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Comparative study of artificial insemination versus natural mating methods on the quality of Bali cattle calves

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Abstract

The aim of this research is a comparative study of artificial insemination versus natural mating methods on the quality of Bali cattle calves. A total of 571 calves were born from 74 cows in 2007; 99 cows in 2008; 100 cows in 2009; 99 cows in 2010; 100 cows in 2011; and 99 cows in 2012, respectively. Breeding has been implemented with a program of selecting livestock based on body weight at the age of one year. The scope of this research was as follows: the impact of the implementation of the artificial insemination (AI) program compared to natural mating (NM) on the improvement of calf performance (birth weight, weaning weight, and one year body weight) of Bali calves. The results showed that the mean birth weight of male Bali calves using the mating method (AI vs NM) were 17.88 ± 1.59 kg and 18.01 ± 1.44 kg, respectively, while the calf birth weight of calves Bali females, was: 18.09 ± 1.26 kg, and 17.78 ± 1.21 kg, statistically not significantly different ($P < 0.05$). Likewise, the weaning weight of male Bali calves and female Bali calves was not significantly different ($P < 0.05$). The average body weight of one year old Bali male calves using AI and NM methods were: 137.75 ± 24.29 kg and 135.08 ± 25.25 kg, while the one-year body weight average for female Bali calves was: 118.00 ± 17.46 kg and 118.52 ± 17.11 kg, respectively. It was concluded that the daily weight gain before weaning of male Bali calves using the AI mating method was higher than that of NM.

Keywords: Birth weight, weaning weight, calves

Introduction

Bali cattle (*Bos sondaicus*) is a local Indonesian cow that has many advantages. The most prominent advantage of Bali cattle is high fertility^[1, 2]; high reproduction; sufficiently good adaptability to new environments, especially the ability to adapt to low quality feed^[18] high percentage of carcass and good quality of meat^[14].

Based on these advantages, Bali cattle are very feasible to be improved and developed, both in terms of genetic quality and population^[20]. In addition, the government has designated Bali cattle as the main local beef producer to support the beef self-sufficiency program in Indonesia. To improve the body weight performance of Bali cattle, two main factors that must be considered are improving the genetic quality and providing the environment, especially feed according to their needs^[13]. Good genetic quality will not be expressed if it is not supported by an optimal environment/feed. Conversely, if the genetic quality is bad/not good, the optimal environment/feed will not provide maximum performance^[20]. The action needed to improve the genetic quality of Bali cows is to select and mate within the Balinese cattle breed, so that the purity of the calves can be maintained.

On the other hand, nowadays it is alleged that Bali cattle have experienced genetic degradation. This can be seen from the body weight of Bali cattle and size of adult male and female Bali cattle have decreased when compared to the results of Djagra^[5]. If the decline in genetic quality occurs continuously, and runs for a long time, it is feared that Bali cattle, as one of Indonesia's native germplasm, will be threatened by its existence^[20].

Efforts to improve the genetic quality of Bali cattle can be carried out by implementing a selection program and application of artificial insemination technology^[13]. Artificial insemination (AI) or injection mating is the insertion or delivery of semen into the female genital tract using man-made tools, not with males (naturally). AI technology plays an important role in improving genetic quality in terms of bulls. AI technology is a very effective tool for improving the genetic quality and population of livestock^[13] and^[17]. Kesuma *et al.*^[9] also stated that AI is the most appropriate technique to improve the genetic quality of livestock

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using superior bulls with high genetic quality. The use of this superior bull will increase the intensity of selection. The AI program must be carried out in a directed and sustainable manner, so that superior livestock are produced, both in terms of production and reproduction. The implementation of the AI program is rarely carried out on community farms whose livestock ownership is very limited (2-3 head), because livestock raising is not guaranteed and at any time the livestock can be sold or slaughtered. In addition, there is generally no recording of the characteristics of production or reproduction [10].

The application of AI technology is carried out on livestock that have been selected so that their offspring can be obtained faster, because a bull can serve 5,000 to 10,000 female cows every year [9]. On the other hand, with the NM program, a bull is only able to serve 50 to 70 cows in one year. Thus, AI technology can be used to accelerate the distribution of superior genetic potential through the use of the cement it produces to obtain calves with better genetic quality. Taming *et al.* [17] stated that AI technology can improve the birth weight of livestock. The results of [12] research show that the calf body weight of Bali cattle produced by the AI program is higher when compared to the NM program. This is because the cement used comes from superior bulls.

