

DETECTION OF SERUM MACRO MINERALS CONCENTRATION IN REPEAT BREEDING ACEH CATTLE

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ABSTRACT

The purpose of this study was to determine the macro minerals concentration in serum of repeat breeding (RB) Aceh cattle. In this study 16 Aceh cattle were examined; they consisted of 7 fertile Aceh cattle (P1) and 9 Aceh cattle with RB (P2), all of which were 3-8 years old with a body condition score (BCS) of 3-4. Serum collection was carried out for examining the level of serum minerals, including magnesium, phosphorus, sodium, potassium, chloride, and calcium. Data was analysed using T-test. The levels of magnesium, phosphorus, sodium, potassium, chloride, and calcium in P1 vs P2 were 2.18 ± 0.60 vs. 2.20 ± 0.34 mg/dL; 6.18 ± 1.34 vs. 6.48 ± 0.74 mg/dL; 142.71 ± 5.09 vs. 142.44 ± 2.29 mmol/L; 4.81 ± 0.76 vs. 4.76 ± 0.51 mmol/L; 104.57 ± 4.35 vs. 107.67 ± 7.36 mmol/L; and 9.07 ± 0.45 vs. 9.90 ± 0.60 mg/dL ($P > 0.05$), respectively. It was concluded that the concentration of serum macro minerals do not affect the incidence of RB in Aceh cattle.

Key words: Aceh cattle, macro mineral, repeat breeding

ABSTRAK

Tujuan penelitian ini adalah mengetahui profil mineral darah sapi aceh yang mengalami repeat breeding (RB). Dalam penelitian ini digunakan 16 ekor sapi aceh yang terdiri atas 7 ekor sapi aceh fertil (P1) dan 9 ekor sapi aceh RB (P2), yang berumur 3-8 tahun dengan skor kondisi tubuh (SKT) 3-4. Koleksi serum dilakukan untuk pemeriksaan mineral darah meliputi kadar magnesium, fosfor, natrium, kalium, klorida, dan kalsium. Data dianalisis menggunakan uji T. Kadar magnesium; fosfor; natrium; kalium; klorida; dan kalsium pada P1 vs P2 masing-masing adalah $2,18 \pm 0,60$ vs $2,20 \pm 0,34$ mg/dl; $6,18 \pm 1,34$ vs $6,48 \pm 0,74$ mg/dl; $142,71 \pm 5,09$ vs $142,44 \pm 2,29$ mmol/l; $4,81 \pm 0,76$ vs $4,76 \pm 0,51$ mmol/l; $104,57 \pm 4,35$ vs $107,67 \pm 7,36$ mmol/l; dan $9,07 \pm 0,45$ vs $9,90 \pm 0,60$ mg/dl ($P > 0,05$). Disimpulkan bahwa konsentrasi mineral serum darah tidak memengaruhi kejadian RB pada sapi aceh.

Kata kunci: sapi aceh, makromineral, repeat breeding

INTRODUCTION

One of the reproductive problems that occur in Aceh cattle is the low reproductive efficiency (Thasmi *et al.*, 2016). The low reproductive efficiency indicates the existence of reproductive disorders such as endometritis, anestrus, and repeat breeding (RB) (Bage *et al.*, 2002) which have a negative impact on cattle production and reproductive performance (Prihatno *et al.*, 2013). The high number of cattle that are rejected reaches 35.2%; which results in large economic losses for cattle farmers (Bonneville-Hébert *et al.*, 2011). According to Kumar (2014), the basic causes of reproductive problems in cattle are management, nutrition, and pathological factors.

One of the factors causing the RB is mismanagement, especially nutritional deficiencies (Prihatno *et al.*, 2013). Nutritional deficiencies or insufficient nutritional intake can directly influence reproductive efficiency, resulting in decreased reproductive performance and productivity (Kumar, 2014). In addition, nutritional deficiencies also cause suboptimal ovarian performance or activity, hormonal disturbances, and longer calving intervals, which in turn leads to the appearance of RB disorders (Prihatno *et al.*, 2013).

