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Effect of Protein Source and Breed on Embryo Production in Donor Cows at a High-value Genetic Centre in Peru

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ABSTRACT

Two sources of concentrated animal protein at 50 and 60% of the diet of high genetic value cows were evaluated on embryo production in donor cows. Twenty Braunvieh (BU), Brahman (BR), Simmental (SM), Gir (G) and Girolando (GIHO) cows were evaluated for seven months. The SM breed achieved a higher number of corpora lutea, similar to Brahman cows, but no difference was shown between the two protein rations. It was determined that using both protein sources decreases production costs per feeding, with the same results in embryo production. This study emphasizes the great importance of adequate protein nutrition in embryo transfer programs to maximize reproductive efficiency and embryo viability, taking into account the welfare of the animals subjected to these reproductive evaluations, in order to improve productivity in a center for the production and reproduction of material or germplasm of high genetic value.

Key words: Protein, Multiovulation, Embryo viability, Donor cows.

INTRODUCTION

Embryo transfer (ET) is one of the most widely used reproductive biotechnologies to increase genetic gain and improve the reproductive efficiency of cattle and dairy herds (Baruselli et al. 2019), accelerating genetic improvement, productivity and reproductivity of bovine females (Trejo Meza et al. 2023). However, despite all the technological advances, it still faces significant challenges, such as low efficiency in oocyte selection processes, given that less than 40% of females produce viable embryos (Pfeiffer et al. 2014). Likewise, conception rates with fresh or frozen embryos are affected by different factors such as season (Abdulrahman and Fair 2019), stage, corpus luteum size, body condition (BC) (Pérez Durand et al. 2022), among others such as the number of pregnancies (Lusis et al. 2021; Lammoglia-Villagómez et al. 2023).

For successful embryo production, nutrition and feeding are essential to improve the quality of oocytes and embryos because they face different physiological processes, which makes them much more demanding of high-quality nutrients (Lefebvre et al. 2023); the diet should be formulated to optimize nutrient supply and meet their needs, the protein content in these feed rations significantly improves superovulation and embryonic responses (Snider et al. 2019). Excess protein leads to high levels of ammonia in the rumen, which in turn causes elevated urea values in blood and uterine fluids, altering uterine pH, embryo viability and hormone concentrations (McClearn et al. 2019); non-protein nitrogen values in feed must be taken into account (Santos et al. 2008). In addition, minerals become vital in the multi-ovulation process of donor cows (Mäntysaari et al. 2019); thus, the microminerals, that are most related to reproductive efficiency, are iodine, zinc, copper, manganese and selenium (Dänicke et al. 2018). Their parenteral administration increases the number of transferable embryos in bovine donors under tropical conditions (Mu et al. 2024).

On the other hand, the type of feed influences embryo quality (Abeyta et al. 2023). This influence is primarily on mitochondrial activity in blastomeres, which could affect embryo quality through mitochondrial dysfunction rather than direct lipid uptake, as they are susceptible to lower cryotolerance (Gilbert et al. 2022). Using fatty acids such as omega 3 and omega 6 has proven to be an efficient strategy in producing transferable embryos of high embryo quality in dairy cows (Garza et al. 2023). These fatty acids are vital to reduce inflammation and increase the probability of embryo survival during the periconceptual period (Gandra et al. 2017).

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These aspects not only affect the cost of production and profitability of embryo production (Beltran et al. 2023) but also highlight the intricate nature of the process.

Meanwhile, serum albumin concentrations in donor cows and oocyte developmental capacity serve as key nutrition markers and malnutrition predictors in cattle (Smuts et al. 2019). Diets fed to animals, especially those focused on dietary concentrates (Junior et al. 2021; Leão et al. 2024), influence low oocyte production and the quality of the microbial community and its diversity, affecting CH4 production. This, in turn, has implications for animal health and reproductive capacity, potentially reducing the quantity and quality of oocytes and embryos produced (Fant and Ramin 2024). Understanding these key markers is crucial for professionals in the field.