The purpose of this study is a comparative study of artificial insemination (AI) versus natural mating (NM) methods on the quality of Bali cattle calves (birth weight, weaning weight, and one year body weight).

Materials and Methods

Location and Research procedure

The research was conducted at the BPTU (Superior Livestock Breeding Center) Bali Cattle Breeding Center in Pangyangan Village, Pekutatan District, Jembrana Regency, Bali Province, Indonesia. A total of 571 calves were born from 74 cows in 2007; 99 cows in 2008; 100 cows in 2009; 99 cows in 2010; 100 cows in 2011; and 99 cows in 2012, respectively. Breeding has been implemented with a program of selecting livestock based on body weight at the age of one year. Replacement livestock breeds, was taken from birth in the livestock population at the nursery center and the basic population at Village Breeding Center. The scope of this research was as follows: the impact of the implementation of the artificial insemination (AI) program compared to natural mating (NM) on the improvement of calf performance (birth weight, weaning weight, and one year body weight) of Bali cattle.

Field observations and in-depth interviews with the leadership and technical staff of BPTU Sapi Bali. Weighing the body weight of the Bali Cattle in the nursery center (BPTU Sapi Bali) is carried out from January to December 2012. In addition, records of calves body weight at birth, weaning, and body weight of one year of age from 2007 was used as secondary data to be analyzed. Furthermore, body weight data was grouped based on age groups (birth weight, weaning weight, and one year weight). Furthermore, the phenotypic diversity of livestock body weight in each age group was calculated.

Determination of Data Sources

Sources of research data are: records (recordings) in BPTU Sapi Bali since 2007 as secondary data, and the weighing of all Bali cattle in BPTU Sapi Bali in 2012 as primary data. Cattle production records taken in this study include the following:

- **Birth weight:** Birth weight is the calf weight obtained through weighing at birth or no later than 3 (three) days after the calf was born [3]
- **Weaning weight:** Weaning weight is the calf weight obtained by weighing at the age of 6 to 8 months (160-250 days) and then standardized to 205 days of age. Weaning calf weighing was done at a certain time, while the time of birth is always different, so when weighing the calves, they will not be of the same age. To eliminate the effects of different ages, an adjustment was made to weaning age: 205 days or BS.205 [3] with the formula:

$$BS.205 = \frac{\text{Real body weight-birth weight when weighed}}{\text{Age at weighing (days)}} \times 205 + \text{birth weights}$$

If the birth weight record is not available, then the average birth weight of the breed was used.

One year old body weight (BW): One year old body weight is calf body weight obtained by weighing at the age between: 11 to 13 months (320-410 days) and then standardized to the age of 365 days [3] with the formula:

$$\text{One year old BW.365} = \frac{\text{Real body weights-BW.205}}{\text{Time interval between (days)}} \times 160 + BS.205$$

Furthermore, the data were grouped by gender and method of marriage. Gender was grouped into male (1) and female (2), births were grouped according to first birth (1); second (2); and third (3). Mating methods were grouped into Natural Mating (1) and Artificial Insemination (2).

Data analysis

The influence of sex with parity, as well as the method of marriage with parity on calves body weight (birth weight, weaning weight, one year weight, daily body weight gain before weaning, and daily body weight gain after weaning) were carried out with a factorial variant analysis (2x3) [16], assuming that the maintenance of all livestock was considered the same, including the feed given.

Results and Discussion

Effect of the method of mating on birth weight

The mean birth weight (BW) of male Bali calves using the mating method (AI and NM) obtained in this study were: 17.88±1.59 kg, and 18.01±1.44 kg, while the mean birth weight of female Bali calves was: 18.09±1.26 kg, and 17.78±1.21 kg, respectively (Table 1). The results of the analysis of variance showed that the mating methods (AI and NM) had no significant effect (P>0.05) on the birth weight of male and female calves. The effect of mating methods (AI and NM) on the birth weight of male and female Bali calves from 2007 to 2012 is also shown in Figure 1.

