UNIVERSITI PUTRA MALAYSIA

EVALUATION OF POINT-OF-CARE GLUCOMETERS FOR USE IN GOATS

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It is hereby certified that we have read this project paper entitled "Evaluation of point-of-care glucometers for use in goats", by Nadiah Sakinah binti Muzaffar Shah and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kursus VPD 4999- Projek ilmiah tahun akhir

PENILAIAN PENGGUNAAN GLUKOMETER PADA KAMBING

Oleh

NADIAH SAKINAH BINTI MUZAFFAR SHAH

2020

Penyelia: Dr. Mark Hiew Wen Han

Penyelia bersama: Dr. Mohd Mokrish bin Md Ajat

Kepekatan glukosa biasanya diukur pada haiwan yang sakit bagi menilai tahap kesihatan untuk memberikan rawatan yang sesuai. Selama ini, doktor haiwan telah menggunakan glukometer yang diciptakan untuk manusia pada kambing bagi mendapatkan bacaan kepekatan glukosa yang pantas. Namun, tiada kajian yang meluas telah dilakukan untuk mengesahkan keberkesanan penggunaan glukometer pada kambing. Dalam kajian ini, dua jenis glukometer telah dinilai iaitu Accu-check Guide® dan Accu-check Active®. Bacaan yang diperoleh daripada kedua-dua glucometer dibandingkan dengan bacaan daripada mesin analisa makmal, “Biolis 24i clinical chemistry analyser” yang berfungsi sebagai kaedah rujukan. Tujuan kajian ini

dijalankan adalah untuk menilai penggunaan glukometer yang berbeza dalam mengukur kepekatan glukosa pada kambing dan menentukan ketepatan bacaan antara glukometer dan mesin analisa makmal. Sampel bagi kajian ini terdiri daripada 50 ekor kambing dewasa yang sihat dari 5 ladang di sekitar Selangor. Sampel darah diambil daripada vena jugulum. Setitis darah segera diletakkan di atas jalur ujian untuk kedua-dua glukometer sementara darah selebihnya disimpan di dalam tabung uji natrium fluorida dan kalium oksalat untuk dianalisis dengan mesin analisa makmal. One-way ANOVA menunjukkan bahawa terdapat perbezaan yang signifikan pada bacaan daripada ketiga-tiga kaedah ($P < 0.05$). Purata bacaan kepekatan glukosa darah daripada Accu-check Guide® lebih tinggi (3.24 ± 0.42 mmol/L) sementara Accu-check Active® lebih rendah (2.97 ± 0.53 mmol/L) jika dibandingkan dengan mesin analisa makmal (3.12 ± 0.39 mmol / L). Dengan menggunakan korelasi koefisien Pearson, bacaan daripada Accu-check Guide® ($r = 0.887$) menunjukkan bahawa ia lebih banyak berkorelasi dengan mesin analisa makmal berbanding Accu-check Active® ($r = 0.814$). Oleh kerana bias relatif untuk kedua-dua glukometer berada dalam 5% penyimpangan maksimum daripada nilai rujukan, kedua-dua glukometer boleh digunakan pada kambing. Kesimpulannya, Accu-check Guide® adalah glukometer pilihan kerana ia menghasilkan bacaan yang lebih dekat dengan mesin analisa makmal dengan korelasi yang lebih tinggi dan bias relatif yang lebih rendah.

Kata kunci: glukometer, kepekatan glukosa, kambing, darah.

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment for the course of VPD 4999- Final Year Project.

EVALUATION OF POINT-OF-CARE GLUCOMETERS FOR USE IN GOATS

By

NADIAH SAKINAH BINTI MUZAFFAR SHAH

2020

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Co-supervisor: Dr. Mohd Mokrish bin Md Ajat

Glucose concentration is commonly measured in sick animals to assess their health to provide appropriate treatment. Over the years, veterinarians have been using glucometers developed for humans on goats to obtain fast results. However, few studies have been carried out to validate glucometers as a point-of-care tool for goats. In this study, two types of glucometers were evaluated - Accu-check Guide® and Accu-check Active®. Readings from both glucometers were compared to the standard laboratory-based analyser, Biolis 24i clinical chemistry analyser which served as the reference method. The aim of this study was to evaluate the utility of different handheld

glucometers in measuring glucose concentration in goats and determine the accuracy between the glucometers and the laboratory-based analyser. A total of 50 healthy adult goats were recruited from 5 farms in Selangor. Blood samples were taken through the jugular venipuncture. A drop of blood was immediately placed onto the test strip for both glucometers while the rest of the blood was stored inside a sodium fluoride and potassium oxalate test tube for analysis with the laboratory-based analyser. One-way ANOVA showed that there was a significant difference in readings from all 3 methods ($P<0.05$). Accu-check Guide® overestimated (3.24 ± 0.42 mmol/L) while Accu-check Active® underestimated (2.97 ± 0.53 mmol/L) the mean blood glucose concentration when compared to the laboratory-based analyser (3.12 ± 0.39 mmol/L). Using Pearson's correlation coefficient, Accu-check Guide® ($r=0.887$) was more highly correlated with laboratory-based analyser than Accu-check Active® ($r=0.814$). Since the relative bias for both glucometers were within the 5% maximum deviation from reference value, they could be used in goats. In conclusion, Accu-check Guide® is the glucometer of choice as it produced readings that were closer to the laboratory-based analyser with higher correlation and lower relative bias.

Keywords: glucometer, glucose concentration, goats, blood.

1.0 INTRODUCTION

1.1 STUDY BACKGROUND

Blood glucose concentration is a vital component to assess the health of goats. Carbohydrate obtained via the consumption of food is converted to glucose for energy. The normal glucose concentration in goats is between 2.8–4.2 mmol/L to maintain homeostasis of the body. Diseases such as pregnancy toxemia, periparturient hypocalcemia (milk fever) and lactic acidosis can cause disturbances in the glucose concentration, as it can cause either hyperglycemia or hypoglycemia (Quandt et al., 2018). These two conditions can lead to many other complications if it is not treated early. Thus, it is important for veterinarians to be able to assess the patient's glucose level in a quick and accurate manner in order to administer the necessary treatment.

Glucometer is a device that measures the blood glucose concentration and is mainly manufactured for human home monitoring use (Roche Diagnostics GmbH, Mannheim, Germany). Fast and accurate determination of blood glucose is important when care providers are in the field. Since hand-held glucometers specifically for ruminants are not available, the human version is being used (Katsoulos et al., 2011). These portable glucometers can produce fast results and are relatively cheap compared to laboratory-based analyzers (Okorie-Kanu et al., 2018). However, the accuracy of glucometers for goats is still unknown and needs to be validated before use.

1.2 JUSTIFICATION

Comparing the accuracy of glucose level readings between the two methods (glucometer and laboratory based analyzer) is vital to ensure the improvement of goat's health and to assess the condition of goats that need critical care management. Since results from the laboratory can be time and cost consuming, most veterinarians have been using hand held glucometers to obtain glucose concentrations to assist in formulating treatment plans and to monitor patients. However, the validation of the Accu-chek Active® and Accu-chek Guide® glucometers against the laboratory based analyzer has not been done. Therefore, it is important to test the accuracy of glucometers as an on farm test for goats.

1.3 OBJECTIVE

1. To test the utility of different hand held glucometers in measuring glucose concentration in goats.

2. To evaluate the accuracy of the glucose concentration readings between hand held glucometers and laboratory-based analyzer.

1.4 HYPOTHESIS

Ho: The value of blood glucose measured using hand held glucometer is not significantly different from the laboratory-based analyzer.

Ha: The value of blood glucose measured using hand held glucometer is significantly different from the laboratory-based analyzer.

2.0 LITERATURE REVIEW

2.1 GOAT

Goat *(Capra aegaagrus hircus)* is a domesticated small ruminant mammal that is commonly reared for its meat and milk. Chevon is a term used for adult goat meat while carbito is the term used for young goat meat but in Malaysia both meat types are called mutton. Over the last two decades, the per capita consumption of goat meat in Malaysia is below 1 kg and remains stagnant when compared to other sources of animal protein. However, the consumption is now increasing due to an increase in urbanization, population, income growth and changes in consumer preference. The current self-sufficiency level in Malaysia is only 10% due to the small volume of domestic production and Malaysia being dependent on imported meat (Kaur, 2010). Thus, veterinarians play an important role to ensure the health of goats in order to increase the production and self-sufficiency.