Nutritional deficiency has been reported as a major factor causing reproductive disorders in dairy cows (Sulieman *et al.*, 2017), and buffalo (Akhtar *et al.*, 2014; Saraswat and Purohit, 2016). Ramandani and

Nururrozi (2015) suggested that nutritional deficiency or imbalance can adversely affect various stages of the reproductive process. Akhtar *et al.* (2014) and Saraswat and Purohit (2016) reported that RB disturbance in buffaloes and cows was related to low concentrations of macro minerals such as calcium (Ca), inorganic phosphorus (P), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), cobalt (Co), and selenium (Se).

Macro mineral deficiencies such as Ca, Mg, P, K, Na, Cl, and Se directly result in longer estrus and ovulation time, delayed uterine involution, increased uterine prolapse, dystocia, and placental retention (Yanuartono *et al.*, 2016). Imbalance between Ca and P minerals can affect ovarian function through block action in the pituitary gland which can cause a decrease in reproductive performance, disruption of normal sexual behavior, delay in puberty, silent heat, anestrus, ovarian hypofunction, and low levels of conception (Yasothai, 2014). The role of minerals in the endocrine system and cell integrity is needed for fertility and follicular development (Widayati *et al.*, 2018). Therefore, detection of minerals concentration through clinical and laboratory examinations is needed in diagnosing reproductive disorders due to minerals deficiency, excess or imbalance (Yanuartono *et al.*, 2016). A comprehensive understanding of the role of macro and micro minerals in the reproductive function of cattle is needed to prevent the emergence of reproductive disorders in Aceh cattle.

MATERIALS AND METHODS

In this study 16 Aceh cattle were used; they consisted of 7 fertile and 9 RB cows, aged 3-8 years with 3-4 of body condition score (BCS). Fertile cattle were > 2 months postpartum period with a history of successful pregnancy with one artificial insemination and have two regular estrous cycles, whereas RB cattle were cattle that fail to get pregnant after being inseminated more than three times but have normal estrous cycles (diagnosed as RB).

Estrus Synchronization

Estrus synchronization was carried out in both fertile and RB Aceh cattle by injecting 25 mg PGF₂α (Lutalyse™, Pfizer, Belgium) twice intra-muscularly with eleven-day intervals.

Blood Sample Collection and Preparation

Blood collection was carried out in the morning (07.00-09.00) with an average ambient temperature reaching 23.6° C. Blood samples were taken through the jugular vein, then put in a vacutainer tube and placed in a cool box. The blood sample was then taken to laboratory and preparations were made to obtain serum. The blood samples were left for 30 minutes. The next process was centrifugation of blood samples at 2500 rpm for 15 minutes. The formed serum was then put into a microtube and stored in freezer at -20° C before biochemical analysis.

Macro Minerals Examination of Serum Sample

Examination of Mg, P, Na, K, Cl, and Ca concentrations was carried out using a semi auto chemistry analyzer spectrophotometry (Cornley). As much as 5 ml of serum sample from both groups of cattle were taken and then added with 500 mL of standard solution (according to the mineral levels examined). Afterwards, mixtures of the solutions were put into a spectrophotometer and subsequently the examination results were read.

Data Analysis

Data were analyzed using a t-test.

RESULTS AND DISCUSSION

The level of Mg; P; Na; K; Cl; and Ca in P1 vs P2 were 2.18±0.60 vs. 2.20±0.34 mg/dL; 6.18±1.34 vs.

6.48±0.74 mg/dL; 142.71±5.09 vs. 142.44±2.29 mmol/L; 4.81±0.76 vs. 4.76±0.51 mmol/L; 104.57±4.35 vs. 107.67±7.36 mmol/L; and 9.07±0.45 vs. 9.90±0.60 mg/dL, respectively. Based on the results of statistical analysis it is known that there was no significant difference (P>0.05) in the serum macro minerals levels between fertile and RB Aceh cattle (Table 1).

