Festulolium and annual ryegrass pastures associated with white clover for small-scale dairy systems in high valleys of Mexico

Jair Jesús Sánchez-Valdés¹; Jesús Israel Vega-García¹; Vianey Colin-Navarro¹; Aida Gómez-Miranda¹; María Nayeli Marín-Santana¹; Rodrigo Ávila-González¹; Amalia Susana Jaimez-García¹; Darwin Heredia-Nava²; Ignacio Arturo Domínguez-Vara³; Carlos Manuel Arriaga-Jordán; Felipe López-González¹*

¹ Universidad Autónoma del Estado de México, Instituto de Ciencias Agropecuarias y Rurales (ICAR), Campus UAEM El Cerrillo, El Cerrillo Piedras Blancas, C.P. 50090, Toluca, Estado de México, México.
² Universidad de Guadalajara, Centro Universitario de Los Altos (CUALTOS), Carretera a Yahualica, Km. 7.5, C.P. 47600, Tepatitlán de Morelos, Jalisco, México.
³ Universidad Autónoma del Estado de México, Facultad de Medicina Veterinaria y Zootecnia, Campus UAEM El Cerrillo, El Cerrillo Piedras Blancas, C.P. 50090, Toluca, Estado de México, México.
* Correspondence: flopezg@uaemex.mx

ABSTRACT

Background: The implementation of polyphitic pastures composed of grasses and legumes is an important component of agricultural systems in temperate zones, since grazing pastures which can reduce feed costs— are a viable option for small-scale dairy systems (SSDS).

Objective: To evaluate the continuous grazing of dairy cows in Festulolium pastures associated with annual and perennial ryegrass and with clover in two farms.

Methodology: Two experiments were carried out. The first experiment was established in the municipality of Almoloya de Juárez using eight cows that were divided into two groups of four; the cows grazed on two pastures with Festulolium associated with annual ryegrass and they were fed with 3.6 kg DM/cow/day of commercial concentrate, for 16 weeks. The second experiment was carried out in the Northwest of State of Mexico; six multiparous cows grazed on two pastures, under a cross over design arrangement; one pasture features Festulolium cv Spring Green and the other, annual ryegrass. Milk and body condition ere measured every 3 and 12 d, respectively. Variables from both experiments were analyzed using a split-plot experimental design.

Results: Neither experiment recorded significant differences for the net accumulation of forage, the height of the pastures, and their chemical composition (P > 0.05). No significant differences between treatments (P > 0.05) were recorded regarding the yields and chemical composition of the milk.

Study Limitations/Implications: The study of mixed pastures can be an alternative for feeding grazing cows, helping to reduce costs in SSDS.

Findings/Conclusions: Festulolium/annual ryegrass pastures with white clover are a viable forage alternative for small-scale dairy systems.

Keywords: pastures; milk; grazing; grasses; white clover.
INTRODUCTION

Small-scale dairy systems (SSDS) contribute more than 35% of production in Mexico (Hemme et al., 2009); they have persisted over time and generate a constant income. They are characterised by herds of 3 to 35 cows plus replacements, by farming activities carried out in small farms (Fadul-Pacheco et al., 2013), and by family labour-based production. They have proven to be an option to overcome rural poverty (Espinoza-Ortega et al., 2007).

The economic scale of sustainability is the most vulnerable aspect of these systems, given the high production costs. Feed is the major component of these costs (Fadul-Pacheco et al., 2013; Prospero-Bernal et al., 2017), as a result of the use of large amounts of commercial concentrates, straws, and stubble (Martínez-García et al., 2015), which represent up to 70% of a farm’s expenses (Espinoza-Ortega et al., 2007).

Grazing grass and legume pastures has proven to be a viable option for SSDS. This practice obtains better results than conventional grassland management (mowing and grazing), significantly reducing feeding costs (Pincay-Figueroa et al., 2016; Prospero-Bernal et al., 2017). However, future scenarios about the uncertain availability of irrigation water, changes in rainfall patterns, and an increasing frequency of extreme temperatures—factors which have a direct influence on pasture production—require the development of feeding strategies that enable the optimization of resources within the production unit, seeking the species and varieties of pasture that are best adapted to the agroecological and management conditions of the SSDS (Plata-Reyes et al., 2018).

