ABSTRACT. The study was aimed to decrease the application of mineral fertilizers in the production of triticale-based forage, with the combined use of Ecomic® (arbuscular mycorrhizal fungi) and Fitomas-E®. It was developed on a red Ferralic leachate soil in the Station of Pastures and Forage Genetics Cattle Company (EPG) “Niña Bonita”, Artemisa province. The treatments were: 1. Absolute Control; 2. Application of mineral fertilizers 150, 92 and 120 kg ha⁻¹ of N, P₂O₅ and K₂O; 3. Application of mineral fertilizers 100, 54 and 70 kg ha⁻¹ of N, P₂O₅ and K₂O + Ecomic® (15 kg ha⁻¹) + Fitomas-E® 2 L ha⁻¹. Mycorrhizal variables, the percentage of N, P and K in the leaf tissue and crude protein percentage in forage were analyzed. The dry mass (forage) in kg ha⁻¹. The results showed that a decreased amount of mineral fertilizers did not reduce the quality of the fodder produced, since no significant differences among control treatments production and use of biofertilizer were recorded. Moreover higher percentage intensity and mycorrhizal colonization and the highest number of spores per g⁻¹ soil was recorded. The results showed the establishment of a mycorrhizal association among guest and host. It was recorded savings in production costs with the implementation of these, so it became more profitable to produce forage.

Key words: inorganic fertilizers, biofertilizers, vesicular arbuscular mycorrhizae

INTRODUCTION

The negative impact of climate change on soil fertility and excess mineral fertilization of crops have been seriously affected food security in developing
countries. Because of this, steps should be taken to ensure a change in the sustainability and improvement of food production systems for all species, more in line with agroecology (1).

The livestock development requires the production and use of feed resources. Undoubtedly, fodder production for animal feed is essential for sustainable growth, the contribution in fiber and protein that gives your diet, which makes this type of food something irreplaceable in the production of milk and meat (2), which in turn directly influences human consumption.

However, in the production of biomass as hay, silage or green forage for direct consumption by animals, removal of soil nutrients is considerable, so it is necessary to apply fertilizer to restore nutrients removed by the biomass (3).

Livestock fed mainly with fodder, has been displaced to marginal areas (4). However, intensive forage production is costly with continued use of mineral fertilizers. In addition, to these excessively used, they are a source of contamination of soil and groundwater.

Today, triticale has productivity in grain like wheat in favorable environments and intensive production systems; but their performance excels in production marginal conditions: dry water shortage or temporary, especially problematic soils. Their genetic superiority in biomass production and its resistance to most foliar diseases make it very competitive as fodder grain or plant -in entirely compared to other forage crops (barley or oats). Moreover, triticale with less water use makes it more sustainable than forages such as alfalfa or rye (5).

In response to this direction, the production of fodder needs the application of mineral or organic fertilizer. Also the use of bio-fertilizers which will restore soil nutrients from the extracted and others, as in the case of arbuscular mycorrhizal fungi (AMF) which allow through symbiosis with plants, to transport the necessary nutrients for them and improve the chemical-physical and biological soil conditions.

Furthermore, in previous studies, it has been reported that effective management of mycorrhizal symbiosis, which contributes to reduce nutrient losses and, in fact, high doses of fertilizers that require grasses, may be a promising practice, in ecological terms, economic and environmental (6).

Moreover, there are other bio-fertilizers that are being biostimulants or natural, anti-stress bioregulators, partial substitutes for conventional fertilization, and conducive to the development of the rhizosphere (symbiotic microorganisms living in the roots). It fixes atmospheric nitrogen and mobilize other mineral nutrients; as in the case of FitoMas-E®, whose mechanism of action promotes the comprehensive improvement of soil-plant complex and increasing the vitality of the crop, which protects it from many of common conditions to the stressed systems (7).

For the above, this experiment was developed with the aim of reducing the dose of mineral fertilizers and achieve fodder high yields with the use of bio-fertilizers, EcoMic® and FitoMas-E®. It is based on arbuscular mycorrhizal fungi; one with the Glomus Cubense species recently reclassified (8) and the other is a new derivative of the Cuban sugar industry which acts as a vegetable biostimulant (natural) with marked anti stress influence, most of the botanical species that belong economic crops (7), without reducing production levels, nor the quality.

