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Influence of body weight and serum electrolyte status on sheep electrocardiography

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Abstract

Considering the importance of sheep, economically and in research, it is necessary to explain what normal sheep electrocardiogram values are, and the factors that can cause variations in ECG parameters. The present study, therefore, sought to verify the differences that exist in ECG time scaling between different sub-groupings of sheep. To achieve this, 60 normal sheep were evaluated for the ECG. We reported that at normal ranges of electrolytes and ECG intervals/durations, with mean weights: Ouda = 41.6 ± 7.2 kg (27.8 – 54.0 kg), Balami = 33.2 ± 6.4 kg (22.2 – 43.5 kg); the ewes showed significantly long timing of ECG waves than the rams ($P < 0.001$), the lambs lower than the adult sheep ($P < 0.001$), the Ouda beyond the Balami breed ($P < 0.001$) and the semi-intensive sheep more than the intensively managed ($P < 0.001$). However, when the sub-groups were evaluated for the variations in the PR interval and QTc, with every increase in a unit of the weight, calcium or potassium ions, no discernable difference was noticed between the two sexes. In this, the lambs showed a better slope of these relationships compared to the adult sheep (PR interval: β- coefficient = 0.0010 – 0.0086 v -0.0001-0.0005 and QTc: 0.0006 – 0.0215 v -0.0001 – 0.0143). The Ouda correlated better than the Balami breed, (PR interval: β- coefficient =0.0062 – 0.2527 v -0.0007 – 0.0033 and QTc: 0.0008 – 0.0264 v 0.0001 – 0.0064). And the semi-intensively managed sheep had an enhanced relationship than sheep managed intensively (PR interval: β- coefficient =0.0002 – 0.0123 v 0.0001 – 0,0071 and QTc: 0.0006 – 0.0197 v 0.0001 – 0.0108). We, therefore, conclude from this result, that within the normal serum electrolytes range, both body weight and habitual physical activity may influence atrial and ventricular depolarization and repolarization timing, with the physical activity level playing an overall dominant role.

Keywords: ECG, Ouda, Balami, Ram, Ewe, Lamb, Serum electrolytes, Physical Activity, Management system

Introduction

A healthy sheep is a recipe for good growth, and this will translate into quality meat, hide and skin, as well as the dairy product of the sheep (Banos *et al*., 2019; Thorne *et al*., 2021). Nevertheless, the breed of the sheep and the systems under which they are reared also play a vital role in their health, growth as well as

productivity. The cardiovascular system of the sheep is central to its health (Chalmeh & Karamifar, 2021). And one of the non-invasive, most reliable, easily accessible and user-friendly procedures for assessing sheep's cardiac health is electrocardiography (ECG) (Chalmeh & Karamifar, 2021). The ECG evaluates the electrical activities of the heart, which is the basis of the mechanical activities of the heart that drives its function (Chalmeh & Karamifar, 2021; Saka, 2021). Electrocardiography in a large animal such as sheep has become more popular in scientific research, especially since the properties of their heart is similar to what is obtained in humans (Bischoff, 2019; Dubois *et al*., 2017; Kviesulaitis *et al*., 2018; Tsang *et al*., 2020). In this regard, ECG records may be used to evaluate the body's physiological processes in sheep and can be extrapolated to similar functions in humans (Kviesulaitis *et al*., 2018).

