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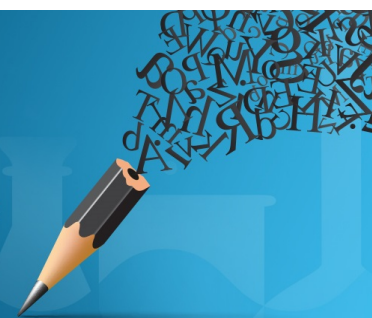


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Utilization of Herbs in Synchronizing Estrus in Cattle

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Abstract. The livestock industry has hit the country groundbreaking year with numerous issues. The economy and futuristic availability for livestock are all in doubt, which has the potential to diminish market prices. Having greater insight into optimal estrus synchronization protocol is critical for animal management capabilities. The utilization of controlled internal drug release (CIDR) to maximize the number of cattle by controlling ovarian function has benefited the population in many ways. Recent research indicates that herbal supplementation is highly promising and could be a viable new practice for reproductive efficiency. Thus, the study was conducted to compare the estrus signs and pregnancy rate derived from CIDR and modified herbs groups. A total of six Charolais Brahman cross cattle were subjected to two estrus synchronization protocols. Group A (CIDR, n=3) received Eazi-Breed CIDR contained 1.38 g of progesterone inserted for seven days and preceded by a 10 ml injection of Lutalyse prior to CIDR withdrawal, whereas Group B (Modified herbs, n=3) herbal fed to the cattle for three days continuously. Estrus signs were monitored following treatment removal by visual observation for 45 minutes at 0700h, 0900h, 1100h, 1300h, 1500h, 1700h, 1900h and 2100h. Cattle were artificially inseminated after 12 hours of estrus onset and pregnancy diagnosis was performed between days 35 and 40 of gestation. The study revealed that the occurrence of estrus signs was higher in CIDR 88.89% than in modified herbs 77.79%. Plus, the estrus signs were the highest in the evening at 61.11% for CIDR and modified herbs compared to morning (38.89% vs 33.33, respectively) and night (55.55% vs 50%, respectively). Pregnancy diagnosis with PregnaDrop test kit found that both groups had a similar pregnancy rate of 100%. Lastly, the study demonstrates that the CIDR is the most efficient tool for eliciting estrus, while herbal supplement offers a novel approach for effective synchronization with high fertility.

INTRODUCTION

The livestock industry is a critical economic activity as it generates supplementary revenue for farmers sustaining their livelihood. In Malaysia, cattle farming is often operated on a small scale and dominated by smallholder farmers owning an average of ten cows. Difficulties arise when the beef crisis has recently been in the headlines owing to incapable of keeping up with increasing demand. Malaysia generates 46,923 tons of beef annually, while the demand was more than 205,000 tons in 2018 [1]. Historical changes in the demand for livestock products by necessity have been primarily driven by human population growth, wage gains, and urbanization, causing beef consumption to rise over time. Owing to this, Malaysia would import approximately 75% to 80% of the beef from different parts of the world to meet domestic consumption [2]. The quest for solutions to this issue is long-standing for almost the past 40 years, and today's disappointing outcome could be attributed to the lack of focus on the industry.

Reproductive failure is one of the most significant factors limiting animal productivity, accounting for millions of annual losses. Previously, there are enormous typical strategies for expanding the beef cattle industry, which has been highly competitive in livestock production. Research on estrus synchronization has effectively aided farmers in detecting estrus signs and allows them to schedule their breeding program systematically. In the history of development breeding programs, progesterone containing intravaginal devices has been considered a critical factor in advancing reproductive schemes by synchronizing estrus in cattle and sheep species for more than 40 years [3]. Despite the considerable efforts in the past, it is worth noting that the exogenous hormones have been identified as practical failings to farmers attributed to high costs, unreliability, and scarcity in rural areas. Therefore, it would be advantageous to have an effective non-invasive method for estrus synchronization that made it feasible in practice. With regard to animal welfare, the underlying mechanisms driving herbal have begun to unfold only recently. Current best practices and emerging novel techniques of completely herbal preparations using *Cinnamomum zeylanicum* (cinnamon), *Piper nigrum* (black pepper), *Zingiber officinale* (ginger) and *Linum usitatissimum L.* (flaxseed) have obtained a greater understanding of their mechanism in regulating the female reproductive system. Each mentioned herb has already been established to increase metabolic products in the body, resulting in increased sexual function enhancers [4,5].

