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Influence of heat stress on pregnancy rate of beef cattle heifers and cows using ovsynch plus CIDR followed by timed artificial insemination in Thailand

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Original Article

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Abstract

This study aimed to evaluate the pregnancy rate in beef cattle heifers and cows in Thailand under different heat stress conditions using the Ovsynch plus controlled internal drug release (CIDR) followed by timed artificial insemination (TAI) protocol. Eighty-one beef heifers and ninety-five postpartum cows were tested. These cattle were divided into three groups. Group 1 (29 heifers and 33 cows) was examined in winter, while Group 2 (25 heifers and 30 cows) and Group 3 (27 heifers and 32 cows) were examined in summer and rainy season, respectively. All cattle were treated with Ovsynch plus CIDR for 7 days followed by TAI protocol. The TAI was performed on Day 10. Pregnancy rate was determined circa 60 days after TAI. Temperature and relative humidity for each season were classified according to the temperature and relative humidity index (THI). Winter was classified as a mild stress condition, while summer and rainy season were categorized as moderate stress conditions. The pregnancy rate of the heifers and cows in the mild stress condition (67.74%) was higher than of those in the moderate stress condition (49.12%) ($P<0.05$). The pregnancy rates of heifers compared to cows, however, were not significantly different in all the seasons year-round. In conclusion, this study suggests that heat stress affects the pregnancy rate of beef cattle in Thailand, namely the pregnancy rate of heifers and cows in the mild stress condition was better than in the moderate stress condition (using Ovsynch plus CIDR followed by TAI protocol).

Keywords: beef cattle, CIDR, estrus synchronization, heat stress, Ovsynch, THI

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Introduction

The weather in Thailand, a tropical country, is characterized by high temperature and humidity. These climate conditions generally cause heat stress in beef cattle and other ruminants. Heat stress induces dramatic physiological effects, i.e. hyperpnea and hyperthermia (Armstrong, 1994), decreased intensity of estrus (De Rensis and Scaramuzzi, 2003), shortened estrus duration and increased number of anestrus cows (Kadokawa et al., 2012; De Rensis et al., 2015), reduced pregnancy rates (Hossein et al., 2013; Khan et al., 2013) and increased embryonic loss (Lòpez-Gatius, 2012). In short, heat stress is harmful to many body systems and processes, especially reproductive performance and embryo development (De Rensis et al., 2015).

Cattle in heat stress conditions have a short estrus period and present lower intensity of estrus behavior. This results in imprecise estrus detection, an increase in the number of days open (Kadokawa et al., 2012; De Rensis et al., 2015), and a low pregnancy rate after artificial insemination, a phenomenon frequently found in beef cattle (Carvalho et al., 2008). Hence, a strategy to enhance pregnancy rate is required to decrease the deleterious effects of heat stress condition. One such strategy is the use of a timed artificial insemination (TAI) protocol including estrus synchronization of gonadotropin-releasing hormone (GnRH) and prostaglandin F2alpha ($PGF_{2\alpha}$) (Pursley et al., 1995).

