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# COMPARISON OF RECTAL THERMOMETRY WITH THE ALTERNATIVE UNDERTAIL, AXILLARY, AND INGUINAL TEMPERATURE MEASUREMENTS IN SHEEP

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Supporting Information

ABSTRACT: This study was conducted to ascertain the suitability of alternative locations for temperature measurement, with reference to rectal thermometry in sheep, using a digital thermometer (DT). The study employed a single-factor multilevel design, considering anatomical location (site) as the main factor. This anatomical location factor had four conditions, including rectal (rectalDTt), undertail (undertailDTt), inguinal (inguinalDTt), and axillary (axillaryDTt) locations. A total of 16 sheep were recruited for the study, and each treatment had eight replicates. The data obtained were descriptively analyzed using means and standard deviations, while inferential statistics included analysis of variance (ANOVA), Pearson's correlation, Tukey's test, t-test, and Bland-Altman plot. The mean inguinal DTt was the highest ( $39.51 \pm 0.31^{\circ}$ C), while the lowest was the mean undertailDTt (38.97±0.45). The effect of anatomical location on temperature readings was statistically significant. The difference between mean rectalDTt and inguinalDTt, or axillaryDTt was not significant. The rectaIDTt measurements were significantly correlated with those of each treatment. Equivalence analysis revealed a non-significant bias between the rectalDTt and inguinalDTt pair. The Bland-Altman plot showed a good level of correlation and considerable agreement between rectalDTt and inguinalDTt measurements. In conclusion, temperature measurement at the inguinal location results in readings that are similar to those of rectal thermometry and thus may be of clinical importance in the future, particularly with digital thermometer application in sheep.



Keywords: Anatomical location, Body temperature, Digital thermometer, Sheep, Rectal thermometry.

# INTRODUCTION

The core body temperature, also known as animal body temperature, is the average temperature of an animal's deep body core (Godyń et al., 2019). Additionally, it is closely related to the animal's metabolism and life activities, which can reflect the physiological and health status of animals (Cai et al., 2023). There are many conditions that cause temperature changes, namely infectious diseases, thermal stress, synchronization and estrus status, onset of lambing, among others (Underwood et al., 2015; Fischer-Tenhagen and Arlt, 2020). It is possible to have a better understanding of the physiological changes that occur in an animal and in-depth analysis of its health by way of body temperature measurement (Cai et al., 2023). Body temperature assessment should be the first procedure to be done when examining sheep, and the results interpreted in conjunction with other clinical signs (Stockler et al., 2021). In view of this, early measurement of this physiological marker would result in timeous decision-making or management of many conditions, and minimizes undue reproductive and economic losses (Godyń et al., 2019; Abigaba and Sianangama, 2023).

The are many body temperature sensors that have been explored for application in sheep, for example, temperature loggers, transponders, clinical digital thermometers (digital thermometer; DT), clinical mercury thermometers (mercury thermometer; MT), and non-contact infrared devices like infrared thermometers and thermal infrared cameras (Pourjafar et al., 2012; Abecia et al., 2015). However, most of these devices are either sophisticated to use, less accurate, expensive, not readily available, or potentially hazardous (George et al., 2014). These drawbacks are of great concern to the farmers, particularly those who live in the rural setting or practice smallholder farming system. Accordingly, the MTs and DTs have been used by many clinicians and some farmers to measure the body temperature of various livestock species, including sheep, for many years. This notwithstanding, the MTs have been sidelined in some countries like USA because of the following drawbacks: generally time consuming, susceptible to breakage, and may cause environmental toxicity (Katsoulos et al., 2016). Recently, the DT device has gained popularity among many users worldwide. This popularity is

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attributed to many factors, namely accuracy and relative rapidity of measurement, nontoxic to the environment, user friendly, less expensive, and availability (Cadioli et al., 2010; Hine et al., 2015).

The DT device is traditionally applied in sheep per rectum, hence it is among the temperature sensors under the group known as rectal thermometers. Temperature measurement per rectum is referred to as rectal thermometry; this method remains the gold standard for the body temperature assessment in sheep (Katsoulos et al., 2016). This notwithstanding, rectal thermometry has been associated with potential drawbacks, such as stress, fomite for disease spread, rectal injuries to animal, inter alia (Katsoulos et al., 2016; Yadav et al., 2017). Notably, some of these disadvantages also apply to the DT device when it is used to measure temperature per rectum (Muhammed et al., 2019). Hence the ability to measure body temperature at the peripheral locations may be helpful and less invasive (Kearton et al., 2020). In view of the foregoing, there is an urgent need to search for an alternative anatomical location that is safer, non-invasive, easy to use, and robust to external variations, particularly with a digital thermometer. This study was conducted to compare rectal thermometry with the alternative temperature that is measured at the minimally-invasive skin locations among adult sheep of both genders.

