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Full Length Research Paper

Impacts of different levels of nitrogen fertilization on yield production, chemical composition and *in vitro* gas production of different varieties of barley (*Hordeum vulgare*) forages

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The performance, chemical composition and *in vitro* gas production of four varieties: Emerald (Eme), Capuchona (Cap), Petunia (Pet) and Cabuya (Cab) of whole plant barley (*Hordeum vulgare*) were evaluated, and fertilized with four levels of nitrogen (I, 80; II, 110; III, 140 and IV, 170 kg N/ha). The variables measured were: Height (dm), bulk (mm), number of leaves and yield production (Tonne DM/ha); chemical composition and *in vitro* gas production were determined using a 4 x 4 arrangement of 16 treatments within a randomized complete block design. The highest yield production (P < 0.001) was in Cap, (5.37 ± 0.35 Tonne/ha) followed by Pet and Cab. The height, bulk and number of leaves were higher (P < 0.001) in Cap (7.4 cm, 3.4 mm and 4.8, respectively) compared to the other varieties. NDF content was lower (P < 0.001) in Eme compared to the rest. ADF in Cap and Eme was lower (P < 0.001) *versus* Pet and Cab. CP content was highest (P < 0.001) in Pet, without differences (P > 0.05) between Cap and Cab (124.3 ± 2.8 g/kg DM), and a lowest content (P < 0.001) in Eme (110.3 g/kg DM). *In vitro* gas production was higher (P < 0.001) in Eme *versus* Cab. Data suggested that the Cap variety has the better production values and nutritional quality *versus* the other varieties.

Key words: Barley, *Hordeum vulgare*, *in vitro* gas production, fertilization.

INTRODUCTION

The use of new varieties of barley has increased its importance in feeding ruminants as a source of alternative energy and protein, because the nutritional quality that may be superior to other feed grains such as oats, triticale or wheat (Cherney and Marten, 1982; Carr et al., 2004). Barley is a cereal that can grow in semiarid areas (Al-Masri, 1998) and sub humid areas (Chen et al., 2004), and can adapt to adverse weather conditions.

The level of nitrogen fertilization is reflected in the nutritional quality and yield production of crops (Moreno et al., 2003; Chen et al., 2004), as shown by Johnson and Raund (2003), who found that increasing levels of N fertilization could be associated with yield production increase in fodder and grain as well as crude protein content. Crop production systems are often based on the use of large amounts of nitrogen (N) fertilizer, frequently in higher amounts than the plants require (Moreno et al., 2003). The technique of *in vitro* gas production (Menke and Steingass, 1988; Theodorou et al., 1994) is used to

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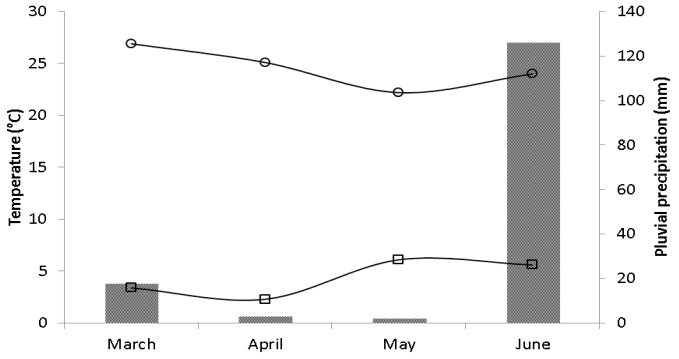

Figure 1. Pluvial precipitation (■), temperatures (ºC) maximum (○) and minimum (□) in Toluca Valley, State of Mexico during the experimental period (INEGI, 2004).

estimate ruminal degradability of feeds is easy to apply and has a high correlation with the *in vivo* evaluation (Getachew et al*.,* 1998). The aim of this study was to identify the variety of barley that has the best indicators of performance, nutritional characteristics and *in vitro* fermentation activities at different nitrogen fertilized amounts.

MATERIALS AND METHODS

Cropping and sampling of plant material

The experiment was carried out in the valley of Toluca, Mexico at 2,600 m above sea level, temperature and mean annual precipitation of 13.7°C and 1000 to 1219 mm, respectively (INEGI, 2000). Four barley varieties, Capuchona (Cap), Petunia (Pet), Cabuya (Cab), which were generated in the CIMMYT (International Maize and Wheat Improvement Center) and Emerald (Eme) as a control, were used with four nitrogen fertilization levels (urea, 46%) I, 80; II, 110; III, 140, and IV, 170 kg N/ha. Fertilization consisted of half N amount at the sowing season; P and K were applied at 130 and 50 kg/ha, respectively; the rest of the N was applied after 30 days, the period when the potential number of ears was determined.