One particularly notable feature of this approach is that the herbal preparations and their active principles are also discovered to possess pharmacological properties, including an anti-oxidant, anti-inflammatory and anti-bacterial agent. Although the debate over herbal reactions is superficial, a study revealed that animals treated with herbs are more effective than CIDR at achieving estrus [6]. Further advancement of herbs is extensively suggested being touted as cheaper, efficient and safe alternatives in treating various reproductive disorders in livestock. However, the herbal preparation in the study is thought to be novel since there is a paucity of information available on estrus synchronization cows. Thus, the ultimate goals of the study were to compare the estrus signs and pregnancy rate derived from CIDR and modified herbs groups.

MATERIALS AND METHODS

Animals and Management

The study was conducted at a local cattle farm in Batu Kurau, Perak, at a longitude of 100° 79'E. A total of six Charolais Brahman cross cattle with a mean body weight of 362.38 ± 26.58 kg and body condition score of 3.5 ± 0.5 (1 to 5 scale) [7] were randomly assigned to two estrus synchronization protocols, Group A (CIDR, n=3) and Group B (modified herbs, n=3) respectively. Cattle were initially chosen based on clinical signs, history, and optimum health score. Group (A) received CIDR, where CIDR intravaginal devices (1.38 g of progesterone) were inserted for seven days in the cattle vagina. Group (B) were fed with modified herbs for three days continuously. Following the removal of CIDR devices and the cessation of herbal feeding, cattle were visually observed for 45 minutes at 0700h, 0900h, 1100h, 1300h, 1500h, 1700h, 1900h and 2100h for indication of behavioural estrus. Then, after 12h of estrus onset, all the cattle were allowed for artificial insemination (AI). Hence, the cattle were reared under an intensive management system. Each cattle had a regular feeding regimen consistent prior to the onset of protocols. The average temperature recorded at the time of the experiment was range from 32°C to 34°C. Each of the cattle was fed with a mixture of PKC and pome in the morning, while rice bran, PKC, and pome were served in the evening to meet National Research Council requirements [8]. Mineral salt and water were offered ad libitum.

CIDR Intavaginal Device

A total of three cattle in group A were conditioned into a seven days CIDR protocol [9]. Each cattle was inserted with 1.38 grams of progesterone (CIDR; 1.38 g progesterone; Pfizer Animal Health, New York, NY) into the cattle vagina irrespective of the estrus cycle stage. The device remained in the vaginal cavity for seven days until the cattle received a 10 mL lulatory intramuscular injection prior to CIDR withdrawal.

Modified Herbs

All of the modified herb components, including *Cinnamomum zeylanicum*, *Piper nigrum*, *Zingiber officinale* and *Linum usitatissimum L.* were purchased in dried powder form from the market in Perak state. A balance scale was used for weighing the mixture of modified herbs and before placed into packaging. The techniques of feeding 10 g of

the modified herbs orally using a syringe to the cattle in group B for three days continuously are similar to the methods described in previous literature [6].

Estrus Synchronization Signs

Estrus signs were considered the total number of females showing estrus within five days after CIDR removal from the total number of females who received CIDR [10]. Following treatment removal, all the cattle in treatment CIDR (n=3) and modified herbs (n=3) were observed for estrus signs. Accordingly, cattle were placed in the individual pen during the visual observation period to allow for a detailed event examination. Only one individual was responsible for visually observing estrus expressions for every session to prevent bias [11]. The most recorded estrus responses were restlessness, sniffs the vulva of other cows, rest her chin on the back of others, mounting and vulva swelling and reddening.