The local indigenous breed in Malaysia is known as Katjang. It is reared for meat and has a high tolerance to the local environment. Other imported breeds such as Jamnapari, Boer and Savanna is not as hardy as Katjang but they are still well adapted to the local environment (Abu Bakar, 2014).

2.2 GLUCOSE

Glucose ($C_6H_{12}0_6$) comes from the Greek word *gleukos* meaning 'sweet wine'. It is a simple sugar that is important to sustain normal body functions and is used as a major energy source in living things. Glucose can be found in carbohydrate that is available in feed. Adequate carbohydrate intake is important to avoid nutritional deficiency but excessive amounts can cause toxicity to occur. Appropriate glucose concentrations are required to maintain health status, avoid disease occurrence and ultimately be able to sustain productive activity (Van Saun, 2000). The normal blood glucose concentration in goats is between 2.8 and 4.2 mmol/L (Quandt et al., 2018).

2.2.1 GLUCOSE METABOLISM AND REGULATION

Insulin, glucagon, amylin and glucagon-like peptide-1 are the glucoregulatory hormones that interplay in glucose homeostasis with the insulin and glucagon being the potent hormonal regulators that work together to maintain the glucose concentration in the circulation. Insulin was discovered in the year 1920 and its main function is to lower blood glucose concentration. It is derived from the B-cells of the pancreas and has become the treatment for diabetes type 1 and a therapy for patient with diabetes type 2. In 1950, glucagon derived from α-cell of the pancreas was discovered. It plays a role as a main stimulus for hepatic glucose production during the fasting state and counteracts the effect of insulin. In 1987, B-cell hormone, amylin, was discovered and it plays a role that complements insulin. In 1970, a hormone from the L-cell of the intestine, glucagon-like peptide-1 (GLP1) which is an incretin hormone, was identified and it contributes to play

a role in the maintenance of glucose homeostasis. Circulating glucose can be derived from intestinal absorption and from the hepatic processes. Glucose regulation involves many hormones from the pancreas and gut that affect multiple target tissues such as the brain, muscle, liver and adipocyte (Aranoff, 2004).

2.2.2 GLUCONEOGENESIS AND GLYCOGENOLYSIS

The source of circulating glucose can be derived from the hepatic process. Gluconeogenesis is a process of glucose formation primarily from amino acid and lactate during the fasting state. Meanwhile, glycogenolysis is the process of glycogen breakdown, which is the polymerized storage form of glucose. The liver and kidneys have the ability to hydrolyze glycogen and release glucose into the circulation because they have the enzyme glucose-6-phosphatase. Endogenous glucose production from the liver is important in the fasting state as the glucose moves out of the circulation at a constant rate. Renal gluconeogenesis only plays a role during a period of extreme starvation. During the first 8 to 12 hours of fasting, glycogenolysis will occur to make glucose available. Glucagon will facilitate this process and promote the presence of glucose in circulation. Long periods of fasting will cause release of glucose produced by gluconeogenesis from the liver. Insulin works as a key regulator hormone of glucose disappearance while glucagon is a glucose appearance major regulator. After eating, high blood glucose concentrations cause insulin to remove the glucose to the skeletal muscle and adipose tissue. Endogenous glucose production is also suppressed by direct action from insulin in the liver portal vein and from paracrine effect which is a direct communication in the

pancreas within α-cell and β-cell that cause glucagon suppression. Blood glucose will slowly decrease several hours after a meal and will return to fasting levels (Aranoff, 2004).

2.2.3 DISEASES ASSOCIATED WITH GLUCOMETER USAGE

Blood glucose concentration is commonly measured in sick animals to identify the health status of the animal and is used to aid in clinical management. Sick animals may develop abnormalities in glucose concentrations due to stress, prolonged starvation or because of the nature of the disease itself while newborn kids commonly develop neonatal hypoglycaemia (Quandt et al., 2018). Common metabolic diseases that occur due to glucose concentration abnormalities are pregnancy toxaemia (pregnancy ketosis or twin lamb/kid disease), lactational ketosis and periparturient hypocalcemia (milk fever) (Van Saun, 2000). Glucometers are also used for goats that suffer from blood parasites such as theileriosis (Ullah et al., 2018). Fast and effective intervention is needed once clinical signs are established which is the reason glucometers are important as an on farm test to analyze the glucose concentration rapidly.

2.3 GLUCOMETER

Glucometer is a device that measures the glucose concentration in the body from whole blood. On the other hand, laboratory based analyzers analyze plasma glucose concentrations. Glucometers available in the market are mostly designed for human use. However, in a farm setting, veterinarians usually use these same machines on animals as they are cheap, small in size, delivers fast results, easy to use, and requires a little amount

of blood for analysis (Okorie-kanu et al., 2018; Quandt et al., 2018). Glucometers play a major role in the early detection, monitoring and diagnosis of diseases in goats especially in the farm setting. However, since glucometers are designed for humans, inaccuracies due to overestimation or underestimation can occur. Inaccurate values may affect the effectiveness of the treatment given to animals (Quandt et al., 2018). Environmental conditions such as humidity, temperature and altitude may cause variability in blood glucose concentration measured by glucometers (Quandt et al.,2018).

2.3.1 ACCU-CHEK ACTIVE®

Accu-chek Active® is a glucometer developed as a point of care testing for glucose in humans at home. It requires little skill to perform and is easy to operate. This system aids in early diagnosing, monitoring and treatment of glucose related problems. Accu-chek Active® consists of a meter, test strip and lancet. However, in goats, blood is withdrawn using a syringe and needle instead of using a lancet. Accu-chek Active® quantitatively measures the blood glucose concentration in fresh capillary blood. The amount of blood required is between 1-2 µL per test. After the blood sample is applied on the test strip, it takes approximately 5 seconds to display the result. The test strip must be stored properly inside the container and closed tightly to avoid incorrect results. The drying agent located at the cap absorbs any moisture present. The test strips are stored in temperatures between 2-30°C, in a dry place and away from sunlight. The test needs to be performed at a temperature between 8 and 42°C and not under direct sunlight. On each strip, there is a test area containing the reagent. When applying blood onto the test area,

the glucose dehydrogenase enzyme will react with the blood glucose and the reaction changes the color of the test area so the meter will register the color changes and convert it into a blood glucose value (Roche Diagnostics GmbH, Mannheim, Germany). The detection limit range is between 10-600mg/dL (Okorie-Kanu et al., 2018).

Figure 1: Accu-chek Active® glucometer

2.3.2 ACCU-CHEK GUIDE®

Accu-chek Guide® is a self-testing device developed for humans with diabetes to check their glucose concentrations at home. Similar to Accu-chek Active®, it consists of a meter and test strips intended to quantitatively measure the glucose in fresh capillary whole blood. Blood can be obtained from venous, arterial or capillary. Blood samples containing anticoagulants and preservatives such as EDTA, sodium heparin and lithium heparin are also accepted but those containing iodoacetate or fluoride are not. Blood must

be tested within 30 minutes of collection to minimize glycolysis effects. A drop of approximately 0.6µL of blood is placed at the test strip and within 4 seconds the result will be displayed. During the pipetting of blood, air bubbles should be avoided in order to obtain accurate results. The test strips must be stored properly at a temperature between 4°C to 30°C and be used at temperature between 4°C to 45°C to ensure that there is no damage that can lead to false readings. The test strips must not be stored in high heat and moisture areas such as in the kitchen and bathroom and used in an environment with humidity around 10 to 90%. Strips need to be used immediately after being removing from the container. The reagent composition in the test strip is made up of non-reactive ingredients (47.2%), buffer (22%), FAD-GDH enzyme (21.3%), mediator (6.6%) and stabilizer (2.3%). When blood is placed onto the test strip, the enzyme FAD-dependent glucose dehydrogenase expressed in *Aspergillus oryzae* will convert the glucose in the blood sample to gluconolactone. A harmless DC electrical current will be created from the reaction which the meter will interpret and the blood glucose result concentration will be displayed. The results corresponded to the blood glucose concentration in plasma (Roche Diagnostics GmbH, Mannheim, Germany). The detection limit range is between 20-600mg/dL (Food and Drugs Administration, 2018)