Perennial ryegrass (*Lolium perenne*)—which is usually associated with white clover (*Trifolium repens*)—is the grass of choice for temperate pastures, but it does not tolerate temperatures above 25 °C or water deficit (Parsons and Chapman, 2000). In this sense, an alternative to the low persistence of perennial ryegrass-based grasslands may be short-duration grasslands based on fast-growing, highly nutritional quality grasses whose lifespan is a long as that of perennial ryegrass grasslands. Grasses that meet these characteristics include annual ryegrass (*Lolium multiflorum*) and hybrids of ryegrass species and species of the genus *Festuca* known as festulolium.

Annual ryegrass is a native species of Europe and North Africa, but it is widely distributed throughout the world. It is a biennial plant used to establish short-term grasslands, because its establishment is faster than other grasses (Humphreys et al., 2003) and it can be grazed within 70 d of sowing. It can achieve high forage yields of excellent nutritional quality and is widely adapted to the different temperate and semi-arid climate regions of Mexico (Hernández-Ortega et al., 2011).

*Fescue* species are more persistent than ryegrass: they have a more developed root system that tolerates low nutrient levels and cold or drought stress. These characteristics improve performance in pastures associated with *Lolium* (Thomas et al., 2003).

Festulolium is an inter-specific hybrid of perennial or annual ryegrass and *Festuca* species. It was developed to have the hardiness and ability to grow in hostile environments of fescue (higher drought tolerance) and the high nutritional value and rapid germination and establishment ability of ryegrass (Touno et al., 2011; Barnes et al., 2013). As a result of its high yield, Festulolium cultivars obtained from the cross between *L. multiflorum* and
Festuca arundinacea have been adapted to Nordic conditions; however, these hybrids have not been evaluated in Mexico.

One of the festulolium varieties available in Mexico is festulolium cv. Barfest, a loliaceum-type festulolium (×Festulolium loliaceum (Huds.) P. Fourn.) resulting from crossing perennial ryegrass with meadow fescue (Festuca pratensis Hudson) (Orloff et al., 2016).

Therefore, the objective of this study was to compare, on the one hand, the continuous grazing of dairy cows on a short-duration fescue pasture associated with annual ryegrass versus a perennial ryegrass pasture. On the other hand, an annual ryegrass pasture was compared with a fescue pasture. All these pastures are associated with white clover and all are located in two small-scale dairy systems farms.

MATERIALS AND METHODS

The work was carried out in two communities in the Toluca Valley, within a rural participatory research framework, specifically through the participatory research approach for livestock technology development. This approach is characterized by on-farm experimentation with the participation of farmers aimed to identify, plan, develop, and establish new management practices that favour the improvement of their production processes and that are disseminated in their communities (Conroy, 2005). Each community was considered as a case study.

Bromatological analyses were performed at the Instituto de Ciencias Agropecuarias y Rurales (ICAR) of the Universidad Autónoma del Estado de México (UAEMex), following the standardized procedures described by Celis-Álvarez et al. (2016).

**Experiment 1**

**Location**

The farm is located in the ejido San Cristóbal, Almoloya de Juárez, in the State of Mexico, Mexico. Its production unit is located at 19° 24’ N and 99° 51’ W, at an altitude of 2650 m.a.s.l. The climate is temperate sub-humid with a rainy season from May to October and a dry season from November to April. The average annual rainfall ranges from 800 to 1,000 mm and the average temperature is 13 °C (Albarrán et al., 2012).

**Experimental specifications**

Eight cows were divided into two groups of four and were randomly assigned to the treatments. Cows were grouped in pairs (blocks), according to the number of calvings, days open, and performance prior to the experiment. The cows in each pair were randomly assigned to each experimental treatment. The experiment lasted 16 weeks.

