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# Preference on silage feed intake and its effect on ovarian activity in *Pecari tajacu*

## Preferencia del consumo de ensilado y su efecto sobre la actividad ovárica del *Pecari tajacu*

**Montes-Pérez Rubén* ruben_montes_p@hotmail.com, Borges-Ventura David david.borges.v@gmail.com Solorio-Sánchez Francisco ssolorio@correo.uady.mx, Sarmiento-Franco Luis luis.sarmiento@correo.uady.mx , Magaña-Monforte Juan jmagana@correo.uady.mx**

Campus of Biological and Agricultural Sciences, Autonomous University of Yucatan. Merida Yucatán, Mexico * Responsible and correspondence author: Montes-Pérez Rubén. Campus of Biological and Agricultural Sciences, Autonomous University of Yucatan. Mérida-Xmatkuil road, km 15.5, Mérida. Yucatán, Mexico, CP. 97315

**ABSTRACT**

The objective of the current study was to evaluate the preference of the consumption of four silage formulations, and their effect of the preferred silage on the ovarian activity of adult collared peccaries subjected to 35 days of *ad libitum* feed intake. In the first experiment, 12 adults *Pecari tajacu* that are non-pregnant females were used to which they were offered four silage mixtures, to measure the preference, through a 4 x 4 Latin square design. In the second experiment, five females were offered a diet with the most favorite silage, pumpkin, and corn, and five others consumed corn and pumpkin. The blood progesterone levels of each female were measured weekly. The first experiment showed that the silage mixture with 55% of *Brosimum alicastrum*, 40% of *Pennisetum purpureum* and 5% of molasses was preferred (P <0.05). All the females in the group that consumed the diet with the preferred silage formulation and the corn-and-pumpkin-based diet showed one to two peaks of progesterone throughout the experiment. It is concluded that females submitted to diets with 40% silage of *P. purpureum*, 55% of *B. alicastrum*, 5% of molasses, corn, and pumpkin do not show alteration of the ovarian cyclicity during 35 days.

**Keywords**: collared peccary; estrous cycle; silage; progesterone; zaino.

**RESUMEN**

El objetivo fue evaluar la preferencia del consumo de cuatro formulaciones de ensilado, y el efecto del consumo del ensilado preferido sobre la actividad ovárica de pecaríes de collar adultas sometidas a 35 días de consumo a libertad. Primer experimento, se utilizaron 12 hembras adultas *Pecari tajacu* no gestantes a las que se les ofrecieron cuatro mezclas de ensilajes, para medir la preferencia, mediante cuadro latino 4 x 4. En el segundo experimento, se ofrecieron durante 35 días a cinco hembras una dieta con ensilaje preferido, calabaza y maíz, otras cinco consumieron maíz y calabaza. Se midieron semanalmente los niveles de progesterona sanguínea de cada hembra. El primer experimento mostró que la mezcla de ensilado con 55 % de *B. alicastrum*, 40 % de *P. purpureum* y 5% de melaza es la preferida (P < 0.05). Todas las hembras del grupo que consumió dieta con la formulación de ensilado preferido y de la dieta a base de maíz y calabaza mostraron de una a dos elevaciones de progesterona en todo el experimento. Se concluye que las hembras sometidas a dietas con ensilados de 40 % de *Pennisetum purpureum,* 55% de *Brosimum alicastrum*, 5% de melaza, maíz y calabaza no muestran alteración de la ciclicidad ovárica durante 35 días.

**Palabras clave:** ensilado; ciclo estral; pecarí de collar; progesterona; zaino.

## INTRODUCTION

In Mexico as of 1997, the Wildlife Conservation and Productive Diversification Program in the Rural Sector (SEMARNAP, 1997) assessed the importance of the wildlife use in the country, identifying the legal markets for the use of live animals, meat and byproducts thereof, especially of various species of artiodactyla; some of these are deer (*Odocoileus virginianus*, *Mazama americana*) and peccary (*Pecari tajacu*) (Briceño 2011, Montes *et al*., 2018). In Peru and Brazil, this type of studies have also been carried out, their progress demonstrated that *P. tajacu* especially has the biological and productive features to be integrated into animal production systems, because they generate meat for human consumption and skin for the fur industry (Rengifo, 2008). Jori *et al*. (2004) reported that a production system in large populations of *P. tajacu* have economic viability, because they generate gross income of $ 32 USD per animal. The main variable that limits generating higher net income is the cost of food, which represents 77 % of the total, due to the use of corn, papaya leaves and Opuntia. Based on this variable, several investigations have been carried out to replace inputs in food, especially grains for human consumption, by fresh forages enriched with urea (Montes-Pérez *et al*., 2012a, Oliveira *et al*., 2009). In this sense, it is necessary to test the forage of native species as food for *P. tajacu*, a main characteristic, is that this type of food is of low cost, compared with the use of commercial feed that is used for pork; native forages represent an option that could overcome this limitation (Ramírez, 2009).

