



Blood Biochemical Indicators in Predicting Retained Placenta in Friesian Holstein Dairy Cows

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Abstract | Retained placenta causes prolonged days of open periods which decrease reproductive efficiency. The study aimed to detect several blood biochemical parameters such as total protein (TP), Blood Urea Nitrogen (BUN), glucose, and calcium (Ca) in the retained placenta (RP). A total of 46 dairy cows were used in this study consisting of 21 cows with RP and 25 cows with non retained placentas (NRP). Blood samples were collected $\pm$ 3 weeks prepartum, $\pm$ 1 week prepartum, 12 hours postpartum, and 3 weeks postpartum via the coccygeal or external jugular vein for biochemical parameter measurement. The results showed that total protein $\pm$ 3 weeks prepartum in RP and NRP were not significantly different (7.76 Vs 7.78 g/dl) then at $\pm$ 1 week prepartum was a decrease in RP (7.72 g/dl) while In the NRP was increase (9.00g/dl) significantly. In the other side, BUN concentrations at $\pm$ 3 weeks prepartum in RP and NRP were not significantly different (12.79 vs 13.20 mg/dl), however at $\pm$ 1 week prepartum, there was increase significant higher in RP compared to NRP (16.60 vs 14.41mg/dl). Glucose levels both prepartum and postpartum showed no significant difference. Meanwhile, calcium levels $\pm$ 3 weeks and $\pm$ 1 week prepartum both in RP and NRP were not significantly different until 3 weeks post partus and still in the normal level. Our results indicated that one week prepartus, BUN level was a significant increase in in RP compared to NRP. Therefore, BUN can be a strong indicator for predicting RP in dairy cows.

Keywords | BUN, Glucose, Calcium, Protein, Retained placenta

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Retained placenta is as failure of placenta expelled due to the problem on separating the fetal villi and maternal crypts after delivery fetal within the normal time of 8-12 hours (Taylor *et al.* 2010). Various losses arise from the retained placenta (RP) due to the emergence of microorganisms in the uterus, which causes inflammation, prolongation of the return to normal and fertile cycles after giving birth to decreased milk production (Tucho and Ahmed 2017) so that this incident is very detrimental to farmers.

Various causes of the retained placenta have been predicted, such as deficiency of Vitamin D and E (El-Shahat and Monem 2011), selenium (Jovanovic *et al.* 2013), calcium, which causes minimal muscle contractions (Mulligan *et al.* 2006) so that the placenta does not expelled after the birth process. The nutrient content of the feed, in the form of protein, selenium, iodine, vitamins A and E, and calcium, must be available in sufficient during the transition period in cattle. The transition period known as period between 3 weeks before parturition to 3 weeks after parturition. Metabolic disorders during the transition period in the cow can be caused by negative energy balance, which is common in postpartum cows (Drackley 2004). Energy reserves from fat and protein stored in the body can be converted into glucose as a reserve energy source by producing metabolic waste in the form blood urea nitrogen (BUN) (Bindari *et al.* 2013). Besides several other minerals, such as calcium, is required to help the birth process (Hadzimusic and Krnic 2012), and selenium to increase immunity during and after giving birth (Ahmed *et al.* 2009).

Blood biochemical parameters are important markers of the physiological or pathological state of the body that can quickly, sensitively, and comprehensively monitor changes in metabolites of the organism (Wishart 2019). Observing the metabolite description of various parameters such as protein, BUN, glucose, and calcium can be used to strongly predict the possibility of placental retention so that early prevention can be carried out. This study aims to analyze the relationship between the biochemical parameters of total protein, BUN, glucose, and calcium during the prepartum period as strong and pathognomonic indicators for predicting the occurrence of retained placenta.

MATERIALS AND METHODS

This research has received ethical clearance with number 226-2022 IPB issued by the Institute for Research and Community Service (LPPM) IPB on January 25, 2022.

RESEARCH ANIMALS

In this research 46 *Friesian Holstein* (FH) cows were used

with a pregnancy of ± 8 months, minimum 3rd lactation, and 5-10 years of age in the area of Lembang District, West Bandung Regency, West Java Province. The Body Condition Score (BCS) of the cattles were 2,75–3,25, a score range of 1-5 is used (England and Commonwealth). The cattle that have been selected are by the management system and breeder care standards. Selected cows are fed 3 times, morning: grass and concentrate, afternoon: grass, and afternoon: grass and concentrate. The criteria for cases of placental retention are caused by maternal weakness, not fetal factors.

