66.92^{b}	64.49^{c}	0.19	47.78
ADF (%)	35.30^{a}	32.54^{a}	31.16^{a}	0.19	21.50	40.70^{a}	36.86^{b}	34.94^{c}	0.17	22.20	45.94^{a}	41.25^{b}	38.91^{c}	0.17	22.15
ASH (%)	8.00^{c}	9.92^{b}	13.28^{a}	0.16	17.60	9.90^{c}	11.44^{b}	12.21^{a}	0.17	17.50	10.90^{b}	12.14^{a}	12.91^{a}	0.19	16.55

DM: Dry matter. OM: Organic matter. CP: crude protein. NDF: neutral detergent fiber. ADF: acid detergent fiber. ^{1}Cut ages of Taiwan grass. T_1: 100% Taiwan grass, T_2: 80% Taiwan grass + 20% duckweed; T_3: 70% Taiwan grass + 30% duckweed. ^{a,b,c} Means that within rows, values with different letters significantly ($P < 0.05$).

Table 2. Dry matter intake (DMI) and *in vivo* digestibility in sheep fed Taiwan grass with or without duckweed.

	PERIOD 1 (P1)				PERIOD 2 (P2)				PERIOD 3 (P3)						
	30 days[1]				45 days[1]				60 days[1]				Significance		
	T_1	T_2	T_3	SEM	T_1	T_2	T_3	SEM	T_1	T_2	T_3	SEM	treat	per	treat*per
DMI															
(g d^{-1})	769.3^{a}	562.5^{b}	462.2^{c}	*15.6*	794.4^{a}	650.0^{b}	603.5^{b}	*28.3*	866.4^{a}	662.1^{b}	570.5^{c}	*23.1*	0.0001	0.0001	0.0539
(g kg^{-1} $BW^{0.75}$)	65.7^{a}	51.8^{b}	41.7^{c}	*1.2*	65.2^{a}	54.6^{b}	54.6^{b}	*1.4*	68.8^{a}	55.1^{b}	45.9^{c}	*1.2*	0.0001	0.0360	0.0639
DMD (%)	60.26^{a}	63.43^{a}	61.95^{a}	*1.68*	58.77^{b}	62.48^{a}	61.40^{a}	*0.57*	54.59^{b}	60.64^{a}	61.19^{a}	*1.30*	0.0191	0.0011	0.0968
OMD (%)	63.25^{a}	65.27^{a}	64.02^{a}	*1.67*	61.82^{b}	65.15^{a}	64.20^{a}	*0.55*	60.75^{b}	64.33^{a}	64.15^{a}	*0.66*	0.0398	0.4578	0.8006
CPD (%)	82.57^{b}	85.65^{a}	85.40^{a}	*0.73*	74.17^{b}	80.90^{a}	82.60^{a}	*0.51*	70.42^{b}	81.78^{a}	81.50^{a}	*0.71*	0.0001	0.0001	0.0001
NDFD (%)	62.49^{a}	68.54^{a}	68.20^{a}	*1.69*	60.73^{b}	65.85^{a}	64.73^{a}	*0.90*	57.27^{b}	66.10^{a}	65.10^{a}	*0.81*	0.0013	0.0070	0.2567
ADFD (%)	58.51^{a}	62.77^{a}	60.00^{a}	*2.54*	58.26^{a}	62.58^{a}	60.15^{a}	*1.24*	53.92^{b}	61.10^{a}	60.53^{a}	*1.39*	0.0299	0.1907	0.3957

NS (not significant), $P > 0.05$; *$P < 0.05$; **$P < 0.01$. ^{a,b} Different letters in the same row are significant different. [1] Cut ages of Taiwan grass. T_1: 100% Taiwan grass, T_2: 80% Taiwan grass + 20% duckweed; T_3: 70% Taiwan grass + 30% duckweed. DMD: dry matter digestibility. OMD: organic matter digestibility. CPD: crude protein digestibility. NDFD: neutral detergent fiber digestibility. ADFD: acid detergent fiber digestibility. SEM: Standard error of mean

Table 3. Rumen pH, NH_3-N, VFA proportions and acetate:propionate ratio in Pelibuey sheep fed Taiwan grass with or without duckweed.

	PERIOD 1 (P1)				PERIOD 2 (P2)				PERIOD 3 (P3)						
	30 days[1]				45 days[1]				60 days[1]				Significance		
	T_1	T_2	T_3	SEM	T_1	T_2	T_3	SEM	T_1	T_2	T_3	SEM	treat	per	treat*per
Rumen pH	6.5^a	6.6^a	6.5^a	*0.05*	6.5^a	6.6^a	6.5^a	*0.09*	6.7^{ab}	6.8^a	6.5^b	*0.05*	0.0806	0.0358	0.3993
NH_3-N (mg dL^{-1})	18.22^b	25.76^a	26.22^a	*1.64*	17.76^b	23.95^a	23.38^a	*1.44*	14.02^b	18.99^a	19.01^a	*0.65*	0.0005	0.0001	0.6936
VFA (mM L^{-1})	76.13^a	95.25^a	77.65^a	*5.57*	60.92^a	61.31^a	56.99^a	*8.27*	56.21^a	60.93^a	68.47^a	*4.95*	0.4910	0.0001	0.1198
VFA (% molar)															