**Establishment of grasslands**

Two 1.0-ha pastures were used. The first one was established in April 2011 with Festulolium cv. Barfest (Lolium multiflorum × Festuca pratense), associated with annual ryegrass cv. Maximus (Lolium multiflorum) and white clover cv. Ladino (Trifolium repens). The seeding rate was 22.5 kg/ha. 15 kg/ha, and 3 kg/ha for Festulolium, annual ryegrass, and white clover, respectively. The second grassland was established 2 years earlier with
perennial ryegrass cv. Bargala, annual ryegrass cv. Maximus, and white clover cv. Ladino, at of 22.5 kg/ha, 15 kg/ha and 3 kg/ha rates, respectively. At the time of the experiment, the cycle of annual ryegrass had ended and this type of grass was non-existent; therefore, the area was considered a perennial ryegrass pasture associated with white clover. For both pastures, the fertilization rate at sowing was 80-80-60 kg/ha. A maintenance application was carried out every 28 d with 50 kg urea/ha.

**Treatments**

The following treatments were evaluated: FL-AR=grazing of fescue and annual ryegrass with white clover; and PR=grazing of perennial ryegrass and white clover. The cows of both treatments were fed 3.6 kg DM/cow/day of commercial concentrate (18% CP).

**Animal variables**

The cows were milked by hand twice a day (5:00 am and 4:00 pm). The stocking rate was four cows (larger livestock units) per ha. Milk yield (RL) was recorded in kg/cow/day once a week with a 20-kg Torino® clock scale, manufactured in Mexico. The protein and fat composition of milk samples collected every 15 days was determined using an Ekomilk® Ultra 40s ultrasound milk analyzer (BULTEH 2000 Ltd., Bulgaria).

Live weight (LW) and body condition (BC) were measured every 15 d. LW was determined using a 1,000-kg electronic scale while BC was estimated on a scale of 1 to 5 (Yabuta et al., 1997).

**Grassland variables**

Net herbage accumulation (NHA) was estimated following the procedure established by Hoogendoorn et al. (2016), using six $0.70 \times 3.0 \times 0.70$ m exclusion cages and cutting $0.25 \times 2.0$ m quadrats inside and outside, at the start and end of each measurement (15 d). The pasture was nominally divided in two.

The grassland was sampled by simulated grazing to determine the chemical composition of the forage (dry matter (DM), organic matter (OM) and crude protein (CP) content), following the standardized procedures described by Celis-Alvarez et al. (2016).

**Statistical analysis**

Grassland and production response variables were analysed with a split-plot experimental design (Kaps and Lamberson, 2004), according to the following model:

$$Y_{ijk} = \mu + B_i + M_j + E_{ij} + p_k + Mp_{jk} + e_{ijk}$$

Where $\mu$=Overall mean; $B$=Block effect (pair) of cows per lactation stage $i=1,2$; $M$=Effect of treatments (major plot) $j=1,2$; $E$=Residual term for major plots; $p$=Effect of experimental period (minor plot) $k=1,...,16$ (for milk yield); $Mp$=Effect of interaction between pasture type and experimental period; $e$=Residual term for minor plots. When significant differences were detected, Tukey’s test was applied ($P<0.05$).
Experiment 2

Location
The farm is located in the northwest of the State of Mexico, at an altitude of 2440 m.a.s.l., with a temperate sub-humid climate, an average temperature of 14 °C, and an average annual rainfall of 800 mm (Plata-Pérez et al., 2020).

Experimental specifications
Six multiparous cows crossbred with Brown Swiss, weighing between 415 and 480 kg, were used under a statistical cross over arrangement. The cows were in the third stage of lactation, grazed nine hours per day, and were subjected to three experimental periods of twelve days each (nine days for adaptation and three days for sampling and data recording). Cows were fed 2.0 kg DM of concentrate per cow/day (16% CP), divided into two 1-kg rations per day. The concentrate consisted of a mixture of ground corn and soybean paste (80% corn, 20% soybean).

Establishment of grasslands
Two 0.75-ha pastures were used: the first was established with Festulolium cv. Spring Green (Lolium perenne/L.multiflorum × Festuca pratense) and the second with annual ryegrass cv. Westerwold. The grasses were sown in each meadow on 25 November 2016, with a density of 30 kg/ha (ryegrass) and 3 kg/ha (white clover cv. Ladino) and a fertilization dose was 58N-30P-00K. Once the grasses were established, a maintenance fertilization was carried out every 28 days with 100 kg of urea.

Treatments
The treatments evaluated (Tx) were: FL=Grazing of Festulolium cv. Spring Green + white clover; and AR=Grazing of annual ryegrass cv. Westerwold + white clover. The cows from both treatments were provided with free access to water.