The germination rate and plant density were adjusted to 110 kg of barley seeds/ha,germination rate was performed previously, using 10 seeds per variety by triplicate, obtaining 100, 93, 85 and 50% germination for varieties Eme, Cap, Cab and Pet, respectively. Crop and seeds were not treated with any insecticide, fungicide and herbicide; it was cropped in a soil classified as pelico vertisol soil of volcanic origin, which is slightly acid (pH 5.6) and has a 1.7% organic matter (OM) content. Sowing took place on 19 March (day zero), 2004 (three replicates per variety and level of fertilization) in plots of 24 m^2, (five rows 4 × 6 m, with two crop rows and 0.8 m between rows). The crop was irrigated by a total cover sprinkler system; the irrigation period was applied after sowing on March 20, continuing with March 26, April 3, 15, 30 and May 20; after that the rainy season completed the water requirements (Figure 1). We manually cut to a height of 5 cm of the soil surface when barley grain was in the milky-dough stage, while for Eme at 52 days and Cap, Pet and Cab at 79 days.

Herbage measurements

The height (dm), stem diameter (mm), number of leaves (NL) and yield production (Tonne DM/ha) were determined by triplicate. Samples were analyzed for DM (#934.01), ash (#942.05), and N (#954.01) according to Association of Official Analytical Chemists [AOAC] (1997). The neutral detergent fiber (NDF, Van Soest et al., 1991) and acid detergent fiber (ADF) (AOAC, 1997; #973.18) analyses used an ANKOM200 Fiber Analyzer Unit (ANKOM Technology Corporation, Macedon, NY, USA). NDF was assayed without use of an alpha amylase but with sodium sulfite in the NDF. Both NDF and ADF are expressed without residual ash. All analysis was performed by triplicate and the average was used for comparison between samples.

Gas production procedure

Three Rambouillet lambs (BW 30 ± 0.5 kg) cannulated in the rumen were used as donors of rumen fluid inoculum for *in vitro* fermentation studies. Lambs were fed *ad libitum* at 09:00 and 16:00 h on a diet based of alfalfa hay and oat straw (50:50) with 2% vitamin-mineral supplement (Gold line Hitec-nutrition Multitec Malta Cleyton). This technique was proposed by Theodorou et al. (1994), and used for determining the *in vitro* gas production. Samples of 800 mg DM (in triplicate) were weighed in 125 ml bottles. The incubation solution (Menke and Steingass, 1988), was prepared and 90 ml were added to each bottle, with 10 ml of rumen fluid previously extracted and filtered in a triple layer of gauze and glass wool, homogenized and gassed with CO_2, and then bottles were incubated in a water bath at 39°C.

There were three series of incubation, which included three bottles without substrate (blank), for the correction of gas production due to fermentation of rumen fluid itself, and oat straw was used as standard. Once the incubation began, gas production was recorded (pressure transducer, Delta; Model HD 8804) at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation. Gas production was adjusted according to the model proposed by France et al. (1993):

$$y = A\,[1 - \exp(-b\,(t - T) - c\,(\sqrt{t} - \sqrt{T}))]$$

Where "*y*" represents the cumulative gas production (ml), "*t*" is time incubation (hours), "*A*" curve asymptote (total gas production, ml), "*b*" (h^{-1}) and "*c*" ($h^{-1/2}$) are constants of gas production. "*T*" represents the delay time (hours) which is the time it takes for bacteria to initiate fermentation.

Statistical analysis

The data were analyzed in a randomized complete block design, 4 × 4 considering the factors, barley variety and level of N fertilization and their interaction, using SAS statistical software (1996) and mean comparisons by Tukey's method ($P < 0.001$).

