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# Potential impacts of dietary *Lemna gibba* supplements in a simulated ruminal fermentation system and environmental biogas production

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ABSTRACT

Enteric methane production from ruminants contributes to current global warming challenges faced by mankind. Supplements that improve nutritive value of diets are potential mitigating strategies that may reduce enteric methane emissions. This study was, therefore, designed to evaluate the potential of duckweed (*Lemna gibba*) supplement to reduce enteric methane emissions using an *in vitro* ruminal gas production technique. In the first of two experiments, *Lemna gibba* from two water bodies (LG1 and LG2), lucerne and ryegrass samples were analyzed for chemical composition and *in vitro* ruminal fermentation parameters. In the second experiment, the two *Lemna gibba* samples were each included in a basal diet at 5, 10, 15, 20 and 25% to create ten dietary treatments. The dietary treatments were also analyzed for chemical composition and *in vitro* ruminal fermentation characteristics as in the first experiment. *Lemna gibba* and lucerne fermentation resulted in similar propionate levels. The inclusion of 15% *L. gibba* had no effect on the ruminal fermentation patterns (volatile fatty acids, acetate:propionate ratio, acetate, propionate and butyrate) and the dry matter and organic matter degradability. These results indicate that *L. gibba* could be used in ruminant diets as an alternative to grains or concentrates with the added advantage of possibly reducing ruminal methane emissions. Dietary supplementation with *L. gibba* in ruminant diets could be an environmentally friendly strategy to reduce feed costs and ensure sustainable production.



## 1. Introduction

Emissions of gases from livestock sector contribute to global environmental greenhouse gases. Livestock contribute 18% of global anthropogenic greenhouse gas emissions (FAO, 2006). When broken down to individual gases, livestock contribute 1.35%, 15%, and 19% of total CO2, $N_2O$, and $CH_4$ anthropogenic emissions, respectively (Knapp et al., 2014). Ruminants reared on tropical pastures contribute the most enteric methane emissions due to the lower quality of feed and the longer time it takes for these animals to reach slaughter weight. Tropical pastures tend to be low in crude protein (CP) and high in fiber content; two characteristics that not only negatively affect the dry matter (DM) intake and digestibility, but also contribute to the production of methane in rumen (Elghandour et al., 2017a, 2017b). Consequently, in developed countries, the contribution of ruminant production to reenhouse gases emissions is lower than the contribution in poor countries or some regions where the quality forages is low and intensive grain

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production is not possible due to adverse climatic conditions.

During fibre fermentation in rumen, relatively large quantities of acetate are produced at the expense of propionate. This fermentation pathway releases $H^+$ ions that are used during mathanogenesis. The utilisation of $H^+$ ions in this manner plays an important role in maintaining carbohydrate degradation, rate of microbial growth and microbial protein synthesis (Knapp et al., 2014; McGinn et al., 2004). Strategies to mitigate enteric methane production include increasing the quality of ruminant diets through the use of grains or concentrates (Elghandour et al., 2017a; Beauchemin et al., 2008). However, the use of grains and concentrates as animal feed is not only expensive but slows down efforts to eradicate food and nutrition security in resource-poor countries. Most grains used in animal diets can also be directly used by humans as food thus creating competition between man and animals, raising demand for grains on the world market and driving prices beyond the reach of the common man. Another drawback of relying on grains for animal feed is that the agricultural processes to produce large volumes of grain such as deforestation, land preparation, and fertilizer application contribute high volumes of greenhouse gases emissions (Beauchemin et al., 2008; Knapp et al., 2014).

