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THE EFFECTS OF AGE ON BIOCHEMISTRY PROFILES OF ACEH CATTLE BLOOD

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ABSTRACT

The purpose of this study was to analyze the effects of age on the biochemistry profiles of aceh cattle, which included examinations of: total protein (TP), albumin, globulin, albumin/globulin ratio (A/G), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), blood urea nitrogen (BUN), calcium (Ca), phosphorus (P) and magnesium (Mg) . Sixteen clinically healthy aceh cattle aged 2-4 years old, and 16 aceh cattle aged >4-6 years old were purposively selected for the study. Blood samples were drawn from the jugular vein and subjected to blood biochemistry measurements using commercial kits. The results showed that serum concentrations of TP, globulin, AST and Ca of aceh cattle were significantly ($P<0.05$) higher in age group >4-6 years old, while ALP activity was significantly ($P<0.05$) higher in age group 2-4 years old. In conclusion, age must be considered as a factor when interpreting the blood biochemistry profiles of aceh cattle.

Key words: aceh cattle, age, blood biochemistry, profile

ABSTRAK

Tujuan dari penelitian ini adalah menganalisis pengaruh umur terhadap profil biokimiawi darah, meliputi protein total, albumin, globulin, rasio albumin/globulin (A/G), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), blood urea nitrogen (BUN), kalsium (Ca), fosfor (P) dan magnesium (Mg) pada sapi aceh. Sebanyak 16 ekor sapi aceh berumur 2-4 tahun dan 16 ekor sapi aceh berumur >4-6 tahun yang sehat secara klinis dipilih untuk penelitian ini. Sampel darah dikoleksi melalui vena jugularis dan dianalisis terhadap parameter biokimiawi darah menggunakan kit komersial. Hasil penelitian menunjukkan bahwa konsentrasi protein total, globulin, AST dan Ca sapi aceh secara signifikan ($P<0.05$) lebih tinggi pada umur >4-6 tahun, sedangkan aktivitas ALP secara signifikan ($P<0.05$) lebih tinggi pada umur 2-4 tahun. Kesimpulannya, faktor umur harus dipertimbangkan saat menginterpretasi profil biokimiawi darah sapi aceh.

Kata kunci: sapi aceh, umur, biokimiawi darah, profil

INTRODUCTION

Aceh cattle are one of the germplasms of local Indonesian beef cattle. Their original geographic distribution is in the Aceh Province of Indonesia, and they have been cultivated in this area for many generations (BSN, 2020). The beef cattle population in the Aceh Province in 2019 was 2.3% of the total beef cattle population in Indonesia in the same year, which was 403.031 heads (Ditjen PKH, 2020). Around 92% of these beef cattle were Aceh cattle (Rasyid *et al*., 2017). This number was low compared to the beef cattle population in Aceh in 2017 which was 627.698 heads (Ditjen PKH, 2020). The number of Aceh cattle is expected to continue to decline (Sofyan *et al*., 2020). For this reason, efforts to increase the population need to be made in order to maintain the sustainability of Aceh cattle. Improving both the health status of the animals as well as the quality of the breed plays an important role in this effort (Kementan, 2020). Monitoring and evaluating the health and nutritional statuses of cattle on a regular basis can help to serve this purpose. This can be achieved through regular examination of their metabolic profiles.

Metabolic profiles can be used to assess the nutritional and physiological status of livestock (Ashmawy, 2015), reveal subclinical disorders (Stojevic *et al*., 2008), identify problems in livestock herds (Singh *et al*., 2020), and assess reproductive status (Bazzano *et al*., 2016); all of which are useful in evaluating the rearing management and performance of livestock (Singh *et al.*, 2020).

The metabolic profile tests were carried out using blood biochemistry as an indicator (Ashmawy, 2015). Age is one of the factors that cause biochemical variations in blood (Alberghina *et al*., 2011; Irfan, 2014; Nagy *et al*., 2014; Kristanto and Widiyono, 2021). Many studies on the metabolic and biochemical profiles of blood in cattle have been carried out (Stojevic *et al*., 2008; Irfan, 2014; Aguirre *et al*., 2018; Dar *et al*., 2019; Choudhury *et al*., 2020). However, until now a study on Aceh cattle has not yet been available. Therefore, the purpose of this study was to analyze the effects of age on the metabolic profiles of Aceh cattle through blood biochemistry examinations. These examinations included measures of total protein, albumin, globulin, albumin/globulin ratio (A/G), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), blood urea nitrogen (BUN), calcium (Ca), phosphorus (P), and magnesium (Mg).