Although quite a few ECG studies have been conducted on sheep previously (Kviesulaitis *et al*., 2018; Mirabad & Rezakhani, 2019; Sudhakara & Sivajothi, 2018), the earlier studies mainly focused on the patterns of ECG, comparison between different sex, as well as age groups (Koether *et al*., 2016; Kviesulaitis *et al*., 2018; Mirabad & Rezakhani, 2019; Pradhan *et al*., 2017; Sudhakara & Sivajothi, 2018). However, the factors that determine the differences or similarities in the ECG of different phenotypes of sheep have not been evaluated in the earlier studies. Importantly, both body weight and habitual physical activity level have been reported to influence ECG waves morphology in human subjects (Storlund *et al*.,2021; Sudhakara & Sivajothi, 2018). In this regard, body mass increase has been reported to cause increase in ECG wave voltage and also thought to scale-up the timing of the electrical activities, hence increase durations (Meijler & Meijler, 2011; Storlund *et al*., 2021). Moreover, serum electrolytes levels are well-recognized to affect the ECG voltage, as well as the durations, at least in human subjects (Chen *et al*., 2018; Kim *et al*., 2018; Ma *et al*., 2019; Yazdanpanah *et al*., 2021). However, how all these interplays to determine the ultimate waveform has not been evaluated in sheep ECG. Further, which of the factors between body weight and habitual physical activity levels, at varying serum electrolyte levels and different sheep weights, influence ECG morphology more than the others, have not been clearly explained in the literature.

In the present study, intensively and semi-intensively managed Ouda and Balami sheep, in Sokoto, Nigeria were evaluated. The animals comprised both sex young and adult. Earlier comparison protocols that the differences in the ECG indices are related to the weight and level of activity of the sheep. The two factors were further evaluated to determine dominant one when both were present.

Materials and Methods

Ethical permission and study population

This study was authorized by the Faculty Animal Research Ethics Committee (FAREC), Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto with reference number UDUS/FAREC/AUP-R16/2019. A total of 60 sheep, sourced randomly from Sokoto state, Nigeria were evaluated in the study. The sheep population comprises four groups of Balami (n=30) and Ouda (n=30) sheep. Each breed consists of 10 lambs (≤ 6 months); 10 non-pregnant adult female sheep (1 – 2 years) and 10 adult male sheep (1-2 years old). In addition, 50% (n=30) of the sheep studied in this research were from a privately owned farm, where the animals are managed intensively and are fed groundnut hay, bean husk and concentrates with mineral supplementation (salt lick), while water was provided *ad libitum*. While the remaining 50% (n=30), were from another farm, where they were managed semi-intensively in open-shed barns with limited access to water and shade. These animals go out to graze in the morning and are fed hay in the evening.

Body weight and physical parameters

Vital parameters were measured according to the methods of Nielsen *et al*., 2021 and the sheep were subsequently weighed using a mechanical weighing scale (HYAN®).

Electrocardiography

The ECG recording for each animal was taken using the standard limb leads (I, II, III) and the augmented limb leads (aVR, aVL, aVF), using a Veterinary electrocardiograph (Edan VE-1010 Veterinary PC ECG®, China) according to the methods of Rezakhani & Edjtehadi (1980). The sheep were physically but minimally restrained without sedation in a standing position on a rubber mat to minimize electrical interference. The device was interfaced with a computer that recorded normal ECG at a paper speed of 50 mm/s and sensitivity of 10 mm/mV for one minute. The machine carried out the automated analysis of the results of each sheep, then saved and later retrieved for data analysis.

Biochemical analysis

Serum for chemistry was obtained from whole blood by centrifugation, at 3000 RPM for 10 minutes. The supernatant (sera) was then carefully decanted into a

sterile vial and was preserved in a refrigerator at −20°C until the period of analysis. Thereafter, well-verified procedures were followed to estimate the serum electrolyte levels, including Na^+, K^+, Cl^-, HCO^{3-} and Ca^{++} (Estridge & Reynolds, 2011).

Statistical analysis

Microsoft Excel 2016, was used for the analysis. Values were presented as mean ± SD, proportions or percentages. Tables, graphs and charts were also used to display the statistical information. Student t-test was used to find the difference in the means of two groups, according to the Microsoft excel protocols.

The slope of the relationship (beta-coefficient) between the dependent and the independent variables as well as the corresponding coefficient of determination (R^2), which represents the percentage of the data series that agrees with the straight line of the graph was also determined, following the Microsoft excel procedures. The slope was hinged on the assumption that a linear relationship between the independent weight and electrolyte levels, with the dependent diastolic electrical activities of the heart, the PR interval and the systolic electrical activities corrected for the heart rate, (the QTc). This was represented as an equation of the linear regression, $y = ax + c$. Where 'a' is the beta-coefficient or slope of the relation that measures the change in y, Δy with every unit change in x, Δx. And c is the value of y at x = 0. The P-values for the data in the present study were pegged at ≤0.050.