A possible strategy to reduce enteric methane emissions is by enhancing the nutritive value of basal diets through the use of readily available, low-cost, non-conventional and nutrient-rich supplements (Hernández et al., 2017a,b; Elghandour et al., 2017c). Such supplements have the potential to improve ruminal digestion, gross energy intake, milk production and composition, daily weight gain, and feed conversion efficiency (Khan et al., 2015). Improved feed utilization results in lower levels of enteric methane emissions and thus reduce the contribution of ruminants to global greenhouse gases emissions. A potential non-conventional, readily available feed resource that can be used as a supplement to enhance the nutritive value of ruminant diets is duckweed (*Lemna gibba*). Duckweed is an aquatic plant from the *Lemnaceae* family whose DM production varies from 10 to 30 tonne/hectare/year (Leng et al., 1995). It is high in crude protein (CP; 15–45%) (Zetina-Córdoba et al., 2012) and low in fiber and lignin content (Chojnacka, 2006). Thus duckweed has potential as supplement that could enhance the quality for ruminant feed thus reducing methane gas production and increasing propionate and butyrate production during ruminal fermentation (Sofyan et al., 2017; Pino and Heinrichs, 2016; Kamel et al., 2018). The successful use of this feed resource would be a step towards profitable and environmentally friendly ruminant animal production.

This study was, therefore, designed to evaluate the effect of including graded levels of *L. gibba* in a ruminant diet on *in vitro* ruminal fermentation parameters. We tested the hypothesis that inclusion of *L. gibba* in ruminant diets would improve the nutritive value (measured in a simulated rumen environment) of ruminant diets and thus reduce enteric methane production.

## 2. Materials and methods

### 2.1. Source of substrates

Samples of stalks and leaves of Lucerne and ryegrass were mechanically (rotary harvester: John Deere® 397, E03970X675701) collected from five different sites randomly chosen in June 2015.

*Lemna gibba* samples were collected from two water bodies (LG1 and LG2) in Aguascalientes, México (longitude 102° 5′ 57.84" W, latitude 21° 49′ 7.17" N), where climatic conditions are semi-warm semi-arid (BS1hw) and warm semi-arid (BS1k"w) according Köppen climate classification, with an annual median temperature of 17.4 °C and average annual rainfall of 526 mm.

Once samples were collected, they were separately dried in a forced air oven at 65 °C until constant weight (DM), and ground in a Thomas-Wiley Mill 4, with 1 mm sieve (Laboratorio Mill, Thomas-Wiley®).

### 2.2. Treatments

In the first of two experiments, four substrates (LG1, LG2, lucerne and ryegrass) were fermented in an *in vitro* ruminal fermentation system (described below) with five runs per substrate. In the second experiment, 10 substrates (plus a basal control) were formulated by including each of LG1 and LG2 in a basal diet at 5, 10, 15, 20 and 25%. A 0% level of inclusion was included as the control. The eleven substrates [5 levels of inclusion of LG1 + 5 levels of inclusion of LG2 + the control (without *L. gibba*)] were also fermented in an *in vitro* ruminal fermentation system as described below with three runs.

### 2.3. Chemical analysis and net energy of lactation

All substrates were analyzed for DM, organic matter (OM), CP, ether extract, ash (AOAC, 2002, 1999), NDF, acid detergent fiber (ADF) (Van Soest et al., 1991), and non-fibrous carbohydrates (NFC).

### 2.4. In vitro ruminal fermentation

#### 2.4.1. Rumen inoculum preparation

The rumen liquid was extracted in the morning from two cannulated Dorper sheep (60 ± 5 kg body weight) feeding on grass-based diets with 760 g/kg DM of barley straw (*Hordeum vulgare*) as forage and 240 g/kg DM of a mixture of grains (primarily corn and soy) as concentrate. Sheep were adapted to the diet for 20 d before ruminal fluid extraction. The chemical composition of the diet was: 1) barley straw: 580 g/kg DM of NDF, 100 g/kg DM of CP, and 441 g/kg DM of crude fiber; and 2) concentrate: 224 g/kg DM of NDF, 160 g/kg DM of CP, and 54 g/kg DM of crude fiber.