Animal variables
The milk yield (MY) was measured on the last 3 days of each experimental period, during the morning and afternoon milkings. One aliquot per day was prepared with the milk from the morning and afternoon milkings, taking the proportion of the yield of each milking. These aliquots were used to determine the protein and fat content with the Ekomilk® Ultra 40s ultrasonic milk analyzer (BULTEH 2000 Ltd., Bulgaria). Live weight (BW) and body condition (BW) were measured every 12 d.

Grassland variables
The NHA (Hoogendoorn et al., 2016) was estimated using six 0.50×0.50×0.80 m exclusion cages in each pasture, cutting 0.40×0.40 m quadrat cuts on the inside and outside, at the beginning and end of each measurement (12 d). To determine the NHA, the pasture was divided into two parts. Pasture heights were recorded every 12 days, using the rising-plate technique described by Hodgson (1994).
Samples were taken from simulated grazing and cut pasture to determine DM, OM, CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) content, following the standardized procedures described by Celis-Alvarez et al. (2016). *In vitro* digestibility of organic matter (IVDOM) was determined by incubation with rumen fluid (López-González et al., 2020). Metabolizable energy (ME) was estimated applying the equation $ME = 0.0157 \times (\text{DOMD})$, where DOMD is organic matter in g/kg DM (AFRC, 1993).

**Statistical analysis**

Grassland variables were analysed with a split-plot experimental design (Kaps and Lamberson, 2004) and animal variables with a Double Crossover design, based on the following mathematical model:

$$Y_{ijkh} = \mu + S_i + C_j(i) + P h(i) + T_l + \epsilon_{ijkl},$$

Where: $\mu$ = Overall mean; $S_i$ = Fixed effect due to sequence; $C_j(i)$ = Random effect due to cow within sequence; $P h(i)$ = Random effect due to period within sequence; $T_l$ = Fixed effect due to treatment; $\epsilon_{ijkl}$ = Experimental error.

When significant differences were detected, Tukey’s test was applied ($P < 0.05$).

**RESULTS AND DISCUSSION**

**Fodder production variables**

Table 1 shows the net herbage accumulation and pasture height in experiments 1 and 2. No significant differences were recorded for the variables evaluated in either experiment ($P > 0.05$). Forage availability in the evaluated pastures is low in both experiments. The NRC (1987) indicates that a forage availability of 2250 kg/ha must be ensured in order to guarantee an adequate intake in grazing cows. The lower forage availability in experiment 2—which was carried out during the dry season—is attributed to the lower ANF (Muñoz-González et al., 2013; Álvarez et al., 2016).

The average NHA in experiment 1 (989 kg DM/ha) is higher than results reported by López-González et al. (2020) for perennial ryegrass (820 kg DM/ha); however, just like the treatments in experiment 2 (807 kg DM/ha), they are lower than the 1747 kg DM/ha in perennial ryegrass and fescue pastures likewise reported by López-González et al. (2017).

The compressed height of the FL treatment (17.3 cm) was greater than BA (12.3 cm) in experiment 2. Pasture height is an indicator of forage availability, Mayne et al. (2000) mention that, in continuous grazing, the meadow should be 5.0-8.0 cm tall to maximize forage consumption (Plata-Reyes et al., 2018). The height of the pastures evaluated in experiment 2 is greater than the heights reported by Plata-Reyes et al. (2018) for festulolium (4.7 cm) and perennial ryegrass (5.5 cm), by López-González et al. (2020) for perennial ryegrass (7.1 cm), and by López-González et al. (2017) for perennial ryegrass cv Bargala (5.5 cm), perennial ryegrass cv Payday (6.1 cm), and Festulolium (5.8 cm).
Table 2 shows the chemical composition of the grasslands evaluated in both experiments. No significant differences (P > 0.05) were recorded for the variables evaluated in both experiments. As a result of low rainfall, the average DM values for FL and BP in experiment 1 are higher than those reported by Plata-Reyes et al. (2018) for Festulolium (212 g/kg) and perennial ryegrass (185 g/kg) associated with white clover.