Estrus synchronization controls the follicular development and luteal phase, resulting in a luteinizing hormone (LH) surge and ovulation (Kasimanickam et al., 2012). In addition, the estrus synchronization protocols in suckled beef cattle are more successful if $PGF_{2\alpha}$, GnRH, and/or a progesterone combination are utilized to induce cyclic and estrus behaviors (Lamb et al., 2001). The protocol using GnRH combined with $PGF_{2\alpha}$ treatment is called Ovsynch. The Ovsynch protocol enables estrus synchronization and optimization for TAI without the need for estrus detection (Pursley et al., 1995; Kasimanickam et al., 2012). The Ovsynch protocol is also used in postpartum beef cows to improve pregnancy rates (Geary et al., 1998). This protocol comprises three steps: a first injection of GnRH in beef cattle, followed by injection of $PGF_{2\alpha}$ on Day 7, and a second injection of GnRH approximately 30 to 48 h after the $PGF_{2\alpha}$. After that the TAI can be carried out in 8 to 24 h (Pursley et al., 1995). According to the mechanism of this protocol, the first GnRH injection will release LH and cause ovulation in the dominant follicle. After the ovulation period, a new follicular wave will reemerge within 2 days. On Day 7, luteolysis is caused by $PGF_{2\alpha}$, and then the re-injection of GnRH also assists in releasing LH. Finally, ovulation will be induced in the new dominant follicle (Bo et al., 2002). Martinez et al. (2002), however, reported that the Ovsynch protocol performed better in cows than in heifers. This previous study used the combination of progesterone with the 7-day Ovsynch protocol to enhance the pregnancy rate in beef heifers followed by TAI (Martinez et al., 2002). In addition, the use of exogenous progesterone combined with the Ovsynch protocol is expected to control the luteal phase between the first GnRH administration and the $PGF_{2\alpha}$ administration (Kasimanickam et al., 2012). The existing exogenous progesterone treatment for successful estrus synchronization in beef cows is in the form of a controlled internal drug release (CIDR) device. Therefore, it is crucial to synchronize estrus in suckled beef cows as quickly as possible after calving in order to replace heifers, leading to estrus behavior at a younger age. In addition, CIDR can synchronize almost 50% of non-cycling cows so that they express obvious estrus behaviors after CIDR removal (Larson et al., 2006). The treatment with intravaginal **C**IDR impregnated with progesterone for 7 to 12 days induces a negative feedback in the hypothalamus and pituitary, which depresses the release of gonadotropin hormone (Barile, 2012). After CIDR withdrawal, large amounts of gonadotropin hormone will be released to stimulate the development of follicles, which results in releasing estradiol hormone, expressing estrus behavior and synchronizing dominant follicles to ovulate (Cerri et al., 2009). $PGF_{2\alpha}$ administration at the time of CIDR termination could lead to dysfunction of the corpus luteum (CL), leading to a successful growth of follicles and estrus induction (Pursley et al., 1995).

The temperature humidity index (THI) can be used to assess the influences of ambient temperature and relative humidity (RH) on heat stress in dairy cows (Armstrong, 1994). However, studies of THI and its effects on pregnancy rates in beef cattle after estrus synchronization followed by TAI have been limited. In Thailand, there are three seasons: winter, summer and rainy season, which differ in temperature and relative humidity. To assess heat stress levels in each season, therefore, the THI was used.

The objective of this study was to evaluate the pregnancy rates of beef cattle heifers and cows under various THI and heat stress conditions in Thailand using estrus synchronization with an Ovsynch plus CIDR followed by TAI protocol.

Materials and Methods

The experiment was approved by the Animal Ethics Committee (Project: VRP-009/2557), Faculty of Veterinary Medicine, Mahanakorn University of Technology (MUT), Bangkok, Thailand. This research was done from November 2014 to October 2015. A total of 81 Brahman beef heifers and 95 postpartum beef cows from a commercial farm in Chonburi province, Thailand (Longitude 100.9695278, Latitude 12.8396525) were initially selected for the experiment. The age of beef heifers ranged from 17 to 22 months (20.30±1.82 months), the average body weight was 343.40±42.81 kg, and the body condition score was about 3 out of 5 scales. Among the cows, the average number of postpartum days was 78.65±21.10. The age of the cows ranged from 3 to 9 years (5.76±1.68 years). The average parity was 3.53±1.53, the average body weight was 445.79±42.42 kg, and the average body condition score was about 3 out of 5 scales. These 176 heifers and cows were healthy, cyclic and free from any reproductive disorders. Before conducting the experiment, each cattle sample was monitored by transducer ultrasonography (7.5 MHz, B-mode, linear-array transducer scanner; Mindray®; Version DP-6600 Vet,

Mindray Medical International Limited, Shenzhen, China) to examine cyclic and reproductive organs. These sampled cattle were housed in a free stall barn and fed daily on roughage. Clean water was available *ad libitum* and the cattle were allowed to walk freely for grazing. The cattle were dewormed four times a year and routinely vaccinated against diseases in order to conform to the standard vaccination program provided by the Faculty of Veterinary Medicine, MUT.