The rumen liquid was filtered through an eight-layered gauze and mixed with a reduced mineral solution in a 1:9 dilution (v/v). The reduced mineral solution contained solution I [$K_2HPO_4$ (6 g/L of water]; solution II [$KH_2PO_4$ (6 g), $(NH_4)_2SO_4$ (6 g), NaCl (12 g), $MgSO_4$ (2.45 g) and $CaCl_2.H_2O$ (1.6 g/L of water)]; 8% sodium carbonate solution [$Na_2CO_3$ (8 g/100 mL of water)]; and reduction solution [L-cysteine (2.5 g), NaOH 2N (15 mL/L), $Na_2S$ (2.5 g) and 0.01% resazurin (1 drop/100 mL of water)] (Cobos and Yokoyama, 1995). Once the rumen inoculum was prepared, it was placed in a 39 °C water bath with continuous purging with $CO_2$ to maintain anaerobic conditions.

#### 2.4.2. Rumen fermentation

The simulated rumen fermentation was conducted using the gas production technique (Menke and Steingass, 1988; Theodorou et al., 1994). Ground (1 mm maximum particle size) 500 mg substrates were placed in 125 mL amber glass bottles to which 90 mL of rumen inoculum was added while ensuring a continuous flow of $CO_2$ in order to maintain anaerobic conditions. Three flasks containing rumen inoculum only were included as blanks. The glass bottles were hermetically sealed and placed in a water bath at 39 °C. The pressure measurements were taken at 0, 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60 and 72 h of incubation using a pressure gauge (0–1 kg $cm^2$; Metron® 63100, Texcoco, México). The gas pressure measurements were transformed into gas volume using the linear regression equation:

$$V = \frac{P + 0.0495}{0.0185}$$

Where: V is the gas volume (mL/g DM); and P, represents gas pressure (kg/cm$^2$).

The fermentation kinetics were obtained from fitting cumulative gas volume data to the logistic model of Menke and Steingass (1988):

$$Vo = \frac{Vmax}{1 + e^{2-4S(t-Lag)}}$$

Where: Vo, is the gas produced during fermentation; Vmax, is the maximum gas volume (mL/g DM); Lag, is the lag phase (h); and S, is the fermentation rate (mL/g DM/h).

Net energy of lactation was determined through the equation: NE = 0.101 gas production +0.051 CP+ 0.112 ether extract (Menke and Steingass, 1988).

Fermentable fractions were determined by first calculating the fractional gas volume (GP) at time intervals from 0 to 8 h (GP at 0–8 h), 8–24 h (GP at 8–24 h), and 24–72 h (GP at 24–72 h) after initiating the *in vitro* incubation. After this, equations were applied to calculate the rapidly fermentable fractions (RF; g/kg) as: GP (0–8 h)/0.4266; medium fermentation (MF; g/kg) as GP (8–24 h)/0.6152; low fermentation (LF; g/kg) as: GP (24–72 h)/0.3453; and total fermentation (Ftot; g/kg) as RF + MF + LF (Miranda et al., 2015).

At the end of the incubation period, the residues were filtered and dried at 65 °C for 48 h in order to calculate *in vitro* ruminal degradability of DM (IVDMD). Dry matter residues were incinerated at 540 °C for 4 h in order to determine the ash content and to calculate *in vitro* ruminal degradability of the OM (IVOMD).

#### 2.4.3. Volatile fatty acids and ammonia nitrogen

At 24 h post-inoculation, 2 mL of the liquid phase was taken from the fermentation bottles and mixed with 0.5 mL of metaphosphoric acid to 25%. The mixture was then centrifuged at 2500 × g for 20 min. The supernatants were used to determine the total volatile fatty acids (VFA), the proportions of acetate, propionate and butyrate (Erwin et al., 1961) and N-$NH_3$ (McCullough, 1967) concentration.

### 2.5. Statistical analysis

Statistical analysis was performed using the SAS package (Statistical Analysis System, version 9.2).

The dependent variables were statistically analyzed using a one-way analysis of variance in a completely randomized design using the general linear models procedures (Proc GLM) of SAS package (SAS, 2002) according to the following linear model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y = the dependent variables (L, S, Vmax, RF, MF, LF, Ftot, DMD, OMD, VFA, acetate, propionate, butyrate, A:P ratio, and N-$NH_3$); $\mu$ = overall mean; $T_i$ = i-th treatment effect; $\varepsilon_{ij}$ = random error. Means were compared using the Tukey test ($P < 0.05$).