**MATERIALS AND METHODS**

The experiment was conducted in Genetic Livestock Production Enterprise (EPG) "Niña Bonita" in Cangrejeras, Bauta, Artemisa province. On a soil that was classified as Nitisol Ferralic Lixic, Eutrico, Rhodic (9). Its characteristics (the arable horizon), complemented with the number of residents spores per gram of soil are reflected in Table I.

Rainfall in the EPG "Niña Bonita" was 1261 mm, which was 83,1 % during the rainy season and 16,9 % (November to April) occurred in the dry season with the development of the experiment in question. An average temperature of 21,77 °C was observed and the average relative humidity was 73,91 %. The meteorological variables that were reported during the experiment showed a similar behavior to historical averages in the area.

For the production of forage crop used as triticale (x. Triticosecale Wittmack), cv. INCA TT-7 (10), due to its good adaptation to the conditions of western and central Cuba and high agricultural yields in dry matter forage and grains. The standard used was planting 100 kg ha⁻¹ of seed chorrillo along the groove.de semilla a chorrillo

An experimental design of random blocks followed with three treatments and ten repetitions to achieve the objectives proposed in the study.
The experimental plots have a total area of 29.4 m² in a planting of 7 x 0.70 m (six rows). The calculation area was 14 m².

Treatment 1 (T1) consisted of absolute control without application of mineral fertilizers and biofertilizers. Treatment 2 (T2) was the application of mineral fertilizer in doses of 460 kg ha⁻¹ with the most balanced formula 14-20-26 187 kg ha⁻¹ of urea (46-0-0 formula), which represents an application of 150, 92 and 120 kg ha⁻¹ of N, P₂O₅ and K₂O. Finally the treatment three (T3) had mineral fertilizer application in doses of 270 kg ha⁻¹ of balanced formula 14-20-26 + 135 kg ha⁻¹ of urea (46-0-0 formula), which represents an application of 100, 54 and 70 kg ha⁻¹ of N, P₂O₅ and K₂O + EcoMic®10 kg ha⁻¹ + FitoMas-E® 2 L ha⁻¹.

The EcoMic®, biofertilizer based on arbuscular mycorrhizal fungi (AMF) was used in the experiment. It was applied by the technique of coating seeds when planting, 10 % of the seed weight spores at 30 g of solid substrate⁻¹ (11).

FitoMas-E®, natural biostimulant growth biostimulan te natural de crecimiento, composed of high-energy biochemicals, mainly amino acids, bioactive oligosaccharides, with marked influence anti stress was applied in doses of 2.0 L ha⁻¹, dissolved in water for foliar sprinkler⁶. The phase of the culture where FitoMas- E® was sprinkled, was Z.29 Zadocks scale (12), 31 days after germination.

The variables analyzed in the experimental work were:

- Biomass (kg ha⁻¹) from the dry mass (DM), (13). For this fresh mass (FM) of the aerial part of the plants that were in the area calculation weighed each plot with a scale of 0.5 kg precision. A sample of 20 plants was taken per treatment to each replica, which took a stove air circulation at 60 °C until a constant mass to determine the percentage of dry mass (DM) using this formula:

  \[
  \text{DM} \, (\%) = \frac{\text{DM sample (g)}}{\text{fresh sample mass (g)}} \times 100
  \]

DM yield was estimated from the yield of MV and percentage of DM, using the following formula:

\[
\text{DM (kg ha}^{-1}\text{)} = \left[\frac{\text{FM (kg plot}^{-1}\text{)} \times \text{DM} \, (\%)}{100}\right] \times f
\]

being:

\[
f = 0.48 \text{ (factor to convert DM performance of kg plot}^{-1}\text{ kg ha}^{-1}\text{in the plots of 14 m}^2\text{ of experiment}).
\]

- The percentage foliar nutrient concentrations; nitrogen (N), phosphorus (P) and potassium (K) were determined according to the methodologies described in the Manual of Laboratory of the National Institute of Agricultural Sciences (INCA) (14).