RESULTS

Herbage measurement

Cap had the highest peak (Figure 2) being higher ($P < 0.001$) compared to the rest (6.1 ± 0.18 dm). For the stem diameter, Pet and Cap (3.3 ± 0.1 mm) were higher ($P < 0.001$) than Eme, while the NL was higher ($P < 0.001$) for Pet and Cap over Cab. Yield production Cap was higher ($P < 0.001$) followed by Cab and Pet. There were no differences regarded to the level of fertilization among treatments (Figure 2). For the interaction between variety

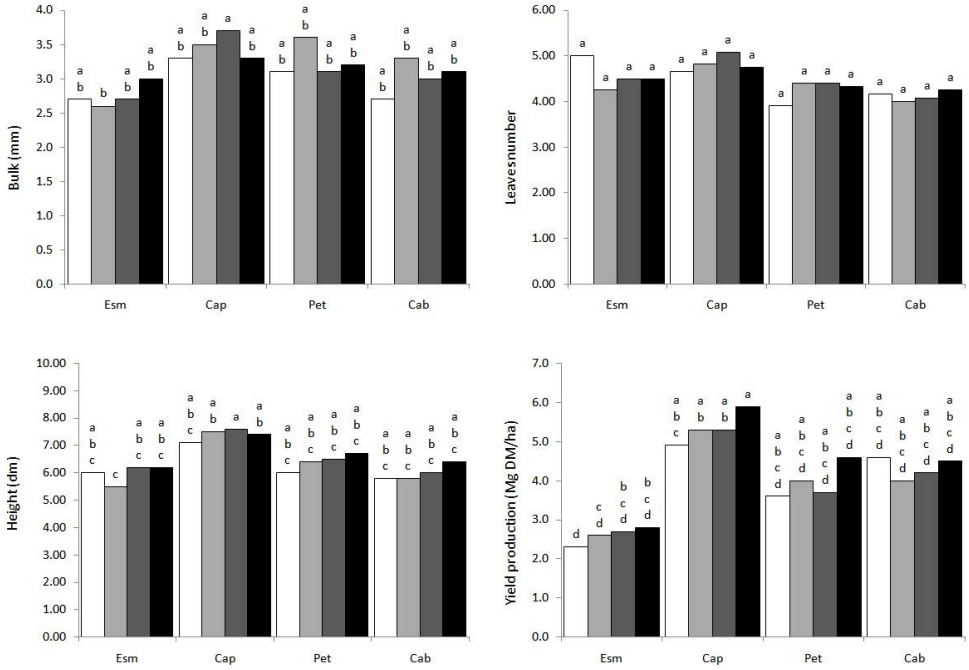

Figure 2. Agronomic parameters of height, weight, number of leaves and dry matter production of four varieties of barley (Emerald = Eme, Capuchona = Cap, Petunia = Pet and Cabuya = Cab) and its interaction with fertilization level (80 = □, 110 = ▭, 140 = ▩ and 170 = ■ kg N/ha, respectively). a >b >c >d showed significant differences (P <0.05).

and fertilization level (Figure 2), height (dm) was higher (P < 0.04) for Cap 110, 140 and 170, vs. Eme 110, while the stem bulk (mm) Cap 140 was higher (P < 0.04) than Eme 110. No differences (P > 0.05) were observed in NL; while the yield production (Tonne DM/ha) of Cap 110, 140 and 170 was higher (P < 0.03) than Eme 80 and 110.

Chemical composition

OM content (Figure 3) was higher for Eme *versus* Pet (P < 0.001), while the CP content was higher (P < 0.001) in Pet than Eme. NDF content was lower (P < 0.001) in Eme compared to the rest (625.3 ± 9.8 g/kg DM). ADF content was lower (P < 0.001) in Cap and Eme *versus* Cab. Regarding to the level of fertilization there were no differences (P > 0.05) among treatments (Figure 3), while the interactions between variety and fertilizer level increased CP (g/kg DM) in Pet (P < 0.05) than Eme. NDF (g/kg DM) content of Cab 140, 170, 110; Pet and Cap 110 were higher (P < 0.05) *versus* Eme 110, 140 and 170, while the ADF (g/kg DM) increased in Cab than Eme (140 and 170, P < 0.05).

In vitro gas production

The highest volume of gas produced (Table 1) was in Eme at the fertilization level of 110 kg N/ha. Regarding the *in vitro* gas production of the variety (Figure 4), the highest gas production (P < 0.05) was in Eme (304.8 ml gas/g DM) *versus* Cap, Cab and Pet (Figure 4). *In vitro* gas productions were not different (P> 0.05) between Cap, Pet and Cab. There were not significant differences (P > 0.05) according to the fertilization level, although the highest gas production was for 110 kg N/ha (Table 1).

