

## EVALUATION OF FRIESIAN HOLSTEIN GRADE COWS FERTILITY BASED THE LEVEL OF MILK UREA

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### ABSTRACT

This study was conducted to evaluate fertility grade of Friesian Holstein cows based on milk urea levels. A number of eight cows which fulfill the criteria of not pregnant and in second to sixth lactation, produced milk of 2.5 liters/day, and body weight ranging from 400-450 kg were used in this research. The cows were given fodder that consisted of 30 kg of forage and 10 kg of a mixture of concentrate (pollard, pulp out, and molasses), in the morning and afternoon. Milk and blood samples were taken once a week for five weeks in the morning before fed. Milk urea levels were measured using the urea FS (DiaSys) and blood samples were tested using enzyme linked immuno-sorbent assay (ELISA) with the progesterone hormone kit (DRG, Germany). The variables measured were pregnancy rate, service per conception (S/C), days open, and progesterone levels. Reproduction and milk urea levels data were analyzed by linear regression to determine the relationship between two variables. Progesterone data were analyzed descriptively. The results showed that the average calving interval, days open, and S/C were 607.5±170.27 days; 341.25±187.65 days; and 2.41±2.32 days, respectively. The average of milk urea levels was 14.16±2.55 mg/dL. Regression analysis showed a significant effect ( $P<0.05$ ) and negative correlation between milk urea level and S/C. The average of non-pregnant progesterone hormone level was 12.04±7.13 ng/mL. In conclusion, the fertility grade of Friesian Holstein cows decreased when the milk urea level at 14.16 mg/dL and non-pregnant progesterone at 12.04±7.13 ng/mL.

Key words: fertility, Friesian Holstein, milk urea level, progesterone

### ABSTRAK

Penelitian ini bertujuan mengevaluasi fertilitas sapi perah peranakan Friesian Holstein (PFH) berdasarkan kadar urea susu. Delapan ekor sapi PFH dengan kriteria berat badan 400-450 kg, laktasi ke-2-6, produksi susu 2,5 liter/hari, dan tidak bunting digunakan dalam penelitian ini. Pakan yang diberikan berupa 30 kg hijauan dan 10 kg campuran konsentrat (pollard, ampas tahu, dan molasses), diberikan pada pagi dan siang hari. Sampel susu dan darah diambil seminggu sekali sebanyak lima kali pada pagi hari sebelum diberi pakan. Kadar urea susu diukur dengan kit urea (urea FS, DiaSys Diagnostic Sistem, Jerman). Sampel plasma darah diuji menggunakan enzyme linked immuno-sorbent assay (ELISA) dengan kit hormon progesteron (DRG, Jerman). Variabel yang diukur adalah angka kebuntingan, jumlah kawin per kebuntingan (S/C), masa kosong, dan kadar progesteron. Data reproduksi dan kadar urea susu dianalisis dengan regresi linear untuk mengetahui hubungan antara kedua variabel. Data hormon progesteron dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa rata-rata jarak beranak; masa kosong; dan S/C masing-masing adalah 607,5±170,27 hari; 341,25±187,65 hari; dan 2,41±2,32 kali. Rata-rata kadar urea susu adalah 14,16±2,55 mg/dl. Hasil analisis menunjukkan terdapat pengaruh ( $P<0,05$ ) dan korelasi negatif antara kadar urea susu dan S/C. Rata-rata hormon progesteron non-pregnant adalah 12,04±7,13 ng/ml. Disimpulkan bahwa sapi perah PFH memiliki fertilitas yang rendah rendah pada kadar urea susu 14,16±2,55 mg/dl dan hormon progesteron non-pregnant 12,04±7,13 ng/ml.

Kata kunci: fertilitas, PFH, kadar urea susu, progesteron

### INTRODUCTION

A low reproductive activity of dairy cows is indicated by days open and calving interval as well as a high number of service per conception. This condition could be caused by low nutrition and imbalance of energy, protein, minerals, and vitamins. Therefore, the adequate availability of these substances will improve the animal condition.

The protein that consumed by ruminants will be destroyed by ruminal microbes to produce ammonia. This substance is a main resource of nitrogen and vital for ruminal microbes protein synthesis. In the rumen, ammonia is not all used by microbes. Some of them will be absorbed through ruminal walls and a few part will be taken to other organs via gut tunnel. The absorbed ammonia is brought by blood into the liver and converted to urea (Castillo-González *et al.*, 2014). Gunaretnam *et al.* (2013) has suggested that in cow the quality of liquid in utery can be modulated to negative in two ways of change of pH in this organ that caused by the increase of ammonia and toxic effects directly in endometrium after the increase plasma uric and

ammonia concentration. Therefore, it is needed to evaluate the grade of fertility of Friesian Holstein cows based on milk urea level and to measure the level of non pregnant progesterone.

