

IMMUNOCASTRATION WITH VACCINATION AGAINST GONADOTROPIN-RELEASING FACTOR (GnRF) ON THE PRODUCTION PERFORMANCE OF RAMS

Immunocastración con Vacunación en Contra del Factor Liberador de Gonadotropinas (GnRF) Sobre el Comportamiento Productivo de Carnerillos

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ABSTRACT

The objective of this study was to investigate the immunocastration using against gonadotropin-releasing factor (GnRF) with Bopriva® (Zoetis) on the performance of production variables, meat quality, docility, and scrotal circumference in young rams, from Sheep production center "Tupac Amaru", Junin region, Perú. A total of 60 animals were divided into four treatments: T1, T2, and T3 with 0.50 mL, 0.75 mL, and 1.0 mL of doses with Bopriva®, respectively, and T4 (control - 1.0 mL of placebo (distilled water)), with fifteen animals in each. The variables were measured every 15 days for three months (September to November 2018). Datasets were assessed by one-way ANOVA and subsequent posthoc Tukey's test to detect significant differences among treatments. The results indicated differences found between treatments with the immunocastration compared with non-castrated animals for final weight, carcass weight and performance, scrotal circumference, and docility. Growth performance and meat quality characteristics were not adversely affected by immunocastration. Likewise, immunocastration with Bopriva proved to be effective to stop scrotal development and consequently reduces the sexual and aggressive behavior of young rams. The dose of 0.5 mL seems to be adequate due showed better performance for most variables compared to other treatments

Keywords: GnRF, Immunocastration, Vaccine, Young rams

RESUMEN

El objetivo de este estudio fue investigar la inmunocastración usando el factor liberador de gonadotropina (GnRF) con Bopriva® (Zoetis) sobre las variables de producción, calidad de la carne, docilidad, y circunferencia escrotal de carneros jóvenes del centro de producción de ovejas "Tupac Amaru", de la Región Junín, Perú. Un total de 60 animales fueron divididos en 4 tratamientos: T1, T2, y T3 con 0.50 mL, 0.75 mL, y 1.0 mL de dosis con Bopriva®, respectivamente, y T4 (control - 1.0 mL de placebo (agua destilada)), con 15 animales en cada tratamiento. Las variables fueron medidos a cada 15 días por tres meses (setiembre a noviembre de 2018). Los datos obtenidos fueron evaluados por análisis de varianza ANOVA y subsecuente post-hoc Tukey test para detectar diferencias significativas entre los tratamientos. Los resultados indicaron diferencia significativa entre los tratamientos con inmunocastración comparado a los animales no castrados para el peso final, peso de la carcasa y desempeño, la circunferencia escrotal y docilidad. El crecimiento y las características de la calidad de la carne no fueron adversamente afectados por la inmunocastración. Asimismo, la inmunocastración con Bopriva provo ser efectivo para parar el desarrollo escrotal, consecuentemente reduciendo el comportamiento sexual y agresividad de carnerillos jóvenes. La dosis de 0.5 mL parece ser el más adecuado debido a que mostró mejor desempeño para la mayoría de las variables comparado a los otros tratamientos.

Palabras clave: GnRF, Inmunocastración, Vacuna, carnerillo

INTRODUCCION

Surgical castration in lambs is frequently performed to control the genetics of the flock, reduces aggressive and sexual behavior, improved carcass and meat quality, and weight gain (Amatayakul-Chantler et al., 2013; Gökdal et al., 2010; Sales, 2014). However, the castration involves different surgical procedures (rubber ring, short scrotum, and Burdizzo), which are painful, distressing to the animals, and may cause wound infections and increased incidence of mortality (Melches et al., 2007; Morales et al., 2017).