MATERIALS AND METHODS

The study was conducted at the Livestock Breeding Center for Excellent and Forage Animal Feed (BPTU-HPT) in Indrapuri, Aceh. The biochemical examinations of the blood were carried out at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University.

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A total of 32 clinically healthy Aceh cattle (16 males and 16 females) with ages ranging from 2-6 years old were selected for this study. The cattle were separated into two groups based on age, with one group consisting of cattle ranging from ages 2-4 and the other ranging from >4-6 years old.

A 10 mL blood sample was taken from the jugular veins of each cow using a venoject. The samples were then put into vacutainer tubes without anticoagulant, and each tube was labeled with a sample code. The samples were then centrifuged for 12 minutes at 3000 rpm. The resulting serum that formed was separated from the blood clot and put into a microtube, which was then tightly closed and labeled. Serum samples were stored at -20° C until analysis was performed (Kessell, 2015; OIE, 2018).

The serum was analyzed against certain blood biochemical parameters using commercial kits (Abaxis® Large Animal Profile) with photometer principles (Vetscan® VS2, Abaxis, Germany). The parameters used included the total concentrations of protein, albumin, globulin, BUN, Ca, P, and Mg; as well as AST, GGT, ALP, and CK activities; and the albumin to globulin ratio (A/G).

Data Analysis

The data was statistically tested using the Mann-Whitney test and the Spearman Rank correlation test to determine the effect and correlation of age on blood biochemical parameters.

RESULTS AND DISCUSSION

The metabolic profiles of Aceh cattle based on age are presented in Table 1. The results of statistical analysis showed that age has a significant effect ($P< 0.05$) on the average concentrations of total protein, globulin, ALP, AST and Ca. Correlation analysis showed that age is positively correlated with total protein, AST and Ca; and negatively correlated with ALP. Previous research studies have stated that age is significantly correlated with the concentrations of total protein, globulin, albumin, ALP, CK, Ca and P in the blood of African buffaloes (Couch *et al*., 2017) and the concentrations of total protein, albumin, creatinine, Ca in the blood of African donkeys (Sow *et al.* , 2017); however, Gwaze *et al*. (2012) stated that there is no correlation between age and the concentrations of AST, ALT, CK, and GGT in the blood of goats.

The data in Table 1 shows that the average concentrations of total protein and globulin in the age group >4-6 years old (9.39±0.31 and 4.89±0.31 g/dL, respectively) was higher and significantly different compared to the age group 2-4 years old (8.49±0.45 and 4.39±0.42 g/dL, respectively). Albumin concentrations were also higher in the age group >4-6 years old (4.52±0.40 g/dL) compared to the age group 2-4 years old (4.09±0.45) but not significantly different. This higher total protein concentration in the older age group is in accordance with studies that have been carried out on male cattle (Irfan, 2014), calves (Nagy *et al*., 2014; Souza *et al*., 2020), Brahman cattle (Kristanto and Widiyono, 2021), African buffaloes (Couch *et al*., 2017), goats (Antunović *et al*., 2020), donkeys (Sow *et al*., 2017), camels (Ahmadi-hamedani *et al*., 2014), dogs (Brenten *et al*., 2016; Lee *et al*., 2020), pigs (Yu *et al*., 2019), and broiler chickens (Tothova *et al*., 2019). Tothova *et al*. (2016) stated that age is one of the most important factors that affect the concentrations of serum protein and its fractions (albumin and globulin). According to Ahmadi-Hamedani *et al*. (2014) and Couch *et al*. (2017), animals are born with underdeveloped immune systems and liver function, which is reflected in lower total protein and globulin concentrations in young animals. These concentrations will increase with immune system growth and maturation. The average concentrations of total protein, albumin, and globulin in Aceh cattle in the two age groups of cattle in this study were higher than the normal reference values used. Similar results were obtained from other studies on Indonesian local cattle; such as a study on Aceh cattle (Prayogi *et al*., 2020) and on Bali cattle (Tombuku *et al*., 2017). In both of these studies the average concentrations of total protein, albumin, and globulin were also found to be higher than the normal reference values. The pathological and physiological statuses of animals cause variations in albumin and globulin concentrations