Results

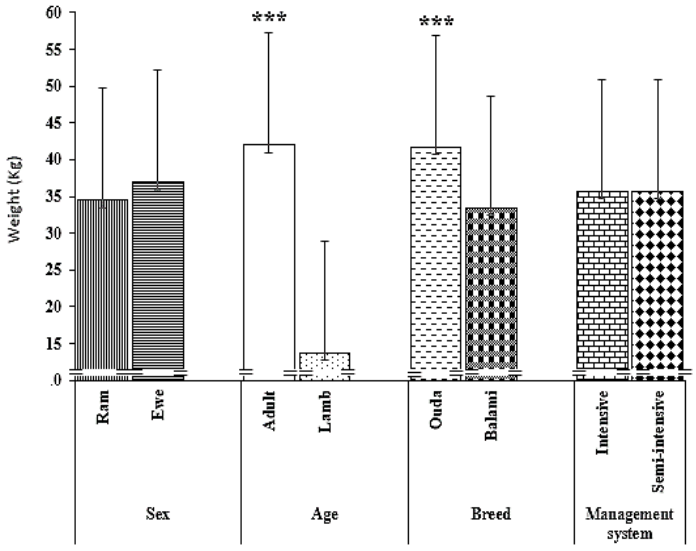

Figure 1: The weights of sheep compared along sex, age, breed and management lines
P<0.000 (***)

In Figure 1, it was shown that the ewe weighs more than the ram, but not significantly (36.9 ± 15.1 kg v 34.5 ± 15.2 kg, p≥ 0.05 respectively). However, when the adult sheep and lamb were compared (Figure 1), there was a statistically significant difference in their weights (42.0 ± 10.5 kg v 13.8 ±2.2 kg, p≤ 0.05 respectively). Similarly, comparing the weight of the Balami breed and the Ouda revealed that the Ouda weighs significantly more (41.7 ± 12.7 kg v 33.5 ± 13.8 kg, p≤ 0.05). However, the study revealed that whether sheep is managed intensively or semi-intensively, it does not result in any significant weight differences. This can be seen in the same Figure 1 as, 35.7 ± 12.7 kg v 35.7 ± 19.4 kg, p≥ 0.05

The following results were obtained at normal ranges of electrolytes and ECG intervals/durations. In brief, mean weights: Ouda = 41.6 ± 7.2 kg (27.8 – 54.0 kg), Balami = 33.2 ± 6.4 kg (22.2 – 43.5 kg); mean K^+: Ouda = 4.7 ± 0.8 mMol/L (3.6 – 6.4 mMol/L), Balami = 4.6 ± 1.0 mMol/L (3.0 – 6.1); mean Ca^{++}: Ouda = 1.8 ± 0.1 mMol/L (1.6 – 2.0 mMol/L), Balami = 1.3 ± 0.1 mMol/L (1.1 – 1.5 mMol/L); mean PR intervals: Ouda = 0.10 ± 0.02s (0.09 – 0.10 s), Balami = 0.06 ± 0.00 s (0.05 – 0.06 s), and mean QTc durations: Ouda = 0.31 ± 0.03 s (0.27 – 0.37 s), Balami = 0.22 ± 0.02 s (0.18 – 0.26 s).In Figure 2, the ECG parameters of the sheep were compared based on sex, as ram and ewe. The results revealed that the ewe, being higher in terms of weight has significantly higher ECG parameters (p≤ 0.05). However, the difference was not significant in the P wave (0.068 ±0.007 v 0.063 ±0.008, p≥0.05), even though the ewe still showed higher P wave duration. But, the QRS duration and QTc were significantly higher in the ram. A comparison between lamb and adult sheep is shown in Figure 3. The adult sheep demonstrated significantly higher values in only the P wave. Although the adult sheep also showed higher PR waves, it was not significant (p≥0.05). However, the lamb demonstrated significantly higher values compared to the adult sheep in terms of QRS, QT and the corrected QT (QTc).