Collar peccaries have the ability to consume fresh forage, because they generate volatile fatty acids from the fermentation of the forage fiber (Montes-Perez *et al*., 2012a); the use of conserved forage is an ideal alternative in situations when the low availability of this occurs periodically, such as occurs in regions with warm subhumid climates, with native vegetation of low deciduous forest, where there is a shortage of forage at the time of drought (Durán and García, 2010; Flores *et al*., 2010). A method of conservation of low cost and that allows maintaining the nutrients of the fodder is the silage (Reyes *et al*., 2009); There is little information on the silage forage use for the feeding of *P. tajacu* (Borges-Ventura *et al*., 2014), and there are no reports of the effect of its consumption on reproductive function, an aspect that is important to evaluate in captive populations fed with forage conserved in microsilos.

The evaluation of the effect of diets based on silage on ovarian activity in *P. tajacu* will allow determining its effect on the reproductive activity, as an animal feeding strategy at the level of agroecosystems, especially for small producers at critical times or for the establishment of new productive systems of wild fauna in captivity.

The objective of the present work was to evaluate the preference of silage forage consumption with different inclusion percentages based on *Leucaena leucocephala, Pennisetum purpureum and Brosimum alicastrum* and the effect of the favorite silage consumption on the ovarian activity of *Pecari tajacu*.

## MATERIAL AND METHODS

### Site of the experiments

The experiments were carried out in the Xmatkuil Wildlife Management and Conservation Unit of the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Yucatan, Mexico, located at 200 51' 20" North latitude and 890 36' 55" West longitude; 10 meters above sea level. The climate is warm sub-humid type, Awo classification, with rains in summer and beginning of winter and dry season the rest of the year (Montes-Pérez *et al*., 2018).

### Preparation of silage treatments for the preference test

The diets were formulated from the crude protein (PC) requirements; greater than 10% reported by Sowls (1997) for *P. tajacu*.

The bromatological determination of the formulation ingredients was made for the offered food, according to the AOAC techniques (1990); they included the analysis of Crude Protein (PC), neutral detergent fiber (NDF) and dry matter (DM).

The forages of *Pennisetum purpureum* (hereinafter referred to by its common name Taiwan pasture), *Leucaena leucocephala* (called Huaxin) and *Brosimum alicastrum* (called Ramón) were processed in fresh with a mechanical mincer; subsequently, the mixture of the ingredients was carried out according to the treatments to be tested (Table 1). All the silages were added with 5% molasses, according to what was reported by Valencia *et al*. (2011).

**Table 1. Composition of the treatments that show the inclusion values of each ingredient.**

| Treatments | Forage (% of inclusion) | Grass (% de inclusion) |
|---|---|---|
| T1 | Ramón (45) | Pasto Taiwan (50) |
| T2 | Ramón (55) | Pasto Taiwan (40) |
| T3 | Huaxín (25) | Pasto Taiwan (70) |
| T4 | Huaxín (30) | Pasto Taiwan (65) |

All treatments contain 5% molasses

The microsilos of 20 kg of capacity were opened to the 90 days after its elaboration to be used in the period of adaptation, the test of preference and the consumption to freedom during 35 days to evaluate its effect on the ovarian cyclicity.

### Adaptation period

Prior to the experiment of preference, silage was offered during a period of adaptation of seven days to 12 adult females, average age of 4.5 (± 1.5) years and weight of 17.5 (± 0.6) kg; the different treatments were offered during a period of four hours a day, before delivering the daily food based on fruits and vegetables of the season (papaya, pumpkin or cucumber and eventually corn).

### First experiment or preference test

The same non-pregnant adult females were separated into two groups of six, housed in pens of 100 $m^2$. The food was supplied separately using plastic containers. A 4 x 4 Latin box design was applied (Table 2) offering the four silage treatments in four different positions for four days in the two pens. 4 kg of each

treatment was offered for four hours, the rejection was heavy to calculate daily consumption per animal on a dry basis. After this time, the animals received the basic diet described above. Four replicates of the experiment were practiced using the same conditions.