SAMPLING BLOOD

Blood sampling was carried out at ± 3 weeks prepartum, ± 1 week prepartum, and after partus and 3 weeks postpartum via the coccygeal vein or external jugular vein of 5 ml using a 10 ml syringe with a 23G needle (Dewi and Durachim, 2014) carried out at 10.00 WIB with a distance of $\pm 4-5$ hours from morning feeding. Blood samples were then placed in EDTA vacutainer tubes (Zhejiang, Gongdong, China). The tube samples were labeled, then placed in a transport bag at a temperature of 4°C, then taken to the laboratory and stored for ± 1 week in the refrigerator before biochemical analysis.

ANALYSIS OF PROTEIN, GLUCOSA, BLOOD UREA NITROGEN (BUN), AND CALCIUM

Blood samples in EDTA vacutainer tubes (Zhejiang, Gongdong, China) were centrifuged (LC – 04B PLUS, Jiangsu, China) at 3000 rpm for 15 minutes. After centrifugation, 2 mL of the supernatant as blood plasma was collected. For the biochemical analysis (protein, BUN, glucose, and calcium), 0.5 mL of blood plasma was placed in a tube and then analyzed with Spectrophotometer Fujifilm Dry-Chem NX 500i (China). The spectrophotometer will suck and drip the sample into the Fuji Dry-Chem Slide. The test results can be seen on the screen, and the results are in the form of numbers with units of total protein (g/dL), BUN (mg/dL), glucose (mg/dL) and Ca (mg/dL).

DATA ANALYSIS

The levels of total protein, BUN, glucose, and calcium in the RP and NRP events were tested statistically using the ANOVA test. If the results showed very significant differences, then it was continued with the Duncan test (Steel and Torrie 1997).

RESULTS

The transition period in cow management is 3 weeks before birth (parturition) to 3 weeks after parturition, which is a critical period for dairy cows, resulting in various kinds of metabolic changes occurring in the blood, that reflect an

Table 1. Total Protein (TP) and BUN values of FH cows with cases of Retained Placenta (RP) and Non-Retained Placenta (NRP).

Diagnosis	N	Parameter	Preriod			
			3 Week Prepartum	1 Week Prepartum	1 Day Postpartum	3 Week Postpartum
RP	21	TP (g/dL)	7.76±1.17 ^{Aa}	7.72±1.49 ^{Ba}	8.09±1.50 ^{Aa}	7.75±1.20 ^{Aa}
		BUN (mg/dL)	12.79±3.68 ^{Ab}	16.60±2.60 ^{Aa}	12.10±3.34 ^{Ab}	11.88±3.36 ^{Ab}
NRP	25	TP (g/dL)	7.78±1.11 ^{Aa}	9.00±1.45 ^{Ab}	8.26±1.61 ^{Aa}	8.24±1.54 ^{Aa}
		BUN (mg/dL)	13.20±3.22 ^{Aab}	14.41±3.13 ^{Ba}	12.10±3.34 ^{Ab}	11.30±3.18 ^{Ab}

Information : Different superscript capital letters in the same column indicate a significant difference (p-value <0.05) in the diagnosis; Different superscript lowercase letters on the same line indicate a significant difference (p-value <0.05) in the period.

indication of the animal's condition. The research showed that of the 46 cows used, 21 had retained placenta (RP), and 25 cows had non-retained placentas (NRP).

The results showed that total protein ± 3 weeks prepartum in RP and NRP was not significantly different (7.76 ± 1.17 g/dL Vs 7.78 ± 1.11 g/dL) then at ± 1 week prepartum there was a decrease in RP (7.72 ± 1.49 g/dL) meanwhile NRP cases increased (9.00±1.45g/dL) with significantly different values (P<0.05). Furthermore, total protein levels at parturition in RP and NRP (8.09±1.50 g/dL vs 8.26±1.61g/dL) decreased at 3 weeks postpartum in RP and NRP (7.75±1.20g/dL vs 8.24±1.54g/dL) experienced no significant difference in values (P>0.05) as shown in [Table 1](#).

The BUN concentration at 3 weeks prepartum in RP and NRP cases had a value that was not significantly different (12.79 ± 3.68 mg/dL vs 13.20 ± 3.22 mg/dL). However, at ± 1 week prepartum, there was a significantly higher increase in RP compared to NRP (16.60 ± 2.60 mg/dL vs 14.41±3.13 mg/dL). Furthermore, the BUN concentration gradually decreased in RP and NRP cases (12.10 mg/dL ± 3.34 vs 12.10 ± 3.34 mg/dL) until 3 weeks postpartum in RP, and NRP cases (11.88 ± 3.36 mg/dL vs 11.30 ± 3.18 mg/dL) experienced an insignificant decrease.