Table 1. Metabolic profiles of Aceh cattle based on age

Parameter	Age (y.o)		Referral value	Correlation
	2 - 4	>4-6		
TP (g/dL)	8.49 ± 0.45[a]	9.39 ± 0.31[b]	5.7 - 8.1[1]	0.580*
Albumin (g/dL)	4.09 ± 0.45[a]	4.52 ± 0.40[a]	2.1 - 3.6[1]	0.306
Globulin (g/dL)	4.39 ± 0.42[a]	4.89 ± 0.31[b]	2.4 - 4.0[2]	0.414
A/G ratio	0.96 ± 0.15[a]	0.93 ± 0.14[a]	0.8 - 1.9[2]	-0.180
ALP (U/L)	146.00 ± 29.48[b]	94.37 ± 41.99[a]	0 – 200[1]	-0.609*
AST (U/L)	61.31 ± 9.00[a]	67.87 ± 10.01[b]	60 – 150[2]	0.471*
GGT (U/L)	13.50 ± 5.80[a]	15.87 ± 3.64[a]	0 - 36.0[2]	0.269
CK (U/L)	179.50 ± 90.49[a]	161.75 ± 32.31[a]	35 – 280[1]	0.037
BUN (mg/dL)	21.38 ± 3.34[a]	21.37 ± 6.00[a]	6.0 - 27.0[1]	-0.232
Ca (mg/dL)	9.14 ± 0.69[a]	9.56 ± 1.27[b]	9.7 - 12.4[1]	0.387*
P (mg/dL)	9.08 ± 1.12[a]	9.34 ± 1.81[a]	5.6 - 6.5[1]	-0.058
Mg (mg/dL)	2.60 ± 0.27[a]	2.62 ± 0.46[a]	1.8 - 2.3[1]	0.075

[a,b]Different superscripts on the same line show significant differences P<0.05), *statistically significantly correlated (P<0.05), [1]Constable *et al.* (2017), [2]Kessell (2015). TP= Total protein, A/G = Albumin/globulin ratio, ALP= Alkaline phosphatase, CK= Creatine kinase, BUN= Blood urea nitrogen, Ca= Calcium, P= Phosphorus, Mg= Magnesium

(Bobbo *et al*., 2017). It has been found that dehydration can cause an increase in the concentrations of total protein and albumin in the blood, while inflammation can cause higher concentrations of globulins to occur (Kessell, 2015). The physiological statuses that can cause variations in the concentration of total protein, albumin and globulin in cattle include: region nation (Irfan, 2014; Choudhury *et al*., 2020), age (Alberghina *et al*., 2011), nutrition, climate/season, rearing system, stages of lactation, and parity (Cozzi *et al*., 2011).

The data in Table 1 shows that the mean ALP activity of Aceh cattle in the 2-4-year-old age group (146.00±29.48 U/L) was higher and significantly different than the >4-6-year-old age group (94.37±41.99 U/L). These results are in accordance with studies that have been carried out on African buffaloes (Couch *et al*., 2017), goats (Gwaze *et al*., 2012; Antunović *et al*., 2020), and dogs (Brenten *et al*., 2016). All of these studies found that adult animals have lower ALP activity compared to young animals. ALP activity is related to the process of bone calcification that accompanies growth; thus, there will always be higher values in younger animals than adults (Gwaze *et al*., 2012). The ALP activity in this study was within the reference range of normal values. ALP enzymes are found in abundance in the plasma membranes of hepatocytes, especially in the bile duct canaliculi and gallbladder epithelium. ALP enzymes are also found in the intestines, kidneys, liver, and bones. The ALP enzyme will increase when tissue damage occurs in these organs (Otter, 2013; Sepulveda, 2013).

The data in Table 1 shows that the mean AST activity of Aceh cattle in the age group >4-6 years old (67.87±10.01 U/L) was higher and significantly different compared to the age group 2-4 years old (61.31±9.00 U/L). Previous studies on African buffaloes (Couch *et al*., 2017), Bangladeshi cattle (Mamun *et al*., 2013), Bali cattle (Kendran *et al*., 2012), and Labrador retriever dogs. Brenten *et al*., 2016 also mentioned that age affects AST activity and as age increases, AST activity also increases. However, this result contradicts the studies conducted by Gwaze *et al*. (2012) on goats, Pošiváková *et al*. (2019) on sheep, and Irfan (2014) on bulls; all of which stated that AST activity is not affected by age, but there is a tendency for it to increase at older ages.

AST enzymes are distributed throughout various tissues with significant amounts being found in the liver, kidneys, and skeletal muscles (York, 2017). Increased AST activity occurs when there is damage to these organs (Otter, 2013). Both the metabolic load of digestive organs and incidences of mild acidosis in fattened cows can cause mild damage to liver parenchymal cells, which then results in increased AST activity (Dokovic *et al*., 2010). The AST activity in this study was within the reference range of normal values, which showed no indication of liver, kidney and skeletal muscle damage in the Aceh cattle used in this study.