CP, NDF and FDA content are important parameters of forage quality that determine intake and digestibility of forage. High protein content increases milk yield and milk protein, while NDF and FDA are related to digestibility (Yang et al., 2017).

Table 2. Chemical composition of grazed grassland, cut grassland, and concentrates.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FL-AR</td>
<td>PR</td>
</tr>
<tr>
<td>DM (g/kg)</td>
<td>246.0</td>
<td>251.3</td>
</tr>
<tr>
<td>Ash (g/kg MS)</td>
<td>114.0</td>
<td>113.2</td>
</tr>
<tr>
<td>OM (g/kg MS)</td>
<td>882.5</td>
<td>847.5</td>
</tr>
<tr>
<td>CP (g/kg MS)</td>
<td>170.8</td>
<td>182.2</td>
</tr>
<tr>
<td>NDF (g/kg MS)</td>
<td>615.7</td>
<td>626.1</td>
</tr>
<tr>
<td>ADF (g/kg MS)</td>
<td>320.2</td>
<td>331.7</td>
</tr>
<tr>
<td>DIVOM (g/kg MS)</td>
<td>722.1</td>
<td>723.6</td>
</tr>
<tr>
<td>ME (MJ/kg MS)</td>
<td>9.6</td>
<td>10.4</td>
</tr>
</tbody>
</table>

FL-AR: grazing of fescue and annual ryegrass with white clover; PR: grazing of perennial ryegrass and white clover; FL: Festulolium associated with white clover; AR: annual ryegrass associated with white clover; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; DIVOM: in vitro dry matter digestibility; ME: metabolizable energy; SEMMP: Standard Error of the Mean of the main plot; SEMSP: Standard Error of the Mean of the split plot; NS: Not Significant (P > 0.05).
The CP values of experiment 1 are slightly higher than those reported by Opitz et al. (2006), Dierking et al. (2008), and Touño et al. (2011), who evaluated different festulolium cultivars and, at different stages, obtained a CP content of 138 to 152 g/kg DM. Nevertheless, they fall within the values previously reported for these systems, which range from 176 g/kg DM (López-González et al. 2017), to 209 g/kg DM (Heredia-Nava et al. 2007). The CP content of the treatments in experiment 2 is below that reported by López-González et al. (2017) and Heredia-Nava et al. (2007). The average height of the pastures evaluated by López-González et al. (2017) did not exceed 6.0 cm, while the height of the treatments (14.8 cm) in experiment 2 indicates that the pastures had higher amounts of structural carbohydrates and, therefore, lower CP content. According to Arriaga-Jordan et al. (1999), CP contents for pasture ranging from 160 g/kg DM to 280 g/kg DM are sufficient to cover the requirements of cows with moderate milk production.

The NDF (620.9 g/kg DM) and FDA (326.0 g/kg DM) contents of the treatments in experiment 2 are higher than those reported by López-González et al. (2017) in festulolium and perennial ryegrass (515 g/kg DM for NDF and 265 g/kg DM for FDA) and by Plata-Reyes et al. (2018) in festulolium (485 g/kg DM for NDF and 234 g/kg DM for FDA) and perennial ryegrass (524 g/kg DM for NDF and 219 g/kg DM for FDA).

The DIVMO of the treatments in experiment 2 (722.8 g/kg DM) and ME (10.0 MJ/kg DM) are similar to those reported by López-González et al. (2017): a DIVMO of 721.5 g/kg DM and ME of 11.2 MJ/kg DM in festulolium and perennial ryegrass pastures.

Table 3 presents the results for milk yield and chemical composition, PV, and CC for experiments 1 and 2, showing that there were no significant statistical differences between treatments (P>0.05) for both experiments.

In experiment 1, the FL-BA treatment presented an RL of 17.1 kg/cow/day and a BP of 18.9 kg/milk/day, while the average milk fat and protein content was 31.9 g/kg and 30.0 g/kg, respectively. Cow weighed 544 kg (FL-BA treatment, with a CC of 1.6) and 524 kg (BP treatment, with a CC of 1.8).

The RL in experiment 2 was 10.1 kg/cow/day for the FL treatment and 10.6 kg/cow/day for the BA treatment, while the average milk fat and protein content was 39.1 g/kg and 30.8 g/kg, respectively. On average, cows in the FL treatment weighed 450 kg with a CC of 2.5, while cows in the BA treatment weighed 448 kg with a CC of 2.5.

