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Full Length Article

Artificial Insemination Success Rate on Limousine Crossbred Lactation Cows

Muchamad Luthfi[1,2*], Dicky Pamungkas[1], Sri Wahyuningsih[3], Gatot Ciptadi[3] and Trinil Susilawati[3]
[1]*National Research and Innovation Agency, Indonesia*
[2]*Doctoral Program Animal Science, University of Brawijaya, Malang, East Java, Indonesia*
[3]*Faculty of Animal Husbandry, University of Brawijaya, Malang, East Java, Indonesia*
[*]For correspondence: muchamad.luthfi@brin.go.id
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Abstract

This study aimed to determine the effect of the success rate of artificial insemination (AI) in lactating Limousin crossbred cows. The materials used were 30 Limousin crossbred cows divided into two physiological statuses: lactating and non-lactating. The cows used had body condition scores (BCS) 4 and 5 on a scale of 1–9, 3–5 years old. Supplementary feeding was given for seven days at 1 kg/head/day post-AI pregnancy examination by ultrasonography (USG) after AI at 42 days of age. Variables observed were feed consumption, estrus appearance (temperature, pH, vulva color, and cervical mucus), AI results (non-return rate) NRR_1, NRR_2, pregnancy rate (PR) and post-AI ovary size. Data were analyzed with *t*-test using Microsoft Excel 2019 program, while AI results were analyzed with descriptive method. The results showed that the consumption of feed nutritional value was not significantly different between the two treatments ($P > 0.05$); temperature, pH, vulva color, mucus, and BCS in non-lactating cows had higher values than lactating cows. However, the results of statistical analysis showed that only the appearance of cervical mucus was significantly different ($P < 0.05$); non-lactating cows had higher NRR_1, NRR_2, and PR than lactating cows, and ovary size of lactating cows was smaller than non-lactating cows. In conclusion, the AI success rate in lactating cows was lower than in non-lactating cows, probably due to estrus display, low AI result display (NRR_1, NRR_2, PR) and small ovary size (length = 1.87 ± 0.13 cm; width = 1.23 ± 0.01 cm).

Keywords: Limousin crossbred cow; Artificial insemination success; Lactation

Introduction

Livestock reproductive performance concerns the reproductive ability of livestock to produce offspring. The success of mating, either naturally or through AI, is influenced by several factors, including the male or semen used, inseminator officers, the accuracy of estrus detection, and the timing of mating (Tophianong *et al.* 2014). The high case of embryo implantation failure on smallholder farms at the beginning of pregnancy is suspected to be due to the cows still breastfeeding their offspring (not yet weaned). This is due to pre-implantation embryonic development that is less than optimal due to inadequate circulating progesterone concentrations (Larson *et al.* 2007).

Beef cattle productivity is influenced by feed, as feed has the most significant influence (60%). The influence of feed proves that high livestock production can only be achieved with feeding that meets quality and quantity requirements (Nurwahidah *et al.* 2016).

Improving the reproductive process can be done by providing adequate nutrition throughout the maintenance of livestock, especially before mating, pregnancy and Lactation (Amin 2014). Nutritional needs must be fulfilled at the beginning of Lactation (Bilal *et al.* 2016). Protein and Total Digestible Nutrient (TDN) feed can affect the production and quality of milk that will be produced so that if the nutritional intake is not sufficient for both the mother itself and the calf will remodel food reserves in the body to cause a decrease in body weight (Abdillah *et al.* 2015), it also results in decreased productivity and impaired reproductive organ function. Supplementation in the form of feed containing sufficient nutrients to support the production and reproductive improvement of beef cows (Rohmah *et al.* 2017).

Therefore, information related to the effect of success rate of AI on lactating Limousin crossbred cows was needed to anticipate pregnancy failure due to mismanagement of cattle maintenance.

Materials and Methods

Location

The research was conducted at a breeder, Senggreng

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Village, Sumberpucung Subdistrict, Malang District, East Java Province, Indonesia.

Sample and reproductive management

Sample: The samples used were 30 Limousin crossbred cows divided into 15 cows that were lactating and 15 cows that were not lactating. Sampling of cows using purposive random sampling technique with the criteria of cows used BCS 4 and 5 scale 1–9, age 3–5 years. Supplementary feeding was given for seven days at 1 kg/head/day post-AI, with nutritional values (Table 1).