The experimental animals were divided into three groups by means of simple randomization. In Group 1, the sampled cattle (n=62; 29 heifers and 33 cows) were examined in the winter of Thailand from November 2014 to February 2015, when the average (mean±SD) temperature and relative humidity (RH) were 27.9±1.12°C and 65.75±4.86%, respectively. In Group 2, the sampled cattle (n=55; 25 heifers and 30 cows) were examined in the summer, between March and June 2015, when the average temperature and RH were 30.55±0.79°C and 71.00±1.41%, respectively. The remaining sampled cattle (n=59; 27 heifers and 32 cows), which constituted Group 3, were examined in the rainy season, between July and October 2015, when the average temperature and RH were 29.78±0.73°C and 73.25±4.35%, respectively. The temperature and relative humidity were recorded everyday (round the clock daily) by Meteorological Department, Chonburi province, Thailand.

All selected samples were estrus synchronized with the Ovsynch plus CIDR protocol, as shown in Figure 1. They were provided with the first GnRH injection (100 µg Buserelin; Receptal®, Intervet International B.V., Bangkok, Thailand) on Day 0 (starting the protocol), followed by intramuscular treatment with 500 µg PGF2α, (Cloprostenol; Estrumate®, Intervet International B.V., Bangkok, Thailand) on Day 7, and a second GnRH injection (100 µg Buserelin; Receptal®) intramuscularly on Day 9 (in the evening). In addition, the cattle were treated with intravaginal CIDR devices (1.9 g progesterone CIDR®; Eazi-Breed®, Pfizer Animal Health, Hamilton, New Zealand) for 7 days; these were inserted on Day 0 immediately after the first GnRH injection. The TAI was completed on Day 10 in the morning, approximately 15 hours after the second GnRH injection. Pregnancy rate was determined by transrectal ultrasonography (7.5 MHz, B-mode, linear-array transducer scanner; Mindray®; Version DP-6600 Vet) approximately 60 days after the TAI was conducted.

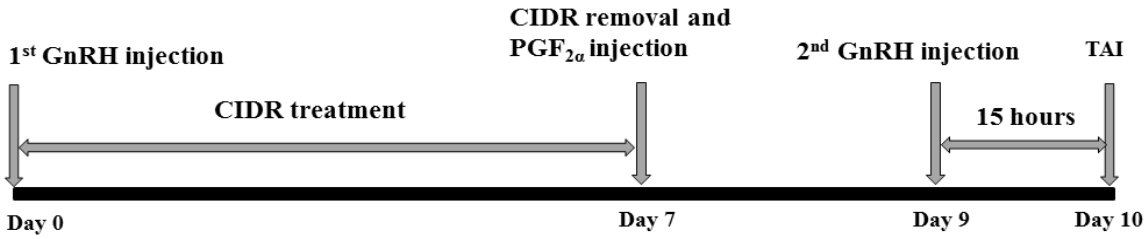

Figure 1 Ovsynch plus CIDR treatment followed by timed artificial insemination (TAI) protocol in beef heifers and cows

Statistical analysis: Pregnancy rates were compared between the different treatment groups in three different seasons and stress conditions which were calculated using the temperature humidity index (THI). The different pregnancy rates between the groups were statistically analyzed by using χ^2 test. A probability of $P<0.05$ was considered significant.

Results

The pregnancy rates of the two types of cattle (i.e. heifers or cows) using the Ovsynch plus CIDR treatment followed by TAI protocol in the winter, summer and the rainy season of one year are presented in Table 1. All year round (winter, summer and rainy season), the pregnancy rates of the beef heifer group and the cow group were not significantly different. The three seasons in Thailand displayed diverse degrees of temperature and relative humidity. The averages of temperature and relative humidity of each season, from November 2014 to October 2015, in the experimental region are illustrated in Figure 2. The heat stress conditions for cattle were calculated from the average temperature and relative humidity by using the THI values. As for the THI values, the average temperature and relative humidity in the winter (27.9±1.12°C and 65.75±4.86%) of this region were classified as a mild stress condition (approximately 77 of THI values), while those in the summer (30.55±0.79°C and 71.00±1.41%) and the rainy season (29.78±0.73°C and 73.25±4.35%) were classified as a moderate stress condition (approximately 82 and 81 of THI values, respectively). The pregnancy rates of the heifers and cows in the mild stress condition did not differ significantly, nor did the pregnancy rates of the heifers and cows in the moderate stress condition. On the other hand, when the summarized results of the numbers between heifers and cows were arranged into one group (Table 1), the pregnancy rate of those in the mild stress condition was higher than of those in the moderate stress condition ($P<0.05$). The pregnancy rate of the heifers in the mild stress condition tended to be higher than of those in the moderate stress condition ($P=0.10$). Similarly, the pregnancy rate of the cows in the mild stress condition also tended to be higher than of those in the moderate stress condition ($P=0.09$).