### MATERIALS AND METHODS

A number of eight cows with the criteria of not pregnant and at second to sixth lactation, produced milk of 2.5 liters/day, and body weight of 400-450 kg were used in this research. The cows were given fodder that consisted of 30 kg of forage and 10 kg of a mixture of concentrate (pollard, pulp out, and molasses), in the morning and afternoon.

Milk and blood samples were taken once a week during five weeks in the morning before fed. Milk urea levels were measured using the urea FS (DiaSys) and milk samples were collected into the microtube of 1.5 ml. The lactoplasm was obtained using centrifuge of 12.000 rpm for 10 minutes. An amount of 0.1 mL of supernatant was collected using micropipette, and then placed into microtube of 1.5 mL. Then, the samples were analysed for the content of milk urea nitrogen

(MUN). The level of milk urea was measured using analysis kit (urea FS, DiaSys Diagnostic System, Germany). The level was measured using spectrophotometer of 570-600 nm. The concentration of milk urea was calculated using the following formula:

$$\text{Concentration} = \frac{\text{Sample absorbance} \times \text{Standard concentration (50 mg/dL)}}{\text{Standard absorbance}}$$

Blood samples were tested using enzyme linked immuno-sorbent assay (ELISA) with the progesterone hormone kit (DRG, Germany). The fertility was measured based on the variables of *postpartum* oestrus, pregnancy rate, mating per pregnancy (S/C), days open, and progesterone levels.

### Data Analysis

Reproduction and milk urea levels data were analyzed by linear regression to determine the relationship between two variables. Progesterone data were analyzed descriptively.

## RESULTS AND DISCUSSION

The results from this study showed that in average, the calving interval of dairy cows was at  $607.5 \pm 170.27$  days (Table 1). The average of this result is above normal. It is longer than the calving interval reported by Wahyudi *et al.* (2013) that found at  $472.19 \pm 156.45$  days. A shorter day has been reported as well by Iskandar and Farizal (2011) in heifers at 377 days. Osterman (2003) suggested that the average of calving interval in cows raised conventionally is between 12-18 months.

Based on the value of coefficient determinant and significance, it was found that at different calving interval the percentage level of milk urea were 0.237, 0.056, or 5.60 and 0.572 (Table 2). This results showed a lack relationship between the calving interval and the level of milk urea ( $P > 0.05$ ). However, there was a correlation between the fertility index and level of milk urea (Table 3). In particular, in Gradiol cow was found that the longest calving interval at 28 months (840 days) and the milk urea level at 11.67 mg/dL. On the

other hand, in Dahlia cow was found that the shortest calving interval at 12 months (360 days) and the milk urea level at 13.33 mg/dL. The value of coefficient determinant showed that the effect of calving interval is 5.60%. The rest of 94.4% is affected by external factors, such as climate, location, cattle house design, the quality and quantity of fodder, as well as animal health.

The calving interval is a method that can be used for evaluation of reproductive performance of dairy cows. Longer calving interval resulted in the decrease of cow productivity. An increase of length of calving interval could be affected by an increase of days open. Days open in dairy cows in this study was found at  $341.25 \pm 187.65$  days (Table 1). In average, it is mean a long days open. Wahyudi *et al.* (2013) has suggested that an average of days open is  $202.45 \pm 165.84$  days for cattle. In contrast, the cow days open reported by DeVries (2006) is at 112-166 days, which is a little bit longer (85-115 days) than the observation made by Izquierdo *et al.* (2008).

Based on the value of coefficient determinant and significance, it was found that the value between days open and the amount of milk urea is 0.252 and 0.064 or 6.40% and 0.547 (Table 2), subsequently. This results suggested that a correlation between days open and the amount of milk urea is very little, although not significant ( $P > 0.05$ ). The observation on fertility index and the amount of milk urea has obtained a longest days open in Gradiol cow (212 months or 630 days) (Table 3). Then, the amount of milk urea was at 11.67 mg/dL. On the other hand, the Dahlia cow showed days open for three months (90 days) and the amount of milk urea at 13.33 mg/dL. The value of coefficient determinant was resulted the effect of days open is 6.40%, and the rest of 93.6% affected by external factors as mentioned above.

