SALIVA UREA NITROGEN (SalUN) OF BALI CATTLE (*Bos javanicus*) FED GRASS OR COMPLETE DIET: A PRELIMINARY STUDY ON THE UTILIZATION OF SALIVA AS A NON-INVASIVE SPECIMEN

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**ABSTRACT**

The purpose of this study was to examine the possibility of saliva to be used as a medium for the measurement of blood urea (BUN) levels, as an indicator of the sufficiency of protein intake in ruminants. Six 2-years-old female Bali cattle (*Bos javanicus*) which divided into two groups i.e., I (n = 3) were given a ration of 90% concentrate with 10% forage (complete diet), and group II (n = 3) fed King grass *ad libitum*. Urea levels in the saliva (SalUN) and the BUN were measured photometrically, using a commercial kit available on the market, commonly used in a standard laboratory. There were no significant differences (P> 0.05) between SalUN and BUN in the same treatment group. Urea concentrations differed significantly (P<0.001) between two groups. In the group I, SalUN levels were 40.07 ± 1.86 mg/dl and BUN 40.50±1.32 mg/dl, which were significantly higher (P<0.001) compared with group II (8.33 ± 2.62 mg/dl in the saliva and 9.67 ± 3.69 mg/dl in the blood). It was concluded that saliva can be used as a non-invasive medium to measure the levels of urea as an indicator of diet quality fed to ruminants. Further study needs to be carried out with a larger sample and wider area of coverage.

Keywords: Saliva, urea, *Bos javanicus*, Bali cattle, non-invasive
INTRODUCTION

Determination of urea concentration is one of the important analyses in ruminants because urea levels may reflect the sufficiency of protein consumed by the ruminant, which means it can be used as an indicator of feed quality and physiological status of the animal. Urea concentration commonly evaluated is the urea levels in the blood either in plasma or serum, known as the level of blood urea nitrogen, BUN (Butler, 1998). In the last few decades, efforts have been made to evaluate urea nitrogen levels in the ruminants using materials other than blood, such as measuring the levels of milk urea nitrogen (MUN). One of the advantages of using MUN is the decrease in animal suffering and stress as the result of blood withdrawal; hence the method by utilizing the milk can be categorized as a non-invasive method (Gustafsson et al., 1993).

Despite its advantages, the utilization of milk as the specimen can be a problem for non-dairy cows. For that reason, saliva has recently received widespread attention to be used as a specimen of non-invasive test including the test for urea nitrogen levels (Piccione et al., 2006). Saliva is ideal because saliva is directly related to the feed digestion process. In ruminants, saliva has a primary function as a buffer of pH level in the rumen and reticulum, as well as lubrication in the process of mixing the feed with regard to the process of regurgitation (Parish et al., 2009).

The results of several studies showed that the level of urea in the ruminants’ saliva is generally lower than in the serum (Piccione et al., 2006). This was similar to the results of the examination of milk urea nitrogen (MUN) in dairy cows (Nozad et al., 2011 and 2012). Although the average value was lower, the researchers agreed that when the data were readily available in more extensive and comprehensive manners, the use of saliva as a source of physiological information is most likely applicable.

In this paper we report the results of a preliminary study on measuring the levels of urea in the saliva of Bali cattle (Bos javanicus) fed complete diet or King grass ad libitum. Bali cattle are the native cattle of Indonesia which have long been used as a source of beef and a draught power for plowing the rice paddy fields. In
the efforts to increase its potential, monitoring of the protein or nutrition intake of Bali cattle is crucial. The use of saliva to analyze the protein intake is practical and acceptable to farmers because it poses little risk and do not cause suffering for their animals.

In this study saliva urea nitrogen (SalUN) levels were measured using a commercial kit that is based on the kinetic reaction between urease and glutamate dehydrogenase (Sampson et al., 1980). This method is widely available commercially at relatively low cost and is commonly performed in the standard clinical laboratories; and can be done in less than one hour. The data obtained from the present study can be used as initial information for the efforts to use saliva as specimen for a noninvasive test, and also to study the Bali cattle as one of the Indonesia native germplasms in more depth.

