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Original article

Total protein, albumin, and globulin levels of blood serum in repeat breeder Holstein Friesian cows

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ABSTRACT

This study aimed to determine the relationship between total protein, albumin, and globulin in the blood with the incidence of repeat breeding in dairy cows. Ten normal cycling cows and ten repeat breeder cows were taken randomly based on the reproductive records of the Tani Wilis Village Cooperative, Sendang District, Tulungagung Regency. Questionnaire data in the form of farmers' knowledge about dairy cows' reproductive systems and feed treatment is displayed descriptively. Blood samples were taken from the jugular vein (6 mL) in the morning before feeding and milking to be measured their serum total protein, albumin, and globulin levels using a Chemistry Blood Analyzer. Data were analyzed using Student t-test and Chi-square test according to the data properties, using the Statistical Product and Service Solution software at a significance level of 5%. The results showed that serum total protein, albumin, and globulin levels in normal dairy cows and dairy cows with repeat breeding were not significantly different ($p > 0.05$). However, in normal cycling cows, most (80-100%) of the total protein, albumin, and globulin levels are within the normal range. Meanwhile, the majority (60-70%) of repeat breeder cows had total protein, albumin, and globulin levels lower than the normal range ($p < 0.05$). It could be concluded that the incidence of repeat breeding was related to the total protein, albumin, and globulin levels in the blood serum of dairy cows. Therefore, it is suggested to raise the awareness of smallholder farmers about proper feeding and feeding management to reduce repeat breeding cases.

Keywords: albumin, globulin, Holstein Friesian, repeat breeder, total protein

INTRODUCTION

The dairy industry and its derivative products are increasing, supported by small and large-scale dairy farming. Smallholder dairy farming is applied in rural areas with small-scale dairy cow ownership, resulting in low business efficiency (Nurdiyansah *et al*., 2020). The productivity of dairy cows is highly dependent on the reproductive efficiency of dairy cows to periodically calving every 12-13 months, also high and continuous milk production. The

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reproductive efficiency may be influenced by reproductive disorders, one of which is the incidence of repeat breeding (Temesgen *et al*., 2022).

Repeat breeding is a condition in which female cows have normal estrus cycles and have been mated more than three times with fertile males or artificial insemination but do not become pregnant without clinical symptoms (Maulana *et al*., 2022). Physiologically, repeat breeding can be caused by anatomical abnormalities of the reproductive tract, abnormalities in the ovulation process, failure of fertilization, failure of implantation, and early embryonic death (Yaginuma *et al*., 2019). Repeat breeding can be caused by nutritional imbalance and lack of feeding. Nutritional deficiency can cause physiological, biochemical and hormonal disturbances, and long calving intervals (Pérez-Marín *et al*., 2023). Lack of knowledge in feed management for dairy cattle contributed to low productivity and fertility. The biochemical component of blood illustrates nutrition fulfillment in dairy cows (Khan *et al*., 2016).

Evaluation of blood biochemical profiles such as total protein and protein fractions provides a basic description of dairy cows' physiological condition and metabolic status (Bobbo *et al*., 2017). The quality of feed intake affects serum total protein and protein fraction levels. The low total protein level in dairy cows is a sign that dairy cows lack protein intake (Erickson and Kalscheur, 2020). Protein fractions consist of albumin and globulin. The blood albumin levels, which are synthesized in the liver (Moman *et al*., 2022), and globulin, one of the main protein fractions in the blood, depend on dairy cows' protein intake (Bobbo *et al*., 2017).

The comparison of protein components in the blood between repeat breeder cows and normal cows that have been published are on the total protein (Widayati *et al*., 2018; Jung *et al*., 2021), albumin (Widayati *et al*., 2019), and BUN as a product of protein metabolism (Ahmed *et al*., 2017; Jung *et al*., 2021). However, no publication has been found about the relationship between total protein, albumin, and globulin in the blood with the incidence of repeat breeding in dairy cows.

MATERIALS AND METHODS

This research was conducted at the Tani Wilis Rural Cooperative, one of the large Cooperatives with good management; animal health and reproductive records were available for secondary research data. Nearly 90% of the villagers in Sendang District are dairy farmers cows with an ownership of 3-8 dairy cows (Izza and Ihsan, 2023). They were sources of primary data, direct observation of dairy cows, and blood sampling. The evidence rate of dairy cattle's repeat breeding at Tani Wilis once reached 45.7% (Setyadi *et al*., 2019).

Dairy cows

This study procedure was approved by the proposal examination commission of the Faculty of Veterinary Medicine of Universitas Airlangga. The population of cows in this study were healthy cows aged 2-8 years, weighing around 450 kg and had ever been calving. Dairy cows feed consisted of elephant grass and concentrate with the nutritional composition as shown in Table 1.