In Perú, Junín sheep breed production is mainly performed in the Andean regions of Perú, where most are bred for wool and meat production that posteriorly are introduced into local and/or international markets (INTA, 2009; Valerio et al., 2015). The Junín breed shows great adaptation to grazing in the native alto Andine meadows. It also presents a good precocity, muscular conformation, great elevation, strength, a wide and deep chest that highlights its butcher's ability. In total, about 80% of rams at a young age are brought to fattening, whose process is carried out under extreme conditions and natural grassland as the main food (Leon-Velarde et al., 2004). Then, to improve the wool and meat production, most of these animals in all production systems are castrated. A similar practice is also conducted in other Latin American countries (Muñoz et al., 2019; Teixeira et al., 2018). Therefore, there is an urgent need for the development and implement new castration methods taken into consideration animal welfare.

Immunization against gonadotropin-releasing factor (GnRF) represents a friendly alternative to the physical castrations of animals. In males and females, GnRF plays a crucial role in the regulation of sexual activity and energy balance (Shahjahan et al., 2014). GnRF is essential for the functioning of the hypothalamic-pituitary-gonadal axis, and immunocastration blocks its action but does not affect FSH (follicle-stimulating hormone) and LH (luteinizing hormone) receptors, only their secretion from the pituitary (Amatayakul-Chantler et al., 2013; Ciechanowska et al., 2016).

Bopriva® (developed by Zoetis) is an anti-GnRF vaccine designed and applied effectively in cattle (Balet et al., 2014). Based on its antibody production may suppress scrotal function more effectively and for a longer duration than other anti-GnRF vaccines (Pfizer, 2018). The effectiveness of vaccination against GnRF with Bopriva® has been demonstrated as a simple approach for the immunocastration in several studies. For instance, using immunization against GnRF with Bopriva®, Janett et al. (2012) evaluate the effect of in prepubertal bull calves on body weight and scrotal circumference; Wicks et al. (2013) assessed the effect on the scrotal function of boars, Amatayakul-Chantler et al. (2013) investigated the effect on carcass and meat quality in bulls; Hirsbrunner et al., (2017) evaluated the suppression of cyclic activity in cattle, and Janett et al., (2012) assessed the scrotal development, serum testosterone levels and physical activity of pubertal bulls.

However, there is little knowledge about the implications of its use, results, and consequences in sheep raised in high Andean areas, for this reason, the present study aimed to evaluate the effects of vaccination with Bopriva® in young rams on production variables (weight initial, weight final, weight gain/day, and dressing percentage), meat quality (pH, total

protein, ash, L*, a*, and b*), docility and scrotal diameter in young rams from Junin region, Perú.

MATERIALS AND METHODS

Animals and treatments

From September to November 2018, sixty healthy young rams (mean weight 29.02 ± 1.87 kg and ages between 10-12 months) of breed "Junín" from a sheep production center called "Sais Túpac Amaru" (latitude -11.980080 and longitude -75.724765) were selected randomly (of a total of 750 animals). Sais Túpac Amaru is located in the Junin Region - Perú, at 3587 m.a.s.l., and has average temperature and relative humidity of 8 °C, and 70%, respectively. All animals here are fed exclusively on natural grassland, being the *Festuca humilior* and *Alchemilla Pinnata* species the more abundant.

The 60 young rams selected were divided into four treatment groups randomly, with fifteen animals in each. Then, the animals of the first three treatment groups were vaccinated subcutaneously with 0.5 mL (T1), 0.75 mL (T2), and 1.0 mL (T3) with Bopriva® (400 µg/mL GnRF-protein conjugate, Pfizer Animal Health, Parkville, Australia), while the animals belonging to the four treatment (T4: control) were vaccinated with 1.0 ml of placebo (distilled water). In total were carried out two subcutaneous vaccinations with an interval of 21 days according to the recommendation of the product.