Meanwhile, the parameters of glucose analysis result in cas-

es of RP and NRP ± 3 weeks prepartum did not show significantly different values (44.14 ± 6.101 mg/dL vs 44.48 ± 7.79 mg/dL) than at ±1week prepartum, there was an increase in RP and NRP (47.91 ± 7.63 mg/dL vs 48.92±8.27 mg/dL), until birth in RP and NRP (51.48±9.23 mg/dL vs 53.68±9.43 mg/dL). A significant increase in glucose concentration occurred from 1 week before birth until parturition (Table. 2). However, 3 weeks postpartum, glucose concentrations decreased again in RP and NRP, approaching prepartum concentrations (45.81 ± 7.99 mg/dL vs 49.24 ± 7.26 mg/dL). Glucose concentrations in RP and NRP cases during the transition period were not significantly different.

The results of the study of calcium concentrations in RP and NRP ± 3 weeks prepartum showed values that were not significantly different (6.95 ± 1.99 mg/dL vs 6.79 ± 1.84 mg/dL). Both concentrations decreased ± 1 week prepartum (6.43 ± 1.78 mg/dL vs 6.78±1.51mg/dL) until birth (6.03±1.39 mg/dL vs 5.85±1.51 mg/dL). After that (3 weeks postpartum), calcium concentration increased with values that were not significantly different (6.45 ± 1.64 mg/dL vs 6.70 ± 1.60 mg/dL). Changes in calcium concentration, both prepartum and postpartum, were within the normal range. [Figure 1](#) Illustrates a graph based on the data shown in [Table 1](#) and [Table 2](#).

Table 2. Glucose and Ca concentrations in FH cows with cases of retained placenta (RP) and non-retained placenta (NRP).

Diagnosis	N	Parameter	Preriod			
			3 Week Prepartum	1 Week Prepartum	1 Day Prepartum	3 Week Postpartum
RP	21	Glucose (mg/dL)	44.14±6.101 ^a	47.91±7.63 ^a	51.48±9.23 ^b	45.81±7.99 ^a
		Calcium (mg/dL)	6.95±1.99	6.43±1.78	6.03±1.39	6.45±1.64
NRP	25	Glucose (mg/dL)	44.48±7.79 ^a	48.92±8.27 ^a	53.68±9.43 ^b	49.24±7.26 ^a
		Calcium (mg/dL)	6.79±1.84	6.78±1.51	5.85±1.51	6.70±1.60

Information: Different lowercase superscript letters on the same row indicate significant differences (p-value<0.05) over the period.