Table 1 Nutrient content (%) of elephant grass and concentrate for dairy cows in Tani Wilis

nutrient content	elephant grass	concentrate
dry matter	20.29	87.39
ash	9.12	7.00
crude protein	6.26	16.76
crude fat	2.06	9.34
neutral detergent fiber	32.60	3.07
nitrogen-free extracts	41.82	40.76
total digestible nutrient	54.90	63.62

The cow samples were ten normal cows and ten cows with repeat breeding, which were taken randomly based on the reproduction records of Tani Wilis. Normal cows in this study were sexually mature, had ever been calving, had normal and healthy conditions clinically and

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reproductively (normal estrus cycle), had never repeatedly bred, and were not currently pregnant. Repeat breeder cows' criteria were sexually mature cows, had ever been calving, had been mated more than three times without being pregnant, even the condition of the cows being healthy, had a normal estrus cycle, and not being pregnant. The day before blood collection, the cows were examined for pregnancy using the rectal palpation to ensure that the cows were not pregnant.

Measurement of serum protein levels

Six mL of cows' blood samples were taken from the jugular vein in the morning before feeding and milking, collected in tubes, and left until the blood clot. Then, tubes were centrifuged at 3000 rpm for 15 minutes; the serum was transferred into a vial, and stored in a cooling box with ice pack to transport to the laboratory. Serum total protein, albumin, and globulin levels were measured using a Chemistry Blood Analyzer (I-UBIO series I-Chem 535). The Blood Analyzer was turned on by pressing the on button, waiting for the initialization process and operator login. A new work list was selected to start a new measurement, waiting for the analyzer to start the start-up process until the analyzer was ready. The reagents to be used were controlled and calibrated, after the analyzer was ready for use. The serum vial was inserted into the analyzer, the type of examination to be performed was selected. The Chemistry Blood Analyzer works automatically and the test results will be displayed and printed.

Data analysis

Questionnaire data was displayed descriptively (Table 3). Data on the age of dairy cows, parity, and frequency of dairy cows receiving artificial insemination services, as well as total protein, albumin, and globulin levels of serum, were analyzed by independent sample t-test. Data of normal cows and repeat breeder cows with lower and normal range levels of total protein, albumin, and globulin were analyzed by Chi-square test. All statistical analyses used the Statistical Product and Service Solution software at a significance level of 5%.

RESULTS

Data on the age of dairy cattle and parity between the normal cows and repeat breeder groups were not significantly different ($p > 0.05$). While the services per conception (S/C) of dairy cattle between the normal cows and repeat breeder cow groups was significantly different ($p < 0.05$) (Table 2).

Table 2 Age of dairy cows (years), parity, and services per conception (S/C)

	age		parity		S/C	
	range	means ± SD	range	means ± SD	range	means ± SD
NC (n=10)	2 - 8	5.00 ± 1.94	1 - 6	2.70 ± 1.89	1 - 1	1.00 ± 0.00 [b]
RBC (n=10)	3 - 7	4.20 ± 1.32	1 - 4	1.80 ± 1.14	3 - 21	8.50 ± 5.25 [a]

NC= normal (non-repeat breeder) cows, RBC= repeat breeder cows; different superscripts in the same column was significantly different ($p < 0,05$).

There are similarities in the knowledge of the owner of normal cows and repeat breeder cows about the estrus cycle and the right time to inseminate estrus cows artificially. However, the quantity of feeding and drink supply in both cows group differed in percentage (Table 3).

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Table 3 Questionnaire results (%) on dairy farmers

	parameters	NC	RBC
1.	understanding of the estrus cycle	100	100
2.	understanding of the signs of estrus	100	100
3.	knowing the right time to inseminate	60	100
4.	cows' feeding was unrestricted	100	30
5.	the forage of elephant grass for cows' feeding	100	100
6.	concentrate feeding	100	100
7.	drinking water available unrestricted	90	70

NC: normal cows (non-repeat breeder); RBC: repeat breeder cows.

They were not significantly different ($p > 0.05$) in levels of total protein, albumin, and globulin in normal dairy cows and those with repeat breeding (Table 4).