Production variables analyses

To evaluate treatment effects, animals were examined every fifteen days for three months, being in a total of 6 periods. At each period/occasion, young rams were weighted throughout a calibrated cage-type balance (Scales LLC Model VS-660, Animal Scale, USA) with a capacity of 100 kg. Weight increases were obtained by subtracting the final weight minus the initial weight and divided by the days of fattening. After the final period, the animals were weighed and sent to slaughter. Then, the hot carcass weight and fat covering (scores were based on the tissue thickness (both fat and lean tissue) at the Girth Rib (GR) site) were recorded. Subsequently, the carcasses were sent to cooling for 48 h, at room temperature. After cooling, the dressing percentage was measured by dividing the carcass weight by the live weight and multiplied by 100 (Sobrinho et al., 2013). Each right carcass side underwent the removal of a sample (~15.0 cm) of meat (in total 15 samples), which were deboned, labeled, vacuum packed, and sent to the Laboratory (in total 15 samples of meat) in iceboxes for analysis.

Meat quality analyses

The meat quality analyses were conducted after 48 h post-mortem in the laboratory of Food of the Faculty of food industries from the Universidad Nacional del Centro del Perú (UNCP). In the laboratory, each sample was cut into 3 cm-thick steaks (three steaks by muscle). The meat still fresh was cleaned for subcutaneous fat and examined for instrumental color, followed by pH, total protein, fat, and finally the ash content analysis.

Color measurements were in three different points (randomly) on the freshly cut surface of the steaks, exposed to 3 °C for 1

h with a CR Minolta 400 Chroma Meter colorimeter (Konica Minolta Optics, Inc., Osaka, Japan), that work on the principle of meat color comparison regarding standard color values. These measurements are following the specifications of the Commission International on Illumination (CIE), which list three values: Lightness (L^*) indicates lightness, range 0 (black) to 100 (white), redness (a^*) show red-green ratio, and yellowness (b^*) blue-yellow ratio (AMSA, 2012). For meat evaluation were used the following values: $L^* = 0 - 30$ (poor luminosity), $31 - 60$ (normal meat), $61 - 100$ (altered meat), if the values are negative, the meat is definitely of low quality. For $a^* = 0$ to 6 (bad meat), $7-10$ (low quality meat), $11 - 35$ (apparently normal meat), $36 - 50$ (normal meat), $51 - 100$ (bloody meat), negative values indicate a green color that corresponds to a meat in a state of decomposition; and $b^* = 0 - 10$ (normal meat), $11 - 25$ (apparently normal meat), $26 - 50$ (meat apparently in decomposition), $50 - 100$ (meat decomposition several days, poor quality), and negative values indicate meat with bruises or in a state of decomposition.

The pH was measured 24 h after slaughter using an LP16 Mettler-Toledo penetration electrode, which was introduced into the center of the steaks (three measurements per steak). Total protein, fat, and ash were determined by proximal analysis (AOAC, 2006).

Docility and reproduction variable analyses

Scrotal circumference was measured using a metal tape; it starts moving testicles firmly to the neck of the scrotum and is measured at the widest diameter (Unchupaico et al., 2018). The flight speed (FS) test developed by (Burrow, 1997) was used to measure the docility of the animals. In this case, a corridor of 5 m was set up to measure in m/s the time taken by an animal to move a set distance after exiting a weighting scale through the corridor covered on both sides without seeing other

animals until its two forelimbs cross the finish line. Lower speed in m/s refers to quieter animals, while higher speeds refer to more nervous animals.

All animal experimentation was performed following approval from the local Ethics Committee of the Universidad Peruana Los Andes – Junín, Perú. Besides, animal welfare protocols and permission to use the animals from the company were obtained.

Statistical analysis

The statistical analysis was conducted with four types of treatment for animal performance information, production variables (carcass characteristics), meat quality attributes (pH, total protein, fat content, ash, and color), docility, and scrotal circumference. One-way analysis of variance (ANOVA) and subsequent posthoc Tukey test with 95% significance were performed to determine differences among treatments. Normality was assessed through the Shapiro-Wilk test. All analyses were performed using R software, version 3.3.6 (R Team Core, 2019).