Correlation between variables was obtained using correlation procedure (Proc Corr) of SAS package.

## 3. Results

### 3.1. Chemical composition

Table 1 presents the chemical composition of lucerne, ryegrass, LG1 and LG2. The OM, ether extract, and NFC were statistically similar in the four samples. Ryegrass had lower CP and more NDF and ADF than lucerne and *L. gibba*. However, both *L. gibba* samples had more ash content than lucerne and ryegrass.

### 3.2. Fermentation parameters of L. gibba, lucerne and rygrass substrates

Lower Vmax values were observed for both *L. gibba* substrates compared to lucerne and ryegrass substrates (Table 2). However, LG1 and LG2 had higher rate of fermentation (S) than ryegrass but these values were lower than those in lucerne. The lag phases of *L. gibba* LG1 and LG2 were shorter than for lucerne and ryegrass. Lucerne, LG1 and LG2 substrates had higher RF and lower LF than ryegrass, but the IVDMD of ryegrass was higher than that of LG1, LG2 and lucerne. The IVOMD of both *L. gibba* substrates was lower than for lucerne and ryegrass.

Table 3 shows that LG1, LG2 and lucerne promoted similar $NH_3$-N concentration in fermentation media, which were higher than in ryegrass. Ryegrass had the lowest A:P ratio while the ratio was similar in lucerne, LG1 and LG2 substrates. Butyrate production was higher in both *L. gibba* substrates than in lucerne and ryegrass.

### 3.3. Fermentation parameters of Lemna gibba-supplemented diets

The chemical composition of basal diets supplemented with *L. gibba* is presented in Table 4. Crude protein content was similar among the treatments but the addition of *L. gibba* decreased the NDF content of the basal diet (Table 5).

*In vitro* ruminal fermentation kinetics, fermentable fractions and 72 h degradability of basal diets supplemented with graded levels of *Lemna gibba* are presented in Table 6. The IVDMD presented a positive linear increase as the RF and MF carbohydrate proportions increased ($R = 0.73$ and 0.94; $P < 0.0001$). Increasing the inclusion levels of LG1 and LG2 in the basal diet tended to decrease RF, MF and IVDMD. Diets supplemented with 5–20% LG1 and those supplemented with 5–15% of LG2 had similar IVDMD. Inclusion of LG1 and LG2 did not influence the IVOMD of the basal diet.

## 4. Discussion

### 4.1. Chemical composition and fermentation patterns

In this study, lucerne and *L. gibba* samples had more CP and less NDF and ADF than ryegrass. The NDF component is associated with lower *in vitro* ruminal gas production, IVDMD, and IVOMD of plant matter (Salem et al., 2006, 2007; Musco et al., 2016).

**Table 1**
Chemical composition (g/kg DM) of *Lemna gibba*, lucerne and ryegrass substrates.

| | *L. gibba* 1 | *L. gibba* 2 | Lucerne | Ryegrass |
|---|---|---|---|---|
| Organic matter | 714.8 | 700.5 | 856.3 | 873.1 |
| Crude protein | 309.2 | 208.2 | 248.9 | 68.1 |
| Ether extract | 11.9 | 19.9 | 23.7 | 18.8 |
| Neutral detergent fiber | 297.9 | 275.4 | 347.1 | 568.6 |
| Acid detergent fiber | 276.3 | 218.4 | 254.5 | 399.8 |
| Non-fibrous carbohydrates | 188.9 | 282.4 | 26.4.0 | 243.6 |
| Ash | 192.2 | 214.2 | 116.3 | 100.9 |
| Net energy of lactation (Mcal/kg) | 12.7 | 12.7 | – | – |

**Table 2**
Fermentation kinetics, fermentable fractions and degradability of *Lemna gibba*, lucerne and ryegrass substrates after 72 h of incubation.

