Livestock and Animal Research
Accredited by Directorate General of Higher Education, Research, and Technology No. 152/E/KPT/2023

Open Access
Livest. Anim. Res., March 2024, 22(1): 105-112
p-ISSN 2721-5326 e-ISSN 2721-7086
https://doi.org/10.20961/lar.v22i1.67613

Original Article

The success rate of artificial insemination in Limousin crossbred heifer with additional concentrate

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Received: November 23rd, 2022; Accepted: December 29th, 2023; Published online: March 29th, 2024

Abstract

Objective: This study aimed to determine whether the addition of concentrate to heifers could be the same as the success of AI in calving cows in terms of Return Rate (NRR), Conception Rate (CR), and Pregnancy Rate (PR).
Methods: The material used in this study was 28 Limousine Crossbred cows with 13 cows and 15 heifers. There were two treatments, namely T0: Cows that had given birth with control feed (control without added concentrate) + BIO ATP after AI, while T1: Heifers with control feed and the addition of 1kg/day concentrate for seven days after AI + BIO ATP. Samples were selected by purposive sampling with the criteria of BCS 3-5 (1-9) and aged ≥ 2 years. The artificial insemination method uses rectovaginal with 4+ deposition at the 2nd and 8th hour after estrus, then injection of Bio ATP brand "Rheinbio" intramuscularly as much as 10 ml.
Results: The results showed that the reproductive performance of Limousine Crossbred Cow that had given birth (T0) was NRR1 84.62%, NRR2 84.62%, CR 53.33%, and PR 66.67%, while in heifers (T1), it was NRR1 86 .67%, NRR2 60%, CR 20%, and PR 20%.
Conclusions: The addition of concentrate feed of 1 kg/head/day for seven days before AI in heifers (T1) showed lower reproductive success than in cows that had given birth (T0) even without the addition of concentrate.

Keywords: Artificial insemination**;** Conception rate; Heifers; Limousin Crossbred cow; Pregnancy rate

INTRODUCTION

Beef cattle are one of the essential ruminant commodities that supply the needs of meat of livestock origin. Problems arise because the fulfillment of protein originating from beef cattle has yet to be fulfilled so far, as well as population growth that has not been able to balance with needs. Beef production in Indonesia in 2020 is 437,780 tons, while the current demand reaches 696,956 tons Badan Pusat Statistik [1]. It shows that there is still a beef deficit of 259,176 tonnes. Limousin Crossbreed Cattle is a type of cattle that many breeders keep. The advantages of this type of Limousin cattle are that it is easy to adapt to various environments, is resistant to ectoparasites and endoparasites, and has excellent mothering abilities Ihsan *et al*. [2]. Increasing livestock population and production can be done by applying reproductive technology in a more efficient direction, such as artificial insemination (AI) Wahyudi *et al*. [3]. Several factors influencing artificial insemination's success are parity, breed, insemination frequency, and insemination time Inchaisri *et al*. [4]. Annashru *et al*. [5] added that performing AI at intervals

of 0-4 hours after estrus gives better results than 8-12 hours after estrus.

Age has a role in influencing pregnancy success, livestock productivity, and the period at first giving birth. If the age of the cattle when they are first mated is too young and it is puberty for the first time, then the cattle tend to experience difficulties during childbirth. In addition, it is difficult for livestock to reach maximum body weight, and the resulting offspring will experience the same thing Zainudin *et al*. [6]. Management of broodstock and prospective broodstock (heifers) must consider their reproductive performance, such as the fertility of the reproductive cycle, the age of the first marriage, and the period of the heifer at the time of first calving. Female cows that can give birth once a year are hopeful for better use of resources to support productive livestock Baco *et al*. [7].