RESULTS

Production variables

Table 1 is presented the effect of immunocastration treatments with Bopriva® at three different doses (T1: 0.5 mL, T2: 0.75 mL, and 1.0 mL) and the control group (T4: 1 mL distilled water (Placebo)). No differences ($p > 0.05$) were found among treatments for the initial weight (Table 1). Likewise, treatment 1 (0.5 mL Bopriva®), showed better performance for most production variables (except final weight). Carcass weight and dressing percentage no showed a significant difference ($p > 0.05$) among treatments (Table 1).

Table 1. Effect of immunocastration treatments with Bopriva® and control on production variables.

Variables	T1 (n=15)	T2 (n=15)	T3 (n=15)	T4 (n=15)	ANOVA p-value ^A
Initial weight (kg)	28.3 ± 5.1 ^a	28.1 ± 2.6 ^a	30.0 ± 3.6 ^a	29.7 ± 4.7 ^a	0.12
Final weight (kg)	35.9 ± 4.3 ^a	35.1 ± 2.8 ^a	37.1 ± 3.6 ^b	35.1 ± 2.8 ^a	***
Weight gain/day (g)	84.4 ± 23.0 ^a	78.0 ± 19.7 ^b	79.6 ± 17.0 ^b	60.8 ± 27.4 ^c	***
Total increase (kg)	7.6 ± 2.0 ^a	7.0 ± 1.7 ^b	7.1 ± 1.5 ^b	5.4 ± 2.4 ^c	***
Carcass weight (kg)	13.5 ± 0.6 ^a	13.0 ± 0.5 ^a	13.3 ± 0.8 ^a	11.7 ± 0.2 ^b	***
dressing percentage (%)	38.1.0 ± 4.4 ^a	37.3 ± 3.0 ^a	36.1 ± 4.0 ^a	34.1 ± 4.8 ^b	***

^A Values on each horizontal line followed by the same letter do not differ significantly ($p > 0.05$). T1: 0.5 mL Bopriva®, T2: 0.75 Bopriva®, T3: 1.0 mL Bopriva®, and T4 (control): 1.0 mL Placebo (distilled water).

Meat quality

In Figure 1 are shown the meat quality variables for both, nutritional content (pH, total protein) and meat quality (lightness (L^*), redness (a^*), and yellowness (b^*)) for each treatment and the control group. For nutritional content, the higher and lower pH value was observed in the treatment 2 and control group, respectively. Significant differences ($p < 0.05$) were found among treatment 2 and the other treatments and control group. The total protein showed similar values for all treatments. Likewise, ash presented similar values for all treatments, but

with a significant difference among treatment 2 and the other treatments.

For meat quality variables, treatment 1 showed higher a^* (14.6 ± 0.06) and b^* (6.7 ± 1.0) values compared to other treatments and group control, but minor value for L^* (14.6 ± 0.06). Significant differences were found between treatments 1 and 3 compared to group control for both L^* and a^* variables (Figure 2). Significant differences were found among treatments for all variables (L^* , a^* , and b^*)