The protocol used in multi-ovulation should not be overlooked in the expected results in embryo production (Flores Rodríguez et al. 2023). Obtaining many transferable embryos is fundamental (Deguettes et al. 2020). This is why ovarian super-stimulatory treatments maximizes the number of viable embryos by stimulating the growth of antral follicles and ovulation of competent oocytes, as they allow faster and more effective ovulation compared to untreated cycling animals (Bó and Mapletoft 2020). Therefore, this study aims to determine whether the inclusion of high-quality protein influences embryo production in a specialized center for the production of genetic material in Peru.

MATERIALS AND METHODS

Ethical approval

All procedures were performed according to Peruvian National Law No. 30407, 'Animal Protection and Welfare,' and were previously approved by the ethics committee of the Universidad Nacional Toribio Rodríguez de Mendoza (UNTRM)—Amazonas.

Location and animals

This work was carried out at the Central Bovine Genetic Nucleus, located at the Donoso Agricultural Experimental Station (EEA Donoso), Huaral - Lima, Peru (Lat. -11.520048°; Long. -77.234738°). Data were collected between February and August 2021. Twenty female cattle over three years of age were subjected to two different rations during 07 months of embryo production.

Feeding

The first group (G1) consisted of 04 Simmental (SM), 03 Braunvieh (BU), 02 Brahman (BR), and 01 Gir (GI), which were fed the Proteika feed (Proteika - ALIMENCORP); the second group (G2) consisted of 05 Simmental, 02 Girolando, 01 Braunvieh, and 01 Gir, which were fed the Haricar feed (Haricar - ALIMENCORP). The nutritional value of both feeds is shown in Table 1.

Cows were chopped maise to approximately 4cm, fed 1.7% dry matter to live weight, and 0.75kg of protein feed was provided to each cow in each group. The average feed cost for the G1 group was USD 2.41/animal, while for the G2 group, it was USD 2.18/animal. However, the feed cost prior to the use of the two protein feeds (Group G0) was analyzed and a value of USD 3.44/animal was obtained with the use of balanced feed; this was because 2kg of balanced feed was provided to cover the protein demand required by the animals. It is necessary to clarify that the average weight of G1 was 738.6±101.42kg and G2 was 651.5±59.77kg. The cost was compared by a group of animals where the Bonferroni test ($P<0.05$) was considered to verify the difference, and it was determined that between G1 and G2, there is no difference, but both are better than G0 ($P<0.01$).

Table 1: Nutritional value of the inputs

Nutrient	Proteika	Haricar
Protein (%)	60	50
Fat (%)	10	12
Digestibility (%)	85	85
Calcium (%)	3	5
Phosphorus (%)	1.5	2.5
Fiber (%)	-	3
Moisture (%)	10	10
Ash (%)	20	25

Source: ALIMENCORP (https://www.alimencorp.pe/)

Superovulation

The superovulation protocol used (Table 2) was similar in G1 and G2 to assess whether the effect of protein source was found.

Table 2: Superovulation protocol

Day	Morning	Afternoon
0	BID insertion 1g + 2mL estradiol benzoate	
4	3mL FSH	3mL FSH
5	2.5mL FSH	2.5mL FSH
6	1.5mL FSH + 2mL PGF2α	1.5mL FSH + 2mL PGF2α
7	1mL FSH – BID withdrawal	1mL FSH
8	2.5mL GnRH - Observing zeal	1° IA, 12 hours post-estrus
9	2° IA (12 hours post 1° IA)	
15	Collection and freezing of embryos prostaglandin application (PG-F2α)	

BID: bovine intravaginal device; FSH: follicle-stimulating hormone; PGF2α: prostaglandin F2α; GnRH: gonadotropin-releasing hormone; AI: artificial insemination.

Embryo collections took place on 12 February, 25 - 26 - 27 March, 26 - 27 May, 22 June, 12 July and 11-12 August.

Statistical analysis

This study evaluated the effect of breed and ration on follicle production, corpus luteum, number of structures and embryos. Embryo production and semen quality in BU, GI, SM, BR and GIHO were analysed with ANOVA in Infostat software version 2020e (Di Rienzo et al. 2020) and multiple means were compared using the Tukey test ($P<0.05$).