Reproductive management: AI is implemented after the cow shows signs of estrus (cervical mucus discharge). Furthermore, for pregnancy examination, rectal palpation and or USG was performed after 42 days of AI. Measurements of ovaries and follicles using measuring the average of two diameters of the horizontal and vertical lines of the ovarian and follicles were done (Keskin *et al.* 2016).

Statistical analysis

The observed variables were feed consumption, estrus appearance (vulva temperature, vulva pH, vulva color, and cervical mucus); AI results (NRR_1, NRR_2 & PR) and post-AI ovary size. The data were analyzed with a T-test using Microsoft Excel 2019 program to compare the success of AI of lactating and non-lactating cows, while the analysis of estrus percentage used a descriptive method.

Table 1: Feed Nutritional Value Data

Parameter	%
Dry Matter (DM)	92.16
Organic Matter (OM)	9.84
Crude Protein (CP)	11.20
Crude Fiber (CFb)	22.69
Crude Fat (CFt)	1.23
Nitrogen-free extract (NFE)	55.04
Total Digestible Nutrient (TDN)	75

Results of Analysis Lab. Nutrition, faculty of animal science, Brawijaya University

Table 2: Consumption of feed nutritional value

Parameter	Lactating cows	Non-lactating cows	*P*
Dry Matter (DM)	10.44 ± 1.98	9.27 ± 2.27	*P* = 0.372
Organic Matter (OM)	8.52 ± 1.15	7.07 ± 1.05	*P* = 0.477
Crude Protein (CP)	0.69 ± 0.09	0.69 ± 0.18	*P* = 0.963
Crude Fiber (CFb)	3.08 ± 0.50	2.36 ± 0.33	*P* = 0.228
Crude Fat (CFt)	0.17 ± 0.05	0.14 ± 0.03	*P* = 0.390
Nitrogen-free extract (NFE)	4.57 ± 0.58	3.87 ± 0.66	*P* = 0.679
Total Digestible Nutrient (TDN)	5.59 ± 0.75	4.54 ± 0.75	*P* = 0.612

Value = Mean ± SEM (standard error of mean), $P > 0.05$ (non-significant)
DM (kg) = 8.50; CF (kg) = 0.82; TDN (kg) = 5.0; ME (Kcal/kg) = 0.45 Mcal/Kg (NRC 2001)

Results

Feed consumption analysis

Based on the results showed that feed consumption, including DM, OM, CFb, CFt, NFE and TDN in lactating and non-lactating cows was a non-significant effect ($P > 0.05$) (Table 2).

Estrus appearance and BCS analysis

Statistical analysis showed that the appearance of estrus in lactating and non-lactating cows for temperature, pH and vulva color were non-significant effects ($P > 0.05$). Contrarily, the appearance of estrus showed significant effect ($P < 0.05$). Likewise, the BCS of lactating and non-lactating cows was a non-significant effect ($P > 0.05$) (Table 3).

Low AI results display analysis.

The results showed that non-lactating cows had higher NRR_1, NRR_2 and PR than lactating cows. Likewise, the ovary size of lactating cows was smaller than non-lactating cows (Table 4 and Fig. 1).

Discussion

One of the efforts to improve the body condition of livestock is to increase the quantity and quality of feed so that livestock are ready to carry out reproductive stages, which include pregnancy, calving, and lactation. Rohmah *et al*. (2017) reported that improving body condition in beef cows before mating and optimizing the livestock's reproductive process can increase pregnancy success and reduce repeated matings. In addition, Susilawati and Yekti (2018) stated that the success of AI is influenced by environmental factors, namely maintenance, especially in the quality and quantity of feed given to cows.

Based on the consumption of food substance content (Table 2), protein consumption is still below the standard of both livestock groups. Pradhan and Nakagoshi (2008) stated that livestock's nutrients influence livestock's reproductive fertility and plays an important role in the reproductive cycle. Lack of nutritional intake has adverse effects on livestock, both in production and reproduction. Nuryadi and Wahjuningsih (2011) added that feeding nutrition before and postpartum will affect the next partum cycle. In addition, sufficient body energy is needed to produce Luteinizing Hormone (LH). This hormone stimulates follicular growth (activates ovarian function) so that postpartum estrus occurs (Winugroho 2002). In other words, if energy reserves are low, postpartum estrus will be long. Sows entering the early dry period of the houses have a low BCS of < 3, which means that during lactation, the cows use nutritional reserves to produce milk and maintain pregnancy (Chebel *et al.* 2018).