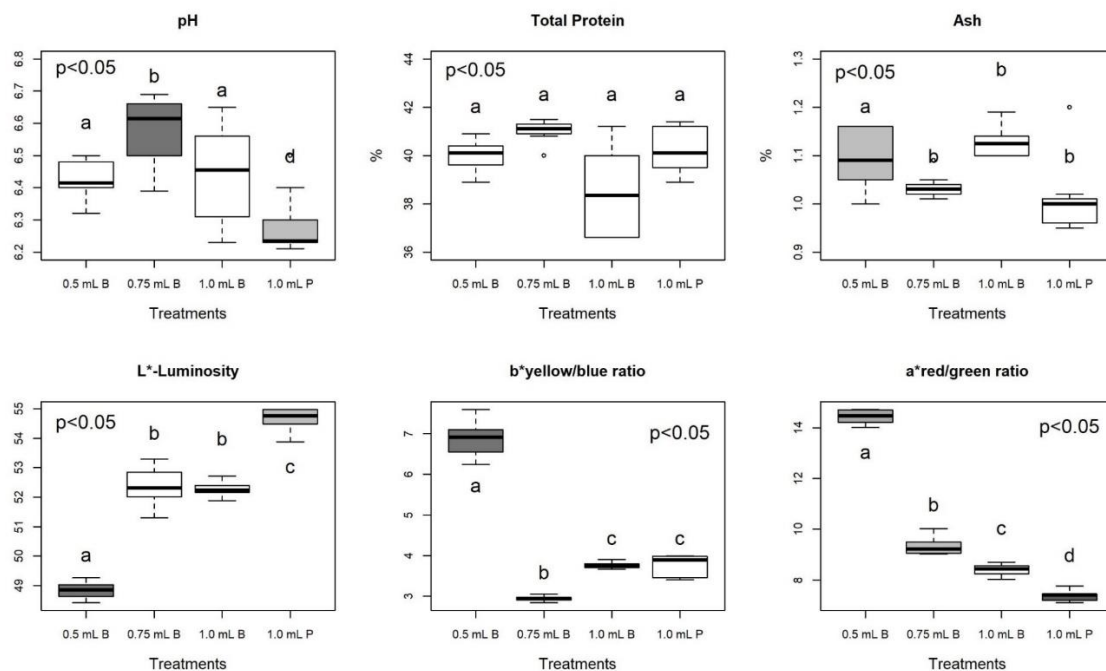


Figure 1. Boxplot means \pm standard deviation (S.D) of meat quality variables for each treatment and control group. Means with the same letter and color are not significantly different (Tukey multiple comparisons of means, $p > 0.05$). B: Bopriva®, P: Placebo (distilled water).

Docility and reproduction variable

The effects of the immunocastration treatment groups with Bopriva® on the docility variable (flight speed or reaction test) and the reproduction variable (scrotal circumference) are shown in Figure 2. It's observed that all treatments significantly

($p < 0.05$) influenced both parameters compared to the control group. This indicates greater docility in the animals to which the treatments were applied. However, among treatments for both variables were not observed significant differences.

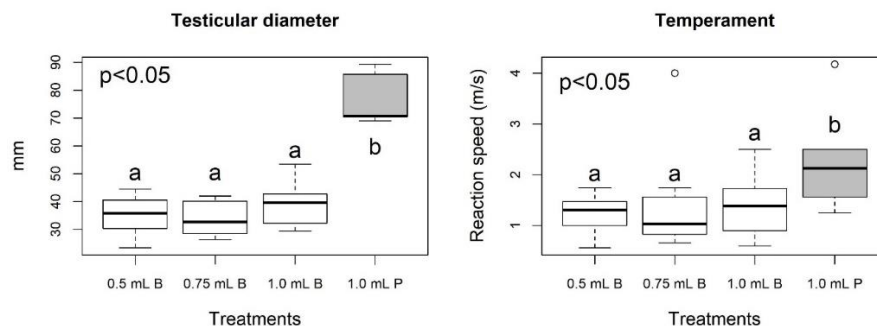


Figure 2. Boxplot means \pm standard deviation (S.D) of docility variable and scrotal circumference for each treatment and control group. Means with the same letter and color are not significantly different (Tukey multiple comparisons of means, $p > 0.05$). B: Bopriva®, P: Placebo (distilled water).

DISCUSSION

This is the first study to evaluate the efficacy of immunocastration with Bopriva® in young rams from the Peruvian Andes. The immunocastration and the doses used in the present investigation were established to preserve the welfare of the animals and guarantee that the antibodies affect the sexual hormones throughout the fattening period. For this, three doses with intervals of 3 weeks have been used to achieve the desired effect based on the recommendation of the product.