Artificial Insemination (AI)

Subsequently, all the cattle were artificially inseminated with high fertility frozen semen after 12 hours of expressing estrus signs. This is due to the highest pregnancy rates were observed when cows were inseminated between 6 and 24 hours prior to ovulation. The timing has been a concern because cattle probably should be bred after the onset of estrus, so the chance for successful fertilization may not be missed. Following mating, cattle were handled in accordance with standard farm practices.

Pregnancy Diagnosis

Pregnancy diagnosis was conducted between days 35 and 40 of post artificial insemination. The results were recorded as positive and negative for all of the cattle under experiment using the PregnaDrop test kit. The PregnaDrop Test Kit is a chemical reagent derived from a unique formulation that efficiently diagnoses early pregnancy. A pregnancy test using PregnaDrop is performed by dropping five drops of reagent into 3 ml of cattle urine. The sample generated white cloud sediment from non-pregnant cattle. On the other hand, pregnant cattle made no modifications to the test. The pregnancy rate was calculated with the following formula [12]:

$$\frac{\text{Number of pregnant females}}{\text{Number of mated females}} \times 100 \quad (1)$$

Outcome Measure and Analysis

Each group has at least three replications. Statistical analyses on the categorical variables (estrus signs and pregnancy rate) were analyzed using a two-tailed student t-test. The analysis was performed using standard software Statistical Package for the Social Sciences (SPSS) version 25. All data obtained were expressed as mean, mean \pm standard error of the mean (SEM). P values of <0.05 will be considered significant.

RESULTS AND DISCUSSION

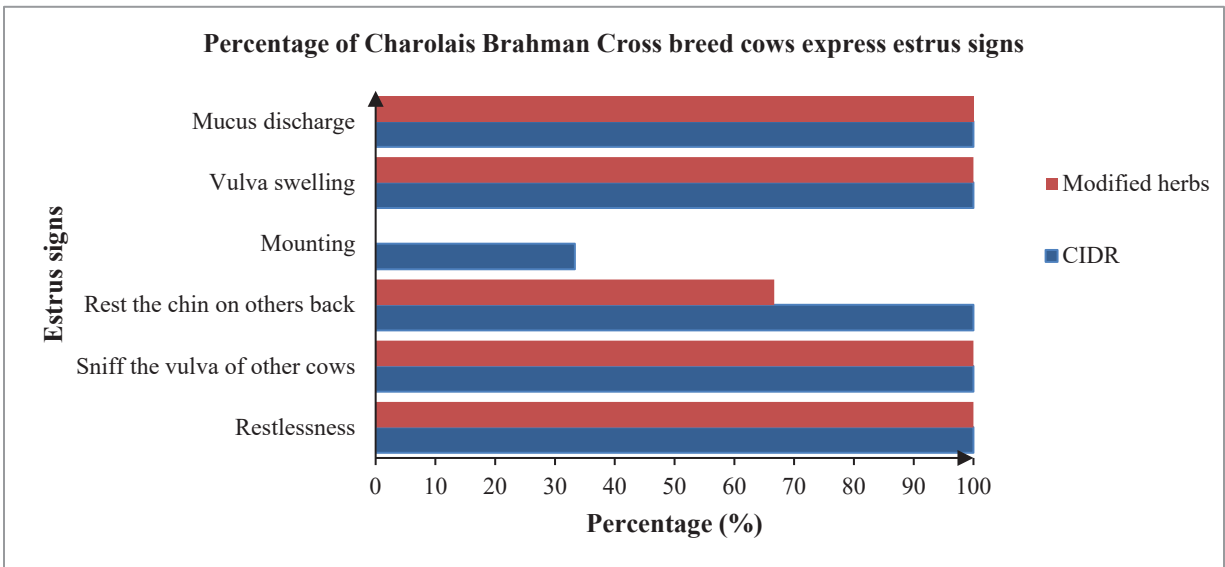


FIGURE 1. The pattern of estrus signs exhibited by Charolais Brahman Cattle in CIDR and modified herbs groups