Feed is one factor that determines the development of the reproductive organs, especially in the period before puberty and in the mother after giving birth. Heifers are generally more sensitive to feed effects than adult cattle because young cattle are still growing Feradis [8]. The feed factor is related to the physiological condition of female cattle. The feed has a role in ovarian growth, uterine condition, and hormone synthesis. Hormone synthesis in livestock will stimulate the stimulation of hormone secretion so that signs of estrus appear in livestock Bindari *et al*. [9]. The nutritional content in feed needs to be considered to meet protein and energy needs for livestock production and reproduction Malda *et al*. [10]. The concentrate is an additional necessary feed to supplement protein obtained from forage feed. Based on this description, it is essential to know the success rate of artificial insemination in Limousin Crossbred heifer with the addition of concentrate in terms of reproductive success parameters, namely Non-Return Rate (NRR), Conception Rate (CR), and Pregnancy Rate (PR).

MATERIALS AND METHODS

Time and location

This research was conducted from 21st April 2021 to 24th August 2021. It was carried out in Senggreng Village, Sumber Pucung District, Malang Regency, East Java, Indonesia.

Materials and methods

The material used in this study was 28 Limousin crossbred cows with 13 cows and 15 heifers. The sample was selected with the criteria of an adult cow that is not pregnant and has a Body Condition Score (BCS) 3-5 (scale 1-9) aged ≥ 2 years. The frozen semen of Limousin Cattle used came from the Singosari National Artificial Insemination Center (SNAIC). The concentrate consists of 300 kg of rice bran, 200 kg of copra, 200 kg of CGF, 100 kg of coffee skin, 200 kg of polar, 10 kg of salt, and 10 kg of minerals containing a crude protein content of 11.20%. Bio ATP, which is used as a vitamin, is adenosine triphosphoric acid 0.100 g, magnesium aspartate 1.500 g, potassium aspartate 1.000 g, sodium selenite 0.100 g, cyanocobalamin (Vit B12) 0.050 g, excipients q,s 100ml.

The method used in this study is field experiments. The artificial insemination technique is rectovaginal with 4+ cement deposition in the cornua uteri. The thawing technique is 30 second at 27°C. Artificial insemination uses a double dose with insemination at the 2nd hour after estrus (one dose) and the 8th hour after estrus (one dose). Then injected, "Rheinbio" as a BioATP vitamin as much as 10 ml/head and given 1 kg of concentrate feed (in treatment one) per day for seven days after insemination. The research sample was divided into two treatment groups, namely:

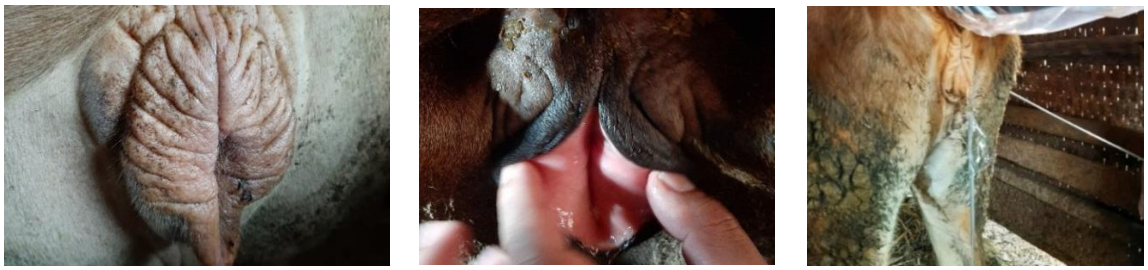
T0 = Cows have given birth by giving basal feed (control without added concentrate) + BIO ATP after AI

T1 = Heifers with basal feed + concentrate 1kg/day for 7 days after AI + BIO ATP after AI

Variables

1. Non-Return Rate (NRR)

The Non-Return Rate is the percentage of AI acceptors who do not return to estrus after insemination. NRR_1 was observed on days 19-21, and NRR_2 was observed on days 38-40



(a) (b) (c)

Figure 1. (a) Swelling vulva, (b) Reddening vulva, (c) Cervical mucus

Table 1. Non-return rate (NRR) of Limousin crossbred cows based on different treatments