**Table 2. A 4x4 Latin box design showing the placement of the positions by corral, days and treatments mentioned in Table 1**

| Day | Position | | | |
|---|---|---|---|---|
| | North | South | East | West |
| 1 | T1 | T2 | T3 | T4 |
| 2 | T2 | T3 | T4 | T1 |
| 3 | T3 | T4 | T1 | T2 |
| 4 | T4 | T1 | T2 | T3 |

The response variable was the average consumption per individual of silage on a dry basis per animal per day. The results were analyzed by the ANOVA for a Latin box design; the multiple means comparison was performed with the Tukey test, with the Statgraphics Centurion XVI software (Statpoint Technologies, Inc., 2013) to evaluate the results.

## Second experiment

The animals were offered two diets for 35 days: control diet (tr1, n=5) based on 76.4% of local pumpkin and 23.6% of ground corn; Silage-based diet (tr2, n=5) composed of 50% of the pumpkin and corn mixture, with 50% silage from Taiwan grass (*Pennisetum purpureum*) and Ramon (*Brosimum alicastrum*). The silage consisted of 55 % Ramón, 40 % Taiwan and 5 % molasses. The individual average consumption per week of each treatment group was measured.

## Bromatological composition of the preferred silage and the control diet

Table 3 shows the amounts of the nutrients in the two treatments. The amount of PC contributed by each tr1 treatment (pumpkin and corn) and tr2 (pumpkin, corn and silage) was greater than 10%, which is the minimum requirement for *P. tajacu* (Sowls 1997).

**Table 3. Bromatological content of treatments tr1 and tr2**

| Treatment | PC (% DM) | NDF (% DM) | DM (%) |
|---|---|---|---|
| **tr1 (pumpkin and corn)** | 10.52 | 7.64 | 26.48 |
| **tr2 (silage, pumpkin and corn)** | 11.49 | 21.54 | 36.95 |

PC is crude protein, NDF is neutral detergent fiber, DM is dry matter

During the 35 days of the test, samples of 1 ml of blood were taken every 7 days per animal, after physical and chemical containment (Montes-Pérez *et al*., 2014). The blood samples were placed in test tubes with aqueous solution of 10% EDTA, centrifuged at 3000 X G (HERMLE, USA) for 3 minutes. The plasma was stored at -12 °C until the determination of progesterone levels. The blood samples were processed according to Montes-Pérez *et al*. (2014), using the Coat-A-Count kit, Siemens, USA (TKPG1), to estimate blood progesterone levels.

The blood progesterone levels of each animal were plotted against the sampling period. The response variables were: average individual consumption in dry matter per week, blood progesterone levels per animal for 35 days and number of animals

that cycled and therefore showed elevations of blood progesterone above 10 ng/ml during at least one sampling (Montes-Pérez *et al*., 2012b).

Fisher's exact test was applied to evaluate the differences in the number of females that showed ovarian cycle between treatments, the t-student test was used to evaluate the difference of the means of progesterone levels between treatments. The Kolmogorov-Smirnov test was used to calculate the maximum distance between the cumulative distributions of blood progesterone levels between the two groups. Statgraphics Centurion (Statpoint 2013) was used to evaluate the significance of the differences.

## RESULTS AND DISCUSSION

### Preference for the consumption of the four silage formulations

The variance analysis of dry matter consumption among the four silage mixtures showed significant differences. The 95% confidence intervals between the treatments are shown in figure 1. There is a significant difference ($p < 0.01$) between T1 and T2 and of these with respect to the rest; except between T3 and T4 ($p > 0.05$). The silage treatment that showed the highest consumption by peccaries was T2.

The T2 was chosen, silage containing: Ramón (55%), Pasture Taiwan (40%) and with Molasses (5%); To test consumption ad libitum for 35 days on the response in blood progesterone levels, from this moment it is called tr2.