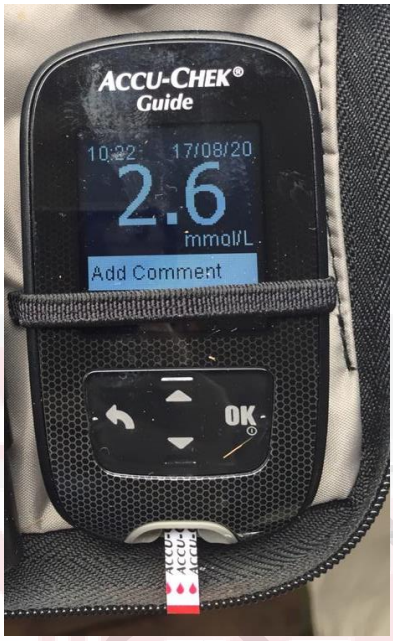

Figure 2: Accu-chek Guide® glucometer

2.4 LABORATORY-BASED ANALYZER

The laboratory based analyzer used in this study is Biolis 24i clinical chemistry analyzer, also known as CLC480 or MGC 240 chemistry analyzer, with the BioREX Mannheim reagent. It is a quantitative determinant of glucose from serum, plasma and urine. This machine measures glucose via glucose oxidase peroxidase (GOD-PAP), an enzymatic colorimetric test. The glucose, oxygen (O_2) and water (H_20) combination will be converted to gluconate and hydrogen peroxide (H_2O_2) in the presence of glucose oxidase (GOD). Then, the combination of 2 H_2O_2, 4-aminophenazone and phenol will be converted to 4-(*p*-benzoquinone-mono-imino) phenazone and 4 molecules of water (H_20) in the presence of peroxidase (POD). These two equations are reversible. The serum, heparin plasma or EDTA plasma must be separated within 1 hour of blood collection.

However, the stability in plasma can be achieved after adding a glycolytic inhibitor such as fluoride, monoiodacetate and mannose. The plasma can be separated later depending on the storage: 2 days at 20-25°C; 7 days at 4-8°C; 1 day at -20°C. The stability in serum without adding glycolytic inhibitor and no hemolysis occurring can be achieved if they are separated within 8 hours at 25°C and within 72 hours if stored at 4°C. Specimens can be frozen if separation of serum or plasma cannot be done immediately but freezing can only be done once and contaminated specimens must be discarded (Pointe Scientific Inc, Michigan, United States).

2.5 POTASSIUM OXALATE AND SODIUM FLUORIDE BLOOD TUBE

Grey top blood tubes contain potassium oxalate, to prevent the clotting of blood, and sodium fluoride which acts as a glycolysis inhibitor (Greiner Bio-One International GmbH, 2020). It is used for special chemistry test and to preserve glucose in whole blood. Blood tubes that contain anticoagulant additives prevent blood clotting when mixed properly. The tube is gently inverted at least 8 times immediately after the tube has been filled with blood to prevent clotting. Transportation of samples in the tube can be done by placing the sample inside an appropriate shipping container with a frozen coolant pack and the temperature maintained around 2-8°C. The sample must be placed in a manner that they do not come in direct contact with the coolant pack (Intermountain Healthcare, 2015).

3.0 MATERIALS AND METHODS

3.1 ANIMALS

A total number of 50 healthy adult goats (41 females and 9 males), were recruited from five farms in the state of Selangor. There was an average of 27.8 animals per farm with one of the farms practiced semi-intensive rearing system while the rest practiced an intensive rearing system.

Samples were collected via convenience sampling method. Sample size were determined by using an online sample size calculator. As of 2018, the population of goats in Selangor was 20 170 (Department of Veterinary Services, 2018). Upon calculation with 95% of confidence level and 5% of confidence interval, the number of sample size recommended is 377. Higher sample size used will lead to a higher test power. However, sample must be reduced due to some limitations such as time and cost-constraint and amount of goat available. Due to Covid19 pandemic outbreak, there were limited access to goat farms.

3.2 BLOOD COLLECTION

Goats were restrained with minimal excitement in a standing position with the head turned to one side to expose the jugular vein. Next, the age (via dentition), sex, breed, tag number and the body condition score were determined and recorded. Digital pressure was applied proximal to the thoracic inlet to occlude and distend the jugular vein. To

confirm that the distended vessel was the jugular vein, the vessel was strummed to see the oscillation of the blood. The area above the finger was swabbed with alcohol. A 21G needle attached to a 5 mL syringe was introduced with the bevel facing up at an approximately 30° angle to withdraw 3mL of blood from each goat. Since goat erythrocyte is prone to hemolysis due to its small size, blood was withdrawn using a needle instead of vacutainers to minimize this effect. The needle was then removed, pressure applied on thoracic inlet was released and placed on the punctured site for one or two minutes to prevent formation of hematoma (Newcomer et al., 2020).

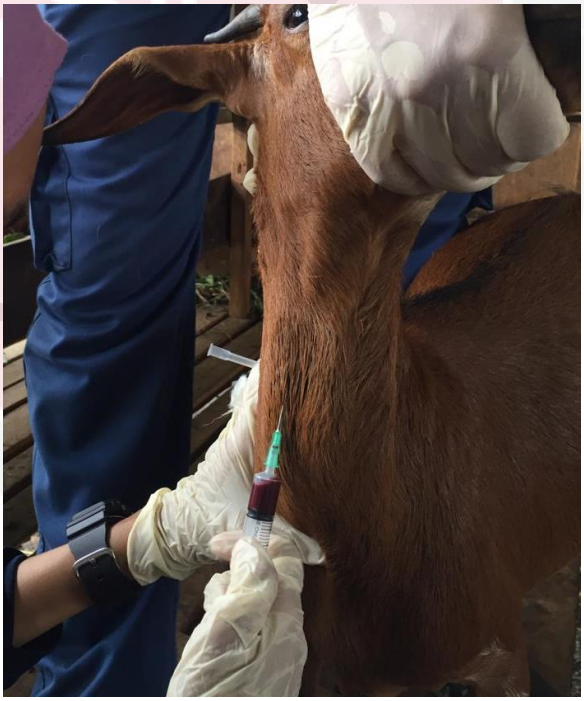

Figure 3: Blood collection via the jugular vein

3.3 AGEING

Mature goats have 32 teeth which consist of 24 molars and 8 incisors. All of the incisor teeth are located on the lower jaw. Since there is no upper incisor, a hard dental pad on the front part of the upper jaw plays a role as the teeth (Pope, 1943).

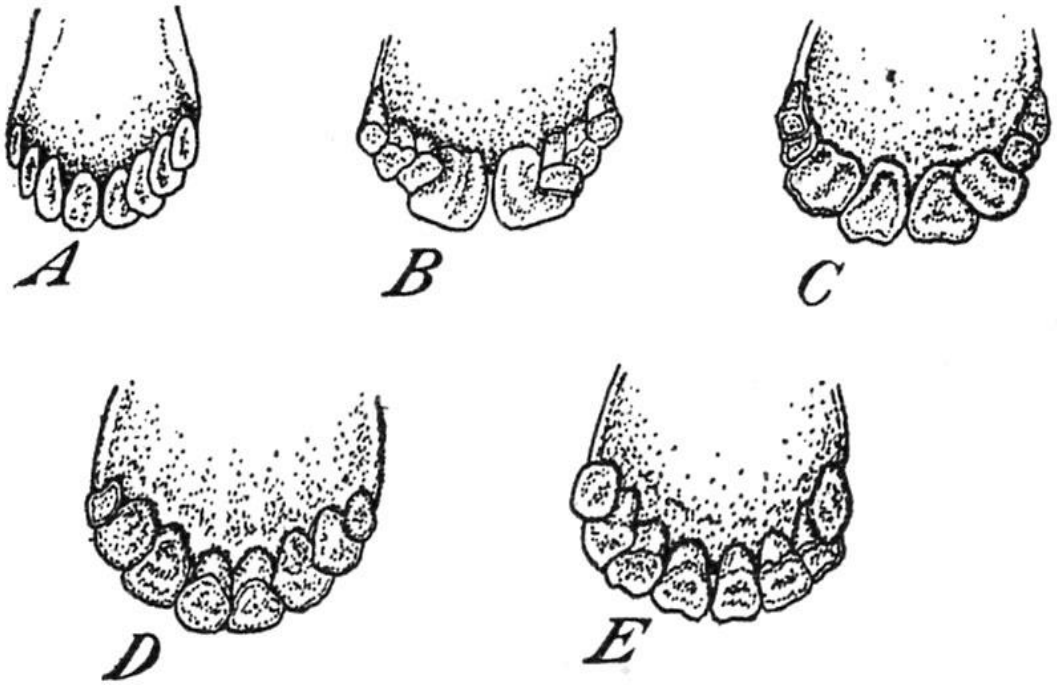

Figure 4: A: 3 months; B: 12 to 15 months; C: 2 years; D: 3 years; E: 4 years (Pope, 1934).