López-González et al. (2017) and Plata-Reyes et al. (2018) obtained lower yields in grasslands of Festulolium cv. Spring Green associated with white clover cv. Ladino under similar production systems than the yields of experiment 1 (15.8 kg/cow/d and 15.4 kg/cow/d, respectively). However, their yield was higher than the LR of experiment 2. The same authors reported cows that weighted 519 kg and 495 kg, respectively.

Another factor that directly influences the LR is the physiological state of the cow and the third stage of lactation (when the cow is in production), which has an impact on the chemical composition of the milk.

A quality ryegrass pasture can cover the energy requirements for intensively grazed cows with milk yields of up to 30 kg/cow/d (Arriaga-Jordan et al., 1999). Work in small-scale systems has obtained values of 19.0 kg/cow/d (Heredia-Nava et al., 2007) and 14.6 kg/cow/d
(Plata-Reyes et al., 2018), so the results of experiment 1 for the BP treatment are within those reported by these authors.

The average values of milk chemical composition (fat and protein), fall within the parameters acceptable by the Mexican standard -NMX-F-700-2004 COFOCALEC (fat ≥32g/kg and protein ≥31g/kg), which regulates Mexican standards. The fat and protein content of experiment 1 and the protein of experiment 2 result lower than those reported by López-González et al. (2017) (34.3 g/kg for fat and 31.76 g/kg for protein) and Plata-Reyes et al. (2018) (34.2 g/kg fat and 31.8 g/kg protein), however, the milk fat content of experiment 2 is above those reported by these authors.

According to Arriaga-Jordán et al. (2010), ryegrass can be used as a strategy to enrich the feed of dairy cows while decreasing the feed cost and thus increasing the level of sustainability (Juárez-Morales et al., 2017).

### CONCLUSIONS

The FL-AR treatment (Festulolium cv. Barfest and annual ryegrass) associated with white clover, from experiment 1, produced more forage compared to the rest of the treatments evaluated in both experiments.

As there were no differences in yield, milk chemical composition and nutritional quality of the grasslands evaluated in both experiments, it is concluded that the integration of the grasslands evaluated in these production systems represents a good alternative as a feed base for dairy cows.

---

**Table 3. Productive response of cows in small-scale dairy systems.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FL-AR</td>
<td>PR</td>
</tr>
<tr>
<td>MY (kg/cow/day)</td>
<td>17.10</td>
<td>18.90</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>32.40</td>
<td>31.40</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>29.10</td>
<td>30.90</td>
</tr>
<tr>
<td>PV (kg)</td>
<td>544.20</td>
<td>524.50</td>
</tr>
<tr>
<td>CC (1-5)</td>
<td>1.60</td>
<td>1.80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>FL</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MY (kg/cow/day)</td>
<td>10.1</td>
<td>10.6</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>40.0</td>
<td>38.3</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>30.8</td>
<td>30.8</td>
</tr>
<tr>
<td>LW (kg)</td>
<td>450.8</td>
<td>448.7</td>
</tr>
<tr>
<td>BC (1-5)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

FL-AR: grazing of fescue and annual ryegrass with white clover; PR: grazing of perennial ryegrass and white clover FL: Festulolium associated with white clover, AR: annual ryegrass associated with white clover, MY: Milk yield, LW: Live weight, BC: Body condition, SEMMP: Standard Error of the Mean of the main plot, SEMSP: Standard Error of the Mean of the split plot; NS: Not Significant (P>0.05).
ACKNOWLEDGEMENTS

The authors would like to thank the farmers for their active participation and commitment to this research, whose privacy is respected and whose names are not mentioned.

Funding. This research was carried out thanks to funding from the Universidad Autónoma del Estado de México. Conflict of interest. The authors confirm that there are no known conflicts of interest associated with these publications.

Compliance with ethical standards. This research was approved by the Institutional Committee for the Care of Laboratory, Teaching, Research, Service and Production Animals, following the procedures approved by the Universidad Autónoma del Estado De México.

Data availability. Data are available from the correspondent upon reasonable request.

REFERENCES