## MATERIALS AND METHODS

## **Ethical consideration**

This study used animals that were physically healthy; the procedures conducted on them were non-lethal and inflicted little or no distress to the study animals. The animal handling, including restraint, experimentation, inter alia, was done with strict supervision by the institutional committee on the animal research. The procedures were done in accordance with the guide for the care and use of agricultural animals in research and teaching (ASAS, 2020).

## Study area

This study was conducted during the month of July, 2023, at the farm station that is owned by the School of Veterinary Medicine, The University of Zambia, located in Lusaka, Zambia. Zambia is located at a latitude S  $14^{\circ}$  20' 0" and longitude E  $28^{\circ}$  30' 0" according to the GeoNames geographical database Google Earth-2023. The country lies, in the tropics, within the Southern-African region. In terms of its weather conditions, Zambia receives an average annual precipitation that ranges from 800 to 1400 mm, while the temperature ranges from 10 to  $20^{\circ}$ C during winter and 20 to  $30^{\circ}$ C in the hot-dry seasons (Bailey et al., 2021). The average ambient temperature and humidity conditions at the field station ranged from 21.5 to 22.5°C and 41 to 51%, respectively, during the study period.

#### **Experimental animals**

This study included physically-healthy sheep that belonged to the School of Veterinary Medicine, the University of Zambia. The weight of these sheep ranged from 43 to 74 kg, with an average of 56 kg. Their age ranged from 1 to 3.5 years, with an average of 2.4 years. All the animals were kept under a semi-intensive rearing mode; they were mostly grazing within the paddocks with minimal supplementation using a commercial concentrate. The concentrate was supplied by National Milling Limited, Lusaka, Zambia. Water was provided to the sheep *ad libitum*.

#### **Experiment design**

The study employed a single-factor multilevel design to determine differences between temperature measurement methods in sheep. According to the design, anatomical location was the main factor considered. This factor had four levels (conditions), namely rectal, axillary, undertail, and inguinal locations, and the measurement conducted at each location was regarded as a measurement method. In this case the measurements were performed at the rectal (rectal temperature; rectalDTt), undertail (undertail temperature; undertailDTt), inguinal (inguinal temperature; inguinalDTt), and axillary (axillary temperature; axillaryDTt) locations. Additionally, the rectalDTt was considered as the reference measurement method. A total of 16 adult sheep were used for this study, and each condition had 8 replicates. In this study, each animal was assessed for all the measurement locations.

#### **Temperature measurement**

Prior to the temperature measurement, each sheep was physically restrained according to the procedures by Stockler et al. (2021). Then, temperature readings (DTt) were taken from each sheep after 20-minutes lapse; this was done to minimize the potential effects of psychogenic fever on the study results. Temperature measurement was conducted on the sheep in a semi-temperature controlled indoor facility, which aimed to minimize variations in the environmental conditions like temperature changes. Furthermore, the measurement of temperature was done using a functional veterinary digital thermometer (DT; GB Kruuse digital thermometer, Taipei, Taiwan). The measuring range of this thermometer was 30.0 - 43.9°C, and its resolution was 0.1°C. Temperature measurement, at the various anatomical locations considered, was done following an order that was determined using a simple random selection. In this case, selection was done using folded papers that bore the name of each anatomical location; these were tossed followed by randomly picking one at time without replacing it. This selection procedure was intended to minimize the bias; hence it was repeated for each sheep under study. Additionally, all the temperature data were obtained at the different anatomical locations on the same day.