In a newborn goat, none of the teeth will appear yet. However, sometimes the two central incisor teeth, called pinchers, and the first pair of intermediate teeth will press through the gums or even have a cut through. In a few days, the second pair of intermediate incisors will appear followed by temporary corner incisors. By the age 3-

months-old, a complete full set of temporary incisor teeth will appear. The temporary incisor teeth appear smaller in size and has a milky whiteness color that can be distinguished from the permanent teeth (Pope, 1934).

When the goat reaches between 12 and 15 months of age, two permanent teeth will replace the temporary pinchers. At the age of 2-years-old, there is shedding of the first temporary intermediates and further replaced by the permanent teeth. At the age of 3-years-old, the secondary intermediates will be replaced by the permanent teeth. At the age of 4-years-old, the temporary corner incisors teeth will be replaced by permanent teeth and the goat now has a 'full-mouth'. To identify goats of more than 4 years of age, a progressive increase in the space between the teeth can be observed. They will gradually be worn to stubs as well as have uneven and unnatural length. In older animals, some teeth may be loose or broken and the animal is said to have a broken mouth (Pope, 1934).

3.4 BODY CONDITION SCORE

Body condition is used to define the body reserves in goats that consists of lipid that is present in fat and protein that is present in the muscle. The function of body reserves is for the production, reproduction and maintenance of the body. Body condition score (BCS) is determined by the amount of fat and muscle around and over the vertebrae at several skeletal checkpoints including spinous process, transverse process, hooks, pins and tail head. The scoring ranges from 1.0 to 5.0 with 0.5 increments. It is an easy and simple procedure used to judge the health status and to evaluate the feeding program in a

goat. Evaluating the BCS has many advantages over estimating the body weight of a goat because weight does not reflect the animal's condition. To do the scoring, the goat must be palpated and felt and it cannot be done by mere observation. The fat and muscle layer over and around the vertebrae must be palpated to feel for the fullness of muscle and fat layer (Ghosh et al., 2019).

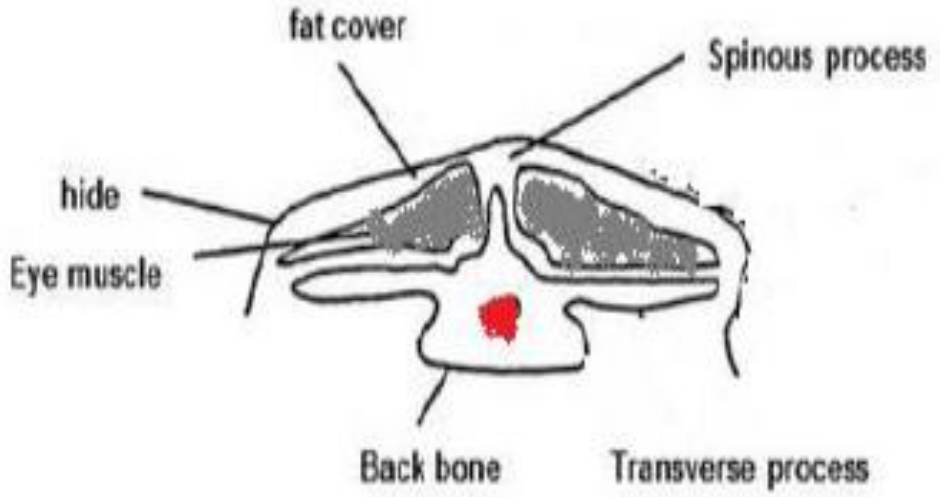

Figure 5: Checkpoints for fat and muscle cover (Ghosh et al., 2019).

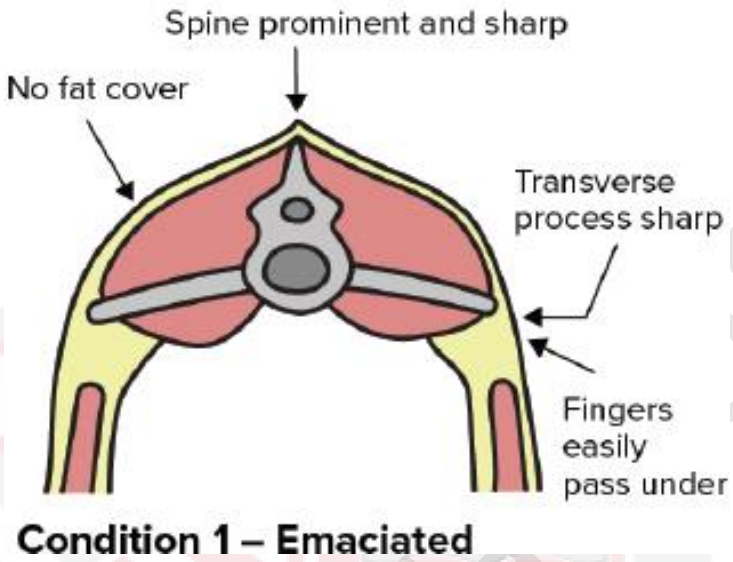

Figure 6: Body condition score 1 (Meat and livestock Australia, 2017).

For BCS 1.0, the physical appearance is characterized by an emaciated and debilitated goat, highly visible back portion, hollow flank, clearly visible ribs, no fat cover and a finger can easily penetrate into the intercostal spaces. Assessment of the skeletal checkpoints shows a very prominent spinous process, bony appearance, in between the skin and the bone there are little muscle layer and no fat, prominent depression in between the spinous process and transverse process and half of the transverse process is clearly visible. Lastly, the joint and cartilage that joins the rib and sternum can be easily felt (Ghosh et al., 2019).

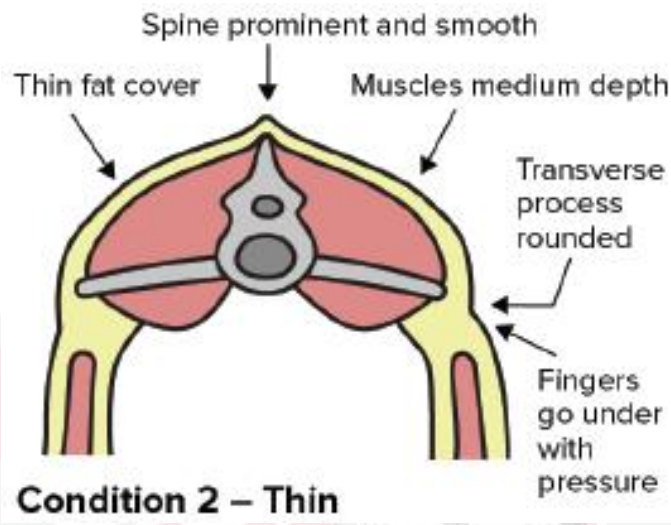

Figure 7: Body condition score 2 (Meat and livestock Australia, 2017).

In BCS score 2.0, the characteristics are moderately visible backbone with prominent ridge, ribs can be felt, little fat cover on the ribs and the intercostal spaces are smooth but can still be penetrated. For the skeletal checkpoints, there is a prominent ridge appearance on the spinous process, 1/3 of the transverse process is visible and a finger can be passed under with some pressure, between the spinous and transverse process there is a moderate muscle layer and a thin fat covering them and hollow flank (Ghosh et al., 2019).