Magnesium concentration showed no significant difference (P>0.05) between fertile and RB cattle, with concentrations of 2.18±0.60 and 2.20±0.34 mg/dL, respectively. Those concentrations did not differ in the two groups were likely related to Ca concentrations which were also not significantly different. Sharma *et al.* (2004) reported that Mg affected the absorption of Ca and P. Additionally; low Mg concentrations are associated with anovulation, which is one of the causes of RB (Das *et al.*, 2009).

Calcium concentrations in both groups of Aceh cattle were normal. Hamali (2008) reports that the Ca concentration in RB cattle is <5 mg/dL. Hypocalcaemia after estrus is very rare. Calcium plays a role in increasing the number and size of preovulatory follicles, ovulation rates, and gonadotropin regulation (Carnegie and Tsang, 1984; Hamali, 2008). Similar to Ca, Mg concentrations in this study were also different from previous reports, which reported lower Mg concentrations in animals with RB (Das *et al.*, 2009; Akhtar *et al.*, 2014).

In this study, there was no significant difference (P>0.05) of P level between fertile and RB cattle with concentrations of 6.18±1.34 and 6.48±0.74 mg/dL respectively. Phosphorus deficiency in cattle will result in service per conception caused by impaired ovarian function (Morrow, 1969 cited by Amin, 2014). In RB cattle, impaired ovarian function can be ignored because the cattle estrous cycle is normal. Therefore, P levels that were relatively similar in the two groups indicated that there was no impairment of ovarian function.

Different results were reported by Amle *et al.* (2014), Akhtar *et al.* (2014), Kumar (2014), that there is a significant difference in P concentration between fertile and RB cattle. This result is reinforced by the statement of Bhaskaran and Patil (1982) that P deficiency will result in ovulation disorders. Ovulation disorders are one of the causes of RB. Based on the opinion above, it can be stated that RB in this study was not caused by ovulation disorders due to P deficiency. Phosphorus concentrations in

Table 1. Concentration of serum macro minerals in fertile and repeat breeding Aceh cattle

Macro Minerals	Aceh cattle	
	Fertile (n = 7)	Repeat Breeding (n = 9)
Magnesium (mg/dL)	2.18±0.60 ^a	2.20±0.34 ^a
Phosphorus (mg/dL)	6.18±1.34 ^a	6.48±0.74 ^a
Sodium (mmol/L)	142.71±5.09 ^a	142.44±2.29 ^a
Potassium (mmol/L)	4.81±0.76 ^a	4.76±0.51 ^a
Chloride (mmol/L)	104.57±4.35 ^a	107.67±7.36 ^a
Calcium (mg/dL)	9.07±0.45 ^a	9.90±0.60 ^a

^aSuperscripts within the same row indicate non significant differences (P>0.05)

fertile and RB cattle are 5.40 ± 0.30 and 4.44 ± 0.17 mg/dL (Amle *et al.*, 2014); 5.56 ± 0.11 and 3.71 ± 0.19 mg/dl (Akhtar *et al.*, 2014); 6.01 ± 0.32 and 4.46 ± 0.30 mg/dL (Kumar, 2014).

Phosphorus is a mineral that plays a role in normal sexual behavior (Kumar, 2003). This mineral level in fertile cattle in this study was lower than in RB cattle. However, the P levels in both groups of cattle were still within normal limits, because normal concentration of P in cow blood plasma ranges from 4.24-7.58 mg/dL (1.4-2.5 mmol/L) (Hadzimusic and Krnic (2012) and 4.5-8.0 mg/dL (1.45-2.58 mmol/L) (Anderson and Rings, 2009).

Phosphorus deficiency results in reproductive disorders such as anestrus, low conception, long calving intervals, embryonic death, calf stillbirth, and delayed sexual maturity (Chaudhary and Singh, 2004; Ceylan *et al.*, 2008). Moderate P deficiency results in recurrent mating events. According to Ali *et al.* (2014), serum P levels dropped significantly in Cholistani cattle that are experiencing anestrus and RB. This is probably due to the large role that P plays in ATP transfer. The deficiency is thought to cause disruption of the fertilization process, so that it can result in premature embryonic death. This phenomenon is likely to cause anestrus and RB (Kumar *et al.*, 2010). Conversely, excessive levels of P will cause the endometrium to be more sensitive to infection (Chaudhary and Singh, 2004).