Acetate (A)	61.29^{a}	60.79^{a}	62.61^{a}	*1.32*	67.99^{b}	70.92^{a}	68.26^{ab}	*0.72*	70.00^{a}	69.52^{a}	68.21^{a}	*0.96*	0.7679	0.0001	0.0841
Propionate (P)	28.76^{a}	29.48^{a}	27.53^{a}	*1.12*	25.39^{a}	22.14^{b}	23.46^{ab}	*0.52*	22.55^{a}	22.76^{a}	23.04^{a}	*0.78*	0.4683	0.0001	0.1258
Butyrate	6.64^{a}	8.28^{a}	7.31^{a}	*0.38*	6.62^{a}	6.94^{a}	8.28^{a}	*0.55*	7.45^{a}	7.72^{a}	8.76^{a}	*0.46*	0.1135	0.0118	0.4862
A: P Ratio	2.13^{a}	2.06^{a}	2.27^{a}	*0.12*	2.68^{b}	3.20^{a}	2.91^{ab}	*0.08*	3.10^{a}	3.05^{a}	2.96^{a}	0.15	0.6020	0.0001	0.0514

NS (not significant), $P>0.05$; $*P<0.05$; $**P<0.01$. [a,b,c] Different letters in the same row are significant different. [1] Cut ages of Taiwan grass. T_1: 100% Taiwan grass, T_2: 80% Taiwan grass + 20% of duckweed; T_3: 70% Taiwan grass + 30% of duckweed. SEM: Standard error of mean.

Table 4. Nitrogen balance of Pelibuey sheep fed Taiwan grass with or without duckweed

	PERIOD 1 (P1) 30 days^{1}				PERIOD 2 (P2) 45 days^{1}				PERIOD 3 (P3) 60 days^{1}				Significancce		
	T_1	T_2	T_3	SEM	T_1	T_2	T_3	SEM	T_1	T_2	T_3	SEM	trat	per	trat*per
Feed N, (g d^{-1})	9.23^{b}	10.51^{a}	10.18^{ab}	*0.29*	8.26^{b}	9.50^{b}	12.16^{a}	*0.44*	8.59^{b}	10.66^{a}	10.94^{a}	*0.32*	0.0015	0.8460	0.0001
Faecal N, (g d^{-1})	2.11^{a}	1.75^{ab}	1.66^{b}	*0.11*	2.46^{a}	1.95^{b}	2.24^{ab}	*0.09*	2.71^{a}	1.99^{b}	2.07^{b}	*0.08*	0.0014	0.0001	0.0277
Absorbed N, (g d^{-1})	7.12^{b}	8.76^{a}	8.52^{c}	*0.24*	5.80^{c}	7.55^{b}	9.92^{a}	*0.37*	5.89^{b}	8.66^{a}	8.87^{a}	*0.29*	0.0001	0.0256	0.0001
Absorbed N, (% feed N)	77.18^{b}	83.39^{a}	83.69^{a}	*0.95*	70.20^{b}	79.48^{a}	81.61^{a}	*0.60*	68.53^{b}	81.28^{a}	81.07^{a}	*0.77*	0.0001	0.0001	0.0001
Urine N, (g d^{-1})	1.04^{a}	0.47^{b}	0.61^{b}	*0.10*	0.58^{a}	0.34^{a}	0.44^{a}	*0.14*	0.79^{a}	0.58^{a}	0.84^{a}	*0.11*	0.0175	0.0110	0.2693
Total N loss, (g d^{-1})	3.15^{a}	2.22^{b}	2.27^{b}	*0.15*	3.04^{a}	2.29^{b}	$2,68^{b}$	*0.14*	3.49^{a}	2.58^{b}	2.91^{b}	*0.12*	0.0001	0.0016	0.4629
N retained, (g d^{-1})	6.08^{b}	8.29^{a}	7.92^{a}	*0.24*	5.22^{c}	7.21^{b}	9.48^{a}	*0.39*	5.10^{b}	8.08^{a}	8.03^{a}	*0.29*	0.0001	0.1165	0.0002
Retained N, (%	65.9^{b}	78.9^{a}	77.7^{a}	*1.4*	63.2^{b}	75.9^{a}	78.0^{a}	*1.7*	59.4^{c}	75.8^{a}	73.4^{b}	*1.2*	0.0001	0.0013	**0.0125**

feed N)

NS (not significant), $P>0.05$; *$P<0.05$; **$P<0.01$. [a,b,c]Different letters in the same row are significant different. [1] Cut ages of Taiwan grass.

T_1: 100% Taiwan grass, T_2: 80% Taiwan grass + 20% duckweed; T_3: 70% Taiwan grass + 30% duckweed.

SEM: Standard error of mean.