The Effect of GnRH on Reproductive Performance and Progesterone Hormone Levels in Buffalo in West Sumatera

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ABSTRAK
Penelitian ini bertujuan untuk mengetahui dosis GnRH terbaik, onset, lama estrus, dan kadar progesteron pada Kerbau Rawa penghasil dadih di Indonesia. Materi yang digunakan yaitu 16 ekor induk Kerbau Rawa dengan bobot badan rata-rata 500 kg dan usia antara 3-5 tahun. Metode penelitian menggunakan Analisis of Variance (Anova) dari RAL (Rancangan Acak Lengkap) dengan berbagai tingkatan dosis FSH yang digunakan pada Kerbau Rawa. Kerbau Rawa diatur berahinya dengan melakukan sinkronisasi dengan injeksi hormon GnRH sebanyak 5 ml pada hari pertama dan ke-11, pada hari ke-10 sampai ke-12 semua kerbau donor diinjeksi dengan GnRH. Injeksi GnRH menggunakan 4 dosis sebagai perlakuan yaitu 200, 225, 250, and 275 ml/kerbau, setiap perlakuan terdiri dari 5 ulangan. Kecepatan estrus, lama estrus dan kadar progesteron merupakan variabel yang diukur. Hasil penelitian menunjukkan bahwa dosis GnRH secara nyata (P <0,05) meningkatkan kecepatan estrus dan lama estrus. Kadar progesteron meningkat secara signifikan mulai dari sebelum perlakuan sampai setelah perlakuan dengan GnRH. Disimpulkan bahwa dosis GnRH terbaik untuk sinkronisasi estrus pada kerbau rawa penghasil dadih adalah 225 ml dengan kecepatan estrus 18,2 jam dan panjang estrus 18 jam.

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ABSTRACT

This study aims to determine the best dose of GnRH, onset, duration of estrus and progesterone level on the swamp buffalo production of dadih in Indonesia. The materials used were 16 Swamp Buffalo with an average body weight of 500 kg and aged between 3-5 years. The research method using analysis of variants (ANOVA) of the CRD (completely randomized design) with different levels of FSH dose used. Swamp Buffalo is synchronized with the injection of 5 ml of GnRH hormone on the first and 11th day, on the 10th to 12th day all donor buffaloes are injected with GnRH. Four different doses of GnRH (200, 225, 250, and 275 ml/buffalo) and each treatment was replicated five times. The speed of estrus and estrus length were the measured variables. The results showed that the doses of GnRH significantly (P<0.05) increase of estrus speed and estrus length. Progesterone levels have increased significantly starting from before treatment until after treatment with GnRH. It is concluded that the best GnRH doses for estrus synchronization in the swamp buffalo producing of dadih was 2.25 ml/buffalo with estrus speed 18.2 h and estrus length 18 h.

INTRODUCTION

Swamp buffalo are animals commonly raised for meat throughout SEA Asia. The implementation of reproductive technologies, especially artificial insemination (AI) of buffalo in Indonesia still has many obstacles, characterized by high service per conception (S/C) ratio and repeated breeding (Ihsan, 2011; Ismaya, 2014). AI which is seldom performed in buffalo, because of the weakness of estrus symptoms and the variability of estrus length is very difficult (Yendraliza et al. 2011). Even though these animals are chiefly raised for their meat rather than as dairy animals, farmers in West Sumatra make a yoghurt-like fermented food from buffalo milk known as dadih which has 43% lower cholesterol and 65% higher calcium than that made from cows. It contains antioxidants, that have benefits for human health.

The market for dadih is at present limited to West Sumatera but has the potential to be widened especially with the current interest in probiotic foods. Dadih was traditionally only fermented in bamboo, however, over the food processing technology has made its manufacture more efficient.

This decline of buffalo population is due to uncontrolled marital management, difficulty in providing superior males, and maintenance goals are still considered as side jobs. When buffalo are regularly milked estrus is delayed further. Environmental management limitations are a lack of knowledge and application of reproduction technology. In many parts of Asia, farmers know very little about how to detect estrus (Jessie et al. 2016).

Problems with livestock systems and farmers' skills and knowledge limitations can be improved through training, counseling and mentoring (Chaikhun et al. 2010). Buffaloes often experience silent heat meaning that they do not clearly show signs of estrus so that the farmer does not know the right time to mate the buffalo either naturally or with AI at the right time. The condition of silent heat in buffalo makes it difficult for farmers to develop buffalo cattle. This condition was natural and cannot be genetically removed. The condition of silent heat results in the difficulty of farmers in detecting estrus in buffaloes, so that the success
of AI implementation is still relatively low and birth intervals (Redhead et al. 2018).