The mean GGT activity of Aceh cattle in this study showed no significant difference between the age group 2-4 years old (13.50±5.80 U/L) and the age group > 4-6 years old (15.87±3.64 U/L) (Table 1). The studies conducted on bulls (Irfan, 2014), African buffaloes (Couch *et al*., 2017), goats (Gwaze *et al*., 2012; Antunović *et al*., 2020), and sheep (Onasanya *et al*., 2015) also obtained similar results that support the argument that age has no significant effect on GGT activity. The high specificity of the GGT enzyme allows for its use in detecting the presence of chronic liver lesions (Moreira *et al*., 2012). GGT activity is often used as an indicator of bile duct epithelial proliferation, cholestatic disorders, liver cirrhosis, chronic and toxic hepatopathy, fasciolosis, metabolic disorders, ketosis, acidosis, and urea poisoning (Davoudi, 2013). The GGT enzyme activity in Aceh cattle in this study was within the reference range of the normal values used.

The data in Table 1 shows that the CK enzyme activity of Aceh cattle was not significantly different between age groups (P>0.05), which means that age did not affect CK enzyme activity in this study. Similar results were also obtained from research conducted by Onasanya *et al*. (2015) in sheep and Antunović *et al*. (2020) in goats, but the study by Couch *et al*. (2017) in African buffaloes stated the opposite,. The CK enzyme activity in this study was within the reference range of normal values used. Increased serum CK activity in cattle commonly occurs in selenium deficiency, vitamin E, muscle injury and disease. Larger animal species also frequently experience increased serum CK activity due to prolonged positions when lying down or pressure-induced muscle necrosis (Hoffmann and Solter, 2008).

The data in Table 1 shows that age did not affect the concentration of BUN (P>0.05) in Aceh cattle. This is in accordance with the studies conducted by Irfan (2014) on bulls, Mamun *et al*. (2013) on Bangladeshi cattle and Couch *et al*. (2017) on African buffaloes. Different results were obtained by Brenten *et al*. (2016) in dogs, Kendran *et al*. (2012) in Bali cattle, Pošiváková *et al*. (2019) in sheep and Antunović *et al*. (2020) in goats, that stated that age does affect BUN concentrations. The concentrations of BUN in this study were within the normal reference range of the values used. According to Varanis *et al*. (2021), the amount of protein in feed can affect the concentrations of BUN. Approximately 70% of protein is converted to ammonia in the rumen and used by rumen microorganisms for protein synthesis, which is then absorbed in certain amounts and converted to urea after reaching the liver. High BUN concentrations indicate inefficient uses of nitrogen intake for growth and milk production (Gulinski *et al*., 2016). The amount of urea nitrogen in the blood is alsoone of the parameters used for evaluating renal function and glomerular filtration (Murayama *et al*., 2013).

The data in Table 1 shows that age affected Ca concentrations, but did not affect P and Mg concentrations in Aceh cattle. These results are in accordance with the research conducted by Irfan (2014) on bulls and Pošiváková *et al*. (2019) on sheep, which

stated that age has a significant effect on Ca but has no significant effect on P and Mg. Different results were obtained by Gwaze *et al*. (2012) which stated that there is a negative linear relationship between age and P concentration, while Ca and Mg do not have a significant relationship with age in goats. Kristanto and Widiyono (2021) stated that age affects the concentrations of Ca, P, and Mg in Brahman cross cattle. Brenten *et al*. (2016) stated that there is no correlation between plasma Ca concentrations and age in dogs. The study conducted by Mamun *et al*. (2013) on Indian cattle stated that there is no significant effect between age and the concentrations of Ca, P and Mg. The average concentrations of Ca in this study was lower than the normal reference value used, while the average concentration of P and Mg was higher than the normal reference value. Hafid *et al*. (2013) stated that the concentrations of the minerals Ca, P and Mg in serum are influenced by age, reproductive status and season in goats. According to Besung *et al*. (2019), mineral concentrations of Ca, P and Mg in Bali cattle depend on the island and soil type.

CONCLUSION

Age affects the average concentrations of total protein, globulin, ALP, AST and Ca in Aceh cattle. The concentrations of total protein, globulin, AST and Ca were higher in the age group >4-6 years old, while ALP activity was higher in the age group 2-4 years old.

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