Table 1: Average Birth Weight of male and female Bali calves using the AI and NM mating method at BPTU Bali Cattle from 2007-2012

Years	Mating Method											
	AI						NM					
	Male			Female			Male			Female		
	n	BW (kg)	SD (kg)	n	BW (kg)	SD (kg)	n	BW (kg)	SD (kg)	n	BW (kg)	SD (kg)
2007	16	17,06 ^a	2,05	6	18,33 ^a	1,63	28	16,79 ^a	1,03	24	17,17 ^a	1,49
2008	12	19,25 ^a	1,22	15	19,07 ^a	0,88	37	19,08 ^a	1,38	35	18,54 ^a	1,15
2009	12	18,67 ^a	1,61	11	17,91 ^a	0,94	36	18,47 ^a	1,28	41	18,12 ^a	1,19
2010	11	18,18 ^a	1,25	14	18,21 ^a	1,12	38	18,34 ^a	1,70	36	18,00 ^a	1,26
2011	11	17,36 ^a	0,81	13	17,23 ^a	1,09	40	17,78 ^a	1,07	36	17,53 ^a	1,00
2012	15	17,20 ^a	0,86	11	17,64 ^a	1,36	34	17,26 ^a	0,67	39	17,15 ^a	0,49
Total	77	17,88 ^a	1,59	70	18,09 ^a	1,26	213	18,01 ^a	1,44	211	17,78 ^a	1,21

Note: n = Number of livestock/sample; AI = artificial insemination; NM = Natural mating; BW = birth weight; SD= Standard deviation; The same superscript on the same line shows statistically nonsignificant difference ($P>0.05$)

BPTU Sapi Bali is a government-owned institution that aims to improve the genetic quality of Bali cattle and the preservation of native Indonesian livestock germplasm. To achieve this goal, the effort that has been done is to carry out the breeding of Bali cattle by means of netting Bali cattle belonging to the people in several areas in Bali for performance tests on prospective bulls or cement sources, followed by a progeny test. The best bull from the results of

this test is used as a cement source for the AI program, while the rest was used for the NM program^[15]. Based on this, it can be said that in implementing the AI program, it does not only include cement deposition into the female genital tract, but also includes the use of superior bulls^[6]. Failure to detect and interpret signs of estrus correctly will contribute to the loss of livestock business.

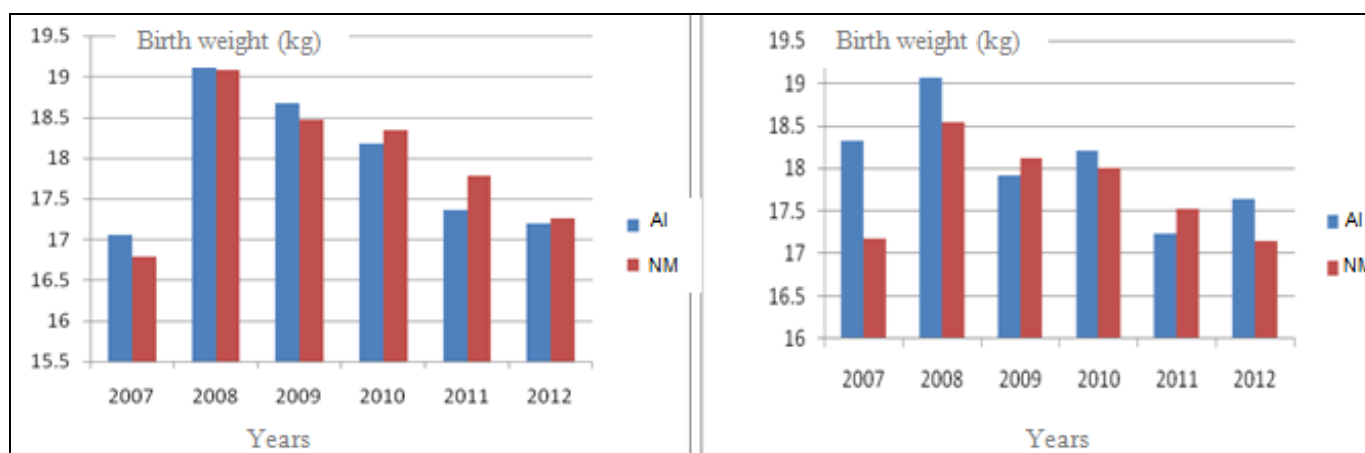


Fig 1: Average birth weight of male Bali calves (left) and females (right) using the AI and NM mating methods at BPTU Sapi Bali in 2007-2012.