| | *L. gibba* 1 | *L. gibba* 2 | Lucerne | Ryegrass | $R^2$ | CV (%) | SEM | P-Value |
|---|---|---|---|---|---|---|---|---|
| Fermentation kinetics | | | | | | | | |
| Lag phase (h) | 0.33$^{c}$ | 0.52$^{bc}$ | 2.25$^{a}$ | 0.80$^{b}$ | 0.96 | 20.67 | 0.16 | <0.0001 |
| S (/h) | 0.04$^{b}$ | 0.04$^{b}$ | 0.05$^{a}$ | 0.03$^{c}$ | 0.97 | 3.02 | 0.001 | <0.0001 |
| Vmax (mL/g DM) | 171.2$^{d}$ | 202.2$^{c}$ | 262.6$^{b}$ | 305.2$^{a}$ | 0.95 | 5.45 | 9.91 | <0.0001 |
| Fermentable fractions (g/kg DM) | | | | | | | | |
| RF | 192.7$^{d}$ | 230.3$^{c}$ | 313.2$^{a}$ | 284.4$^{b}$ | 0.97 | 4.04 | 7.98 | <0.0001 |
| MF | 133.0$^{c}$ | 159.0$^{b}$ | 236.3$^{a}$ | 245.7$^{a}$ | 0.97 | 6.09 | 9.11 | <0.0001 |
| LF | 139.7$^{c}$ | 163.0$^{bc}$ | 179.9$^{b}$ | 297.8$^{a}$ | 0.97 | 7.54 | 11.37 | <0.0001 |
| TF | 465.4$^{d}$ | 552.3$^{c}$ | 729.4$^{b}$ | 827.9$^{a}$ | 0.96 | 5.53 | 27.51 | <0.0001 |
| Degradability (g/100 g DM) | | | | | | | | |
| IVDMD | 59.5$^{b}$ | 67.9$^{a}$ | 56.3$^{b}$ | 70.0$^{a}$ | 0.94 | 3.07 | 1.51 | <0.0001 |
| IVOMD | 56.0$^{d}$ | 59.3$^{c}$ | 69.9$^{a}$ | 66.6$^{b}$ | 0.95 | 2.75 | 1.34 | <0.0001 |

$^{a,b,c,d}$In a row, means with a common superscript do not differ ($P > 0.05$). $R^2$, coefficient of determination; CV, coefficient of variation; SEM, standard error of the mean; S, fractional fermentation rate; P-Value, probability value of F test; Vmax, maximum gas volume produced; DM, dry matter; RF, fraction of carbohydrates rapidly fermented; MF, fraction of carbohydrates fermented at a medium rate; LF, fraction of carbohydrates slowly fermented; TF, total fermentable carbohydrates; IVDMD, *in vitro* ruminal dry matter degradability; IVOMD, *in vitro* ruminal organic matter degradability.

**Table 3**
Ammonia N- concentration and volatile fatty acids (VFA) profile after 24 h of *in vitro* ruminal fermentation of *Lemna gibba*, lucerne and ryegrass substrates.

| | *L. gibba* 1 | *L. gibba* 2 | Lucerne | Ryegrass | $R^2$ | CV (%) | SEM | P-Value |
|---|---|---|---|---|---|---|---|---|
| Total VFA (mM/L) | 20.0$^{b}$ | 18.4$^{b}$ | 23.2$^{ab}$ | 25.8$^{a}$ | 0.72 | 12.36 | 2.94 | 0.0041 |
| VFA proportions (%) | | | | | | | | |
| Acetate (A) | 46.0$^{b}$ | 44.6$^{c}$ | 49.4$^{a}$ | 42.2$^{d}$ | 0.96 | 1.48 | 0.73 | <0.0001 |
| Propionate (P) | 38.6$^{c}$ | 40.3$^{b}$ | 38.3$^{c}$ | 44.8$^{a}$ | 0.99 | 1.04 | 0.46 | <0.0001 |
| Butyrate (B) | 15.4$^{a}$ | 15.2$^{a}$ | 12.3$^{c}$ | 13.0$^{b}$ | 0.96 | 2.23 | 0.34 | <0.0001 |
| A:P ratio | 1.19$^{b}$ | 1.11$^{c}$ | 1.29$^{a}$ | 0.94$^{d}$ | 0.97 | 2.59 | 0.03 | <0.0001 |
| Ammonia -N (mg/dL) | 17.3$^{a}$ | 13.8$^{a}$ | 15.3$^{a}$ | 9. 9$^{b}$ | 0.8 | 13.65 | 2.09 | <0.0001 |