- The calculation of the percentage of net protein (crude) was performed using the formula:

\[
\% \text{ Protein} = \% \text{ Nitrogen} \times 5.27
\]

(conversion factor for wheat) (15)

**Mycorrhizal variables**

Ten samples of rhizosphere soil were taken which were homogenized and rootlets after washed were extracted. When they were dried, also they were stained by the staining technique roots (16). Besides the frequency and intensity of colonization was determined by the methodology intercepts (17).

The amount of mycorrhizal spores taking a sample of 50 g of rhizosphere soil was determined, and the methodology described for extraction followed by species (18).

Index Fertilization Efficiency Scheme which was calculated using the formula (19) was used to calculate the share of biofertilizers and mineral nutrition fertilization triticale:

\[
\text{IEEF (\%)} = \left[\frac{\text{Yield DM (t ha}^{-1}\text{)} treatment inoculated and fertilized - DM yield (t ha}^{-1}\text{)} \text{Witness}}{\text{DM yield (t ha}^{-1}\text{)} \text{of control}}\right] \times 100
\]

---

Data were analyzed by SPSS 11.5 statistical processor (IBM) for Windows (20). All experimental data met the assumptions of normality and homogeneity of variance, so we proceeded to run the ANOVA model for simple classification. The Docimo dócima Duncan’s multiple range was used (with a significance of p <0.05) for discriminating means (21).

**ECONOMIC ANALYSIS**

The economic analysis was based on the experimental results, taking into account the comparison of costs of mineral fertilizers and the biofertilizers applied in treatments in cuestión², shown in Table II, as well as the cost of farming associated with work performed (22).

| Table II. Indicators of basic information for the calculating costs of the mineral fertilizer and bioproduct application |
|---|---|---|---|
| Indicator | Unit | CUP | References |
| Cost of labor for mineral fertilization (machinery, fuel and labor) | CUP ha⁻¹ | $38,40 | 19 |
| Balanced fertilizer price (Formula: 14-20-26) | CUP t⁻¹ | $395,35 | 20 |
| Price of fertilizer Urea. Formula: (46-0-0) | CUP t⁻¹ | $334,50 | 20 |
| Price of the mycorrhizal inoculant EcoMic® | CUP kg⁻¹ | $2,50 | 12 |
| Price of Fitomas-E® | CUP L⁻¹ | $1,45 | 12 |

**RESULTS AND DISCUSSION**

Table III shows the results of the variables studied in experimental work. For the percentage of macronutrients contained in the feed, the results show that was 1,86 % for N; 0,237 % to 1,901 % phosphorus and potassium for treatment 3 (T3) and 1,81 % N; 0,237 % to 1,89 % phosphorus and potassium for treatment 2 (T2) with 150, 92 and 120 kg ha⁻¹ of N, P₂O₅ and K₂O without being different among them. In the absolute control treatment (without application of mineral fertilizers and bioproducts), significantly lower concentrations of N, P and K from the rest of the treatments were observed.

The results achieved with treatment 3, then allow considerably reduce the mineral fertilizer that is made in EPG “Niña Bonita”, showing no significant differences in treatment 2, where mineral fertilizers applied in high doses.

The above stated is explained by the effect of an effective inoculation of AMF, as it allowed improving the concentrations of N, P and K in the aboveground biomass of forage, which demonstrates the importance of bio-fertilizers based arbuscular mycorrhizal fungi in making and assimilation of nutrients by plants (23).

Moreover, it can be considered that the use of natural growth stimulant (leaf application) influenced positively with their contribution macronutrients in the results of this treatment, which coincides with other authors (6), who expressed respect that, FitoMas-E® allows to increase the nutritional content of crops and increase agricultural yields thereof.

It can also be seen (Table III) that the application of bioproducts did not negatively influence the nutritional quality of forage.

In this regard, various authors (24, 25) indicate that the use of bioproducts in forage production does not diminish the nutritional quality of them and their use can significantly reduce application of mineral fertilizers, preventing pollution and damage to the ecosystem causing use in high doses.

As noted, the percentage of net protein (crude) shows that the treatment that most contributes to the quality of forage was the reduced with the implementation of AMF and FitoMas-E® fertilization, without differentiating significantly from the treatment of higher fertilization provided.