The absence of differences in mating methods (AI and NM) on the birth weight of male and female Bali calves in this study indicated that the quality of bulls used for AI was not different from the quality of bulls from semen sources in the AI program. The bull used for NM is the bull that follows the progeny test program. This program aims to find bull candidates that will be used as cement source bulls in the AI program to replace the previous AI bulls.

Effect of Mating Method and Parity on Birth Weight of calves

The mean birth weight of Bali calves using the AI and NM mating methods at parity I obtained in this study were: 17.89±0.26 kg and 17.52±0.14 kg, respectively. The mean birth weight of Bali calves using the AI and NM mating methods at parity II obtained in this study were: 17.86±0.25 kg and 17.53±0.14 kg, respectively. Furthermore, the mean birth weight of Bali calves using the AI and NM mating methods at parity III obtained in this study were: 17.68±0.25 kg and 18.03±0.17 kg, respectively. The mean birth weight of Bali calves with the AI and NM mating methods at parity I,

II, and III at BPTU Bali cattle is presented in Table 2. The analysis of variance shows that the mating method (AI and NM) has no significant effect ($P>0.05$) on Bali cattle birth weight at parity I, II, and III.

Table 2: The Average birth weight of Bali calves using the AI and NM mating method at Parity I, II, and III at BPTU Sapi Bali

Parity	Mating Methods					
	AI			NM		
	n	BW (kg)	SD (kg)	n	BW (kg)	SD (kg)
I	27	17,89 ^a	1,37	84	17,52 ^a	1,17
II	28	17,86 ^a	1,69	87	17,53 ^a	1,19
III	28	17,68 ^a	1,61	60	18,03 ^a	1,35

Note: n = Number of calves; AI= artificial insemination; NM= Natural mating; BW= birth weight; SD= Standard deviation; The same superscript on the same line shows statistically nonsignificant difference ($P>0.05$)

This shows that the quality of bull as a source of cement in the mating method with AI and NM has the same quality. All bulls to be used as bulls in the NM and AI methods have been selected based on the progeny test.

Effect of the method of mating on the weaning weight of calves

The average weaning weight of male Bali calves using the AI and NM mating methods obtained in this study were: 96.39 ± 16.20 kg and 92.30 ± 17.57 kg, while the average

weaning weight of female Bali calves was: 89.37 ± 14.20 kg and 90.08 ± 13.11 kg, respectively (Table 3). The results of the analysis of variance showed that the mating method (AI and NM) had no significant effect ($P > 0.05$) on the weaning weight of male and female Bali calves.

Table 3: Average weaning weight of male and female Bali calves using the mating method AI and NM at BPTU Sapi Bali 2007-2012

Years	Method of Mating											
	n	WW (kg)	SB (kg)	n	WW (kg)	SB (kg)	n	WW (kg)	SB (kg)	n	WW (kg)	SB (kg)
2007	16	90,38 ^a	23,94	6	99,00 ^a	15,86	27	88,62 ^a	20,57	23	87,43 ^a	17,38
2008	12	103,25 ^a	12,88	13	85,62 ^a	18,03	29	93,17 ^a	21,80	31	87,81 ^a	17,47
2009	11	106,55 ^a	11,27	8	83,50 ^a	18,29	34	86,03 ^b	18,31	37	89,03 ^a	11,87
2010	11	91,73 ^a	15,25	13	91,38 ^a	14,68	32	94,09 ^a	20,74	28	88,93 ^a	12,87
2011	11	95,55 ^a	14,65	13	87,31 ^a	9,34	38	93,71 ^a	12,68	36	90,69 ^a	11,34
2012	15	93,20 ^a	7,15	11	91,82 ^a	6,38	34	96,56 ^a	8,36	39	93,82 ^a	7,88
Total	76	96,39 ^a	16,20	64	89,37 ^a	14,20	194	92,30 ^a	17,57	194	90,08 ^a	13,11

Note: n = Number of calves; AI = artificial insemination; NM = Natural mating; WW= weaning weight; SD= Standard deviation; The same superscript on the same line shows statistically nonsignificant difference ($P > 0.05$)

There was no significant effect on the mating method (AI and NM) on the weaning weight of male and female Bali calves, proving that the overall quality of bull semen source in the AI program was not different from the quality of bulls used for mating NM. The bulls used for mating with NM are bulls that undergo a progeny test. If the progeny test result is higher than the AI result, then the bulls used for NM will replace the cement source bulls in the AI program. In addition, the appearance of cattle at weaning weight is very dependent on the condition of the cow which includes the production of cow's milk which can be utilized by the calves.

