



Validation of Electrochemical Biosensor based Hand-held Device for the Measurement of Blood Glucose in Lactating Buffaloes (*Bubalus bubalis*)

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ABSTRACT

Background: Bovine ketosis is a major metabolic disorder emerging as a result of negative energy balance in the post calving period and subsequent lactation of lactating dairy animals. The measurement of the concentration of blood glucose was reported to be helpful for the early diagnosis of subclinical ketosis in buffaloes. Also, blood glucose will provide the overall energy status of the buffalo.

Methods: The experiment was conducted on 56 healthy lactating Murrah buffaloes. Blood samples were collected in two slots for two consecutive days in the morning hour in an anticoagulant-added sodium fluoride bulb and in clot activator plain tubes by jugular venipuncture. Also, glucose concentration was measured by a handheld device ("Gluco One", Model BG-03) by puncturing the small branches of the ear vein and the results were recorded. The estimation of blood glucose plasma and serum was done by standard laboratory enzymatic method i.e. Glucose Oxidase- Peroxidase (GOD-POD) on an automated chemistry analyzer.

Result: There was no significant difference between the mean values of plasma glucose measured by the GOD-POD method and by the biosensor. But the regression analysis showed the blood glucose in plasma do not have a significant correlation with that measured by biosensor. The glucose measured in serum is significantly less than in plasma and measured by the biosensor. It is advisable to use fluoride-added preservative anticoagulant tubes and not to use a serum for the estimation of blood glucose.

Key words: Biosensor, Buffaloes, GOD-POD, Glucometer.

INTRODUCTION

Ruminants particularly dairy cows and buffaloes have a special position with respect to glucose metabolism because of the massive demand of their mammary gland for the biosynthesis of lactose during the onset of lactation and also permanent alterations between pregnancy and lactation. Most of the glucose is generated by gluconeogenesis through glucogenic precursors like propionate, alanine, valerate, and isobutyrate. Oikonomou *et al.* (2008) reported the significant influence of glucose concentration on reproductive performance, ovarian cyclicity, and postpartum uterine infections. Glucose concentration has been proposed as a plausible biomarker for the health and reproduction of postpartum dairy cows (Senosy *et al.*, 2012; Garverick *et al.*, 2013; Bicalho *et al.*, 2017). Garverick *et al.* (2013) found that at 3 weeks postpartum cows with blood glucose concentration >3.33 mmol/L had a greater chance of pregnancy at first insemination than cows with lower blood glucose concentration.

As with other dairy animals, buffalo also have a heavy demand for the synthesis of lactose at the early parturient period for milk production. Insufficient supply of glucose due to hypoglycemia to the mammary gland leads to a negative energy balance and eventually subclinical or clinical ketosis.

The measurement of the concentration of blood glucose was reported to be helpful for the early diagnosis

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of subclinical ketosis in buffaloes. Also, blood glucose will provide the overall energy status of the buffalo. Currently, diagnostic tests used for estimating blood glucose levels are based on glucose oxidase-peroxidase enzymatic method (GOD-POD), a laboratory-based method for determining plasma glucose concentration, routinely used, and is considered a gold standard. But this required a

costly analyzer and a technical person for the collection, preservation, and analysis of blood samples, time-consuming and relatively expensive. To overcome this and to estimate blood glucose at farm level/ field condition, the electrochemical biosensor method which is routinely used in human beings for estimation of blood glucose in diabetic patients and also in some pet animals can be use, however it is not validated in buffaloes. Some researchers have used hand-held meters designed for glucose monitoring in diabetic human patients to measure cow-side blood glucose concentrations (Galvão *et al.*, 2010; Gordon *et al.*, 2017; Ruoff *et al.*, 2017). Present study was conducted to validate electrochemical biosensor method for the estimation of blood glucose in lactating buffaloes.

MATERIALS AND METHODS

Experimental animals and methodology for blood collection

A total 56 healthy lactating Murrah buffaloes, irrespective of their lactation number and stage were used for the study. Blood samples were collected in two slots for two consecutive days in the morning hour in an anticoagulant added sodium fluoride bulb and in clot activator plain tubes by jugular venipuncture. Also, at the same time, to obtain blood glucose concentration by a hand-held device, the small branch of the ear vein was punctured with a 21G disposable needle to obtain a single blood drop was taken on the sensor as per the manufacturer's instructions and the results were recorded. The blood samples were transported on ice packs in a thermocol box to the laboratory and then centrifuged at 1700xg for 15 minutes to obtain clear plasma and serum. The estimation of blood glucose was done by standard laboratory enzymatic method *i.e.* GOD-POD.