Treatments	Total acceptors (n)	NRR 1		NRR 2	
		Number of acceptors did not show heat (n)	(%)	Number of acceptors did not show heat (n)	(%)
T0	13	11	84,62	11	84,62
T1	15	13	86,67	9	60,00

Susilawati [11]. The NRR percentage can be calculated using the formula:

$$\%NRR = \frac{A- B}{A} \times 100\% \quad \ldots\ldots(1)$$

With:
A = Total inseminated acceptors
B = Total re-insemnated acceptors

2. Conception Rate CR)

The Conception Rate is the percentage of pregnancy produced during the first insemination Jainudeen *et al*. [12]. The following formula expresses the CR value based on Jainudeen *et al* [12]:

$$\%CR = \frac{C}{D} \times 100\% \quad \ldots\ldots(2)$$

With:
C = Total pregnant acceptors from the first insemination
D = Total inseminated acceptors

3. Pregnancy Rate (PR)

The Pregnancy Rate shows the percentage of pregnant cattle from the total inseminated cows. The following formula expresses the PR value based on Jainudeen *et al* [12]:

$$\%PR = \frac{E}{F} \times 100\% \quad \ldots\ldots(3)$$

With:
E = Total pregnant acceptors
F = Total inseminated acceptors

Data Analysis

The data obtained were tabulated using Microsoft Excel and then analyzed using a qualitative descriptive analysis method to determine the percentage of successful artificial insemination in each treatment.

RESULTS

Non-return rate (NRR) of Limousin crossbred cows based on different treatments

Evaluation of the success of AI can be known through NRR observations. NRR observation is based on the assumption that the cow is post-AI and is no longer in heat, so the acceptor is considered pregnant Wiranto *et al*. [13]. NRR observation is done by checking for signs of heat, as shown in Figure 1. Based on the research results presented in Table 1. it is known that the NRR_1 value at T1 shows a higher impact, namely 86.67%, compared to T0, which is 84.62%. In comparison, the results of the NRR_2 at T0 showed higher results, namely 84.62%, compared to T1, which was 60.00%. At T0, it showed the same results between NRR_1 and NRR_2, while T1 experienced a decrease in the percentage of NRR_2 by 26.67%.

Conception rate (CR) of Limousin crossbred cows based on different treatments

The conception rate (CR) is the number of pregnant acceptors in the first AI divided by

Table 2. Conception rate (CR) of Limousin crossbred cows based on different treatments

Treatments	Total acceptors (n)	Conception Rate	
		Number of pregnant acceptors (n)	(%)
T0	13	8	53,33
T1	15	3	20,00

Table 3. Pregnancy rate (PR) of Limousin crossbred cows based on different treatments

Treatments	Total acceptors (n)	Pregnancy Rate	
		Number of pregnant acceptors (n)	(%)
T0	13	10	66,67
T1	15	3	20,00

the number of acceptors in the AI multiplied by 100%. This study carried out a pregnancy examination using the rectal palpation method, which was carried out 60 days after insemination. According to Jaśkowski *et al*. [14], pregnant cows have a larger corpus uteri size than non-pregnant cows. This can be confirmed by examining pregnancy using the rectal palpation method on days 35 – 40. Based on Table 2, the percentage of CR in T0 is higher, namely 53.33%, compared to T1, which is 20.00%.

Pregnancy rate (PR) of Limousin crossbred cows based on different treatments

Based on the results shown in Table 3, T0 has a higher PR value of 66.67% compared to T1, which is 20%. A pregnancy examination is carried out using rectal palpation. Rectal palpation is carried out by feeling around the uterus to find out if there is an enlargement of the female reproductive organs during pregnancy Lestari *et al*. [15].