Discussion

The findings of this study revealed that the Ovsynch plus CIDR followed by TAI protocol during all three seasons of the year performed effectively to produce pregnancy rates in the beef heifers (60.49%) and postpartum cows (51.58%), which is consistent with the findings of Martinez et al. (2002). These findings are also congruent with those of

Kasimanickam et al. (2008) in terms of beef cow pregnancy rates and those of Martinez et al. (2002) in beef heifers. Moreover, this study found that there was no statistically significant difference between the pregnancy rates of the heifers and the cows over the course of the year (winter, summer and rainy season). In other words, the Ovsynch plus CIDR followed by TAI protocol provided similar results in terms of enhancing the pregnancy rates of beef heifers and those of postpartum beef cows in both mild and moderate stress conditions. In accordance with the present results, the Ovsynch plus CIDR treatment followed by TAI performed better in enhancing the pregnancy rates of either group of cattle (heifers and cows). Furthermore, the pregnancy rates of the heifers compared to the cows in the same stress condition were not significantly different. In contrast, when the number of heifers and cows were summarized into the same group, the different stress conditions, as evaluated by the THI values, had impact on their pregnancy rates. It was also found that the pregnancy rate of the heifers and cows in the mild stress condition were higher than that of the heifers and cows in the moderate stress condition. In addition, the location of experimental farm in Chonburi province had no effect on the THI values because this study used the average temperature and relative humidity of Chonburi province recorded by Meteorological Department for calculation of the THI values.

Table 1 Pregnancy rates of beef cattle treated with Ovsynch plus CIDR protocol followed by TAI year-round

Cattle type	Season	Stress condition classified by THI	Pregnancy rate (No. pregnant cattle)	P value
Heifers	Winter, summer and rainy season	Mild and moderate	60.49% (49/81)	0.24
Cows	Winter, summer and rainy season	Mild and moderate	51.58% (49/95)	
Heifers	Winter	Mild	72.41% (21/29)	0.46
Cows	Winter	Mild	63.64% (21/33)	
Heifers	Summer and rainy season	Moderate	53.85% (28/52)	0.36
Cows	Summer and rainy season	Moderate	45.16% (28/62)	
Heifers+cows*	Winter**	Mild	67.74% (42/62)[a]	0.02
Heifers+cows*	Summer and rainy season**	Moderate	49.12% (56/114)[b]	
Heifers	Winter	Mild	72.41% (21/29)	0.10
Heifers	Summer and rainy season	Moderate	53.85% (28/52)	
Cows	Winter	Mild	63.64% (21/33)	0.09
Cows	Summer and rainy season	Moderate	45.16% (28/62)	

[a,b]The averages (±SD) of pregnancy rates in the same column are significantly different (P<0.05).
*Heifers+cows indicates the summarized numbers of heifers and cows to be the same group.
**Winter is categorized as mild stress condition, while summer and rainy season are categorized as moderate stress condition by THI values (Armstrong, 1994).

However, the pregnancy rate of the beef cows in the mild stress condition (winter) was similar to that found in the study by Sakase et al. (2005), in which the Ovsynch plus CIDR treatment followed by TAI protocol was utilized with suckled Japanese Black beef cows in Japan. Likewise, the pregnancy rate of the heifers in the mild stress condition was similar to the findings of Kawate et al. (2004), in which the Ovsynch plus CIDR treatment followed by TAI protocol was used with suckled Japanese Black beef cows from autumn to winter (or from September to February) in Japan.