Table 4 Levels of total protein (g/dL), albumin (g/dL), and globulin (mg/L) of normal cows and repeat breeder cows.

	total protein	albumin	globulin
normal range	7.70 - 9.40	2.87 - 4.29	3.03 - 5.73
NC.01	7.21	3.27	6.11
NC.02	8.24	4.23	3.19
NC.03	9.25	**2.25***	**2.31***
NC.04	7.19	3.23	3.92
NC.05	6.23	4.25	5.37
NC.06	7.27	3.15	3.16
NC.07	8.32	**1.35***	**2.09***
NC.08	7.18	3.25	5.65
NC.09	9.32	4.31	4.91
NC.10	7.17	3.12	5.48
means	7.74	3.24	4.22
SD	1.00	0.93	1.47
RBC.01	**5.09***	**2.08***	5.15
RBC.02	**4.30***	3.35	**2.92***
RBC.03	**6.36***	**1.51***	**2.11***
RBC.04	**6.11***	**2.39***	**2.82***
RBC.05	**5.29***	**1.32***	**3.15**
RBC.06	**6.31***	**0.35***	**2.21***
RBC.07	7.28	**2.29***	**1.79***
RBC.08	6.97	3.31	3.39
RBC.09	8.16	3.15	**2.26***
RBC.10	6.8	**1.23***	**2.58***
means	6.27	2.10	2.84
SD	1.14	1.00	0.95

Normal range references (CMRI, 2022); NC: normal cows; RBC: repeat breeder cows; **bold**

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number*: lower than the normal range; NC and RBC had no significant difference (p >0.05) in the mean total protein, albumin, and globulin levels.

The number of normal cows with total protein, albumin, and globulin levels within the normal range was more significant than the number of repeat breeder cows (p <0.05). On the other hand, the number of repeat breeder cows with lower levels of total protein, albumin, and globulin was higher than normal non-repeat breeder cows (p <0.05) (Table 5).

Table 5 Number of normal and repeat breeder Holstein Friesian cows based on the normal range of total protein (g/dL), albumin (g/dL), and globulin (mg/L) levels

serum protein	NC	RBC	p
total protein (g/dL)			
normal	10	4	<0.05
low	0	6	
albumin (g/dL)			
normal	8	3	<0.05
low	2	7	
globulin (mg/dL)			
normal	8	3	<0.05
low	2	7	

NC: normal cycling cows; RBC: repeat breeder cows; p: probability.

DISCUSSION

The normal cow and repeat breeder cow's groups age and parity data distribution was homogeneous. The difference was found in S/C, one in the normal cows and 8.50 ± 5.25 in the repeat breeder cows. The knowledge of farmers on disease or reproductive disorders positively correlated with the evidence of repeat breeders (Setyadi *et al*., 2019). However, in this study, all dairy farmers understood the estrus cycle and signs of estrus in cows. The owner's percentage of normal cows who knew the right time to inseminate their cows was fewer than the owners of repeat breeder cows (60% vs. 100%). This could not be related to the incidence of repeat breeder cows in Tani Wilis because if a cow was in estrus, the farmer only needs to report the time (hour) of estrus, then the inseminator determines when the right time for insemination (Setyadi *et al*., 2019). The success of artificial insemination followed by pregnancy depends on the reproductive health of the cow, the accuracy of estrus detection, semen quality, the right time to inseminate, and inseminator skills (Tadesse *et al*., 2022). All cows in this study were in a healthy condition and normal estrus cycle. All farmers also understand the reproductive cycle and signs of estrus as the basis for detecting estrus. Artificial insemination of dairy cows at Tani Wilis used certified frozen semen produced by the Singosari Artificial Insemination Center, Malang Regency, Indonesia. As well, the inseminators were certified and experienced.

In this study, the unrestricted feeding on normal cows was 100%; however, it was only 30% on repeat breeder cows. The feed provided includes elephant grass and concentrate produced by Tani Wilis. Elephant grass contains 6.26% crude protein (Dumadi *et al*., 2021), and the protein content of concentrate is 16-18% (Pratama *et al*., 2019). Inadequate feeding is one of the main causes of low fertility of dairy cows (Khan *et al*., 2016). The risk factors for repeat breeding, among others, are quantity, quality, and feeding frequency (Maulana *et al*., 2022). Dairy cows in this study were fed elephant grass and concentrate with a crude protein content of 6.26 and 16.76%, respectively (Table 1). Based on the average body weight of the dairy cows of 450 kg, the intake of elephant grass (10% body weight) was 45 kg, and the concentrate intake was 4.5 kg (1% body weight). Total protein intake from elephant grass was 2.82 kg (6.26% multiplied by 45 kg), and 0.75 kg daily from concentrate (16.76% multiplied by 4.5 kg), to get 3.57 kg daily.