Sodium and potassium concentrations in both fertile vs. RB cattle were 142.71 ± 5.09 vs. 142.44 ± 2.29 mmol/L and 4.81 ± 0.76 vs. 4.76 ± 0.51 mmol/L, respectively. The Na concentration in this study is in accordance with Akhtar *et al.* (2014) that Na concentration in fertile buffaloes and RB buffaloes showed no significant differences, namely 139.9 ± 1.69 and 138.2 ± 1.22 mmol/L, respectively. Sodium deficiency affects the normal physiology of reproduction through prevention of protein and energy use, while K deficiency will affect reproductive tract muscles which cause reproductive disorders (Yasothai, 2014). The minerals Na, K, and Cl are the most important ions in regulating the balance of acid-base (pH) and osmotic pressure in the body (Lawton, 2013). Sodium deficiency associated with hyperkalemia are assumed to result in decreased fertility because they can disrupt the estrous cycle, endometritis, and follicular cysts (Pradhan and Nakagoshi, 2008).

Potassium deficiency can cause a decrease in the working power of muscles including uterine muscles, so that it can indirectly cause inhibition of the use of protein and energy that will result in reproductive disorders. This is results in weakness in uterine muscle tone, making it susceptible to metritis and retention of the placenta (Chaudhary and Singh, 2004; Sattler and Fecteau, 2014). Feeding with excessive amounts of element K (5% dry matter) is thought to cause delayed puberty and ovulation, impaired corpus luteum development and increase anestrus in cattle (Kumar, 2014; Velladurai *et al.*, 2016), and uterine disorders (DeGaris and Lean, 2008).

The results of research on Ca levels showed that Ca in the blood of fertile cattle was lower than in that of RB Aceh cattle, which were 104.57 ± 4.35 and 107.67 ± 7.36 mmol/dL, respectively. Abnormalities of Ca levels in cattle blood can affect the reproductive performance of Aceh cattle. High levels of Ca in the blood will cause reproductive disorders through decreased absorption of other minerals such as P, Mn, Zn, and Cu in the rumen (Yasothai, 2014). The results of research by Ceylan *et al.* (2008) showed that high Ca levels resulted in an increase in RB incidence in cattle, while the results of Ahlawat and Derashri (2010) research showed that high Ca levels resulted in the occurrence of anestrus in dairy cows. But on the contrary, according to Martinez *et al.* (2012), one of the risks of the causes of metritis is the low concentration of serum Ca in dairy cows. Low levels of Ca in the blood are also related to the incidence of anestrus. In addition, Akhtar *et al.* (2014) reported that buffalo with RB also had low Ca, P, and Mg concentrations.

Calcium deficiency or hypocalcaemia during birth until several days after giving birth is a common case, especially in dairy cows. Deficiency will result in changes in the ratio of Ca : P, so that it affects the function of the ovaries through the blocking action of the pituitary gland (Yasothai, 2014). This results in longer estrus and ovulatory times, delayed uterine involution, increased uterine prolapse, dystocia incidence, and placental retention (Habib *et al.*, 2007).

Excess or deficiency of minerals can result in RB cattle (Das *et al.*, 2009). Reproductive failure can be caused by a deficiency of one or several types of minerals and an imbalance between one mineral and another (Gupta *et al.*, 2005). Deficiency, imbalance, and toxicity of certain minerals can cause reproductive disorders (Sharma *et al.*, 2007). An imbalance between certain minerals can also affect ovarian function through the blocking action of the pituitary gland (Yasothai, 2014). The role of minerals in the endocrine system and cell integrity is needed for fertility and follicular development (Ceylan *et al.*, 2008).

CONCLUSION

Based on the results of the study, it was concluded that the serum macro minerals concentrations did not affect the incidence of RB in Aceh cattle.

ACKNOWLEDGMENT

The authors would like to thank the Chairperson of Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM) and the Chancellor of Syiah Kuala University for funding the research through Professor Grant Program 2016.

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