$^{a,b,c,d}$In a row, means with common superscripts do not differ ($P > 0.05$). *$R^2$, coefficient of determination; CV, coefficient of variation; SEM, standard error of the mean; P-Value, probability value of F test; VFA, volatile fatty acids.

**Table 4**
Ingredients and chemical composition of the basal diet.

| Ingredients | g/kg DM |
|---|---|
| Rolled corn | 350 |
| Soybean meal | 80 |
| Canola seeds | 30 |
| Wheat bran | 70 |
| Molasses | 60 |
| Commercial mixture | 10 |
| Oat hay | 50 |
| Lucerne hay | 350 |
| Chemical composition[a] | |
| CP | 171.0 |
| Ash | 69.5 |
| NFC | 483.0 |
| NDF | 252.0 |
| ADF | 168.0 |
| NEL (Mcal/kg) | 16.0 |

[a] Chemical composition: DM, dry matter; CP, crude protein; NFC, non-fibrous carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; NEL, net energy for lactation.

Cell walls are primarily composed of cellulose and hemicellulose, whose potential as energy sources is limited not only because of the β-1, 4 bonds linking the monomers of sugars to form the polymers, but also because of the lignin, which restricts the degradation of the other cellular polysaccharides (Jung and Casler, 2006).

In ruminal environment, gas production is a result of the combined action of microorganisms and endogenous enzymes such as cellulases (*i.e.* endo-β-glucanases, exo-β-glucanases or cellobiohydrolases and β-glucosidases) and xylanases (*i.e.* arabinofurosidases, acetyl xylan esterases, glucuronidases, β-xylosidases and endo-β-xylanases) that break cell wall linkages and promote the fermentation of both structural and non-structural carbohydrates (Tirado-González et al., 2016; Menke and Steingass, 1988). Ruminal fermentation of fat and protein produces very little gas (Deutschmann et al., 2017) despite that fact that these compounds can affect the ruminal fermentation (Beauchemin et al., 2008; Salem et al., 2014).

Ellis et al. (2007) modeled the effects of some nutritional

**Table 5**
Chemical composition (g/kg DM) of basal diets supplemented with graded levels of *Lemna gibba*.

| Treatment | Level (%) | Ash | Crude protein | Neutral detergent fiber | Acid detergent fiber |
|---|---|---|---|---|---|
| *Lemna gibba* 1 | 5 | 75 | 189 | 235 | 186 |
| | 10 | 81 | 169 | 231 | 192 |
| | 15 | 88 | 172 | 245 | 205 |
| | 20 | 89 | 178 | 248 | 211 |
| | 25 | 93 | 189 | 232 | 215 |
| *Lemna gibba* 2 | 5 | 77 | 157 | 235 | 174 |
| | 10 | 82 | 157 | 212 | 178 |
| | 15 | 86 | 167 | 219 | 174 |
| | 20 | 94 | 168 | 231 | 175 |
| | 25 | 99 | 169 | 228 | 189 |

**Table 6**
*In vitro* ruminal fermentation kinetics, fermentable fractions (g/kg DM) and 72 h degradability (g/100 g DM) of basal diets supplemented with graded levels of *Lemna gibba*.