The average weaning weight with different mating methods obtained in this study is lower than the results of [8], who obtained the average weaning weight of Bali calves of 97.16 ± 6.47 kg for natural mating and 97.28 ± 4.25 kg for AI results. This is caused by environmental factors that are not supportive, especially cow feed that is not in accordance with the needs, so that the performance of the calves cannot perform optimally.

Effect of Mating Method on One Year Body Weight of Calves

The results showed that the mean birth weight of male Bali calves using the mating method (AI vs NM) were: 17.88 ± 1.59 kg, and 18.01 ± 1.44 kg, while the mean birth weight of female Bali calves were: 18.09 ± 1.26 kg, and 17.78 ± 1.21 kg were not statistically significant ($P < 0.05$). Likewise, the weaning weight of male Bali calves and female Bali calves were not significantly different ($P < 0.05$).

The one-year body weight average of male Bali calves using the AI and NM methods were: 137.75 ± 24.29 kg and 135.08 ± 25.25 kg, while the one-year weight average for female Bali calves were: 118.00 ± 17.46 kg and 118.52 ± 17.11 kg, respectively. The one-year body weight (BW1) average of male Bali calves using AI and NM methods obtained in this study were: 137.75 ± 24.29 kg and 135.08 ± 25.25 kg, while the one-year body weight of female Bali calves was: 118.00 ± 17.46 kg and 118.52 ± 17.11 kg, respectively and statistically showed no significant difference ($P > 0.05$) (Table 4).

Table 4: One year average body weight of male and female Bali calves using the mating method AI and NM at BPTU Sapi Bali 2007-2011

Years	Mating method											
	AI						NM					
	Male			Female			Male			Female		
	n	BW1 (kg)	SD (kg)	n	BW1 (kg)	SD (kg)	n	BW1 (kg)	SD (kg)	n	BW1 (kg)	SD (kg)
2007	9	160,78 ^a	31,50	-	-	-	15	155,87 ^a	21,91	-	-	-
2008	12	140,33 ^a	13,67	11	116,09 ^a	20,06	29	128,62 ^a	27,22	29	114,66 ^a	18,40
2009	11	121,09 ^a	17,39	8	113,00 ^a	24,00	33	128,21 ^a	14,98	36	117,19 ^a	18,69
2010	11	133,82 ^a	31,86	13	122,23 ^a	19,89	32	137,91 ^a	39,38	28	121,57 ^a	22,40
2011	11	135,18 ^a	3,63	13	117,23 ^a	3,00	37	134,14 ^a	4,24	36	119,14 ^a	6,83
Total	54	137,75 ^a	24,29	45	118,00 ^a	17,46	146	135,08 ^a	25,25	129	118,52 ^a	17,11

Note: n = Number of calves; AI = artificial insemination; NM = Natural mating; BW1 = One year average body weight; SD= Standard deviation; The same superscript on the same line shows statistically nonsignificant difference ($P > 0.05$).

There is no difference in the mating methods (AI and NM) to the one-year body weight of Bali calves, indicating that the quality of the bulls used for NM is not different from the quality of the bulls whose semen is used for AI. On the other hand, a calf that is one year old has been released from the influence of the cow, so its appearance to grow is a reflection of its own ability.

The one-year body weight average for calves obtained in this

study was lower than the results of [8] study on Bali calves, which obtained one year average weight were: 144.37 ± 11.48 kg for NM, and 145.47 ± 9.98 kg for AI results regardless of sex. This is due to environmental factors, namely feed that is not in accordance with their needs, so that the Balinese calves in BPTU Sapi Bali have not been able to show their maximum performance.

Effect of mating method and parity on Calves body weight after one year of age

The mean body weight of one year old Bali calves using the AI and NM mating methods at parity I were: 120.27±5.20 kg and 124.72±2.77 kg, respectively. Furthermore, the one-year body weights of Bali calves using the AI and NM mating methods at parity II were: 121.75±4.51 kg and 127.34±2.85 kg. The average body weight of Bali calves with the AI and NM mating methods at parity III were: 139.60±5.20 kg and 122.34±3.41 kg, respectively. More details are presented in Table 5.