MATERIALS AND METHODS

a. Animal samples

In this study six 2-years-old female Bali cattle (Bos javanicus) had been specifically reared to conduct a research on the effects of type and composition of rations on Bali cattle growth in the "Teaching Farm" of Faculty of Animal Science, University of Mataram. The animals were non-pregnant and non-lactating cows with good body condition score. The cows were housed in a barn with individual pen and were divided into two groups based on the feed given. The two treatment groups namely group I (n = 3) were given complete diet (67% corn, 30% soybean meal, 1.5% mineral mix, and 1.5% calcium) and 10% King grass (Pennisetum purpureum), while group II (n = 3) were given ration of 100% King grass. Feed and water were given ad libitum, twice a day. The King grass was chopped manually into a size of about 15-25 cm then mixed with or without the concentrate according to the treatment group.

b. Collection of Saliva

Saliva collection was carried out during mid-day about 3-4 hours after the cows were fed. Saliva was collected directly from the cows’ oral cavity, using a sponge (10 x 10 x 5 cm), by trained technicians with their hands wearing gloves. Once
absorbed by the sponge, the saliva was collected into 50ml conical tubes (Corning) by squeezing the sponge in order to let the saliva flow into the tubes. Collected saliva was then added with NaN₃ 0.2% (v/v) as bactericide. Different sponge was applied for each individual cow; hence the possible of mix up among cows saliva was controllable. Tubes which had been given a number according to the number of cows and have been filled with saliva were placed into a Styrofoam box containing ice cubes and immediately delivered to the laboratory for processing at Immunobiology Laboratory, Mataram University.

In the laboratory, the saliva was centrifuged using TOMY MX-160 centrifuge at 5000xg for 5 min at 4º C to separate the dirt and food debris from the cattle oral cavity. Supernatant from each tube was collected in a new conical tube, and then frozen at -20º C or -60º C until used for further experiments.

c. Collection of Blood Serum

To obtain the blood sera, the blood sample was collected from the jugular vein using Venoject tubes (BD Vacutainer) in accordance with standard procedures. The collected blood were kept at 4º C for 1 hour, and then centrifuged at 5,000xg, for 10 minutes. Sera was then taken and separated from the blood cells and then frozen at -20º C for further processing.

d. Measuring of saliva pH

Measuring of saliva pH was performed using a pH meter (Beckman) according to the manufacturer’s instructions. Measurement was done in duplicate for each animal.

e. Measuring of Blood and Saliva Urea

Determination of urea concentration in the sera and saliva was performed using a commercial kit UREAL-Cobas® (Roche) which is produced for the quantitative determination of urea/urea nitrogen in serum, plasma or urine. The measurements were carried out according to manufacturer’s instructions. This test kit is based on the kinetic reaction between urease and glutamate dehydrogenase (Sampson et al., 1980). In this reaction, initially urea is hydrolyzed by urease to form ammonium and carbonate. The presence of glutamate dehydrogenase (GLDH) and coenzyme NADH
furthers the reaction of ammonium to react with 2-oxoglutarate, which then produces L-glutamate. In this reaction, for each mole of urea hydrolyzed, two moles of NADH are oxidized to NAD. The rate of reduction in NADH concentration during the reaction is directly proportional to the concentration of urea present in the samples tested, which can then be measured photometrically.

f. Data Analysis

The data obtained were tabulated and analyzed by a simple arithmetic mean (Mean ± SEM) for all parameters in the blood and saliva samples. To reveal the differences among parameters, student t-test was applied as presented by Piccione et al., (2006).

RESULTS AND DISCUSSION

In this study, the cows used were specifically kept for the study on the effect of diet quality on Bali cattle growth and development from the weaning to maturity. In regards to the study of urea levels in saliva and the blood, the cows have been given two different types of diets i.e. complete ration (with a 90% concentrate, 10% forage), and 100% forage without concentrate for more than 8 months. Of the total saliva collected, the average volume of saliva from cows fed high concentrate has a total volume lower than those of the group fed forage only, i.e. 10–15 ml and 25–40 ml per collection time respectively. It must be noted here that this is actually not a reflection of the real total volume of Bali cattle saliva; because the volume is relevant to the time of collection only. Thus the results obtained should just be used as a rough estimate of the volume of saliva from each treated cow. The results are in line with the explanations of Broderick (2006) and Beauchemin et al., (2008) who reported that ruminant diets with high forage stimulate rumination, chewing, and salivation.

The results of pH measurement showed that there was no significant (P>0.05) difference in pH between the two treatment groups. In the group fed with high concentrate rations, the average salivary pH was 8.56 ± 0.08 while the group fed without concentrate was 8.54 ± 0.10.
The results were in line with results of previous studies both in cattle (Meot et al., 1997) as well as in goats and sheep (Sunagawa et al., 2008). This is understandable because for the ruminants, saliva plays an important role in the feed buffering process, especially in the process of regurgitation. The most important buffer in ruminants saliva is bicarbonate (Imamidoost and Cant, 2005) serving as a buffer, so it is clear why the pH is relatively high and there is no difference between treatments. The rumen fluid generally has an acidic pH of 5.8 to 6.5 in grain-adapted cattle (Nagaraja and Titgemeyer, 2007). In the process of regurgitation, digested feed that re-enter into the oral cavity will experience the process of neutralization by saliva (Reynolds and Kristensen, 2008). The benefit of such process is in term of preventing ruminal acidosis (Beauchemin et al., 2008).