Test principle of hand-held device

The test principle of the hand-held devices is on the amperometric biosensor technology. The glucometer consists of two components. The biosensor strip is designed to analyze a little blood sample of about 0.5 μ l. The other meter components process the data and display the concentration. After the application of blood to the sensor, the electrochemical reaction starts. Glucose is oxidized by glucose oxidase and converted the glucose into gluconolactone. The electrons from glucose are transferred to the oxidized form of a mediator on the electrode surface. The current generated is monitored by the meter and is directly proportional to the glucose concentration in the blood sample.

Specifications of hand-held glucometer

The handheld glucometer used in the present study was Dr. Morepen's "Gluco One" blood glucose monitoring system, (Model BG-03). It is manufactured by Morepen laboratories Ltd, Morepen village, Malkamajra, Nalgarh, Dist. Salan, Himachal Pradesh India with R and D South

Korea. The specifications of the meter include its test range of 20-600mg/dl., sample size required is 0.5 μ l capillary whole blood, operating temperature range from 50-104^oF with hematocrit range of 35-50% and result will display in 5 second.

Blood Samples analysis

Approximately 2 h after collection, the samples were centrifuged at 1700 xg, for 15 min. Clear plasma and serum were separated and stored into 2ml Eppendorf microtubes. Analysis of the samples was done immediately after separation. Plasma and serum samples were analyzed on an automated chemistry analyzer (Falcon, ARK Diagnostic) at Central Instrumentation Laboratory, Department of Veterinary Biochemistry, Mumbai Veterinary College, Parel, Mumbai, India. Control was run along with the samples during each assay. Intra-assay and inter-assay coefficient of variance was also calculated.

Statistical analysis

The one-way ANOVA followed by Duncan's Mean Range Test (DMRT) was carried out to analyze the difference between the groups. The linear regression analysis was also carried out to analyze the correlation between the serum glucose or plasma glucose and the glucose measured using the biosensor. All the analysis was carried out in the statistical 'R' software as described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

There is no significant difference between the mean values of plasma glucose and glucose measured by the biosensor. However, the glucose measured in serum is significantly less than in plasma and measured by biosensor as depicted in Table 1. The regression analysis showed that, there is a significant correlation (40.73%, $p < 0.05$) between changes in blood glucose measured in serum, plasma with that measured by biosensor (Fig 1 and 3). On the other hand, the changes in blood glucose measured in plasma do not have significant correlation (15.94%, $p = 0.24$) with that measured by biosensor (Fig 2)

Mean $\pm$ SE (mg/dl) of glucose levels and range obtained by biosensor glucometer and plasma glucose by GOD-POD method are similar to the levels reported by various researchers. Saha and Singh, (1998); Maurya and Singh

Table 1: Mean $\pm$ SE of capillary blood glucose, plasma and serum levels (mg/dl) measured by standard GOD-POD and biosensor using hand-held device in buffaloes.

Method of analysis	Sample	Mean $\pm$ SE Blood Glucose
Biosensor	Capillary blood	62.89 $\pm$ 1.09
GOD-POD	Plasma	64.08 $\pm$ 1.47
GOD-POD	Serum	50.48 $\pm$ 1.42

The means bearing different superscript differs significantly ($p < 0.05$).

(2015), Hagawane *et al.* (2009) and by Galdhar *et al.* (2021) in buffaloes by GOD-POD method and Ajit *et al.*, (2020) by using biosensor method. However, Sushanta *et al.* (2020) reported higher plasma glucose levels of during different seasons in dairy buffaloes. Some workers have used biosensor technique and human glucometer for estimation

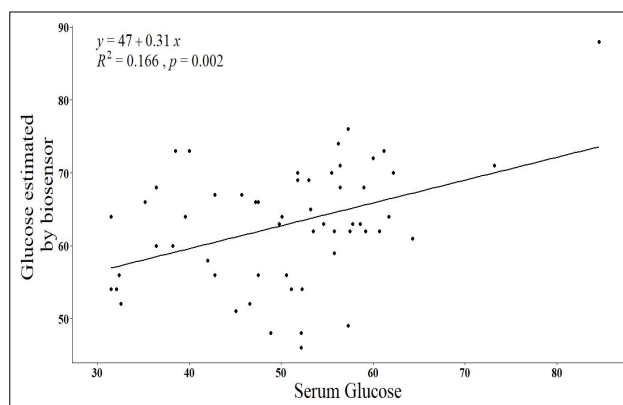


Fig 1: Linear regression analysis correlation between the serum glucose and the glucose measured using the biosensor.