The rectalDTt measurement was performed based on the previous procedure (Pourjafar et al., 2012). In the case of the inguinalDTt and axillaryDTt measurements, the procedures were based on another study (Levy et al., 2020), with a minor modification. Briefly, axillaryDTt measurement was conducted by carefully inserting a DT probe deep into the left axilla, approaching from the caudal aspect, and aiming towards the dorsum. This procedure was done on a study sheep standing with both forelimbs close to its body. Again, a similar standing posture, with both hindlimbs close to the body, was considered during the inguinalDTt measurement. The inguinalDTt readings were obtained by inserting a DT probe deep in the left inguinal area, approaching from the cranial aspect, and aiming towards the dorsum. With regard to the udertailDTt measurement, the DT probe was introduced in between the ano-triangular surface and tail base, approaching from the lateral aspect, and aiming towards the cranial direction. For each measurement location, the DT placement at the site and temperature recording ranged from 15 to 65 seconds. Additionally, each anatomical location was measured twice and an average of the two (DTt) readings considered as a single datum.

## **Data analysis**

In the Statistical Package for Social Scientists (SPSS® IBM 26 version, USA), data were analyzed using descriptive statistics, including means and standard deviations (SD). The Shapiro-Wilk and Lavene's tests were used to check for normality and homogeneity of the data, respectively. For inferential analysis, selected statistical tests were conducted. A One-way analysis of variance (ANOVA test) between subjects was conducted using Generalized Linear Model, a univariate analysis procedure, to determine the main effect of location factor. The following statistical model was considered;

# $Y_{ij} = \mu + \delta_i + e_{ij}$

Where,  $Y_{ij}$  is the dependent variable denoting the trait measured (DTt reading),  $\mu$  represents the overall mean,  $\delta_i$  signifies the fixed effect of the *i*<sup>th</sup> location (*i* = rectal, undertail, inguinal, and axillary location), and  $e_{ij}$  is the random error term. Tukey test was used to determine the pairs whose means differed. The correlation between the different temperature measurements was determined using a Pearson's correlation test. A one-sample t-test was employed to establish the level of bias between the reference and alternative measurements (locations), while a Bland-Altman analysis was employed to compare selected DTt measurement methods. In all cases, significance was taken at a level of p < 0.05.

## RESULTS

## The mean temperature at different anatomical locations

The results of ANOVA that was conducted to determine the effect of location factor on the temperature readings are presented in this subsection. The Lavene's test showed equality of the groups' variance (F(3,60) = 1.212, p > 0.05). The main effect of location factor on temperature readings (DTt) was statistically significant (F(3,60) = 7.241, p > 0.05,  $\eta_p^2 = 0.266$ ). The observed effective size ( $\eta_p^2$ ) indicated that 26.6% of the variance in the DTt was explained by the location factor. The mean DTt, including rectalDTt, undertailDTt, inguinalDTt, and axillaryDTt, that was measured at the rectal, inguinal, axillary, and undertail locations, respectively, are presented in Table 1. The mean inguinalDTt was the highest (39.51 ± 0.31°C), while the lowest mean value was undertailDTt (38.97 ± 0.45°C). There was no significant difference between measurements at the rectal (rectalDTt) and inguinal (inguinalDTt) area (p > 0.05), or axillary location (p > 0.05). The mean rectalDTt was significantly higher than that of the undertailDTt measurement (p < 0.05).

## The correlation between temperature readings at different anatomical locations

The results from a correlation analysis of the various DTt readings, including rectalDTt, undertailDTt, inguinalDTt, and axillaryDTt, are shown below (Table 2). Considering rectalDTt as the standard measurement, the correlation between rectalDTt and axillaryDTt readings was the stronged (r = 0.928, p < 0.05), while the lowest was observed with the undetailDTt (r = 0.782, p < 0.05). When 'stardard method factor' is not considered, the correlation between inguinalDTt and axillaryDTt readings (r = 0.943, p < 0.05) and that of the undetailDTt and inguinalDTt (r = 0.582, p < 0.05) was the strongest and weakest, respectively.

#### Reliability and comparison of the DTt measurements (methods) in sheep

The results of a reliability analysis that quantitatively analyzed for the potential significance in the mean of differences (bias) between paired DTt readings or measurements, viz. rectalDTt measurement (standard method) with each of the alternative anatomical locations (methods), are presented below (Table 3). The rectalDTt-undertailDTt pair had the largest mean of differences (bias)  $(0.5\pm0.28^{\circ}C)$ , while the rectalDTt-inguinalDTt pair had the lowest bias (-0.03\pm0.15^{\circ}C). The equivalence analysis revealed a significant bias between rectalDTt and undertailDTt measurements (p < 0.05), as well as rectalDTt and axillaryDTt (p < 0.05). The bias between rectalDTt and inguinalDTt measurements was not significantly different (p > 0.05); moreover, the data for the difference values met the normality assumption. The relationship between rectalDTt and inguinalDTt measurements or methods is shown in Figure 1. From the plot, most of the data points are close to the zero line, a similar distribution of the points is observed around the bias (mean of difference) line. Most of the data points are within the agreement limits and not significantly different (p > 0.05, with a 95% confidence interval).