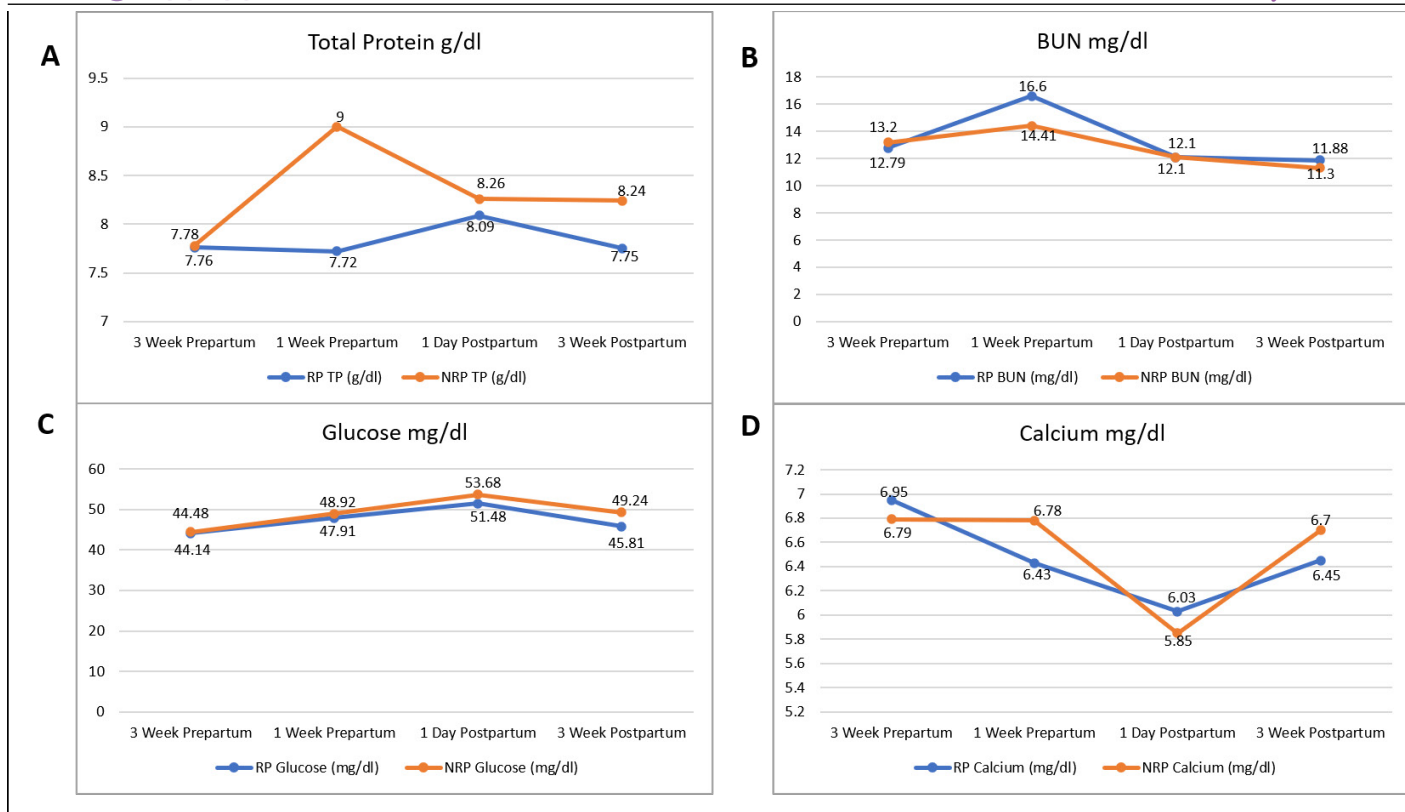


Figure 1. Total protein (a), BUN (b), Glucose (c) and Calcium (d) concentration based on transition time periods: 3 weeks and 1 week prepartum; 1 day postpartum and 3 weeks postpartum in cows retained placenta and non-retained placenta.

Figure 1 describes the changes in each blood biochemical indicator in this study in TP (a), BUN (b), glucose (c) and calcium (d) concentration for each RP and NRP cattle period. The average TP concentration in NRP was higher than in RP. The average BUN concentration in the RP was slightly higher than in the NRP. The average glucose concentration in the RP and NRP did not have a significant difference. Meanwhile, the calcium concentration in the RP showed a decrease from 3 weeks prepartum to 1 day postpartum and increased again at 3 weeks postpartum. The calcium concentration of the NRP experienced a sharp decrease at 1 day postpartum.

DISCUSSION

Before birth, preparations are made by the cows to produce a normal birth by metabolize various kinds of food reserves to prevent a negative energy balance, which will have a destructive impact on the birth and afterward. One of the main sources of food reserves used is protein as an energy source (Lu *et al.* 2020) to compensate for energy deficiencies caused by negative energy balance. Besides that, total blood protein functions to help regulate blood osmotic pressure, which is essential for determining cell membrane permeability (Utari *et al.* 2013) and increasing immunity (Bell 1995). In this study, total protein 1 week before birth in RP had lower results than NRP (7.72 ± 1.49 g/dL vs 9.00 ± 1.45 g/dL). The significant difference in total pro-

tein in RP and NRP is ± 1 week prepartus because total protein in RP has decreased for protein catabolism to provide energy before parturition. This result is different from Lu *et al.* (2020), which showed that RP's total protein was higher than NRP. This difference is likely caused by various factors, such as each individual's level of protein intake (Kaslow 2010) and the individual's stress level before birth (Piccione *et al.* 2012). Furthermore, the total blood protein is divided into 2 fractions, namely albumin and globulin (Grummer 1995). A decrease in albumin content causes inflammation, which can cause retention of the placenta and slow down the rate of tissue repair during placental retention (Kristotomus 2010). Even, a decrease in globulin content can result in immune deficiency, weakening the body's immune system (Kaslow 2010). Albumin production aligns with the protein intake that enters the body, so careful calculations are needed when administering it. Therefore, it is necessary to carry out a more detailed analysis of the total protein components in RP cases.