The result observation of estrus appearance (Table 3), which includes temperature, pH, vulva color, and mucus; As well as BCS in non-lactating cows have higher values than lactating cows, but the results of statistical analysis show that only the appearance of cervical mucus was significant effect ($P < 0.05$). Failure to detect estrus is a major cause of impaired reproductive performance in cows. Reduced signs of estrus not only lead to suboptimal estrus detection, thus

Table 3: Estrus display of lactating and non-lactating cows

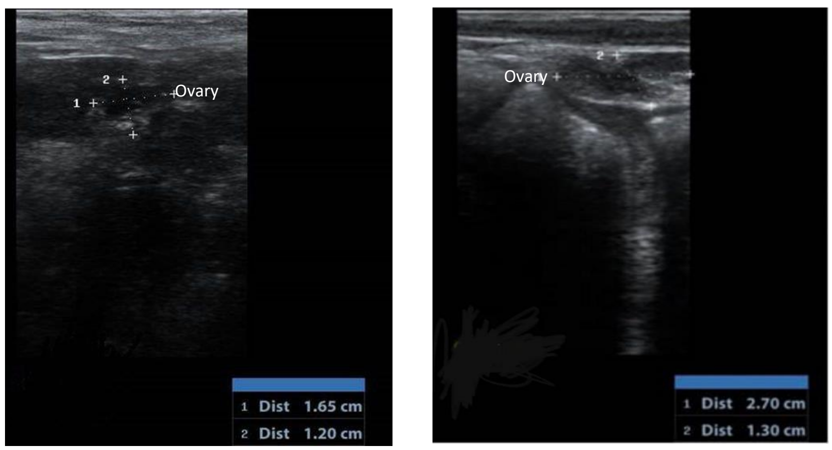
Physiology status	N (heads)	Estrus Display				BCS
		temperature (°C.)	pH	vulva color	Cervical mucus	
Lactating cows	15	38.15 ± 0.13	6.85 ± 0.7	1.70 ± 0.26	2.60 ± 0.31[a]	4 ± 0.18
No Lactating cows	15	38.22 ± 0.21	7.80 ± 0.13	2.3 ± 0.21	4.0 ± 0.10[b]	4 ± 0.20

Value = Mean ± S.E.M.; BCS with a scale of 1-9
Superscripts in different notations (a,b) on the same line indicate significant differences ($P < 0.05$) between treatments
Color 1 = Pink color and peripheral vessels are not visible
2 = Red color (reddish) and peripheral vessels are visible
3 = Dark red color, and the branching of peripheral vessels is very clear (Akbar *et al.* 2020).
Mucus 1 = no mucus comes out
2 = mucus comes out only wetting the vulva area
3 = mucus hanging out up to 2 cm from the vulva area
4 = mucus comes out until it falls (Putra *et al.* 2021)

Table 4: Display of artificial insemination results of lactating and non-lactating cows

Status Fisiologis	N (heads)	NRR (%)		Pregnancy (%)		Ovary Size	
		NRR_1	NRR_2	Pregnant	Non-pregnant	Length (cm)	Width (cm)
Lactating	15	50	40	20	80	1.87 ± 0.13	1.23 ± 0.01
Non-Lactating	15	80	80	90	10	2.38 ± 0.34	1.30 ± 0.16

Notes: NRR_1 = observed on day 19-21 post-AI and NRR_2 = observed on day 40-42 post-AI;



a. Ovary of Lactating Cow b. Ovary of Non-Lactating Cow

Fig. 1: Ovarian Ultrasound of Lactating and Non-Lactating Cows

contributing to low pregnancy rates, but can also lead to false positive estrus detection, as estrus detection is based on sometimes less reliable symptoms. Missed estrus detection and false positive estrus in high-producing cows are eliminated as causes of conception failure in timed AI protocols (Tenhagen *et al.* 2003).

Cows during the dry period experience an increase in body weight due to fetal growth and development until, at the end of the dry period, can achieve BCS of 3.5. Thus, the need for feed consumption will also increase along with the increase in the body weight of the cows (Ruan *et al.* 2019).