# Table 1 - The mean temperature readings measured at the different anatomical locations

Variable	DTt readings			
Measurement site/method	Mean±SD (°C)	Minimum (°C)	Maximum (°C)	
Rectal	39.47 ± 0.35ª	38.85	40.04	
Undertail	38.97 ± 0.45 <sup>b</sup>	38.10	39.60	
Inguinal	<b>39.51 ± 0.31</b> ª	39.05	40.03	
Axillary	39.34 ± 0.34 <sup>a</sup>	38.80	40.25	
Total	39.32±0.41	-	-	

<sup>a,b</sup> Different letters (superscript) within the same column denote a significant difference (p < 0.05), SD: standard deviation, °C: degrees Celsius, DTt: temperature reading by a digital thermometer

Table 2 - Correlation between DTt readings obtained from the different anatomical locations

	rectalDTt	undertallDTt	InguinalDTt	axillaryDTt
rectal	1			
undertailDTt	0.782*	1		
inguinal DTt	0.898*	0.582*	1	
axillary	0.928*	0.640*	0.943*	1

DTt: temperature readings by a digital thermometer, correlation coefficient 0.00-0.10: negligible, 0.10-0.39: weak, 0.4-0.69: moderate, 0.7-0.89: strong, 0.9-1.0: very strong correlation, \*significant correlation at p < 0.05

Table 3 - Shows results of equivalence analysis for the different DTt measurement pairs

Paired sites/methods	DTt measurements (difference)						
		df	t valuo	n valuo	95%	95% Cl	
	Weanitop ( C)	u	t-value	p-value	Lower	Upper	
Rectal - undertail	0.5±0.28	15	7.214	<0.05	0.35	0.65	
Rectal - inguinal	-0.03±0.15	15	-0.8	>0.05	-0.11	0.05	
Rectal - axillary	-0.13±0.13	15	4.134	<0.05	0.07	0.20	
DTt: temperature readings by digital thermometer, CI: confidence interval, SD: standard deviation, df: degrees of freedom, °C: degrees Celsius, <: less than, >: greater than							



Figure 1 - A Bland-Altman plot showing the rectal-inguinal temperature relationship

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# DISCUSSION

The measurement of body core temperature is among the means employed to monitor the health and reproductive status of animals, including sheep (George et al., 2014; Godyń et al., 2019). The notion behind this is that when the measured temperature falls outside the range of temperatures covered by the normal circadian rhythm (Weinert and Waterhouse, 2007), it is regarded as an abnormal body temperature and thus signals changes in the animal status. For this reason, its monitoring provides an early signal regarding the status of the animal in terms of morbidity, thermal stress, synchronization and estrus status, calving onset, and other factors that may impact on rhythmicity of normal body temperature (Fischer-Tenhagen and Arlt, 2020; Kearton et al., 2020). However, many of the temperature sensors fall short of the needed basic qualities, viz. non-invasive, rapid, safer, easy to use, and robust to external variations. Rectal thermometers, including the clinical digital thermometer, are generally robust to external variations and acceptably accurate. Moreover, rectal thermometry, a standard method for the body temperature measurement, relies on the use of these clinical thermometers. Since rectal thermometry is associated with many drawbacks (Abecia et al., 2015; Yadav et al., 2017), the current study has discovered an alternative location on sheep at which temperature measurement may be conducted using a digital thermometer. This measurement location is less invasive and allows manipulation with ease.