The present study is the first to advocate the number of cattle exhibiting estrus signs and the values are expressed as a percentage into CIDR and modified herbs groups. All the estrus signs mentioned in Figure 1 above are classified as secondary estrus signs. The current study found that all the estrus signs did not significantly affect the treatment ($P > 0.05$). The highest percentage of all the estrus signs was in the CIDR group, 88.89% compared to modified herbs 77.79%. The present study obtained that restless behaviour for Charolais Brahman cross breed cattle was 100% for both groups, which was higher than crossbreed cattle 66.67% [13] and Sahiwal cattle 68.96% [14]. In livestock, restlessness signs have been shown to be strong predictors of estrus [15]. Cattle in the midst of estrus are constantly agitated, walking faster, trailing other cattle, and bellowing louder [16]. Thereby, the present finding further supports the idea that estrus cattle expend less time resting than other cattle [17]. It should be highlighted that the estrus cattle generally remain standing and alert while others are resting.

Hormonal variation related to estrus may cause an increase in blood flow to the reproductive organs, resulting in swelling and reddening of the vulva [15]. When the labia are opened, an extreme dark pink to red and heavily moistened vagina is visible [18]. Undoubtedly, a further pinkish swollen appearance attracts the male to scent the genitalia and rub the vulva even more often when the cattle are in estrus. In addition, both groups were observed sniffing and vulva swelling at 100% in the current research as expected. Similarly, previous research discovered 100% sniffing in crossbreed cattle [19]. On the contrary, it was higher than 91.38% for Sahiwal cattle [18]. Moreover, rest the chin on others back was 100% for the CIDR group, consistent with the previous study at 100% on lactating Holstein Friesian cattle [20,21]. For the modified herbs group, estrus signs were recorded at 66.67%.

The presence of an active ingredient may have an effect on the action of herbs on estrus synchronization signs. The study ultimately showed that the most important components contained in cinnamon are cinnamomin and cinnamaldehyde. Simultaneously, black pepper possesses piperine as the primary pungent alkaloid. The notion of cinnamon and black pepper are well established for their potential to enhance reproductive performance by affecting the hypothalamus-pituitary axis and elevating luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone levels [4,22]. On the other hand, ginger has garnered special attention as a dietary supplement for animals. Anti-oxidant activity exhibited by active components of the ginger rhizomes such as gingerols, shogaols and zingerone has reduced the formation of the enzyme xanthine oxide. The enzyme is primarily responsible for the production of reactive oxygen species (ROS) [23]. Previous research has confirmed that low ROS levels have been shown to delay the production of nitrogen-based free radicals, which will positively impact the egg maturation, ovulation and development of the corpus luteum during pregnancy [24]. Generally, flaxseed accumulates a wide variety of

biologically active compounds and elements, including linolenic acid, linoleic acid, polysaccharides and alkaloids. Some unique features of the flaxseed have made it a significant source of omega-3 fatty acids, particularly α -linolenic acid (ALA). Consumption of omega-3 fatty acids potentially improved reproductive efficiency, which may have accelerated folliculogenesis in the ovaries [25]. Therefore, previous study proposed that greater dietary flaxseed supplementation directly affects the ovulation of large follicles in pregnant cattle [26].

Previously, the mounting activity observed in Holstein cattle has been considered to be 80% with a frequency of (2.9 mounts/h) [27]. Contrary to expectations, the average mounting activity observed in the present study was 33.33% (2 mounts/h) for CIDR and 0% for modified herbs groups. However, this was 0.8 lower than the frequency for Charolais cattle (2.8 mounts/h) [28]. This research corroborates with the finding of estrogen greatly stimulates mounting activity while progesterone inhibits it [29]. As a result, the mounting frequency in proestrus and estrus cattle is considerably higher than in non-estrus, with a function of the corpus luteum. Therefore, the lack of estrus manifestation in certain cattle apparently can be related to the optimal timing for administering the luteolytic agent, resulting in a lower prevalence of cattle engaged in the mounting activity. One of the aspects that can increase the arousal potential of mounting activity is temperature. To be more precise, the temperature had a detrimental effect on the number of mounts [30]. It appears that a decrease in LH secretion was resulting in inhibited follicular steroid production, lowering plasma estradiol levels and adding to reduced estrus identification [31]. Alongside the increase in activity on estrus signs, the present study also found that both groups had 100% mucus discharge. By contrast, this result differs from the one obtained in Fogera cattle and heifer at 61.7% [32] and 75.6% in pluriparous Sahiwal cattle [14].