This result shows the possibility of reducing the dose of mineral fertilizers applied in the production of forage triticale based, with proper use of bio-fertilizers used in this study, without diminishing the quality of forage produced. In this sense, the results confirm the view on the importance of optimizing and streamlining the application of mineral fertilizers, with the application of biofertilizers, as the case of the AMF, expressed by different authors (26, 27).

The effect of the treatments on variables frequency and AMF colonization intensity and the number of spores g⁻¹ of soil is observed in Table III. Higher values significantly for T3 were recorded , compared with treatments 2 (T2) and absolute control (T1), who did not differ significantly among themselves, for these variables found in residents AMF.
Table III. Effect of bioproducts FitoMas-E® and EcoMic® application in the experimental variables

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Foliar nutrient concentrations (%)</th>
<th>Crude Protein (%)</th>
<th>Mycorrhizal variables</th>
<th>Agricultural yield dry forage mass (kg ha⁻¹)</th>
<th>Fertilizer Scheme Efficiency Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
<td>Frequency of colonization (%)</td>
<td>Colonization intensity (%)</td>
</tr>
<tr>
<td>1. Absolute control</td>
<td>1,319 b</td>
<td>0,192 b</td>
<td>1,583 b</td>
<td>7,27</td>
<td>31,92 b</td>
</tr>
<tr>
<td>2. Application of 150, 92 &lt;br&gt;y 120 kg ha⁻¹ de N, P₂O₅ y K₂O</td>
<td>1,81 a</td>
<td>0,237 a</td>
<td>1,893 a</td>
<td>9,22</td>
<td>30,68 b</td>
</tr>
<tr>
<td>3. Application of 100, 54 &lt;br&gt;y 70 kg ha⁻¹ de N, P₂O₅ y K₂O + EcoMic® + Fitomas - E®</td>
<td>1,86 a</td>
<td>0,237 a</td>
<td>1,901 a</td>
<td>9,76</td>
<td>60,78 a</td>
</tr>
<tr>
<td>Es x</td>
<td>0,029**</td>
<td>0,004**</td>
<td>0,019**</td>
<td>0,316</td>
<td>1,305**</td>
</tr>
</tbody>
</table>

Means with no common letter in the same column are significantly different at P ≤ 0.05, according to Duncan’s test.

These results demonstrate that application of AMF more FitoMas-E® and reduced dose of mineral fertilizers (T3), responds with increases in frequency and colonization intensity of soil, against fertilization of high inputs. It allows greater absorption of nutrients by the crop and demonstrates the effective partnership between plant and AMF with positive increase thereof, with respect to treatments with high application of mineral fertilizers (T2) and the witness all (T1), which shows the activity of an efficient strain promoting greater plant-AMF relationship, with high application of mineral fertilizers (T2) and the witness all (T1), which shows the activity of an efficient strain promoting greater plant-AMF relationship, with high application of mineral fertilizers (T2).

The values obtained with high application of mineral fertilizers (T2) shows that no positive increase is not produced in the AMF activity by not registering differences, compared to absolute control. It is due to the application of high doses of fertilizers cannot be expected arbuscular mycorrhizal fungi efficient activity in biology rhizosphere of soil, nor in other indicators such as the stability of organic matter, microaggregates soil and improve its physical properties (29, 30).

In this sense when the AMF report, frequency and intensity of higher mycorrhizal colonization, provide nutrient to the cereals, cereals that in turn, substances made to the AMF, as AMF and FitoMas-E®, which are harmless to the environment and ecosystems, as they allow in turn, make the crop, and provide more nutrients to the cereals and in turn, substances made to the AMF and FitoMas-E®, which are harmless to the environment and ecosystems, as they allow in turn, make the crops more nutrient and produce better yields.

By analyzing the values obtained with high application of mineral fertilizers (T2) shows that no positive increase is not produced in the AMF activity by not registering differences, compared to absolute control. It is due to the application of high doses of fertilizers cannot be expected arbuscular mycorrhizal fungi efficient activity in biology rhizosphere of soil, nor in other indicators such as the stability of organic matter, microaggregates soil and improve its physical properties (29, 30).
Scheme Fertilization in percent (IEEF) showed that the response to treatment 3 was higher, without significant differences with treatment 2. However, if they differ both absolute control treatment that is, the non-application of mineral fertilizers or biofertilizers.