The differences in AI results (Table 4) at NRR_1, NRR_2 parameters indicate that the percentage of cows that were not successfully fertilized in a given reproductive cycle. The higher the NRR percentage, the lower will be the reproductive efficiency in the breeding program. In addition, there is an imbalance between the value of PR and the value of NRR in non-lactating cows, and this is likely due to the presence of female reproductive health disorders, one of which is silent heat. According to Varotto *et al.* (2016), NRR is one indicator of livestock fertility to evaluate the reproductive performance of livestock without waiting for calving. The decrease in NRR value can be caused by animals experiencing silent heat, which results in signs of estrus not being detected by farmers, besides that Rao *et al.* (2013) stated that another factor that causes silent heat in livestock is that livestock experience ectoparasitic disorders such as ticks found on the vulva of acceptor livestock, causing stress. Susilawati (2011) stated that livestock will experience stress due to ectoparasites and endoparasites, which can interfere with the reproductive system, one of which is silent heat.

Aji *et al.* (2017) reported that breastfeeding will cause no implantation. Early embryonic death occurs in many lactating cows due to the inhibition of the implantation process in the endometrium due to low steroid hormones and the production of oxytocin (Jousan *et al.* 2005). One of the factors causing low fertility in cows is the concentration of the hormone progesterone during the pre-implantation phase of embryonic development (Sandra *et al.* 2007). Enhanced embryo development is associated with maternal progesterone concentration (Mann and Lamming 2001; Green *et al*. 2005).

The ovary size of lactating cows is smaller compared to non-lactating cows (Table 4), this is in accordance with the results of Ismaya research (2014) which states that the diameter of the ovaries of SimPO (Simental Peranakan Ongole cross) cows have a range of 3–4 cm; furthermore, Jalaluddin (2014) states that species, age, reproductive cycle phase, parity, and feed nutrition level strongly influence the size of the ovaries of each animal. Variations in the ovary and follicle size are relatively high in each individual, even at the same age (Mossa *et al.* 2012).

The size of the corpus luteum (Cl) in normal cows and cases of reproductive disorders can vary depending on the cow's reproductive cycle stage. In normal cows, the size of the Cl can range from 2–5 cm in diameter. However, this size may fluctuate depending on the phase of the reproductive cycle. In the early phase of the estrous cycle (followed by ovulation), the Cl size is usually smaller, while in the late phase of the estrous cycle, or if pregnancy occurs, the Cl size can be larger (Ball and Peters 2004).

Improvements in nutrition provided to livestock must be calculated based on nutritional balance, including vitamin and mineral requirements to meet the very complex coordination mechanisms between nutrients in the reproductive process because if not done carefully, too much or too little feed nutrients provided will negatively affect follicular development, resulting in anestrus (Pradhan and Nakagoshi 2008).

Conclusion

The success rate of AI in lactating mothers is lower than in non-lactating mothers, presumably due to the appearance of estrus, the appearance of AI results (NRR_1,NRR_2 & PR) is low and the size of the ovaries is not normal (small).

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Author Contributions

ML, TS designed the experiment, conducted research and collected the data. ML, DP, SW and GC analyzed the data and finalized the write-up of this manuscript. All authors approved and finalized the manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Data Availability

Data presented in this study will be available on fair request to the corresponding author.

Ethics Approval

The animal care and use committee of Brawijaya University has carefully studied that the proposed research protocol was approved by the institutional review board with decision letter No. 128-KEP-UB-2021.

References

Abdillah F, M Hartono, Siswanto (2015). Conception rate pada sapi perah laktasi di balai besar pembibitan ternak unggul dan hijauan pakan ternak Baturaden Purwokerto Jawa Tengah. *J Ilmiah Petern Terp* 3:98–105

Aji R, A Panjono, A Agus, BP Widyobroto, T Hartatik, IGS Budisatria, I Ismaya, S Bintara (2017). Kinerja reproduksi sapi betina sumba ongole yang di inseminasi dengan semen beku sapi jantan belgian blue. *Bull Petern* 41:379–384

Amin R (2014). Nutrition: Its role in reproductive functioning of cattle-a review. *Vet Clin Sci* 2:1–9

Akbar FA, D Samsudewa, YS Ondho (2020). Tampilan vulva sapi perah yang disinkronisasi dengan hormon prostaglandin pada umur yang berbeda. *J Sain Petern Indo* 15:91–137

Ball PJH, AR Peters (2004). Reproduction in Cattle. Blackwell Publishing, Oxford, UK

Bilal G, RI Cue, JF Hayes (2016). Genetic and phenotypic associations of type traits and body condition score with dry matter intake, milk yield and number of breedings in first lactation Canadian Holstein cows. *Can J Anim Sci* 96:434–447

Green MP, MG Hunter, GE Mann (2005). Relationships between maternal hormone secretion and embryo development in dairy cows on Day 5 of pregnancy. *Anim Reprod Sci* 88:179–189