The result of measurement of urea in saliva and blood is presented in Table 1. In this study, salivary urea levels for group of cows fed high concentrate was significantly (P <0.001) higher compared with the group given forage only, i.e. 40.07 ± 1.86 mg/dl and 8.33 ± 2.62 mg/dl respectively. Similar results were obtained in the blood urea i.e. 40.50 ± 1.32 mg/dl and 9.67 ± 3.69. Limited information is available on the levels of urea in the saliva of cattle, especially Bali cattle. However, data from previous studies especially for blood urea and milk urea can be used as a comparison, which values can be up to 40 mg/dl (Butler et al., 1998, Khalili and Sairanen, 2000, Nozad et al., 2012). The variations of urea concentration can occur between breeds or species, the type of diets, and the physiological status of the animal (Nozad et al., 2012, Piccione et al., 2006, Kohn et al., 2002, Pattanaik et al., 1999, and Butler et al., 1998). Of these factors, effects of nutrition fed to the ruminants seem to be more relevant with the present study i.e. to use blood- or saliva urea as reflection of protein metabolism in cattle. Pattanaik et al., (1999) reported that crossbred (Bos taurus x Bos indicus) male calves fed with concentrate (maize, groundnut cake, and mineral mixture) gave blood urea between 27.1±4.44 mg/dl and 34.0 ± 4.30. These results were quite similar to our data on the group fed with high concentrate. Furthermore, blood- as well as saliva urea for the group fed with forage alone was comparable to the results of Hong et al. (2003). They found that blood urea nitrogen (BUN) and milk urea nitrogen (MUN) of crossbred Holstein-Friesian Cross, fed with cassava hay were 9.0-11.7
mg/dl and 7.6-10.4 mg/dl respectively, while in this study was 5.5-12.5 mg/dl for BUN and 5.9-11.1 for SalUN.

Table 1. Saliva- and blood urea (mg/dl) of Bali cattle fed with two different diets

<table>
<thead>
<tr>
<th>Cow No. (n=3)</th>
<th>Diet I (90% concentrate)</th>
<th>Cow No. (n=3)</th>
<th>Diet II (0% concentrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saliva</td>
<td>Blood</td>
<td>Saliva</td>
</tr>
<tr>
<td>A14</td>
<td>39.90</td>
<td>41.50</td>
<td>D21</td>
</tr>
<tr>
<td>A06</td>
<td>42.00</td>
<td>41.00</td>
<td>D24</td>
</tr>
<tr>
<td>A17</td>
<td>38.30</td>
<td>39.00</td>
<td>D23</td>
</tr>
<tr>
<td>Mean</td>
<td>40.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mean</td>
</tr>
<tr>
<td>SE</td>
<td>1.86</td>
<td>1.32</td>
<td>SE</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Different letters in the row differed significantly (P < 0.001)

Recent research on the measurement of salivary urea (SalUN) is still very limited except the studies on capra or ovis by Muscher <i>et al.</i>, (2010) or Piccione <i>et al.</i>, (2006) compared to the studies that measure milk (MUN) and blood (BUN) urea in <i>Bos taurus</i>. As far as our concern, the present study was the only information on the measurement of saliva urea in regards to diets consumed by Bali cattle (<i>Bos javanicus</i>).

The interesting thing about this study is it turns out that the levels of urea in the saliva of Bali cattle did not differ significantly with blood urea levels in the group of animals with the same treatment. However, the value was incredibly able to discriminate between the cattle fed with high concentrate and those of without concentrate. In other words, saliva is a potential media to substitute the blood as a specimen for determination of urea in cattle. Quoting some ideas that milk urea nitrogen reading will someday be developed at cow side in the milking parlor (Jenkins and Delwiche, 2002, Broderick, 2006); it is possible to speculate that similar thing could be happen for saliva urea measurement as well. The method is very promising and it is very useful for researchers and for farmers as well as for
animal welfare point of view, since the use of saliva is practically and will greatly reduce the suffering of animals that are being examined.

**CONCLUSIONS AND SUGGESTIONS**

Urea levels reflect the quality of the diets that is fed to ruminants. Determination of urea from saliva did not differ significantly with those of measured from the blood. Thus, most likely that saliva can be used as an alternative to replace the blood for examination of blood urea in Bali cattle or other ruminants. To strengthen the present results, further study needs to be done using more samples under the real conditions that exist among the farmers.

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**REFERENCES**