Days open is an efficiency measurement of reproduction and directly affect the calving interval (Atabany *et al.* 2013). This aspect could affect milk production, the pregnancy and calving interval. A good quality of fodder could increase the visualization of oestrous sign. The decrease of nutrition resulted in a reduction of body condition and milk production. Then, the administration of nutritious supplement could

**Table 1.** Average index of fertility and the amount of milk urea in FH cows

Variable	Average
Calving interval (days)	$607.50 \pm 170.27$
Days open (days)	$341.25 \pm 187.65$
Service per conception	$2.41 \pm 2.32$
The amount of milk urea (mg/dL)	$14.16 \pm 2.55$
The level of progesterone (ng/mL)	$12.04 \pm 7.13$

**Table 2.** Index correlation of fertility and the amount of milk urea (mg/dL) using linear regression model, a correlation (R), coefficient determinant ( $R_{\text{square}}$ ), significance (Sig), and regression

Variable	R	$R_{\text{square}}$	Sig	Regression
Calving interval (days)	0.237	0.056	0.572	$Y = 16.331 - 0.107 \text{ CI}$
Days open (days)	0.252	0.064	0.547	$Y = 15.340 - 0.103 \text{ DO}$
Service per conception	0.759	0.577	0.029*	$Y = 16.175 - 0.835 \text{ S/C}$

\* Superscript showed significantly different ( $P < 0.05$ )

increase the ovary physiology, the onset and duration of oestrous as well as the pregnancy (Fitz-Rodriguez *et al.*, 2008; Moonmanee *et al.*, 2015).

The service per conception of dairy cows that observed in this study was at  $2.41 \pm 2.32$  times (Table 1). This result was closer to an observation by Wahyudi *et al.* (2013) at 2.93 times. However, it was higher as compared to observation in earlier studies by Hartatik *et al.* (2009) that found the value of service per conception was at 1.72 times. It has been suggested that a lower value of service per conception could increase the fertility of animals.

Based on the value of coefficient determinant and significance, the results showed that the correlation between the value of S/C and the amount of milk urea at 0.759 and 0.577 or 57.7% and 0.029 (Table 2). These results means that a very significant relationship between these parameters ( $P < 0.05$ ). Then, based on the observation of fertility and the amount of milk urea in individual cow showed different value between samples (Table 3). The highest S/C was found in Krisan cow at 8 times above normal with the amount of urea milk at 10 mg/dL. In contrast, in Mawar cow was 1 time lower with the amount of urea milk at 13.33 mg/dL. The value of coefficient determinant showed the effect of S/C was 57.7% and the rest caused by external factors. In addition to the external factors mentioned above, Baco *et al.* (2013) has suggested the influence of metabolic factors, such as hormone concentration, gonadotropine, estrogen, progesterone, body weight, and nutrition.

The amount of milk urea in dairy cow of FH was at  $14.16 \pm 2.55$  mg/dL (Table 1). In average, the concentration of milk urea in this cow was considered normal. Drudik *et al.* (2007) have showed that the average of milk urea nitrogen (MUN) was 12-18 mg/dL of milk. The variation in individual cow was 8-25 mg/dL of milk. Stoop *et al.* (2007) have reported that the average value of MUN was 12 to 13 mg/dL, although it might be between 1 to 50 mg/dL. Then, Nousiainen *et al.* (2004) and Hutjen and Chase (2007) have observed the value of MUN was 10-14 mg/dL. In addition, Hossein-Zadeh and Ardalan (2011) have also showed the value of MUN from FH cows was 8.5-11.5 mg/dL. In average they have reported the highest value of MUN was 17.97 mg/dL, which lower than the value obtained by Stoop *et al.* (2007) at 20.39 mg/100 g milk.

A higher value of MUN could cause a risk of reduction of pregnancy. In particular, when the cattle in first lactation period, the increase of the value of MUN  $> 18$  mg/dL could decreased the fertility index to 14% (Nourozi *et al.*, 2010). Milk urea is the value of measurement for estimation of the balance between cattle nutrition from appropriate protein and energy (Jilek *et al.*, 2006). The increasing value of MUN can be influenced by several factors. Some of them are inefficiency in rumen protein degradation and protein synthesis in milk tissue, or the change in conversion process in particular locations. The accumulation of ammonia in rumen resulted in the increase of urea forming process in liver and then distributed by the

blood in animal body. This process could compromise the animal reproductive activity (Dhali *et al.*, 2006). The used of MUN value in a new regulation and possibly is related to the increase of it and the decrease of fertility (Melendez *et al.*, 2000).

The results of this study showed that there is an increase of progesterone level in non-pregnant FH cow at  $12.04 \pm 7.13$  ng/ml (Table 1). The level of progesterone in luteal phase of dairy cows is at 6.6 ng/mL and during pregnancy is at 6.6 ng/ml (McDonald, 2000). A higher level of progesterone could be used as an indicator of prolong of oestrous cycle. In turn, this condition resulted in inhibition of production of estrogen, follicle stimulating hormone (FSH), and luteinizing hormone (LH), which caused an absence of ovulation (Tuasikal, 2004).

## CONCLUSION

In conclusion, there is a negative correlation between service per conception and the value of urea in milk. However, a weak positive relationship was observed between calving interval and days open. FH cow has a lower value of milk urea of 14.16 mg/dL and progesterone *non-pregnant* at  $12.04 \pm 7.13$  ng/mL.

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