TABLE 1. The total percentage of Charolais Brahman crossbred cattle exhibited estrus signs during morning, evening and night for CIDR and modified herbs group

Estrus observation	CIDR (%)	Modified herbs (%)
Morning	38.89	33.33
Evening	61.11	61.11
Night	55.56	50.00

%-Percentage

Table 1 displays the percentage for the morning, night, and evening observation times. The percentage of cattle that express estrus signs in morning, evening and night was not affected ($P < 0.05$) by treatment. Overall, the evening has the highest percentage of estrus response, with 61.11% for both groups. A noticeable difference between the estrus observations at night for the CIDR group was higher at 55.56% than the modified herbs group at 50.00%. In addition, the most negligible estrus response was in the morning, with only 38.89% and 33.33% for CIDR and modified herbs respectively. The findings contradict a previous study, which indicated that estrus begins in the late evening, with peak sexual activity occurring at night [33]. It seems possible that the finding is attributed to the fact that night is preferable for the initiation of estrus and the LH surge for animals [34]. In practice, the variation in the number and duration of estrus signs may be attributed to the disconnection of estrus behaviour in secondary signs detected by activity monitors restlessness and standing estrus [35].

TABLE 2. Accuracy of pregnancy diagnosis by PregnaDrop from the first trimester of gestation

Groups	Pregnant urine		
	Positive (+)	Negative (-)	Percentage (%)
CIDR	3	0	100*
Modified herbs	3	0	100*

*-Percentage of true positive

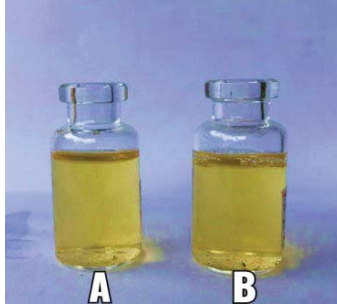


FIGURE 2. Result of early pregnancy diagnosis using PregnaDrop. (A) After (Urine + PregnaDrop), (B) Before (Urine only)

Table 2 illustrates the accuracy of pregnancy diagnosis using the PregnaDrop. PregnaDrop is a chemical reagent that employs a novel formulation to quickly diagnose early pregnancy, rendering reproduction quality control more noticeable. A total of six cattle were examined for pregnancy diagnosis between days 35 and 40. It is apparent from this table that cattle in both groups were confirmed with a 100% of pregnancy rate. The findings agree well with existing studies [6], which found that the CIDR and modified herbs group in goats had a 100% pregnancy rate. However, the finding was greater than 88.9% in suckled beef cows treated with CIDR for 7 days [36]. The above Fig. 2 illustrate that the results obtained from the urine of pregnant cattle remain clear from cloud and sediment incomparable to non-pregnant cattle, which immediately develop white sediment after analysis. Hence, the accuracy of PregnaDrop within one to two months of gestation was reported to be 95% [37]. Insights into this aspect are vital to better comprehend the beneficial feature of non-invasive pregnancy diagnosis using urine. In short, pregnancy diagnosis using PregnaDrop is the current best practice for the farmers as it requires less expertise, minimal instrument and simple techniques.

CONCLUSION

Among the two estrus synchronization protocols used in this study, the CIDR had comparatively higher effectiveness in achieving satisfactory estrus signs as opposed to the modified herbs. The overall pregnancy rate for both groups was similar at 100%, allowing producers to make a precise judgment to implement the best protocol depending on their preferable. The utilization of this natural herbal can result in most cattle exhibiting estrus without the need for a holistic approach. Therefore, meticulous attention to detail in the modified herbs is crucial to create a novel opportunity in the estrus synchronization program. Although both groups were identified as effective in reproductive performance, the experiment can be further improved by increasing the number of cattle and herbal feeding regimens in the future.

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