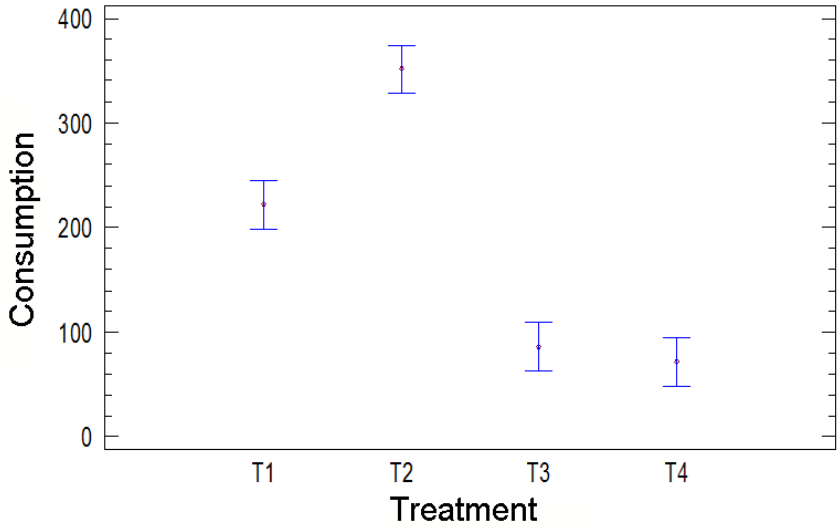


**Figure 1. Daily consumption of dry matter per animal (g), means and confidence intervals in the different treatments applied**

### Dry matter consumption values of the two treatments tested in 35 days

There are significant differences between the consumptions of the weekly total average treatments in dry matter between tr1 and tr2 (Fig. 2) ($p < 0.01$).

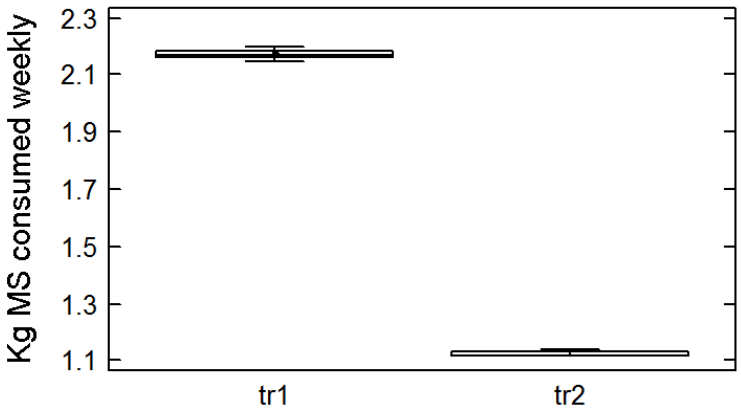


**Figure 2. Individual average consumption per week in kg of dry matter (kg DM consumed) of tr1 (diet based on corn and pumpkin), tr2 (silage, pumpkin and corn).**

## Blood progesterone levels in the two treatment groups

Lower blood progesterone levels ranged from 1 to 9 ng/ml, and those greater than 10 to 70 ng/ml. Figure 3 shows the total blood progesterone levels of the females subjected to the two treatments, which did not show a significant difference (p> 0.05). Fisher's exact test showed that there is no significant difference (p> 0.05) in the amounts of animals that cycled between the two treatments.

Figure 4 shows the Kolmogorov-Smirnov contrast result, which is the distribution of the cumulative percentages of progesterone levels in both treatments, which fluctuate along a linear relationship, whose maximum distance between them is equal to 0.1667 ( p> 0.05); therefore, it indicates similarity of the tendencies in blood progesterone levels in the animals subjected to the two treatments during the study period. All the females of group tr1 (figure 5) and tr2 (figure 6), showed at least one luteal phase, characterized by at least one elevation of blood progesterone greater than 10 ng / ml.