The mean total protein, albumin, and globulin levels of normal cycling and repeat breeder cows in this study were not significantly different. Therefore, it is necessary to evaluate individual cow's total protein, albumin, and

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globulin levels based on their normal range. Normal levels of total protein, albumin and globulin were 6.70-9.40 g/dL, 2.87-4.29 g/dL, and 3.03-5.73 mg/L (CMRI, 2022) respectively. The 80-100% of normal cycling cows have total protein, albumin, and globulin levels within the normal range. Meanwhile, in repeat breeder cows, 60-70% of them have lower levels of total protein, albumin, and globulin than the normal range. This fact matches to the data that the unrestricted feeding on cows with normal cycle was 100%, and only 30% were repeat breeder cows. Evaluation of the biochemical profile of bovine blood individually on total protein and protein fractions (albumin and globulin) can provide a basic description of the type of protein related to the physiological conditions and metabolic status of dairy cows (Tothova *et al*., 2016). Several publications stated varying results regarding the relationship of protein content in the blood with the incidence of repeat breeder cows. A study on Aceh cattle shows that blood protein levels in repeat breeder cows were similar to those in fertile cattle (Thasmi *et al*., 2021). Likewise, there were similarities in total protein and blood urea nitrogen levels in Holstein Friesian cows (Jung *et al*., 2021). Conversely, there were negative correlations between albumin on the post-partum mating (time between first mating after calving) and the S/C of Holstein Friesian cross cows (Widayati *et al*., 2019). In Ongole cross breed cows, low total protein levels were related to high urea nitrogen levels and caused repeat breeders (Widayati *et al*., 2018). The blood urea nitrogen levels of repeat breeder cows were higher than that of the normal cycling cows' Sudanese crossbred cows (Ahmed *et al*., 2017).

Total protein in serum and plasma consisted of albumin (60%) and globulin (40%). Low levels of total protein indicate physiological abnormalities in dairy cows (Sammad *et al*., 2020). Protein deficiency indicates that dairy cows lack protein intake (Schwab and Broderick, 2017). Protein deficiency can also occur due to failure of protein absorption in the digestive system. If protein digestibility is high, protein can be hydrolyzed and appropriately used by the body, and vice versa (Joye, 2019).

Low albumin levels can be caused by decreased synthesis, albumin damage, failure to absorb albumin, and lack of protein intake. Albumin levels correlate with intake of protein consumption (Gounden *et al*., 2022). A lower albumin level directly impacts increasing the value of S/C (Cattaneo *et al*., 2021). Albumin can bind to various lipophilic compounds, including estrogen and progesterone. Dairy cows with low albumin levels had a problem regulating the response of the estrogen and progesterone hormones (Baker, 2002).

Globulin is vital as steroid hormone-binding globulin in steroid hormones transport in the circulation system (Hammond, 2016). Inadequate estrogen levels cannot stimulate LH release from the anterior pituitary, causing follicle failure to ovulate. Repeat breeder cows have a lower FSH (Barui *et al*., 2015) and progesterone level than normal cycling cows (Thasmi *et al*., 2018). Inadequate levels of progesterone resulted in failed implantation of the embryo and early embryo death (Xiao *et al*., 2023). The primary reproductive hormone (FSH, LH, estrogen, and progesterone) levels were lower in repeat-breeder cows (Ahammed *et al*., 2018).

Among other factors, nutritional intake influences the incidence of repeat breeding in cattle (Pérez-Marín and Quintela, 2023). Body weight and Body condition score were lower in repeat breeder cows compared to normal cycling cows (Padder *et al*., 2018). Body condition scores are related to the prevalence of repeat breeding (Eshete *et al*., 2023). Thereby, monitoring body condition scores as an indicator of the nutritional status of cows is essential for reducing the prevalence of repeat breeding cases (Pérez-Marín and Quintela, 2023). The limitation of this study was that only 10 repeat breeder cows were found in the study area. Feed intake and nutritional intake in each sample cow were also not measured. It was suggested future studies involve a larger population of repeat breeder dairy cows with more detailed blood chemistry measurements. Although, it is challenging to determine the

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specific chemical elements that characterize the repeat breeding syndrome (Pérez-Marín *et al*., 2023)

CONCLUSION

There is a relationship between the incidence of repeat breeding with blood total protein, albumin, and serum globulin of repeat breeder dairy cows. Therefore, raising awareness of smallholder farmers about proper feeding and feeding management is suggested to reduce repeat breeding cases.

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AUTHOR'S CONTRIBUTIONS

Siti Iqmallisa Nurrill Asrar (SINA), Rimayanti Rimayanti (RR), Maslichah Mafruchati (MM), Ismudiono Ismudiono (II), Maria Gandul Atik Yuliani (MGAY), Ginta Riady (GR)

SINA: conceived the idea, designed the mainframe of this manuscript, acquisition, analysis and interpretation of data, and manuscript drafting under the supervision of RR and MM. II, MGAY, and GR: critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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