Blood Urea Nitrogen (BUN) is substance as results from protein catabolism (Lu *et al.* 2020), which is reflected in blood circulation. For this reason, in this study, BUN levels were analyzed to differentiate between RP and NRP. This study showed that BUN levels 3 weeks before birth did not show significant differences in RP and NRP (Table 2). However, BUN concentration increased significantly ± 1 week before birth in RP and NRP (16.60 ± 2.60 mg/dL vs

14.41 ± 3.13 mg/dL), which was statistically significantly different. This is RP cases experience protein catabolism which results in high BUN levels. These results align with the research results of [Lu et al. \(2020\)](#), who found an increase in BUN concentration at ±1 week prepartum. High concentrations of BUN are predicted to result in RP due to decreased immunity and postpartum plasma osmotic pressure, resulting in tissue edema, thereby affecting placental detachment. Furthermore, [Raboisson et al. \(2014\)](#) explained that high BUN concentrations can decrease white blood cells and inhibit cell phagocytosis, increasing the risk of experiencing placental retention. However, BUN concentration decreases from birth until 3 weeks after delivery due to decreasing stress factor. The differences in BUN concentrations in previous studies occurred due to several factors, such as the amount of protein contained in the feed, the energy contained in the cow's ration, hydration status at the time of sampling, dry matter intake, and the analytical method used ([Kauffman and St-Pierre 2001](#); [Tshuma et al. 2014](#)). The sampling time conducted by [Lu et al. \(2020\)](#) was in the morning before feeding, when the food content in the intestinal tract was relatively less; meanwhile, in this study sampling time for BUN was not carried out uniformly. It was further explained that the concentration of BUN in the blood is thought to be related to the crude protein content, the dry matter intake of dairy cows during the non-lactation period, and the analytical method used to evaluate the BUN concentration ([Tshuma et al. 2014](#)).

Glucose is the primary source for the formation of immune cells ([Ohtsuka et al. 2006](#)) and an energy source ([Rabiee et al. 1999](#)), which is positively correlated with energy balance ([Reist et al. 2002](#)). Glucose levels in the RP and NRP cows groups did not show significant changes in concentration both ±3 weeks and ±1 weeks before birth. However, after birth, there was a significant increase in both RP and NRP glucose concentrations. Suspected possibility since the birth process requires more energy for expelling the fetus and placenta, so the glucose concentration was increase sharply ([Sundrum 2015](#)). Furthermore, [Lucy et al. \(2014\)](#) explained that glucose requirements increase after birth for milk production at the beginning of lactation. In line with that, [Berlinguer et al. \(2012\)](#) and [Lu et al. \(2020\)](#) stated that the need for glucose after giving birth is twice as significant as before giving birth, so a much larger glucose supply is needed.

One of the causes of RP is that calcium deficiency which can cause a decrease in muscle function and immune function ([Mulligan et al. 2006](#)). Calcium also plays a role in regulating uterine contractions, and low calcium will result in dystocia and placental retention, which causes high cases of uterine inflammation (metritis) so that uterine involution is delayed in cows and buffalo ([Roche 2006](#)). Loss of muscle contractions can cause dystocia and placental retention,

which is supported by decreased immunity ([Panday et al. 2007](#)). In this study, calcium levels from 3 weeks before parturition until birth did not show significant differences and were still in normal levels ranging from 2-10 mg/dL, as stated by [Fadlalla et al. \(2020\)](#) and [Masoero et al. \(2011\)](#). This is because the cattle are given additional feed in the form of adequate calcium intake and controlled by cooperative management according to the cattle's needs. The results of other studies also show that Ca is closely related to the immune system through decreasing neutrophil function ([Lewis 2001](#); [Kimura et al. 2002](#)). Therefore, further analysis is needed regarding the relationship between calcium and the incidence of RP. Meanwhile, RP can occur from this study even though calcium levels are still within normal limits.

Based on the description above of the blood biochemical parameters, the total protein reported by [Lu et al. \(2020\)](#) showed a high total protein was in line with high BUN concentration. Meanwhile, results of this study were not in line with those results. Furthermore, the pattern of glucose changes follows normal changes and was not significantly different for both in RP and NRP. Likewise, the pattern of changes in calcium in the blood was inline with physiological characteristics and was not show differences in either RP or NRP. Interestingly the BUN parameters in this research had special pattern where it was increase sharply in the RP compared to NRP one week before parturition. With an interesting pattern, it might be used as strong predictors of RP.

CONCLUSIONS

Based on the blood biochemical parameters analysis, it indicated that BUN had a marked change pattern one week before parturition with a significant difference between RP and NRP. Therefore, BUN can be used as a strong indicator in predicting retained placenta. However, further research is required by analyzing of changes in levels of more specific types of protein such as albumin or globulin and involvement of other factors in the retained placenta.

NOVELTY STATEMENT

Blood Urea Nitrogen is a blood biochemical parameter as a prediagnostic indicator for prepartum placental retention in dairy cows.

AUTHOR'S CONTRIBUTIONS

All authors contriuted equally to the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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