Ismaya (2014). *Bioteknologi Inseminasi Buatan Pada Sapi dan Kerbau*. Gadjah Mada University Press. Yogyakarta, Indonesia

Jalaluddin (2014). Morfometri dan karakteristik histologi ovarium sapi aceh (*Bos indicus*) selama siklus estrus. *J Medika Vet* 8:66–68

Jousan FD, M Drost, PJ Hansen (2005). Factors associated with early and mid-to-late fetal loss in lactating an non-lactating holstein cattle in a hot climate. *J Anim Sci* 83:1017–1022

Keskin A, G Mecitoglu, E Bilen, B Guner (2016). The effect of ovulatory follicle size at the time of insemination on pregnancy rate in lactating dairy cows. *Turk J Vet Anim Sci* 40:68–74

Larson SF, WR Butler, WB Currie (2007). Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. *Anim Reprod Sci* 102:172–179

Mann GE, GE Lamming (2001). Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 121:175–180

Mossa F, SW Walsh, ST Butler, DP Berry, F Carter, P Lonergan, W Ireland, J ACO Evans (2012). Low number of ovarian follicles ≥ 3 mm in diameter are associated with low fertility in dairy Cows. *J Dairy Sci* 95:2355–2361

NRC - National Research Council (2001). Nutrient requirement of dairy cattle. 7th Revised edn. National Academy Press. Washington DC, USA

Nurwahidah J, AL Tolleng. MN Hidayati (2016). Pengaruh pemberian pakan konsentrat dan urea molases blok (UMB) terhadap pertambahan berat badan sapi potong. *J Imu Ilmu Petern* 2:111–121

Nuryadi N, S Wahjuningsih (2011). Penampilan reproduksi sapi peranakan ongole dan peranakan limousin di kabupaten malang. *J Ternak Trop* 12:76–81

Pradhan R, N Nakagoshi (2008). Reproductive Disorders in Cattle Due to Nutritional Status. *J Intl Dev Corp* 14:45–66

Rohmah NY, S Ondho, D Samsudewa (2017). Pemberian pakan flushing dan non flushing terhadap intensitas birahi. *J Sain Petern Indo* 12:290–298

Rao TKS, N Kumar, P Kumar, S Chaurasia, NB Patel (2013). Heat detection techniques in cattle and buffalo. *Vet World* 6:363–369

Chebel RC, LGD Mendonça, PS Baruselli (2018). Association between body condition score change during the dry period and postpartum health and performance. *J Dairy Sci* 101:4595–4614

Ruan RD, KE Hanna, MW Daniel, AGVK Marina (2019). The relationship between transition period diseases and lameness, feeding time, and body condition during the dry period. *J Dairy Sci* 103:649–665

Sandra FL, WR Butler, WB Currie (2007). Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. *Anim Reprod Sci* 102:172–179

Susilawati T, APA Yekti (2018). *Teknologi Inseminasi Buatan Menggunakan Semen Cair (Liquid Semen): Solusi untuk Daerah yang Tidak Ada/Sulit Nitrogen Cair*. Universitas Brawijaya Press, Malang, Indonesia

Susilawati T (2011). Tingkat keberhasilan inseminasi buatan dengan kualitas dan deposisi semen yang berbeda pada sapi peranakan ongole. *J Ternak Trop* 12:15–24

Tenhagen BA, C Vogel, M Drillich, G Thiele, W Heuwieser (2003). Influence of stage of lactation and milk production on conception rates after timed artificial insemination following ovsynch. *Theriogenology* 60:1527–1537

Tophianong TC, B Agung, MN Erif (2014). tinjauan hasil inseminasi buatan berdasarkan anestrus pasca inseminasi pada peternakan rakyat sapi bali di kabupaten sikka nusa tenggara timur. *J Vet* 32:46–54

Varotto A, R Finocchiaro, JB Kaam, Maurizio, Marusi, G Civati, M Cassandro (2016). Analysis of non-return rate in italian holstein friesian bulls. *Acta Agric Slov* 5:94–98

Winugroho M (2002). Strategi pemberian pakan tambahan untuk memperbaiki efisiensi reproduksi induk sapi. *J Litb Pert* 21:19–23

Putra EY, P Anwar, J Jiyanto (2021). Identifikasi tingkah laku birahi sapi kuantan betina plasma nutfah riau. *J Green Swarn* 10:585-593