DISCUSSION

Evaluation of pregnancy success in this study was seen based on the appearance of cow's reproduction after AI was carried out, namely through the calculation of the Non-Return Rate (NRR), Conception Rate (CR), and Pregnancy Rate (PR). The results showed that NRR_1, NRR_2, CR, and PR percentages at T0 were 84.62%, 84.62%, 53.33%, and 66.67%, respectively. In T1, NRR_1, NRR_2, CR, and PR percentages were 86.67%, 60.00%, 20.00%, and 20.00 respectively. The percentage of NRR in both treatments showed relatively high results. The treatment of concentrate as much as 1 kg/head/day for seven days to T1 acceptors is expected to improve the nutritional status of the acceptor so that it can affect the reproductive performance of the acceptor. Rosita *et al*. [16] explained that the NRR > 50% is still in the excellent category. Observations from NRR_1 to NRR_2 decreased, possibly due to cows experiencing silent heat. Another reason for the low NRR could be the condition of closed cattle sheds, lack of light, not cleaning, and it can stress the cows, which affects their reproductive performance of the cows. Wahyudi *et al*. [3] stated that one of the causes of silent heat and early embryonic death is interference from ectoparasites and endoparasites and not optimal absorption of nutrients, which affect stress on acceptors. Borchardt *et al*. [17] added that the success rate of AI is influenced by several factors such as temperature, climate, weather, and maintenance management, especially housing and feed. Providing additional feed in the form of concentrate 1 kg/head/day for seven days after AI, the aim is to increase the availability of AI acceptor protein to minimize the occurrence of early embryo mortality due to lack of feed.

The value of pregnancy success in this study shows a relatively low number. This is demonstrated by the percentage of CR and PR values in Table 2 and Table 3. The provision of forage to livestock at the study site depended on the season. Handayanta *et al*. [18] stated that the season factor is one of the determining factors for feed availability, mainly forage. The productivity of ruminant livestock during the dry season is generally low because they consume feed of low quality and quantity due to a decrease in the availability of forage. In the rainy season, livestock tends to be given feed through field grass, elephant grass, and corn

stalks. If the feed is in the dry season, the feed given is in the form of agricultural waste, such as rice straw. Rice straw is the result of straw plant waste which can be used as an alternative feed during the dry season Yanuartono *et al*. [19]. The feed given to acceptors at the study site is generally by the breeder's ability, not following the needs of the livestock. Low-quality feed supply, if it occurs continuously for quite a long time, will have a negative impact on livestock productivity.

Giving concentrate feed as much as 1 kg/day for seven days after AI at T1 did not significantly affect livestock reproductive performance. The quantity of feed that is not to the needs will have a negative impact on pregnancy in cows. Cows with insufficient consumption will experience a deficiency of energy used for fetal development and the reproductive cycle in these cows. The content of glucose and amino acids is helpful for the development of the embryo in the womb. Deficiency in the nutrition of the cows feed disrupts the cows production and reproduction because the nutrients obtained are only sufficient to sustain life. In contrast, the cow's reproductive and developmental functions are not adequately fulfilled Olson *et al*. [20]. The quantity of nutrients in the feed in small amounts or does not meet the needs of livestock can decrease the BCS value, which will affect the reproductive status of beef cattle Bindari *et al*. [9].

The average body condition score at T0 was 4.23 ± 0.60, higher than that at T1, which was 3.93 ± 0.46. The Body Condition Score is an indicator to determine the nutritional status of cows. An ideal BCS value in cows can usually run the estrus cycle Mondragon *et al*. [21]. Acceptors can produce optimally if they are given enough feed and meet the nutritional requirements. The condition of low BCS in cows can be used as an indication that these cows have dietary deficiencies which affect the low success of pregnancy. Gaude *et al*. [22] stated that other biological factors could affect the reproductive conditions of cows, namely nutritional status or changes in body condition before and after giving birth. Yekti *et al*. [23] stated that for endocrine functions to function normally, the nutritional needs of livestock must be fulfilled, and the quality and quantity of feed given will affect the synthesis and secretion of reproductive hormones by the endocrine glands.