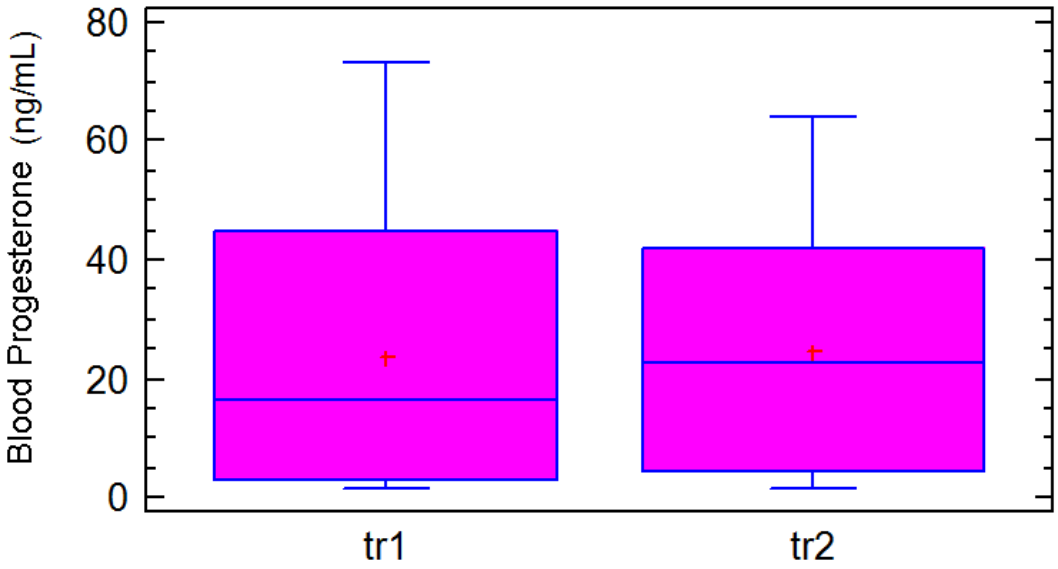


**Figure 3. Blood progesterone levels of *P. tajacu* females subjected to treatments tr1 and tr2**

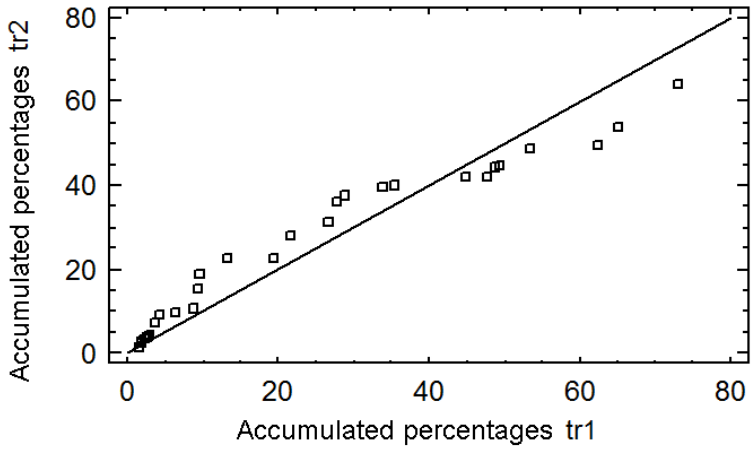


**Figure 4. Quantile of the accumulated percentages of blood progesterone levels in the animals of the two treatments, showing the distribution between them, and whose significance value is p> 0.05**