DISCUSSION

Herbage measurement

Chen et al. (2004), obtained yields ranging from 4.0 to 6.5 Tonne DM/ha similar to those of this study, but lower than Al-Masri (1998) 8.1 Tonne DM/ha, and Colin et al. (2009). With yields ranging 10 to 13 Tonne DM/ha in barley varieties, it is clear that production varies from year to year and between regions; in our case, we assume that the varieties studied are not adapted to the weather conditions in the region, mainly altitude (over 2600 m above sea level), rainfall and temperature, since the time when rainfall was cropped is higher and exceeds the requirements of water, and were harvested at a younger age compared to other authors (Colin et al., 2009).

Chemical composition

The content of CP was similar to Chen et al. (2004) 109 ±20 g/kg DM and those obtained by Yu et al. (2003), with a variety Valier 136 g/kg DM, and lower to Carr et al.

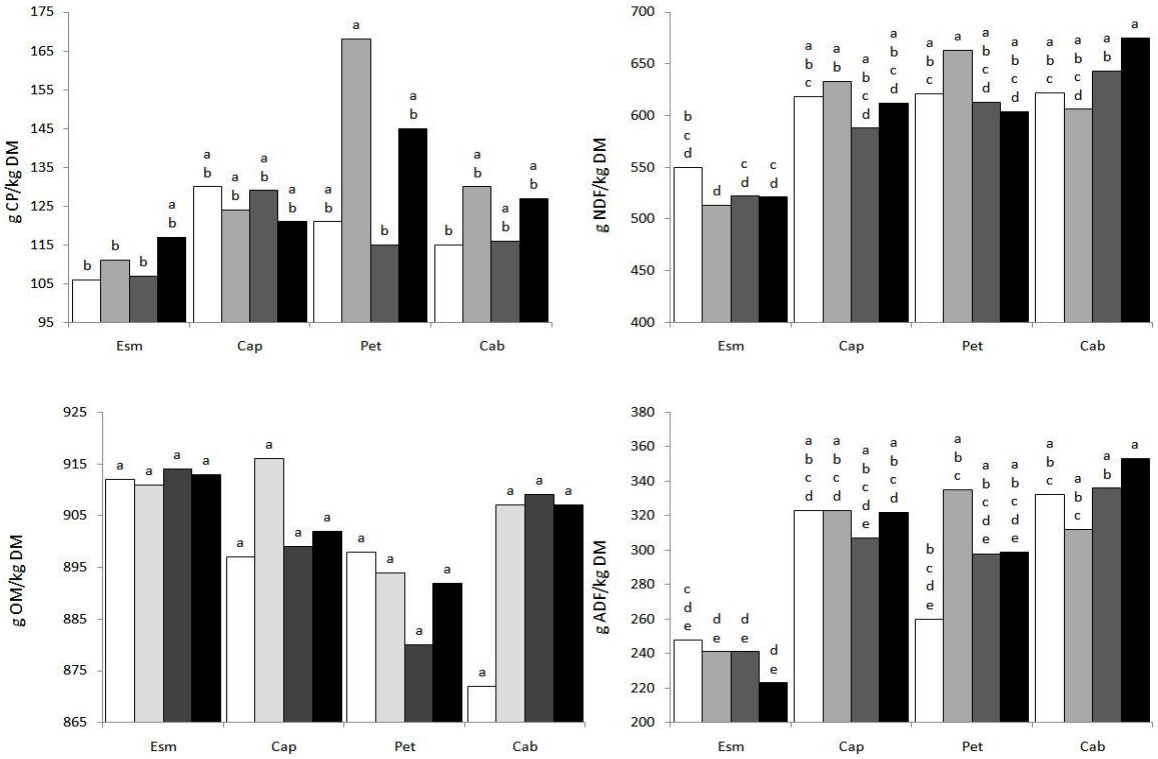

Figure 3. Content of OM, CP, NDF and ADF (g/kg DM) of four varieties of barley (Emerald = Eme, Capuchona = Cap, Petunia = Pet and Cabuya = Cab) and its interaction with fertilization level (kg N/ha) (80 = □, 110 = ▒, 140 = ▓ and 170 = ■). a >b >c >d >e show significant differences (P < 0.05).

Table 1. *In vitro* gas production of four varieties of barley at four levels of nitrogen fertilization (kg N/ha).