Table 5: One year old body weight of Bali calves using the AI and NM mating methods at parity I, II, and III at BPTU Sapi Bali 2007-2011

Parity	Mating methods					
	AI			NM		
	n	BW1 (kg)	SD (kg)	n	BW1 (kg)	SD (kg)
I	15	120,27 ^a	19,35	53	124,72 ^a	16,62
II	20	121,75 ^a	16,20	50	127,34 ^a	25,23
III	15	139,60 ^b	20,44	35	122,34 ^a	18,99

Note: n = Number of calves; AI = artificial insemination; NM = Natural mating; BW1 = One year old body weight; SD= Standard deviation; The same superscript on the same line shows statistically nonsignificant difference ($P>0.05$)

Analysis of variance showed that mating methods (AI and NM) had no significant effect ($P>0.05$) on body weight of one year old Bali calves at parity I and II, but had a significant effect ($P<0.05$) on parity III. The body weight of one year old Bali calves using the AI mating method is higher than that of Balinese calves born using the NM mating method. The use of superior bulls as a source of cement in the AI program will improve the quality of livestock. Based on the potential possessed by these cows, coupled with the ability of cattle at the age of one year, this is a reflection of the ability of the cattle themselves, so that their appearance is better than those from NM bulls.

Table 6: Average ADG before weaning on Bali male and female calves using the AI and NM mating methods at BPTU Sapi Bali 2007-2011

Years	Mating Methods											
	AI						NM					
	Male			Female			Male			Female		
	n	ADG (kg)	SD (kg)	n	ADG (kg)	SD (kg)	n	ADG (kg)	SD (kg)	n	ADG (kg)	SD (kg)
2007	16	0,36 ^a	0,12	6	0,39 ^a	0,07	27	0,35 ^a	0,10	23	0,34 ^a	0,08
2008	12	0,41 ^a	0,06	13	0,32 ^a	0,09	29	0,36 ^a	0,11	31	0,34 ^a	0,08
2009	11	0,43 ^a	0,05	8	0,32 ^a	0,09	34	0,33 ^b	0,09	37	0,35 ^a	0,06
2010	11	0,36 ^a	0,07	13	0,36 ^a	0,07	32	0,37 ^a	0,10	28	0,34 ^a	0,06
2011	11	0,38 ^a	0,07	13	0,35 ^a	0,05	38	0,37 ^a	0,06	36	0,36 ^a	0,06
Total	61	0,39 ^a	0,08	53	0,35 ^a	0,07	160	0,36 ^b	0,09	155	0,35 ^a	0,07

Note: n = Number of calves; AI = artificial insemination; NM = Natural mating; ADG = Average daily gains; SD= Standard deviation; The same superscript on the same line shows statistically nonsignificant difference ($P>0.05$).

Graphs of ADG before weaning for male and female Bali calves using the AI and NM mating methods at BPTU Sapi

Effect of mating method (AI and NM) on daily weight gain before weaning

Table 6 shows that the average daily body weight gain (ADG) before weaning in male Bali calves using the AI and NM mating methods in this study were: 0.39±0.08 kg and 0.36±0.09 kg, respectively. While the average daily body weight gain before weaning in female Bali calves is: 0.35±0.07 kg and 0.35±0.07 kg, respectively.

The results showed that mating methods (AI and NM) had a significant effect ($P<0.05$) on daily body weight gain before weaning on male Bali calves, but had no significant effect ($P>0.05$) on daily body weight gain before weaning on female Bali calves. The average daily body weight gain before weaning of male Bali calves using the AI mating method was higher than that of NM, indicating that the quality of bulls used as a cement source in the AI program was better than the quality of bulls used in the NM program. The difference in bull quality only affects male cattle. According to [11], the appearance of cattle before weaning will depend on the condition of the cow or the production of cow's milk. If the cow during breastfeeding lacks feed, it will have a direct impact on its calves.