Reproductive technology that can be applied is synchronizing estrus by utilizing exogenous hormones including GnRH (Nalley et al. 2011; Jordiansyah et al. 2013). GnRH is a natural hormone produced by the hypothalamus in the brain that can produce another hormone called Luteinizing Hormone (LH) in collaboration with Follicle Stimulating Hormone (FSH) in follicular development and the onset of signs of estrus. (Afriani et al. 2014) GnRH injection of 48 hours caused more ovulation to occur in response to the release of FSH and LH by hypophysis due to GnRH stimulation. GnRH injection in each estrus cycle will ovulate the dominant follicles that exist and the emergence of new follicular waves 2 or 3 days later (Arum et al. 2013; Rabidas et al. 2017).

The reason for choosing this age range was that the average age at first mating for buffalo is been found to be 2.8 ± 0.3 years (Afriani et al. 2018). Buffalo was chosen with purposeful sampling from three breeders using intensive farming methods.

**Research materials and tools**

The material used is GnRH, PGF2α, FSH, physiological NaCl, NaOH, Ethanol, Methanol. The tool used is petridish 35 and 60 mm, pasteur pipette, disposable syringe, glass cover, gas pack. As well as the main tools used include incubators, electric scales, ovens, eppendorf pipettes, refrigerators, centrifuge.

**Experimental design**

A completely randomized study design with 4 treatments and 5 replications. each replication consisted of 5 (five) buffalo as experimental units, namely treatment A. (200 ml/buffalo), B. (225 ml/buffalo), C. (250 ml/buffalo), and D. (275 ml/buffalo). Synchronization was achieved by injecting GnRH on day 0, day 7 then two days later GnRH, after which estrus was detected. The speed of estrus, estrus length, and progesterone levels were the measured variables. For the analysis of buffalo-producing dadih, blood samples were used by using the ELISA method to observe progesterone levels.

**Statistical analysis**

All data were analyzed by analysis of variance (ANOVA) using a general linear model procedure on SPSS software version 16.0. Duncan’s multiple range test was used for the determination of differences between treatment means.
RESULTS AND DISCUSSION

Estrus Speed and Estrus Length

Analysis of variance indicated that four doses of GnRH significantly (P<0.05) influential on the speed estrus in the swamp buffalo producing of dadih. Table 1 shows that the estrus speed of the swamp buffalo producing of dadih on treatment 225 ml/buffalo produced the fastest estrus; 18.2 hours after the second GnRH injection.

Table 1. Average Estrus Speed (Hours) and Length of Estrus (Hours) in The Swamp Buffalo Producing of Dadih After Injection of GnRH With Different Doses

| The Dosages of GnRH (ml/buffalo) | Estrus Speed (h) | Estrus Length (h) |
|----------------------------------|------------------|-----------------|
| A. 200                           | 29.4<sup>b</sup> | 16<sup>a</sup>  |
| B. 225                           | 18.2<sup>c</sup> | 18<sup>a</sup>  |
| C. 250                           | 32.4<sup>a</sup> | 22<sup>b</sup>  |
| D. 275                           | 28.6<sup>b</sup> | 18.6<sup>a</sup>|

Note: Data presented as the mean of 5 replicates, a-c Values in the same column with different superscript letters are significantly different (P<0.05)

Based on Table 1. This was statistically faster than any other dosage trial. The results showed that GnRH injection can affect estrus speed in the swamp buffalo production of dadih. GnRH can stimulate FSH, which function in stimulating follicle growth in the ovary. The appearance of estrus is due to the influence of the increase in the estrogen hormone in the body produced by the ovum. GnRH injection in treatment B (225 ml/buffalo) shows that the speed of estrus emergence most quickly when compared to treatments A, C, and D. This is caused by the injection of GnRH at a dose of 225 ml/buffalo there is a lot of follicle formation. This is similar to that obtained by (Yendraliza et al. 2017) that the magnitude of the percentage change in buffalo behavior given GnRH-PGF2α from buffaloes which do not get additional synchronous hormones, probably caused by the number of follicles formed due to the addition of GnRH. The addition of the GnRH will stimulate follicle growth (Ibrahim 2008). GnRH gave on the first day, PGF2α on day 7th and GnRH on the 9th day showed a significant influence between estrus response and pregnancy in Mediterranean swamp buffalo (Neglia et al. 2016).

The speed of estrus (onset of estrus) is the time when animals show signs of estrus for the first time. The ultimate goal of estrus seizure in the swamp buffalo production of dadih is to increase reproduction with the presence of clear estrus signs so that it can improve time efficiency for AI which will ultimately increase the production of dadih. The average speed of estrus the swamp buffalo producing of dadih in treatment B (225 ml/buffalo) is 18.2 h. The results of this study similar to that obtained by (Yendraliza et al. 2017) synchronization protocol on female buffaloes in Kabupaten Kampar using combinations of GnRH and PGF2α in postpartum period make higher estrus intensity, faster estrus and longer estrus duration, the estrus speed (30.80 hours to 2.5 hours), and estrus length (18.6 hours to 6.5 hours).