| Treatment | Fermentation kinetics | | | Fermentable fractions | | | | Degradability | |
|---|---|---|---|---|---|---|---|---|---|
| | L (h) | S (/h) | Vmax (mL/g) | RF | MF | LF | TF | IVDMD | IVOMD |
| Control | 0.70[a] | 0.049[a] | 291.3 | 345.3[abc] | 238.0[ab] | 194.6[a] | 777.8[ab] | 83.7[a] | 85.9[a] |
| *L. gibba* 1 | | | | | | | | | |
| 5 | 0.76[a] | 0.048[ab] | 288 | 333.1[abcd] | 237.0[ab] | 205.3[a] | 775.4[ab] | 82.8[ab] | 85.7[a] |
| 10 | 0.55[a] | 0.049[a] | 268.9 | 324.3[abcd] | 216.2[ab] | 184.2[a] | 724.6[ab] | 82.7[abc] | 85.5[a] |
| 15 | 0.69[a] | 0.045[ab] | 268.5 | 300.2[bcd] | 221.5[ab] | 203.6[a] | 725.3[ab] | 81.5[abcd] | 84.3[a] |
| 20 | 0.68[a] | 0.045[ab] | 245.3 | 275.4[d] | 201.4[b] | 187.8[a] | 664.6[b] | 81.5[abcd] | 85.7[a] |
| 25 | 0.62[a] | 0.046[ab] | 250.7 | 285.7[cd] | 205.2[ab] | 184.1[a] | 675.0[b] | 78.7[bcd] | 82.2[a] |
| *L. gibba* 2 | | | | | | | | | |
| 5 | 0.52[a] | 0.046[ab] | 323.2 | 370.8[a] | 262.5[a] | 234.1[a] | 867.3[a] | 81.9[abcd] | 84.6[a] |
| 10 | 0.48[a] | 0.046[ab] | 321.5 | 370.5[a] | 259.4[ab] | 236.4[a] | 866.3[a] | 80.5[abcd] | 83.9[a] |
| 15 | 0.44[a] | 0.046[ab] | 305.4 | 352.4[ab] | 245.8[ab] | 224.7[a] | 822.8[ab] | 80.2[abcd] | 85.5[a] |
| 20 | 0.23[a] | 0.044[ab] | 299.4 | 344.9[abc] | 237.2[ab] | 227.2[a] | 809.2[ab] | 78.1[cd] | 80.9[a] |
| 25 | 0.16[a] | 0.044[b] | 298.8 | 344.3[abc] | 236.2[ab] | 227.3[a] | 807.8[ab] | 78.0[d] | 81.2[a] |
| $R^2$ | 0.56 | 0.62 | 0.69 | 0.77 | 0.6 | 0.6 | 0.69 | 0.71 | 0.54 |
| CV (%) | 23.49 | 3.4 | 7.69 | 6.73 | 8.89 | 10.01 | 7.57 | 1.94 | 2.57 |
| SEM | 0.21 | 0.002 | 21.83 | 22.02 | 20.42 | 20.77 | 57.87 | 1.56 | 2.13 |
| P-Value | 0.035 | 0.015 | 0.003 | 0.003 | 0.0242 | 0.022 | 0.003 | 0.007 | 0.058 |

[a,b,c,d]In a column, means with common superscripts do not differ ( P > 0.05). Control, without *L. gibba*; L, lag phase; S, fractional fermentation rate; Vmax, maximum gas volume produced; DM, dry matter; RF, fraction of carbohydrates rapidly fermented; MF, fraction of carbohydrates fermented at a medium rate; LF, fraction of carbohydrates slowly fermented; TF, total fermentable carbohydrates; IVDMD, *in vitro* ruminal dry matter degradability; IVOMD, *in vitro* ruminal organic matter degradability; $R^2$, coefficient of determination; CV, coefficient of variation; SEM, standard error of the mean; P-Value, probability value for F test.

components of ruminant diets on enteric methane production and reported high correlation between NDF as potential energy source and methane production. This is because when sugars released from NDF degradation are fermented to propionate and butyrate in the rumen, four H+ ions are released while acetate production from the fermentation of structural carbohydrates releases two H+ ions (Knapp et al., 2014). These metabolic hydrogen ions are converted to $H_2$ by hydrogenase-expressing bacterial species, and converted to $CH_4$ by *Archaeas* (Jin et al., 2017). Although the complexity of the interaction between cell wall structures and specific ruminal microorganisms is not fully understood (Gado et al., 2011; Salem et al., 2012, 2013; Elghandour et al., 2013), studies of pyrosequencing of ruminal microbiota have provided some evidence of changes in the proportion of major ruminal phyla (*Bacteroidetes*, *Firmicutes*, and *Proteobacteria*), and *archaeas* caused by diets composition. This evidence plays a key role in understanding how bacteria and enzymes directly act to break cell wall polymers and how this promotes methane gas emissions (McCann et al., 2014).