The results showed that mating methods (AI and NM) had a significant effect ($P<0.05$) on daily body weight gain before weaning on male Bali calves, but had no significant effect ($P>0.05$) on daily body weight gain before weaning on female Bali calves. The average daily body weight gain before weaning of male Bali calves using the AI mating method was higher than that of NM, indicating that the quality of bulls used as a cement source in the AI program was better than the quality of bulls used in the NM program. The difference in bull quality only affects male cattle. According to [11], the appearance of cattle before weaning will depend on the condition of the cow or the production of cow's milk. If the cow during breastfeeding lacks feed, it will have a direct impact on its calves.

Bali in 2007-2011 are shown in Figures 2.

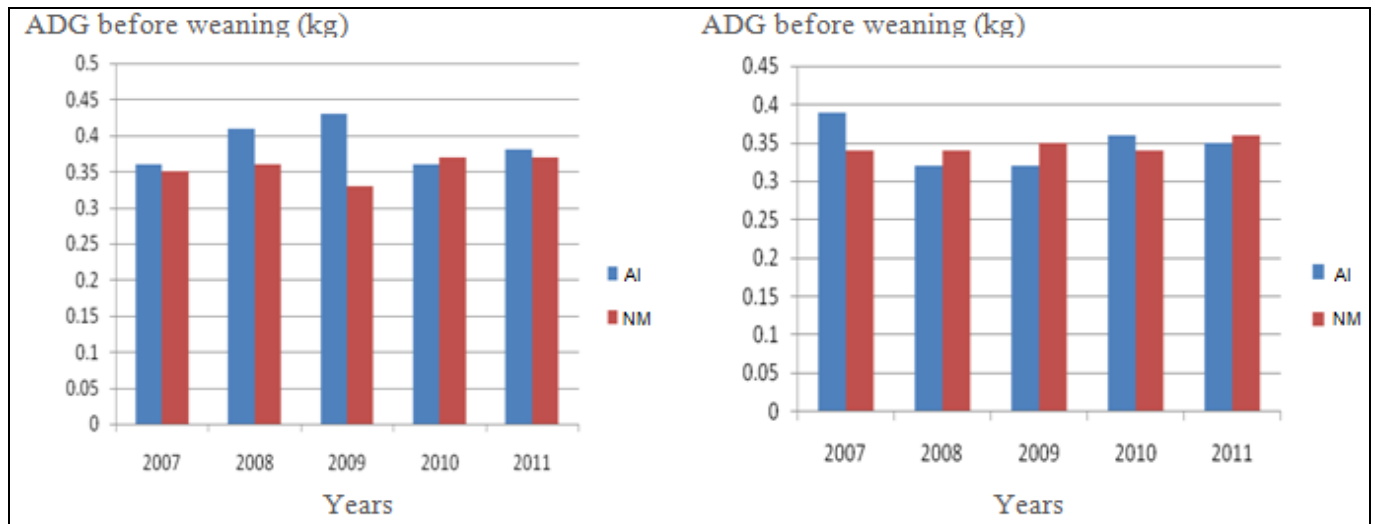


Fig 2: Average daily gain before weaning male (left) and female (right) Bali calves using the AI and NM mating method at BPTU Sapi Bali 2007-2011

Improving the quality of cattle breeds will be achieved more quickly through males, especially with the use of AI mating technology. Naturally, a bull is only able to serve 50 to 70 cows in one year, but with AI technology, a bull can serve 5000 to 10000 cows per year^[6]. Kesuma *et al.*^[9] state that the successful mating rate of AI to produce a calf is quite variable at this time, but for some areas it has worked well. One of the keys to AI success is that cows are intensively maintained by means of cages.

Oka^[12] reported that the calf body weight performance of AI technology was higher than that of NM mating. Research by^[4] conducted in 2005-2009 found that the percentage of cows using the AI method, in fact, were not pregnant between 45-65%. Judging from these results, it turns out that the human resource factor (inseminator) as the operator of AI, also plays a very important role in the success of the AI program^[7]. In addition to skills in insemination, accuracy is also needed in detecting cows that are in heat to be AI^[9]

Conclusion

It can be concluded that the AI and NM mating methods have no effect on body weight of one year old bali calves at parity I and II, but have a significant effect on parity III. The daily weight gain before weaning of male bali calves using the AI mating method was higher than that of NM. Likewise, the body weight of Bali calves at the age of one year is actually higher with the AI mating method compared to the NM mating method.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

All authors have contributed to this Article. Dewi Ayu Warmadewi and I Gusti Nyoman Gde Bidura designed studies, analyzed and interpreted the data until the submission.

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