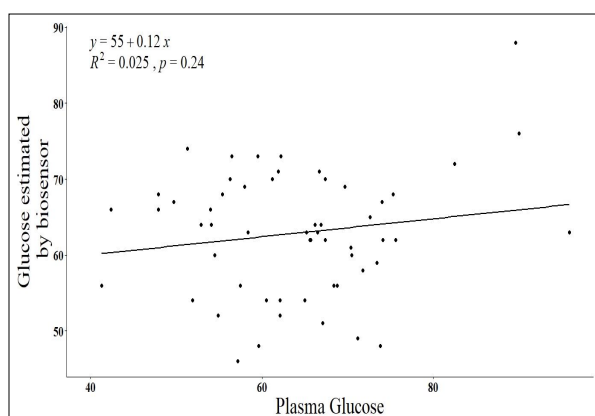


Fig 2: Linear regression analysis between the plasma glucose and the glucose measured using the biosensor method.

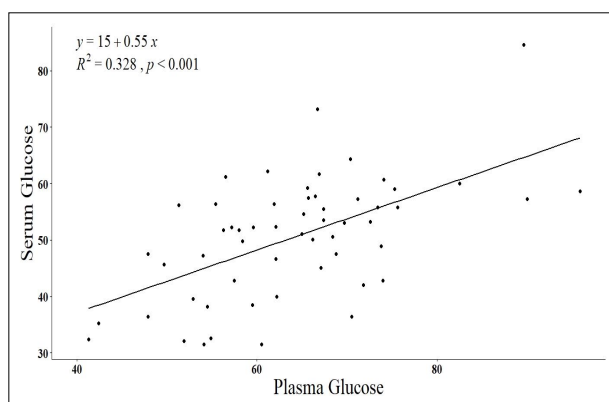


Fig 3: Linear regression analysis between the serum glucose and plasma glucose measured using GOD-POD method.

of levels in cow and they end up with varying results. Lopes *et al.* (2018) evaluated 6 hand-held meters designed for human use by estimation of blood glucose in cows. Important difference was reported across 6 hand-held meters. Precision was accepted in some meters but none of the meter was accurate for the measurement of blood glucose in dairy cows. In contrast to the findings, Mair *et al.* (2016) determined the capillary blood glucose levels using 3 different handheld devices in cows. They found that, out of three devices two were suitable for the measurement of blood glucose in cows. They also recommended these devices to use in veterinary practice. However, they recommended use of cattle specific chip for accurately measure the capillary blood glucose.

In our experiment, there is no significant difference between the mean values of plasma glucose measured by the GOD-POD method and glucose measured by biosensor method. However, the regression analysis showed that, the blood glucose measured in plasma do not have a significant correlation (15.94%, $p=0.24$) with that measured by biosensor. Mean $\pm$ SE of serum glucose level was significantly lower than the sodium fluoride tubes plasma glucose level. This may be due to rapid utilization of glucose *i.e.* glycolysis and lowering the levels in whole blood without preservative added tubes. Barring one estimation (Ajit *et al.*, 2020) no reports are available about the use of biosensor technique for the glucose estimation in buffalo.

CONCLUSION

The electrochemical hand-held device method is minimally invasive. Also, the major advantage is that, the estimation can be carried out at farm without involvement of technical person and use of preservative containing vials. However, the hand-held device is not sensitive method for measurement of blood glucose in buffaloes as describe in our results. It may give the approximate estimate of blood glucose levels which may help in screening the herd for the diagnosis of hypoglycaemia and monitor prognosis of ketosis in lactating buffaloes.

The glucose measured in serum is significantly less than in plasma and measured by the biosensor. As blood glucose levels are declining rapidly in blood serum, it is advisable to use fluoride-added preservatives anticoagulant tubes and not to use a serum for the estimation of blood glucose.

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Conflict of interest

All authors declared that there is no conflict of interest.

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