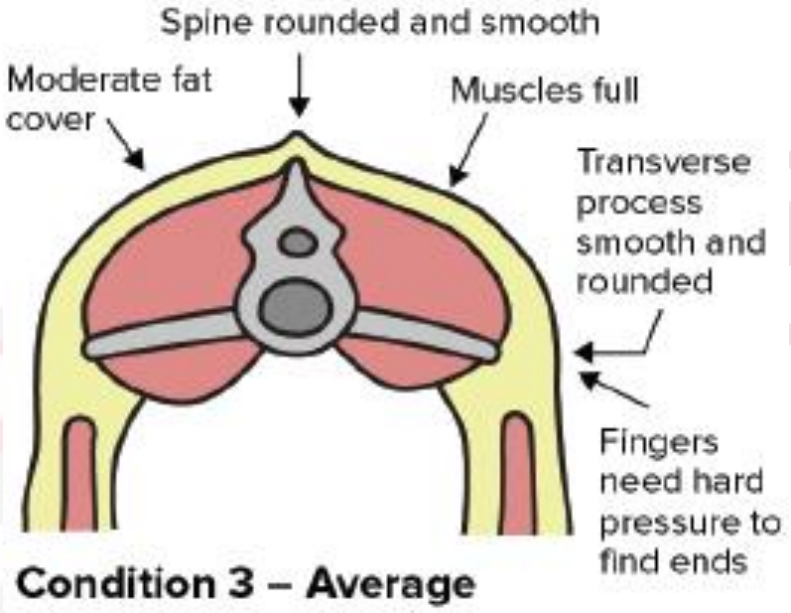

Figure 8: Body condition score 3 (Meat and livestock Australia, 2017).

In BCS 3.0, the physical appearance is characterized by a not so prominent backbone, ribs not clearly visible and covered with a thin layer of fat, and the intercostal spaces can be felt only after applying pressure. For the skeletal checkpoints, smooth and rounded appearance of the spinous and transverse process, the muscle area between the two processes are covered with moderate fat and the hollow region in the flanks are barely concave (Ghosh et al., 2019).

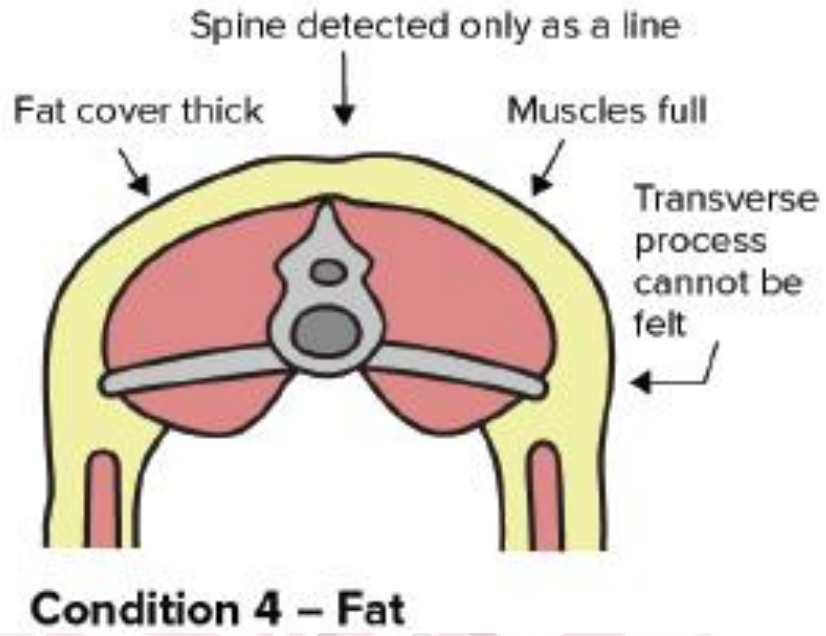

Figure 9: Body condition score 4 (Meat and livestock Australia, 2017).

In BCS 4.0, the backbone and ribs cannot be seen as it is covered with a thick layer of fat and there is a slight rounded appearance on the side of the animal. For skeletal checkpoints, the spinous process appears flat, transverse process is not visible and needs pressure to locate the end of the processes, there is a thick layer of fat covering the muscle area between the two processes and the hollow in flank cannot be appreciated (Ghosh et al., 2019).

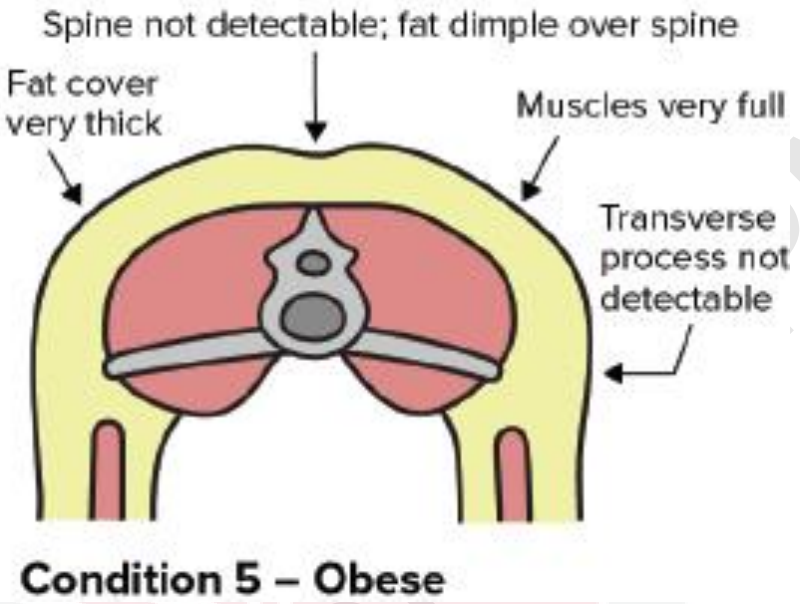

Figure 10: Body condition score 5 (Meat and livestock Australia, 2017).

In BCS 5.0, backbone and ribs cannot be seen as they are completely covered in fats. Hollow and curvature of the flank cannot be seen and there is excessive fat deposition on the sternum and pelvic region. For the skeletal checkpoints, the demarcation on the spinous process are completely lost and it is difficult to feel the transverse process as the muscle and fat layer are so thick, between the two spinous process there are bulging transitions, the sternum is covered with sternal fat and lastly the cartilage and ribs are covered with joining fat (Ghosh et al., 2019).

3.5 BLOOD GLUCOSE EVALUATION VIA GLUCOMETER

In this study, Accu-chek Active® and Accu-chek Guide® glucometers were used. A drop of blood was placed onto each strip and within ~5 seconds, the results were displayed. In Accu-Chek Active®, the minimum amount of blood needed was 1-2µL. When blood was placed onto the test area, the glucose dehydrogenase enzyme will react with the glucose in blood and a chemical reaction changed the color of the test area. The meter will register the color change and convert it into the blood glucose value. Meanwhile for Accu-Chek Guide®, the minimum amount of blood require was 0.6µL. After a drop of blood was placed onto the test strip, the enzyme FAD-dependant glucose dehydrogenase will convert glucose to gluconolactone and a direct current electrical current was formed for meter to interpret the glucose value (Roche Diagnostics GmbH, Mannheim, Germany). Normal readings fall between 2.8 and 4.2 mmol/L (Quandt et al., 2018). The remaining blood in the syringe was carefully transferred into a potassium oxalate monohydrate and sodium fluoride blood tube to avoid red blood cell (RBC) rupture (Newcomer et al., 2020). The grey-top tube contains potassium oxalate that acts as an anticoagulant and sodium fluoride acts as an anti-glycolytic agent that ensures no glucose breakdown occurs after the sample was taken. After blood was transferred into the tube, the tube was inverted 5 times for proper mixing of the anticoagulant with blood (Bryan, 2017). The tube was labeled and placed inside an icebox for transportation. The temperature inside the icebox was maintained around 15-20°C.