Analysis of variance indicated that four doses of GnRH significantly (P<0.05)
influential on the estrus length in the swamp buffalo producing of dadih. Table 1 showed that estrus length of the swamp buffalo producing of dadih on treatment 200 ml/buffalo produced the fastest estrus; 16 h after the second GnRH injection, however, it was not significantly different (P>0.05) from treatment B (225 ml/buffalo) with estrus length is 18 h. The length of estrus is the time interval between the onset of estrus and the completion of the estrus period. The estrus length is also influenced by age, body condition, and the types of hormones used for synchronization or estrus induction (Irmaylin et al. 2012).

The results show that injection of GnRH can affect the estrus duration in the swamp buffalo production of dadih. This is caused by differences in the dose of GnRH injection which can affect the duration estrus of buffalo. The difference in the duration of estrus in female swamp buffalo is caused by the difference in the number of doses of GnRH given which will affect the length of work of PGF2α (Yendraliza et al. 2012). Estrus length is the time shown by buffalo with the first range showing signs of estrus and loss of estrus signs in buffalo. Increasing the dose of GnRH synchronized with PGF2α produced different estrus length (Yendraliza et al. 2012).

Genetic improvement can be obtained by improving feed management and reproduction in buffalo, one of one effort that can be done is injecting GnRH, day 1st, followed by PGF2α, 7th day; then GnRH 9th day; and IB, 10th day. Improve genetic quality and increase livestock populations, requires serious handling and attention in buffalo, because of the phenomenon of difficulty detecting heat related to the phenomenon of silent heat. Reproduction improvement is aimed at improving reproductive efficiency through regulating the marriage system and accurate detection of estrus or estrus striking which ends with marriage which results in a maximum number of pregnancies (Forde et al. 2011; Ismaya, 2014).

In order improve reproductive efficiency, various synchronization protocols for buffalo have been made to regulate the estrous cycle and ovulation. It has been reported that following gonadotropin-releasing hormone (GnRH) associated with PGF 2 administration and timed artificial insemination, the percentage of ovulating buffaloes were 60-90% (Chaikhun et al 2010) with conception rates recorded as 32.7%-60% during the breeding season (Konrad et al 2013). Buffaloes were treated with progesterone (P4)-releasing intravaginal device (PRID) along with pregnant mare serum gonadotropin (PMSG) and PGF 2, the ovulation rate during the low breeding season and breeding season was 58.3% and 91.7%, respectively (Barile et al. 2015) and pregnancy rate was between 28% and 52.7% during the non-breeding season (Neglia et al. 2016; Carvalho et al. 2013). (Neglia et al 2016) observed a pregnancy rate of 45% in buffalo cows synchronized with PGF2α alone and 48.8% when PGF2α was combined with GnRH injection at the time of AI. Similarly, 33.3, 43.7, 36.0, and a 15.0 vs. 51.4% pregnancy rate was recorded in Murrah buffalo, Italian buffalo, Italian cyclic buffalo and Swamp buffalo heifers vs. cows after using the ovsynch.
protocol and timed insemination, respectively. In the current study, the circulating concentrations of progesterone precisely indicated the presence or absence of a CL and reflected its size and activity (Chaikhum et al. 2010; Neglia et al. 2016) The average estrus length of the swamp buffalo producing of dadih in treatment B (225 ml/buffalo) is 18 h. According to Barile (2015) who reported that the average onset of estrous was 54.6 h in buffaloes.

**Progesterone levels**

The levels of the hormone progesterone in each treatment are shown below.

| Doses GnRH (ml) | Progesterone Levels (ng/ml) |
|----------------|-----------------------------|
|                | Days - 0 (A) | Days - 3 (B) | Days - 12 (C) |
| 220            | 4,012<sup>A</sup> | 6,096<sup>B</sup> | 8,667<sup>C</sup> |
| 225            | 2,989<sup>A</sup> | 5,880<sup>B</sup> | 9,587<sup>C</sup> |
| 250            | 3,386       | 6,298       | 8,413       |
| 275            | 2,901<sup>A</sup> | 5,901<sup>B</sup> | 7,988<sup>C</sup> |

Note: ABC superscript value that showed very significant difference (p<0.01)

The results showed that the administration of GnRH at doses of 200 ml, 225 ml, and 275 ml showed significant (p <0.01) results on progesterone levels in buffaloes. However, administration of a 250 ml dose level gives a significantly different increase to buffalo progesterone levels on the 12th day was the high level of progesterone in the 225 ml treatment. The highest level of progesterone in the luteal phase occurred on day 21, amounting to 5.21 ng/ml and the lowest level at the beginning of the phase was 0.40 ng/ml. In the follicle phase, progesterone levels are generally low in sores ranging from 0.07 ng/ml to 0.55 ng/ml (Bearden et al. 2004).

**CONCLUSION**

It is concluded that the best GnRH doses for estrus synchronization in the swamp buffalo producing of dadih was 225 ml/buffalo with estrus speed 18.2 h and estrus length 18 h. Progesterone levels have increased significantly starting from before treatment until after treatment with GnRH.

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