Variety/ N level	Gas production	Degradation rate ($h^{1)}$	Degradation rate ($h^{1/2}$)	Lag time
Eme 80	299.5	0.035	-0.025	0.96
Eme 110	310.4	0.041	-0.030	1.15
Eme 140	304.8	0.036	-0.022	0.99
Eme 170	308.0	0.070	-0.059	1.39
Cap 80	273.8	0.037	-0.041	1.59
Cap 110	278.6	0.037	-0.048	1.82
Cap 140	278.2	0.036	-0.082	1.20
Cap 170	282.6	0.035	-0.088	1.65
Pet 80	281.7	0.035	-0.036	1.48
Pet 110	284.1	0.035	-0.045	2.07
Pet 140	283.6	0.033	-0.038	1.77
Pet 170	269.2	0.032	-0.040	1.88
Cab 80	285.5	0.033	-0.033	1.33
Cab 110	277.1	0.032	-0.029	1.23
Cab 140	279.5	0.034	-0.042	1.66
Cab 170	261.3	0.056	-0.049	2.53
SEM	3.40	0.056	0.057	0.04
P value				
Variety (V)	0.05	0.4	0.6	0.5
Nitrogen level (N)	0.5	0.5	0.6	0.5
VxN	0.5	0.6	0.5	0.7

SEM = Standard error of means. No significant (P> 0.05); Eme, Emerald; Cap, Capuchona; Pet, Petunia; Cab, Cabuya; 80, 110, 140 and 170 kg N/ha.

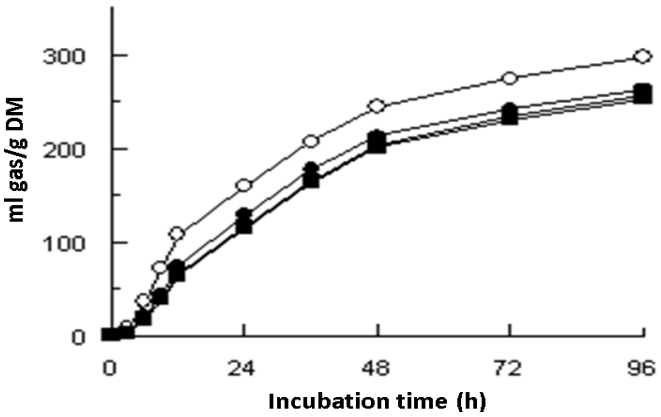

Figure 4. *In vitro* gas production (ml gas/ g DM) obtained by the adjustment of the incubation of different varieties of *barley* (○, Emerald; □,Cabuya; ●, Capuchona and ■, Petunia).

(1998) with the variety Horsford 141 g/kg DM and Colin et al. (2009), (130 to 160 g CP/kg DM) at 88 days of age. Aguilar-Lopez et al. (2013) founded higher CP concentrations (176 g CP/ kg DM) in barley Doña Josefa cropped in the same area; these differences arise due to climatic conditions that favor or limit the fixation of nitrogen applied as fertilizer or environmental (Moreno et al., 2003), as well as the different varieties of study (Ghezeljeh et al., 2011). The cell wall content increased with the maturation of the plants (Ankom, 2003; Tolera and Sundstøl, 1999); Cap, Pet and Cab were harvested at 25 days after Eme, as later maturing, which favors the formation of structural carbohydrates. This was observed primarily with higher NDF content in these varieties.

In vitro digestibility

The results obtained by Arhab et al. (2010) testing fermentation on barley and forage production of gas obtained from 295.5 ml gas/g DM at 72 h, although it took less than the fermentation time compared to the present study (at 96 h). In other studies, Colkesen et al. (2005) tested the *in vitro* fermentation of barley grain in eight varieties, Esterel variety had the highest gas production (400 ml gas/g DM at 96 h) which was higher than the highest level of gas production in the present study with a variety Eme at 110 kg N/ha fertilization level; this one was used in whole plants of barley. Ghezeljeh et al. (2011) evaluate different barley grain varieties, and authors found difference ($P < 0.05$) in asymptotic gas volume, and gas production constant rate among the varieties.

Conclusions

Depending on the sowing conditions in the present study, Capuchona variety had the best growth performance and representing the option to use. The addition of N did not affect the yield production and/or chemical composition of barley, suggesting a total of 80 to 110 kg N/ ha. Higher N inputs could have negative effects, implying environmental contamination and an increase in the cost of forage production.

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ABBREVIATIONS

Eme, Emerald; **Cap,** capuchona; **Pet,** petunia; **Cab,** cabuya; **DM,** dry mater; **CP,** crude protein; **NDF,** neutral detergent fiber; **ADF,** acid detergent fiber; **dm,** decimeter; **mm,** millimeter; **N,** nitrogen; **P,** phosphorus; **K,** potassium; **BW,** body weight; **h,** hours; **NL,** number of leaves; **OM,** organic matter.

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