3.6 LABORATORY BASED ANALYZER

Blood was centrifuged at the laboratory for 5 minutes at 3000rpm using The Hettich® EBA 20 centrifuge machine and the plasma was removed and then transferred into a sample cup. This was done within 4 hours of collection. Plasma glucose concentrations were analyzed with Biolis 24i clinical chemistry analyzer with BioREX MANNHEIM reagent. 2.4 µL of plasma was pipetted into the sample cup and inserted into the machine for analysis. The GOD-PAP method (an enzymatic colorimetric test) was used for the analysis. The 2.4µL of plasma was mixed with 200µL of reagent and was incubated in 37°C for 5 minutes. The enzyme glucose oxidase (GOD) will react with glucose, oxygen and water present in the plasma converting them into gluconate and hydrogen peroxide. Meanwhile the enzyme peroxidase (POD) will react with 2 hydrogen peroxide molecule, 4-Aminophenazone and phenol, converting them into 4-(*p*-benzoquinone-mono-imino) phenazone and 4 molecules of water. The readings obtained were recorded.

Figure 11: The Hettich® EBA 20 centrifuge machine set at 3000 rpm for 5 minutes to separate plasma from red blood cells

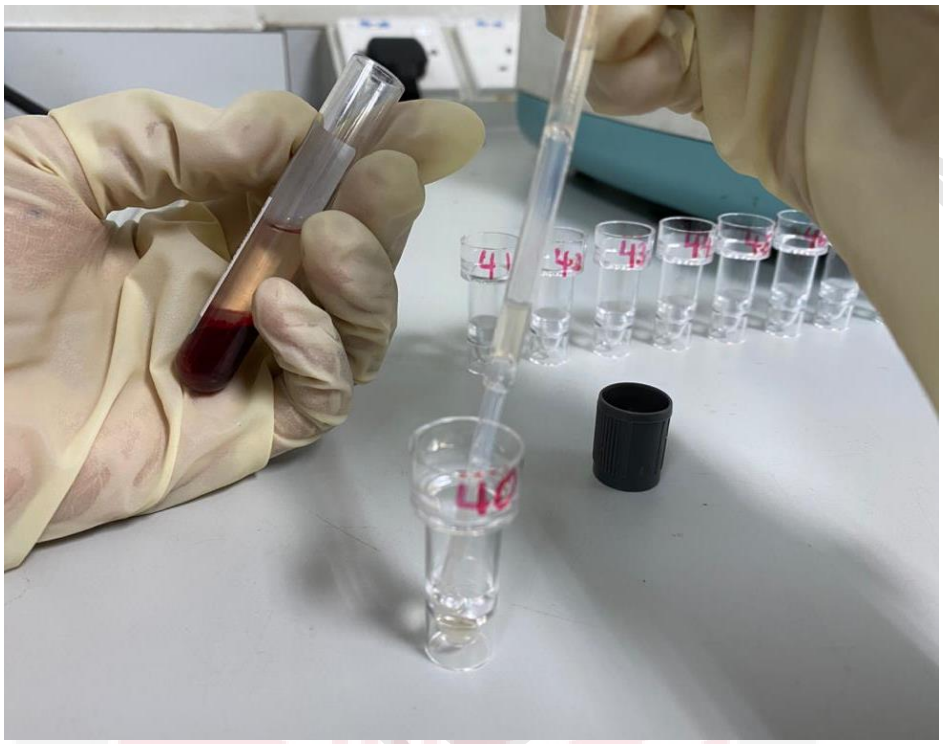

Figure 12: Plasma transferred to sample cups

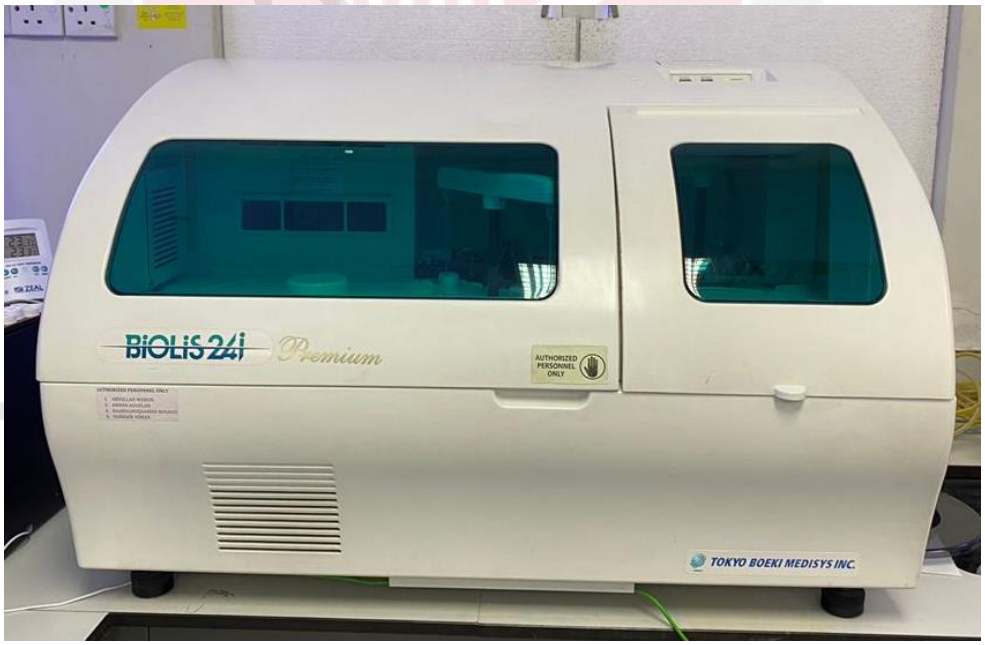

Figure 13: Biolis 24i clinical chemistry analyzer

3.7 STATISTICAL ANALYSIS

The data recorded were analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. One-way ANOVA was used to analyze the biochemistry finding. To use a parametric test, the fundamental assumptions such as the data must be normally distributed, has equal variance and no extreme score must be fulfilled. The normality was tested using Shapiro-Wilk's Test. The data was normally distributed if the P-value was >0.05 which indicates that the null hypothesis is accepted. The variance was considered equal when the P-value <0.05. The extreme score must be zero as it can distort the mean, standard deviation and standard error. Extreme score can be identified by plotting the boxplot and observe any asterisk (*) present. Once all of the fundamental assumptions have been fulfilled, data was further analyzed using one-way ANOVA. The null hypothesis will be rejected if the P-value<0.05, which indicates that there was a significance different between the mean of glucose concentration measured using Accu-chek Guide®, Accu-chek Active® and laboratory-based analyzer. The strength and direction of linear relationship between the different methods available were tested using Pearson correlation coefficient. The parameters were considered significantly correlated if the P-value < 0.01.

Bland-Altman was plotted to compare the two different measurement techniques and to assess the agreement between both methods (Bland & Altman, 1986; 1999). The bias was identified and the relative bias was calculated using the formula: (Glucometer – Laboratory-based analyzer) / (0.5 X [Glucometer + Laboratory-based analyzer]) X 100

L (Quandt et al., 2018). The maximum deviation for relative bias cannot exceed 5% from the reference value for the readings of glucometers to be accepted.

4.0 RESULTS

50 goats (41 females and 9 males) were recruited in the study. The breeds consist of Jamnapari (n=1), Boer (n=7), Saanen (n=6), Katjang (n=12), Jamnapari cross (n=2) and Boer cross (n=22). The normal range for glucose concentration is between 2.8 mmol/L to 4.2 mmol/L (Quandt et al., 2018).

Method	**Laboratory-based analyzer**	**Accu-chek Guide®**	**Accu-chek Active®**
Hypoglycemic (<2.8 mmol/L)	17	7	8
Normoglycemic (2.8mmol/L-4.2mmol/L)	33	43	42
Hyperglycemic (>4.2mmol/L)	-	-	-
Total	50	50	50

Table 1. Blood glucose concentration obtained from different methods

From table 1, the results obtained from Accu-chek Guide® shows that 14% (n=7) were hypoglycemic and 86% (n=43) were normoglycemic. When using Accu-chek Active®, 34% (n=17) were hypoglycemic and 66% (n=33) were normoglycemic. When tested with laboratory-based analyzer, 16% (n=8) were hypoglycemic and 84% (n=42) were normoglycemic. The readings obtained from Accu-chek Guide® were consistently closer with the value of laboratory-based analyzer.