According to the findings of this study, it is highly probable that different stress conditions in the three seasons had impact on the pregnancy rates of beef cattle treated with Ovsynch plus CIDR followed by TAI protocol. To put it another way, the cattle well adapted to the temperature and relative humidity in the mild stress condition attained a greater pregnancy rate than those in the moderate stress condition, in which the weather conditions were more severe. In a tropical country like Thailand, the temperature and relative humidity are high throughout the entire year, and this normally generates greater stress conditions in the animals. As a result, these stress conditions could stimulate the hypothalamus-anterior pituitary-adrenal cortex to release the hormone cortisol into the blood circulation. The increased cortisol could then suppress hypothalamic functions and decrease the release of the hormone gonadotropin, causing ovulation failure, and decreasing the pregnancy rate in animals (Megahed et al., 2008). Furthermore, the low pregnancy rate under heat stress conditions might be related to a reduction in the viability and fertility capacity of spermatozoa after insemination into the reproductive tract of hyperthermic female cattle (Hansen et al., 2001). Also, heat stress could be harmful to the embryo and cause embryonic loss (López-Gatius, 2012). Thus, the beef cattle yielded a higher rate of pregnancy in the mild stress condition than in the moderate condition after the Ovsynch plus CIDR followed by TAI protocol. In addition, there is evidence, strongly supported by Garcia-Ispierto et al. (2007), that when the THI values are higher than 75, the pregnancy rate decreases approximately 10-20%. According to the results from the present study, the pregnancy rates of the heifers

and cows in the moderate stress condition with 81-82 THI values were lower than of those in the mild stress condition (77 THI value). Therefore, the negative effect of heat stress on animal farms could be decreased by the installation of shades, fans or air conditioning (Garcia-Ispierto et al., 2007) and by better management, including the use of the Ovsynch plus CIDR followed by TAI protocol (El-Tarabany and El-Tarabany, 2015). Similarly, as De la Sota et al. (1998) suggested, the decrease of the pregnancy rate of cattle during the heat stress condition in the summer could be dealt with by means of estrus and ovulation synchronization, together with the Ovsynch followed by TAI protocol. An explanation might be that the second GnRH injection of the Ovsynch protocol increased the GnRH hormone level in the blood circulation, compensating for the inadequate amount of GnRH hormone released from the hypothalamus. The increase of the GnRH hormone brought about ovulation and increased the likelihood of pregnancy in cattle.