Our results demonstrate that compared to the control group vaccination of young rams with Bopriva® at the age of 10 -12 months suppressed the scrotal development and reduced aggressive and sexual behavior (docility). Besides, was confirmed that production variables such as weight final, weight gain/day, and dressing percentage were not affected by immunization against GnRF. This confirms the findings reported by Janett et al., (2012b) and Wicks et al., (2013) carried out on prepubertal bull and boars, respectively, where scrotal growth was interrupted, without affecting the body weight. Scrotal development is stopped because GnRF vaccines with Bopriva® act as an effective neutralizer, which induces to stops

the activation and production of LH and FSH, thus as the reduction of testosterone releasing at least three months (Pfizer, 2018).

The aggressive and sexual behavior reduction may be ascribed to the neutralization or lack of sex hormones. For instance, Bolado-Sarabia et al., (2018) assessed sexual, aggressive, and social behavior in Holstein bulls Immunocastrated with Bopriva and placebo (distilled water) and found that bulls treated with placebo showed a higher average of head butts, threats, mounting, sniffing, and flehmen sign compared to bulls treated with Bopriva. Likewise, Janett et al., (2012a) reported decreases in testosterone secretion and physical activity after injection of vaccination against GnRF with Bopriva. Its probably that variables before mentioned have been affected by stress caused by experimental animal management, however, this variable was not assessed.

Meat color is one of the most important variables used by customers to evaluate and decide and select delicious meat and of quality. From these perspectives, in the present study, immunization with Bopriva did not cause any negative effect on meat characteristics (Figure 2). Therefore, we may conclude that colorimetric parameters provide a better performance and meat quality in immunized animals compared to the control group (placebo). Amatayakul-Chantler et al. (2012) evaluated the meat quality in *Bos indicus* Zebu x Brown Swiss bulls using Bopriva and placebo (control-distilled water) and reported improved performance and meat quality on the group where Bopriva was applied. Amatayakul-Chantler et al. (2013) reported that there were no adverse effects on carcass or meat quality when immunocastration (Bopriva) was applied to *Bos indicus* beef bulls. Besides, reported production gains and concluded that the method was safe and effective.

Finally, it is necessary to mention that in most of the parameters measured, more favorable results were found with doses of 0.5 ml with intervals of three weeks, this is probably based on two aspects, one strictly physiological and the other hypothetical. The physiological explanation is supported by the mechanism of action of GnRH antagonists that is based on the permanent occupation of the GnRH receptor and that avoid endogenous GnRH connecting to its receptor (Karten and River, 1986), Besides, immunization with GnRH reduces the number of GnRH receptors in the pituitary. The two doses established in our study seemed to be sufficient to establish this mechanism in animals. The hypothetical aspect is based on aspects related to the product used (Bopriva®). The recommended dose is 1 ml, however, the product is specific for cattle and when used in young rams, which represents only 10% of the weight of a bovine, increases the chances that half the dose will approach appropriately.

CONCLUSIONS

This study demonstrates that immunization against GnRF with Bopriva® at different doses (0.5 mL, 0.75 mL, and 1.0 mL) on young rams (6 – 12 months of age) improved the final weight and dressing percentage. Likewise, immunocastration provided marked improvements on meat quality characteristics (meat color (L*, a*, and b* parameters), ash, and total protein), is the treatment 1 (0.5 mL Bopriva®) showed better results. Furthermore, vaccination led to the suppression of scrotal development and reduction in their aggressivity (docility).

These findings suggest that GnRF immunization with Bopriva® does not have negative effects on these traits.

CODE OF ETHICS

The authors state that the study presented has been conducted following the Code of Ethics for experiments with animals, as reflected in the Regulations: http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

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AUTHOR CONTRIBUTION STATEMENT

IUP Funding acquisition, Methodology, Project administration, Supervision; FAV Conceptualization, Methodology; EFCHZ, and EAG Data curation, Formal analysis, Writing – original draft; JNC Methodology; CQE Formal analysis, Visualization, Writing – original draft, Writing – review & editing; ARHDL Formal analysis, Writing – review & editing.

COMPETING INTEREST STATEMENT

The authors have no conflict of interest to declare.

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