One-way ANOVA was used to analyze the data with 95% confidence interval. Before using the parametric test, three fundamental assumptions must be fulfilled such as the data is normally distributed, has equal variance and no extreme score. The distribution of the data was tested using Shapiro-Wilk's Test and the result shows that they were all normally distributed ($P>0.05$). Next, the variance was tested using Levene's test and the variance was equal ($P>0.05$). Lastly, no asterisk (*) present in the boxplot which indicates that there was no extreme score. In one-way ANOVA, each record data must be independent.

The ANOVA test ($P<0.05$) showed that there was significant difference in the blood glucose concentration mean obtained from Accu-chek Guide® and Accu-chek Active® when compared to standard laboratory-based analyzer, $F(2, 147) = 4.577$, $p= 0.012$. Therefore, the null hypothesis was rejected.

Method	Laboratory-based analyzer	Accu-chek Guide®	Accu-chek Active®
Mean ± standard deviation	3.12 ± 0.39	3.24 ± 0.42	2.97 ± 0.53
Minimum value	2.30	2.40	1.80
Maximum value	4.10	4.20	3.90
Standard error	0.0553	0.0595	0.0745

Table 2: Blood glucose concentration obtained from different methods using statistical analysis, One-way ANOVA.

From table 2, the mean blood glucose concentration value for Accu-chek Guide® overestimated the value from laboratory-based analyzer. On average, the glucose concentration measured by Accu-chek Guide® was 0.12 mmol/L more than laboratory-based analyzer. Meanwhile, the mean value from Accu-chek Active® underestimated the mean value from laboratory-based analyzer with average of 0.15 mmol/L less than the reading from laboratory-based analyzer.

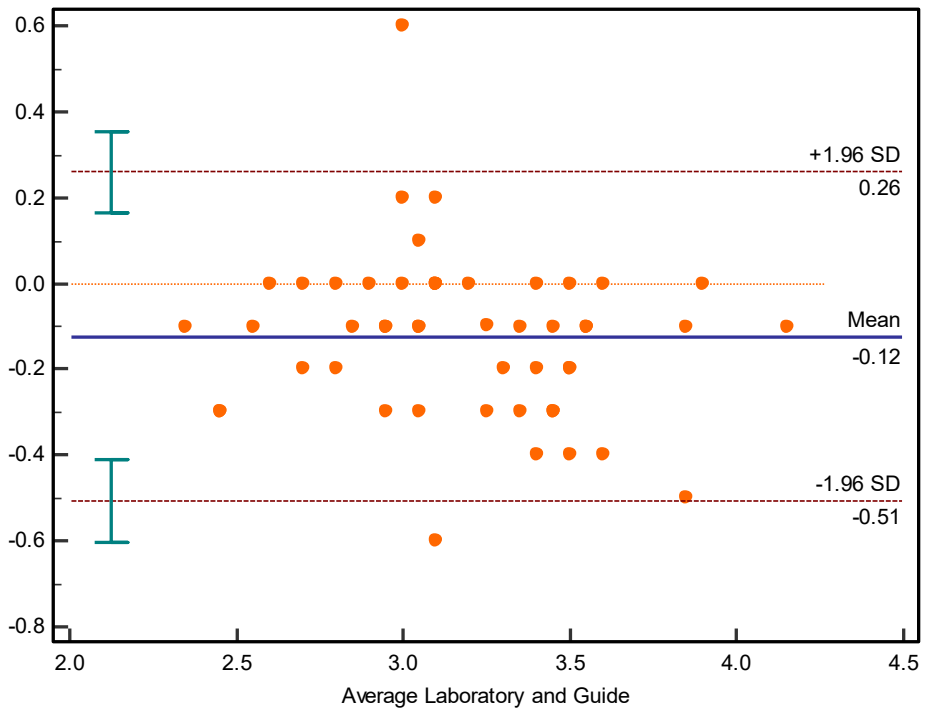

Figure 34: Bland-Atlman plot on Laboratory-based analyzer and Accu-chek Guide® to assess the agreement between both methods.

From Figure 14, the bias (mean difference) was -0.12 which indicates that Accu-chek Guide® overestimates the reading when compared to the laboratory-based analyzer. The relative bias for Accu-chek Guide® and laboratory-based analyzer was 3.8% which was considered acceptable as it was still within the 5% from reference value as recommended by American Diabetes Association (ADA). The 95% confidence interval of limit of agreement (LoA) ranged between -0.51 to 0.26. The r=Pearson correlation was done to evaluate the association between glucometers and laboratory-based analyzer. There was a strong and positive significant correlation between Accu-chek Guide® and laboratory-based analyzer with r=0.887 (P<0.01).

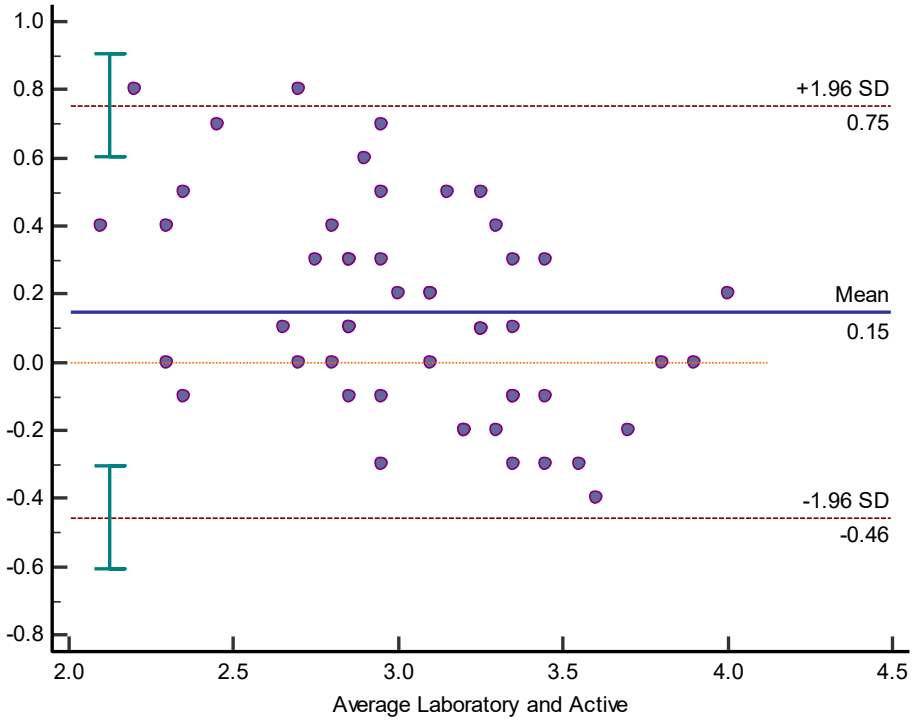

Figure 15: Bland-Altman plot on Laboratory-based analyzer and Accu-chek Active® to assess the agreement between both methods

From the plot, the bias was 0.15 which indicates that the Accu-chek Active® underestimates the readings from the laboratory-based analyzer. The relative bias calculated was 4.8% which was still within the 5% from reference value as recommended by American Diabetes Association (ADA). However, it was slightly higher than Accu-chek Guide®. The 95% confidence interval of limit of agreement (LoA) ranged from -0.46 to 0.75. There was a higher precision when compared to the Accu-chek Active® as all of the values were within the maximum and minimum allowed difference (Δ). There was a strong and positive significant correlation between Accu-chek Active® and laboratory-based analyzer with $r=0.814$ ($P<0.01$).

5.0 DISCUSSION

Glucometers have many advantages when compared to the standard laboratory-based analyzer. They are able to provide rapid results, cheaper, need a small volume of blood and the operator does not need high skills to use it. However, the disadvantage when using glucometers is inaccurate results due to overestimation and underestimation from the glucometer reading (Quandt et al., 2018). Therefore, the validation of glucometer for every species is important to avoid misdiagnosis and inappropriate treatments being given.

In this study, the number of females sampled were higher than males. This was due to the fact that most farms practicing a natural mating system with the male to female ratio of approximately 1:20 (Alves et al., 2014). This gives rise to there being more females on the farm.