Consequently, some strategies to mitigate methane production consist of improving the degradability of NDF in ruminant diets by adding enzymatic supplements (Hernández et al., 2017a, 2017b) or increasing the proportion of good quality feedstuffs (grains or concentrates) (Elghandour et al., 2017a). Both approaches work by modifying rumen fermentation to increase ruminal propionate production at the expense of acetate. Other options include using supplements or ingredients that would directly or indirectly ameliorate methanogenesis such as oils, monensins, ionophores, and yeasts (Elghandour et al., 2017a; McGinn et al., 2004). In this study we opted to evaluate a supplementation strategy using nutrient-rich *L. gibba* to improve the nutritive value of a ruminant diet. *Lemna gibba* supplements promoted better A:P ratio than lucerne, suggesting that the inclusion of *L. gibba* in ruminant diets has potential to reduce enteric methane emissions and thus contribute to environmentally friendly ruminant production. The high CP content of *L. gibba* suggest that it could be an alternative source of dietary protein. The high protein content could have promoted the production of isobutyric acid in the present study (Maccarana et al., 2016) and therefore, a higher butyrate proportion (Sofyan et al., 2017; Pino and Heinrichs, 2016). *Lemna gibba* could be included in the diets of dairy cows to increase the production of fat in milk.

Although it has been established that increasing the proportion of concentrate feeds in ruminant diets can decrease greenhouse gases emissions (McCann et al., 2014; Beauchemin et al., 2008), this is not always the best strategy given the cost and low availability. Indeed, in a number of regions, the climatic conditions tend to adversely affect grain production (Knapp et al., 2014; Beauchemin et al., 2008). As such, the strategies to mitigate methane production should consider the use of readily available, low cost, non-conventional forages such as *L. gibba*, in ruminant diets to improve the NDF digestibility (Khan et al., 2015) thereby reduce enteric methane emissions in a cost-effective and sustainable manner. This approach has the primary objective of modifying ruminant diets in order to decrease the A:P ratio an thus lower enteric methane gas production (Ellis et al., 2007).

The concentration of $NH_3$-N in the *in vitro* ruminal fermentation medium of *L. gibba* was similar to that of lucerne, a commonly used protein supplement in ruminant diets, suggesting that *L. gibba* protein has high ruminal degradability (Khan et al., 2002; Zetina et al., 2013). Additionally, the $NH_3$-N levels in *L. gibba* fermentation medium were within the optimum range for microbial fermentation and growth (12–17 mL/dL) (Anantasook and Wanapat, 2012).

### 4.2. Potential for Lemna spp. in ruminant diets

The amino acid profile of *Lemna gibba* is very similar to that of soybeans suggesting that the quality of duckweed protein could be similar that of the 'gold standard', soybean. However, it is important to note that the amount and quality of *L. gibba* nutrients may vary with its source i.e. the water body in which it grows (Khandaker et al., 2007). The nutritive value of *Lemna* spp. suggests that it could be used as a mineral and protein supplement in order to reduce feed costs, without negatively affect the DM intake or the feed efficiency (Haustein et al., 1994) as well as to reduce the contribution of ruminants to greenhouse gases emissions by reducing enteric methane production. In a related study, Zetina et al. (2013) included *L. gibba* in Pelibuey sheep diets at 20–30%

and reported improved the digestibility of Taiwan grass harvested at 45 and 60 days of age.

## 5. Conclusions

Dietary inclusion of *L. gibba* has the potential to produce low-cost, environmentally friendly ruminant diets that promote lower ruminal methane production The present study suggests that it is possible to include *L. gibba* up 15% of basal diet without negatively affecting the final nutritive value.

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