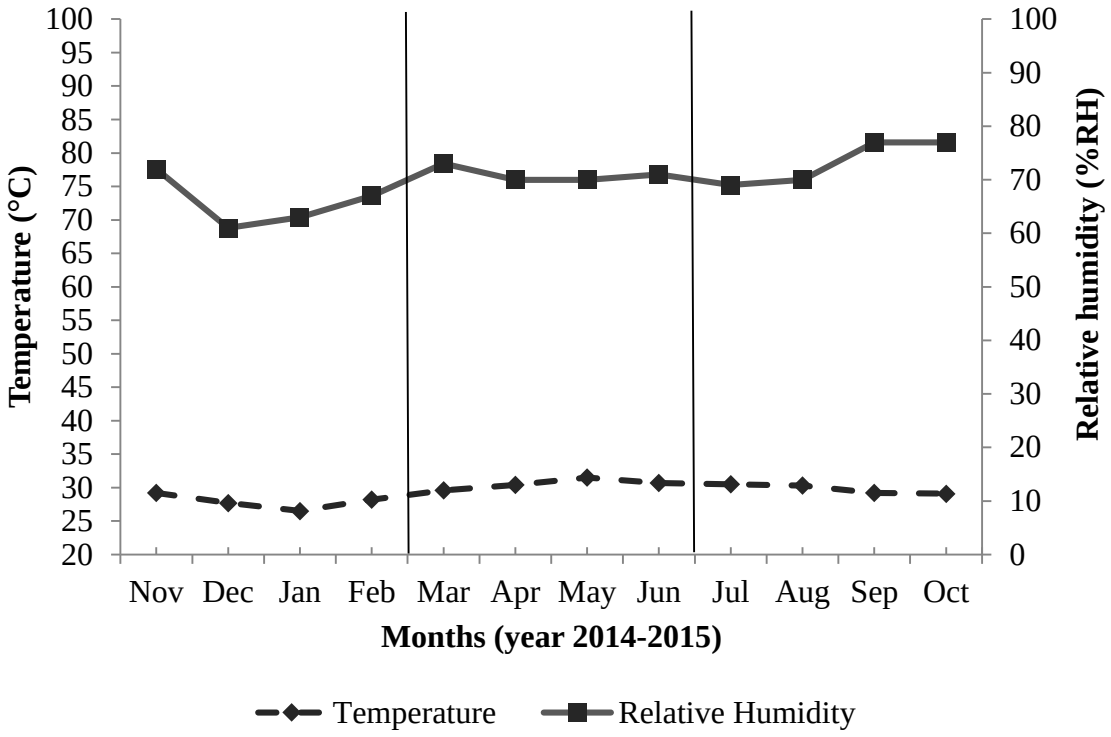

Figure 2 The average temperature (°C) and relative humidity (%RH) of each month divided into three different seasons as follows: winter (November 2014 to February 2015), summer (March 2015 to June 2015) and rainy season (July 2015 to October 2015) of Chonburi province, according to the Meteorological Department, Thailand

Therefore, the findings of the current study could be beneficial to beef cattle producers; they could use the estrus and ovulation synchronization procedure, along with the Ovsynch plus CIDR followed by TAI protocol, to effectively enhance the pregnancy rates of beef cattle in mild or moderate stress conditions. However, it is recommended that they adopt this procedure in the mild stress condition of winter to obtain the most effective results in terms of increased pregnancy rates.

In conclusion, the weather in tropical countries like Thailand causes heat stress in beef cattle, which influences the pregnancy rate. In mild stress condition, beef cattle heifers and cows could attain higher pregnancy rates than in moderate stress condition by using estrus synchronization with the Ovsynch plus CIDR followed by TAI protocol.

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บทคัดย่อ

อิทธิพลของความเครียดจากความร้อนต่ออัตราการตั้งท้องของโคเนื้อสาวและนางที่ถูกเหนี่ยวนำด้วยวิธี Ovsynch plus CIDR แล้วผสมเทียมแบบกำหนดเวลา ในประเทศไทย

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การศึกษาประเมินอัตราการตั้งท้องของโคเนื้อสาวและนางที่ถูกเหนี่ยวนำด้วย Ovsynch กับ Controlled Internal Drug Release (CIDR) แล้วผสมเทียมแบบกำหนดเวลา ในสภาวะเครียดจากความร้อนที่แตกต่างกันของประเทศไทย แบ่งโคสาว 81 ตัวและโคนางหลังคลอด 95 ตัวเป็น 3 กลุ่มดังนี้ กลุ่ม 1 (โคสาว 29 ตัวและโคนาง 33 ตัว) ทดลองในฤดูหนาว กลุ่ม 2 (โคสาว 25 ตัวและโคนาง 30 ตัว) ทดลองในฤดูร้อน และกลุ่ม 3 (โคสาว 27 ตัวและโคนาง 32 ตัว) ทดลองในฤดูฝน ซึ่งโคทุกตัวถูกเหนี่ยวนำด้วย Ovsynch กับ CIDR 7 วัน ผสมเทียมในวันที่ 10 และตรวจการตั้งท้อง 60 วันหลังผสม สภาวะเครียดจากความร้อนในแต่ละฤดูสามารถหาจากดัชนีความสัมพันธ์ระหว่างอุณหภูมิและความชื้น ซึ่งฤดูหนาวจัดอยู่ในสภาวะเครียดน้อย ฤดูร้อนและฤดูฝนจัดอยู่ในสภาวะเครียดปานกลาง การศึกษาพบว่า โคทั้งหมด (โคสาวและโคนาง) ในสภาวะเครียดน้อยมีอัตราการตั้งท้อง (67.74%) สูงกว่าในสภาวะเครียดปานกลาง (49.12%) ($P<0.05$) แต่อัตราการตั้งท้องในทุกฤดูกาลตลอดทั้งปีของโคสาวเปรียบเทียบกับโคนางไม่แตกต่างกัน สรุปได้ว่า สภาวะเครียดจากความร้อนของประเทศไทยมีผลต่ออัตราการตั้งท้อง โดยโค (โคสาวและโคนาง) ที่อยู่ในสภาวะเครียดน้อยมีอัตราตั้งท้องหลังถูกเหนี่ยวนำด้วย Ovsynch กับ CIDR แล้วผสมเทียมสูงกว่าโคที่อยู่ในสภาวะเครียดปานกลาง

คำสำคัญ: โคเนื้อ CIDR การเหนี่ยวนำการเป็นสัด ความเครียดจากความร้อน Ovsynch THI

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