The mean glucose concentration reading from Accu-chek Guide® (3.24 ± 0.42) overestimated the mean glucose concentration reading of the laboratory-based analyzer (3.12 ± 0.39). The overestimation of reading may cause misdiagnosis of their glycemic status that will affect the treatment plan (Okorie-Kanu et al., 2018). On average, the glucose concentration measured by Accu-check Guide® was 0.12 higher than laboratory-based analyzer. Thus, veterinarians must keep in mind of this overestimation and be careful when giving any treatment. Besides that, the difference in time between analysis can also be one of the factors that contributes to overestimation of the glucose concentration. The longer time taken for analysis with laboratory-based analyzer causes

lower readings due to a reduction in glucose by red blood cell metabolism (Quandt, 2018). In this study, the analysis using glucometers was done immediately after blood collection, while the analysis using laboratory-based analyzer was done within 4 hours of blood collection.

The normal range for blood glucose concentration is between 2.8 mmol/L to 4.2 mmol/L (Quandt et al., 2018). The minimum value obtained by Accu-chek Active® was 1.80 mmol/L while the maximum value was 3.90 mmol/L. This glucometer produced consistently lower values compared to the laboratory based analyzer (reference standard in this study). Therefore, the user needs to be careful to not over-diagnose patients with hypoglycemic status. The mean glucose concentration reading of Accu-chek Active® (2.97 ± 0.53) underestimates the glucose concentration value from the laboratory-based analyzer (3.12 ± 0.39). The causes for underestimation of the glucose concentration reading is still unknown but probably occurs due to the lower precision of the glucometer at low blood glucose concentration as the glucometer was designed for humans that generally have a higher blood glucose concentration of 4.4-6.1 mmol/L (Katsoulos et al., 2011; Kazmi, 2017). In Accu-chek Active®, glucose is determined by a reflectance photometry (Roche Diagnostics GmbH, Mannheim, Germany). Photometry is defined as a measurement of the strength of light. In other words, difference in light intensity might disrupt the reflection of light for glucose concentration readings by Accu-chek Active® causing false readings. Thus, it is important to control the ambient lighting by testing the glucometer in the same facility with dim light or away from direct sunlight.

Overestimation and underestimation can also occur due to some variables such as the ambient temperature, altitude and humidity (Quandt et al., 2018). These parameters were not controlled in this study as the glucometers were used in various farms with different environments. Other possible errors are the presence of bubbles and inadequate amount of blood placed on the strip, improper storage of strip, and improper calibration of the meter. Accuracy can also be increased if the user operates them according to the manufacturer's recommendation (Salacinski et al., 2014; Roche Diagnostics GmbH, Mannheim, Germany).

The accuracy of glucometers can be assessed by comparing the readings of the glucometers with the laboratory based analyzer, which is a standard way of analyzing glucose concentration. However, the difference may also occur due to other factors such as different in time during analyzing. Prolonged period of analyzing can cause glucose uptake by red blood cell metabolism but in ruminants the rate of glycolysis in the red blood cell is low (Quandt et al., 2018). In glucometers, the glucose was analyzed almost immediately after blood collection however in laboratory based analyzer, it was analyzed within 4 hours after blood collection.

One-way ANOVA was done to analyze the data using the Statistical Package for the Social Sciences (SPSS) version 25.0. To use the parametric test, the three fundamental assumptions were fulfilled. Firstly, the data distribution was tested using Shapiro-Wilk's Test. The data is normally distributed if $P>0.05$. The P for laboratory-based analyzer was 0.546, Accu-check guide was 0.35 and Accu-check active 0.477.

The first assumption was fulfilled. Secondly, the variance must be equal. In variance, P> 0.05 indicates that the variance is equal. The variance value for laboratory-based analyzer was 0.153, Accu-check guide was 0.177 and Accu-check active was 0.278. The second assumption was fulfilled. The last assumptions state that no extreme score should be presence. Boxplot was plotted to observe asterisks (*). In this study, no asterisks were present which indicates that there was no extreme score.

Once all of the assumptions were fulfilled, the one-way ANOVA test was conducted. The P-value for Accu-chek Guide® with laboratory-based analyzer was 0.000 and the P-value for Accu-chek Active® with laboratory-based analyzer was 0.001. We can conclude that there was a statistically significant difference between the mean glucose concentration measured by laboratory-based analyzer with the Accu-chek Guide® and Accu-chek Active® ($P<0.05$). The null hypothesis was rejected at the α level of significance. Although there was a statistically significant difference in value between the three methods, there was a strong and significantly positive correlation between one another. The correlation was tested using Pearson correlation coefficient to assess the validity. The r-value for Accu-chek Guide® and Accu-chek Active® were 0.887 and 0.814 respectively ($P<0.01$). Based on the results of this study, it is better to choose the Accu-chek Guide® than the Accu-chek Active® although both of the glucometers were significantly and positively correlated with the laboratory-based analyzer. However, the Accu-chek Guide® has a higher correlation value with the reference standard compared to the Accu-chek Active®. Both glucometers can be used in goats with some precautions towards the overestimation and underestimation value that may cause misdiagnosis.

Bland-Altman plot was done to compare the two different measurement techniques and to assess the agreement between both methods (Bland & Altman, 1986; 1999). The bias from laboratory-based analyzer and Accu-chek Guide® was -0.12 which indicates that there was overestimation of Accu-chek Guide®. The bias can also be expressed in percentage (relative bias) and it was calculated using the formula: (Glucometer – Laboratory-based analyzer) / (0.5 X [Glucometer + Laboratory-based analyzer]) X 100 L (Quandt et al., 2018). The relative bias for Accu-chek Guide® and laboratory-based analyzer was 3.8%. According to the American Diabetes Association (ADA), the maximum deviation can only be 5% from the reference value for the readings of glucometers to be accepted. The 95% confidence interval of limit of agreement (LoA) ranged between -0.51 to 0.26.

The bias from comparing the laboratory-based analyzer and Accu-chek Active® was 0.15 which indicates that the Accu-chek Active® underestimates the readings when compared to the laboratory-based analyzer. The relative bias calculated was 4.8% which is higher when compared to the Accu-check Guide® but still within acceptance range of 5% from reference value by the ADA. The 95% confidence interval of limit of agreement (LoA) ranged from -0.46 to 0.75.

Both of the glucometers do not achieve a 100% reliability when compared to the laboratory-based analyzer as the glucometers were calibrated for human use. However, both of them were still within 5% of maximum deviation approved by American Diabetes Association. Thus, they can still be used but operators must be cautious about the

overestimation and underestimation in order to not misdiagnose the patient's glycemic status. (Salacinski et al., 2014).

Based on the glucose concentration value measured, readings from Accu-chek Guide® show a constant higher correlation compared to the Accu-chek Active®. Accu-chek Guide® generated the closest value to the laboratory-based analyzer with relative bias lower than Accu-chek Active®. In a nutshell, Accu-chek Guide® is the glucometer of choice.

6.0 CONCLUSION

In conclusion, there is a statistically significant difference in blood glucose concentration between all 3 methods. The blood glucose concentration readings from Accu-chek Guide® overestimate the value from laboratory-based analyzer while Accu-chek Active® consistently produce lower value and underestimate the reading from laboratory-based analyzer. However, Accu-chek Guide® is more strongly correlated, generated the closest value to the laboratory-based analyzer and has a lower relative bias with laboratory-based analyzer compared to Accu-chek Active®.

Thus, Accu-chek Guide® should be chosen as a point-of-care glucose testing to measure the blood glucose concentration on farm for goats. Before a glucometer is use, they must be validated accordingly to the species for accuracy of diagnosis.

7.0 RECOMMENDATION

Further studies can be done to evaluate the accuracy of Accu-chek Guide® and Accu-chek Active® by doing the laboratory-based analyzer analysis immediately after blood collection. This is to minimize the red blood cell metabolism that will reduce the glucose concentration.

For the sample size, obtaining equal numbers of male and female can be done to avoid biases. In this study, only 50 healthy adult goats were recruited due to the current pandemic situation caused by Covid 19. An increased sample size will help to strengthen the power of the test.

In this study, only healthy goats were tested. Additionally, animals that are known to have hyperglycemic or hypoglycemic conditions could be added to further validate the glucometers.

Besides the time of analysis, environmental conditions also may affect the reading on glucometers. Thus, it is advisable to do the testing for all samples in the same facilities so there is no variation in the environment

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