At T1, the acceptor was a heifer, which showed a low percentage of success compared to T0, which was a cow. Heifers that have reproductive organs that are not optimal cause pregnancy failure. This is following Rohayati *et al*. [24] which state that heifers are female cows that have never given birth. In these cows, pregnancy failure often occurs due to the condition of the reproductive organs. Who have not reached optimal but are already showing signs of estrus that cause infertility. In this study, feeding to support reproductive performance in heifers has not demonstrated a significant effect. Budiyanto *et al*. [25] stated that hormonal disturbances experienced by livestock due to lack of nutrition, stress, and late uterine involution, can cause livestock to experience silent heat caused by insufficient levels of the hormone estrogen so that livestock are unable to show signs of sign of heat.

One of the factors that can affect the success rate of artificial insemination is the physiological condition of livestock, such as the condition of cows in responding to the environment and adapting Utami *et al*. [26]. Acceptors in this study had Limousin blood, a Bos Taurus cattle originating from hot areas. This resulted in decreased reproductive performance due to climate differences and adjustments to the type of feed available. At the research location, tends to have a reasonably hot temperature. Hot temperature conditions will cause neurotransmitters and can cause stress in livestock, causing pregnancy failure. Yekti *et al*. [23] added that the need for optimal maintenance management is one of the reasons for the low percentage of success in artificial insemination. This is indicated by the cramped conditions of cattle pens and the absence of sanitation which can cause ectoparasites or endoparasites which can cause cows to become stressed and cause inhibition-release of FSH and LH hormones caused by the hormone GnIH. The absence of FSH causes no follicles to form, whereas the lack of LH can cause cows to be unable to ovulate.

Based on the results of the pregnancy examination using rectal palpation, there were three cattle with ovarian hypofunction. Masruro *et al*. [27] stated that ovary hypofunction is a reproductive disorder that often occurs in cattle, livestock that experience ovarian hypofunction is characterized by the absence of follicles or a growing corpus luteum so that when examined by rectal palpation, will feel slippery on the surface of the ovary. Hormonal deficiencies and imbalances in the body of livestock cause ovarian hypofunction. This hormonal condition is closely related to the consumption of feed by cows, according to Suartini *et al*. [28] hormonally, hypofunction of the ovaries occurs because the anterior pituitary is unable to secrete sufficient amounts of FSH for the growth and development of follicles, this is due to livestock experiencing nutritional deficiencies. Utami *et al*. [29] stated that ovarian hypofunction in livestock could be caused by low gonadotropin hormones produced by the gonads. This affects the performance of the anterior pituitary to secrete FSH and LH so that the ovaries do not develop optimally.

Artificial insemination implementation time is one factor determining the success of artificial insemination. The timing of AI implementation depends on the accuracy of human resources, in this case, breeders in the heat detection process. The AI time in this study was at the 2nd and 8th hour after the cattle showed signs of estrus. The lack of accuracy by farmers in detecting heat often results in delays in the implementation of insemination, resulting in pregnancy failure due to delays in informing the inseminator about the state of heat Gucht *et al*. [30]. Berdugo-Gutierrez *et al*. [31] added that if the implementation of AI is not on time, there will be the potential for pregnancy failure due to not achieving the ovulation process. Incorrect timing of insemination between the end of sperm capacitation until the sperm enters the ovary does not coincide with the release of the ovary (ovum), failing fertilization which results in pregnancy loss Affandhy *et al*. [32].

CONCLUSION

The addition of concentrate feed of 1 kg/head/day for seven days after AI in heifers shows lower reproductive success than cows that have already given birth. The success rate of artificial insemination in Limousine Crossbred Cow that had given birth was NRR_1 84.62%, NRR_2 84.62%, CR 53.33%, and PR 66.67%, while in heifers NRR_1 86.67%, NRR_2 60%, 20% CR, and 20% PR.

CONFLICT OF INTEREST

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

ACKNOWLEDGMENT

The authors grateful to the Faculty of Animal Science, University of Brawijaya, through the research that gave under the "Professor Grant or Hibah Guru Besar" scheme in the DPA (Budget Implementation Document) funding community of the University of Legal Entities (PTNBH) University of Brawijaya with contract number No. DPA-FPt-251101/1022-1 for funding this research.

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