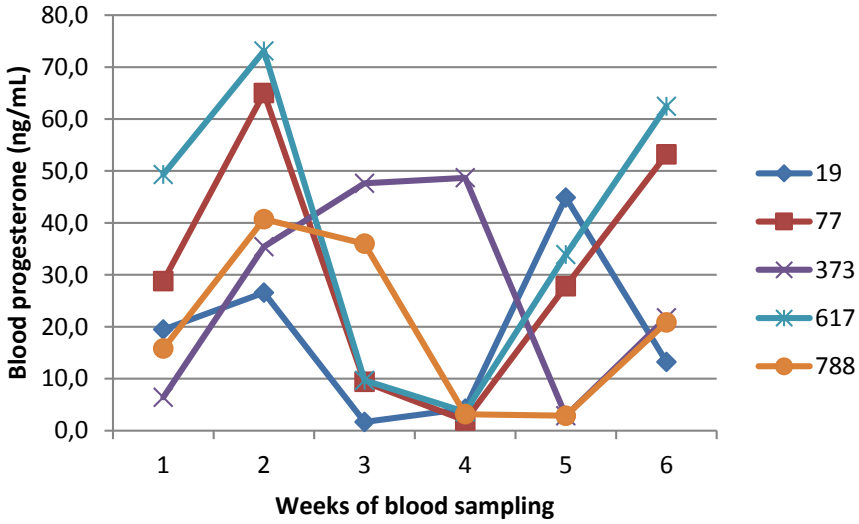


**Figure 5. Profiles of the blood progesterone levels of female peccaries subjected to the consumption of tr1.**

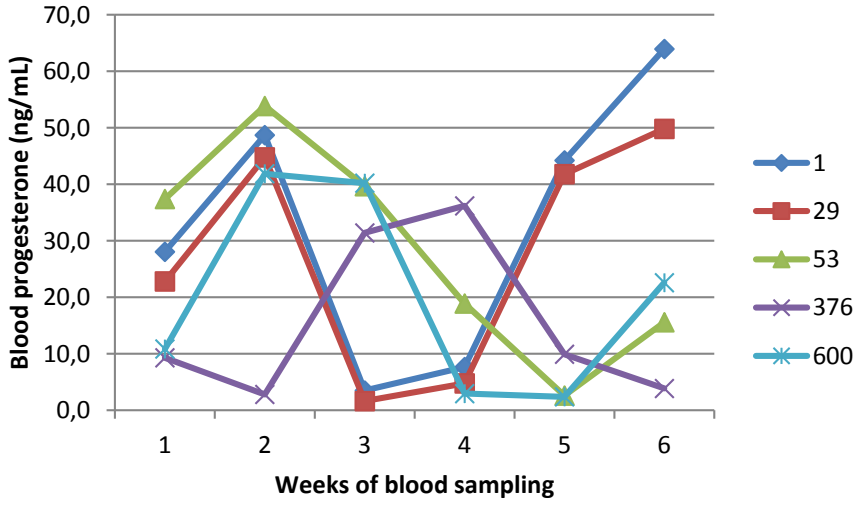


**Figure 6. Profiles of the blood progesterone levels of female peccaries subjected to the consumption of tr2.**

## General discussion of the results

It has been reported that collared peccaries prefer to consume fresh forage of *B. alicastrum* and in smaller amount *P. purpureum* (Montes-Pérez *et al*., 2012a), because *B. alicastrum* has a greater quantity of PC (15.9 vs 12.2%) and greater digestibility (70.8 vs 58.7%), (Plata *et al*., 2009). It has also been reported (Di Marco 2011) that forages with values less than 50% NDF and PC greater than 15%, are of high nutritional quality, and those that contain more than 65% NDF and less than 8% PC they are of low quality. In this work the conditions are met so that the tested foods are considered of high quality.

The results of the consumption preference of the silage formulation of *B. alicastrum* (55%), *P. purpureum* (40%) and molasses (5%) corresponding to T2, it is congruent with the results of Montes-Pérez *et al*. (2012a) for fresh forage, since this formulation contains the highest proportion of *B. alicastrum*; therefore, the preservation of silage allows the nutritional characteristics contained in fresh forage to be conserved, according to Mendoza and Martínez (2010) report a loss of 1 to 2% in silage feed nutrients, when the method is properly applied.

The consumption of the diet with silage in the second experiment is significantly lower than the control diet, this result is important, because it shows that although silage meets the necessary values in PC, the palatability is low compared to the control diet. However, the low consumption of diet formulated with silage apparently does not affect the ovarian activity of the females, because the cyclicity shown by the presence of one to two elevations of blood progesterone with values higher than 10 ng/ml indicate ovulations and therefore growth and maturation of ovarian follicles to become pre-ovulatory follicles, in normal periods of time for what is reported for this species, which is between 18 and 36 days (Montes-Pérez *et al*., 2014).

Four females (80%) of tr1 presented two elevations of blood progesterone, as in tr2, and the maximum values of progesterone in both groups were similar, which indicates that the luteal tissue in the females of both groups have the same capacities endocrine cells to synthesize and release ovarian steroids. In addition, the progesterone decreases reached baseline values between 2 and 9 ng/ml, which indicates luteolytic process after cyclic elevations, which allow the growth and maturation of pre-ovulatory follicles at the end of each stage of the right-handed and during the follicular phase of the cycle ovarian (Montes-Pérez *et al*., 2014).

Ovulations are the product of adequate ovarian functioning when the supply of nutrients is also adequate, since at least the requirement of proteins and energy are covered; otherwise the deficit in some of these requirements stops or alters the ovarian activity, as a compensatory mechanism of the organism to maintain homeostasis in conditions of nutritional stress (Campos and Hernández, 2008).

From these results, it is necessary to propose a second stage of research, which would be to carry out this study in the long term and in different breeding systems, with other forage sources.

## CONCLUSION

Adult non-pregnant females of *P. tajacu* prefer to consume silage formulation containing 55% *B. alicastrum,* 40%, *P. purpureum* and 5% molasses; the *ad libitum* consumption test of silage diets for 35 days did not affect the ovarian activity during the treatment period of both groups (p> 0.05); there were no significant differences in blood progesterone levels and their corresponding cumulative distribution between the two treatment groups (p> 0.05).

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