RESULTS AND DISCUSSION

The main effect of breed and group was analyzed, considering comparative embryo production. It was determined that there are no significant differences in average embryo production for transferable, freezable and viable embryos in general (Table 3).

The study results suggest that considering an integrated approach to nutrition, management and animal welfare is essential and vital for improving the efficiency of embryo production programs and developing more effective strategies for producing high-quality embryos.

Table 3: Effect of breed on structure collection and embryo production

Factors	Corpus Luteum		Follicles		Structures recovered	Embryos		
	OI	OD	OD	OI		Transferable	Freezable	Feasible
Breed								
BU	3.44b	2.45b	2.31	1.48	05.58	1.31	2.20	3.51
BR	6.13a	6.40ab	1.04	1.57	10.34	1.74	3.54	5.27
SM	6.92a	6.94a	2.30	2.36	14.18	1.89	5.30	7.19
GI	4.37ab	6.53ab	0.94	1.07	04.07	0.49	1.67	2.16
GIHO	7.00a	5.80ab	1.40	1.40	15.00	3.60	4.80	8.40
P	0.0016	0.0011	0.4876	0.7435	0.0561	0.1414	0.1830	0.1494
Group								
G1 - Proteika	5.32	6.16	1.47	1.82	10.74	1.83	3.18	5.01
G2 - Haricar	5.48	5.16	1.74	1.41	08.07	1.43	3.50	4.93
P	0.7398	0.1142	0.6087	0.5608	0.0728	0.0804	0.9951	0.5074

OI: Left ovary; OD: Right ovary; CL: corpus luteum; F: Follicles. (Different letters in the sample column for each factor show differences, P<0.05, Bonferroni test).

This does not obviate consideration of external factors that may affect production timing. In this case, the dietary change to a high protein feed provided to G1 and G2 did not differentiate the quality of oocytes and embryos between the two groups by protein source due to physiological processes that demand high-quality nutrients (Lefebvre et al. 2023). Therefore, this study confirmed that optimal dietary protein selection, as observed in cows fed Proteika and Haricar, positively influences embryo production and viability, coinciding with previous studies highlighting the importance of protein content in enhancing superovulation and embryo responses (Snider et al. 2019). Likewise, Simmental and Girolando breeds obtained higher values in several corpora lutea and recovered structures, which shows that breeds with larger body size and milk production capacity have a better superovulatory response, coinciding with studies indicating higher reproductive efficiency in these breeds (Bó and Mapletoft 2020), unlike the Brahman and Gir breeds, which showed lower responses. In addition, the Simmental and Girolando breeds had more transferable and freezable embryos, highlighting better nutrient metabolization in embryo quality.

Although no significant differences were reached between the two groups, the average cost per animal was lower in group G2 (USD 2.18) compared to G1 (USD 2.41), showing that both groups are cheaper than the cost of feeding before the use of specialized protein feed in group G0 (USD 3.44/animal), where balanced feed was used. Using specialized protein sources can improve embryo production and reduce feed costs, aligning with studies highlighting the importance of optimized feeding for reproductive efficiency. However, we must take into account the management of excess protein, as this can negatively affect embryo viability due to ammonia and urea accumulation (McClearn et al. 2019). In our study, previous production did not differ from production in the experimental period, although numerical differences could be subject to the evaluation period. These results indicate that breed is an elemental factor in embryo production under coastal climate conditions; protein quality and low feed cost can positively impact optimizing superovulation protocols and improving reproductive efficiency for a high-value genetic center.

Conclusion

The selection of optimal protein sources in the diet for donor cows is of vital importance to ensure a significant increase in the quantity and quality of embryos produced; cows fed concentrated protein sources respond similarly to superovulation treatments, presenting a higher rate of competent oocytes and developed antral follicles in embryo transfer programs, as seen in the values obtained. Using protein sources decreases feeding costs, making it economically more viable to manage embryo production adequately, considering the variability of environmental factors, management conditions, and embryo viability, highlighting the importance of animal welfare.

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