These results indicate that may occur appreciable amounts of forage by decreasing the amount of mineral fertilizers when EcoMic® and FitoMas-E® are applied which are not aggressive to the environment or to the soil. A correspondence with the increased production of biomass (T3) which became effective mycorrhizal symbiosis, as frequency indices and mycorrhizal colonization intensity and number of spores produced so indicate (Table III), is also observed.

Similar results are related to the increase in agricultural yield fruit when inoculation with AMF is made to plants via symbiosis and the use of FitoMas-E® for its importance in the supply of nutrients in its capacity fitoestimulador growth and development plant (33).

In addition it is confirmed in this work, that the joint action of bio-fertilizers and rational use of mineral fertilizer it possible to obtain satisfactory results in agricultural production (33).

In addition, the results of studies in the cultivation of habichuela beans (Phaseolus vulgaris L. var. Verlili) showed the effectiveness of the products in growth, development and yield, where plants received the EcoMic® + FitoMas-E® (34) combination; ie are confirmed by this study, the positive results of the combined use of these bioproducts in different agricultural crops.

Another important aspect was the variable Index Efficiency Scheme Fertilization (IEEF), where a higher percentage of it is shown, with reduced levels of fertilization plus the application of arbuscular mycorhizal fungi and FitoMas-E® (T3), without decreasing forage production in quantity or quality, about where the higher dose of mineral fertilizers (T2) is applied.

It should be noted that the results for this variable (% of IEEF) coincided with those obtained by decreasing the dose of mineral fertilizers with inoculation of AMF and application of FitoMas-E® in growing Psidium guajava. L cv, Enana Roja Cubana (33).

Moreover, these results confirm studies in the maize cultivation (35). When the AMF and FitoMas-E® were applied, improved the efficiency of crop fertilization scheme (IEEF), based on the knowledge of the contents nutritional soil and the use of these bioproducts, a proper management of fertilization is made, improving nutrition of plants grown and achieving a reduction in the dose of fertilizers used.

In Table IV, the economic impact is shown in the production of fodder with treatment 3, which was higher compared to the traditional production technology in the EPG “Niña Bonita” for the production of fodder, because there were savings 74,61 CUP ha⁻¹, by reducing fertilizer dosage without reducing yields. This meant a cost reduction of fertilization of 27,04 CUP to produce one ton of forage, so it can achieve a positive influence on the environmental and economic environment by reducing the application of mineral fertilizers (31).

In addition, controlling the excessive use of mineral fertilizers and promoting the use of biofertilizers, it promotes better crop development and the economy for producers in different crops is improved, such as cereals (36).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Complete formula 14-20-26</th>
<th>Cost of fertilization (CUP ha⁻¹)</th>
<th>Saving (CUP)</th>
<th>Cost of fertilization / T forage (CUP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application of 150, 92 and 120 kg ha⁻¹ of N, P₂O₅ and K₂O</td>
<td>181,86</td>
<td>62,55</td>
<td>-</td>
<td>244,41</td>
</tr>
<tr>
<td>Application of 100, 54 and 70 kg ha⁻¹ of N, P₂O₅ and K₂O + EcoMic® + FitoMas - E®</td>
<td>106,74</td>
<td>45,16</td>
<td>2,90</td>
<td>15,00</td>
</tr>
</tbody>
</table>
CONCLUSIONS

- The application of arbuscular mycorrhizal fungi and FitoMas-E® product helps to reduce the amount of mineral fertilizer in forage production based on triticale, since there were no differences regarding treatment where standards fertilization where it was developed were applied the experiment.
- The cost of fertilizer per ton of forage produced was less when the AMF and FitoMas-E® were applied so it became more profitable forage production.
- Variables forage quality were similar and as a result of the AMF application, greater presence of these fungi was observed on the soil (where they were applied), reflecting greater biological activity in the rhizosphere of the crop, with a positive possible effect on the soil improvement.

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Received: September 30th ,2014
Accepted: February 2nd, 2015

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