This study revealed that mean temperature at the inguinal location, as well as axillary temperature, was similar to rectal temperature. The observed mean temperature values, particularly at the inguinal and indeed rectal locations, were consistent with the earlier reported rectal temperature  $(39.48\pm0.09^{\circ}C)$  (Katsoulos et al., 2016). Moreover, the mean temperature values for both inguinal and rectal measurements fell within the established body temperature ranges for a normal or healthy sheep  $(39.0-39.75^{\circ}C)$  (Stockler et al., 2021). The consistency between the observed mean inguinal temperature and the reference normal body temperature for sheep points to the potential utility of inguinal thermometry. Currently, rectal temperature is mostly used to estimate the body core temperature of animals, including sheep (Katsoulos et al., 2016). However, the temperature acquired by this method can be affected by digestion, peristaltic movements, fecal masses, muscle tone, and physical activity (Abecia et al., 2015). In terms of the numerical comparisons, the inguinal temperature was generally closer to the body core temperature compared to its rectal counterpart. It is plausible that the closely apposed thigh and abdominal wall (inguinal) was responsible for the aforementioned, since this anatomical predisposition may have minimized heat loss to the environment. It is noteworthy that variations in temperature readings, numerical and or statistical, were observed at different locations. This was consistent with the previous study reports for many animals species, including sheep (Kearton et al., 2020), cattle (George et al., 2014), and chickens (Abigaba and Sianangama, 2023).

The strong correlation observed between rectal and inguinal temperature was consistent with the previous findings in sheep (Katsoulos et al., 2016) and chickens (Abigaba and Sianangama, 2023), but disagreed with those of cattle (unpublished data). Moreover, the mean of difference between the rectal-inguinal temperature pair was not statistically significant (p > 0.05). This points to the reliability of inguinal temperature measurement (method) for body core temperature estimation in sheep. With regard to the rectal-axillary temperature pair, the observed bias was significant despite the similarity in their means and a strong correlation. The current correlation results are consistent with the previous study findings in a related species, which reported a strong correlation between the rectal and axillary temperature (r=0.95, p < 0.01) (Chaturvedi et al., 2004). Discussing about deductions from the foregoing, it can be stated that all odds favour the inguinal location for temperature measurement in sheep compared to axillary location. However, further studies utilizing larger sample sizes, under a more controlled environment, may be needed to validate the current findings.

It should be mentioned that correlation coefficients reveal a relationship between variables or methods but do not determine their agreement (Doğan, 2018). The observed significant bias from the zero-point limit, in the case of rectalaxillary temperature pair, supports this notion. For this reason, the Bland-Altman analysis was conducted for the rectalinguinal temperature pair only, since their mean of differences did not show a significant bias. The results indicated a good level of correlation and or agreement because most of the observed data points were close to the bias and zero lines, respectively, although the observed limits were considerably outside the previously suggested difference of  $\pm$  0.2°C (Fulbrook, 1993). The current findings are similar with those of the previous study in a related species (Abigaba and Sianangama, 2023), although the limits of agreement was generally higher than the case of an earlier study. It is plausible that this disparity in the limits of agreement was attributed to the smaller sample size, species studied, and environmental conditions under the current study. Moreover, this study did not factor in the contested lustiness of rectal thermometry to external variations (Abecia et al., 2015). Hence future studies intended to validate the current findings must factor in these issues. Additionally, a consideration of the potential hyperthermia or fevers that are attributed to localized causes, for example lactation and mastitis (Stockler et al., 2021) will also be crucial. A proper positioning of the thermometer probe at the inguinal location is also suggested to minimize the environmental effects on temperature readings. These may confound the actual estimates of the body core temperature.

## CONCLUSION

Body temperature monitoring contributes to the early detection and management of febrile conditions and changes in the physiological state of animals, including sheep. In an effort to search for a suitable thermometry method, it was observed

that variations in temperature readings existed among the different anatomical locations under study. However, it was revealed that the rectal and inguinal temperature measurements in sheep had similar means, strong correlation, with a non-significant bias between them, particularly when a digital thermometer is employed. Additionally, the observed higher numerical value of the inguinal temperature could suggest a better reflection of the body core temperature than the case is with the standard rectal thermometry. Further studies are needed to validate the current findings, particularly on the inguinal location, for generalization and future application.

## DECLARATIONS

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#### Authors' contribution

R. Abigaba conceived and designed study, collected and analyzed data, and wrote the manuscript, Ph. C. Sianangama designed, supervised study, and reviewed the manuscript, O. Chibinga Supervised study and reviewed manuscript, N. Gulaita designed study and reviewed manuscript, M. C. Sitali supervised study and reviewed manuscript, E.S. Mwaanga conceived and supervised study and reviewed manuscript. All authors read and approved the final manuscript for publication.

#### Availability of data and materials

The additional data from the present study may be provided on request from the corresponding authors

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This study did not receive any funding.

## **Ethical consideration**

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues including consent to publish, misconduct, fabrication of data, and redundancy have been checked by the authors.

## **Conflict of interests**

The authors declare no conflict of interest regarding this publication

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