When the sheep were compared based on their breed into Ouda v Balami, in Figure 4, the results obtained showed that the Ouda had significantly higher values in all the ECG indices evaluated in the study.

Figure 5 is the result of the comparison between the ECG parameters of the sheep, based on the management system under which they were reared. The results showed that semi-intensively reared sheep had predominantly higher values of the parameters. In this regard, the semi-intensively managed sheep demonstrated higher

values in the P, PR, QT as well as QTc parameters. Only in the QRS duration did the intensively managed sheep show higher values, with statistical significance.

Table 1 in the present study displays the changes in some of the ECG parameters that occurred with a unit change in the weight, potassium and calcium ions, represented by the slope of the relationship (β-coefficient), between these independent variables and the dependent PR and QTc. In this regard, the ewe showed significantly higher chances of changes in the ECG parameters with either a unit change of the weight or the electrolyte values, compared to the ram. And this was supported by the higher values of the coefficient of determination of the relationships (R^2). The only exceptions to these findings are the effects of the weight on QTc, where the ram sub-group was noticed to have shown more regression compared to the ewe. When the linear regression analysis was carried out between the dependent and independent variable for the sheep, based on age in Table 2, the lamb consistently showed higher tendencies to changes in the ECG parameters for every unit change in the weight or electrolytes, beyond what was recorded for the adult rams. The only exception in the relationship was with calcium ion to QTc, where the beta-coefficient was significantly higher for the adult sheep.

In Table 3, the Ouda sheep consistently showed more appreciable linear regression, as well as the coefficient of determination, R^2 beyond their Balami counterpart. when their weight and electrolytes were evaluated against the atrial electrical activities (PR) and ventricular electrical activities (QTc). Further, the Balami breed in addition showed negative relations of this evaluation, with reference to the potassium ion.

In Table 4, the slope of the relationship between weight and electrolytes with the ECG indices was assessed. The stratification, however, was based on the management system by which the sheep were reared. Most of the regressions obtained for the semi-intensively managed sheep were negative, they were more appreciable beyond that obtained for the intensively managed sheep. This was also the finding for the coefficient of determination, R^2 of the slopes.

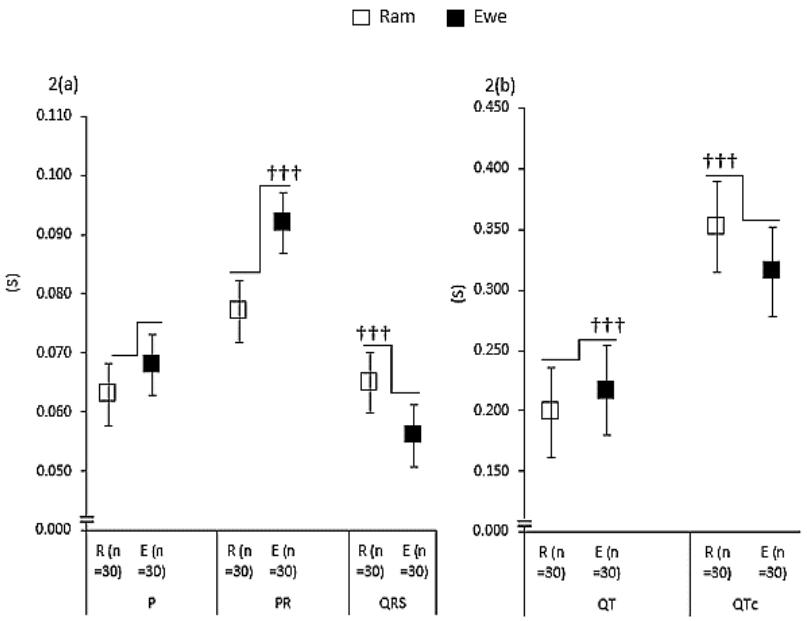

Figure 2: Comparison of the ECG parameters of ram and ewe
Key: ram (R), ewe (E)
P < 0.000 (†††)

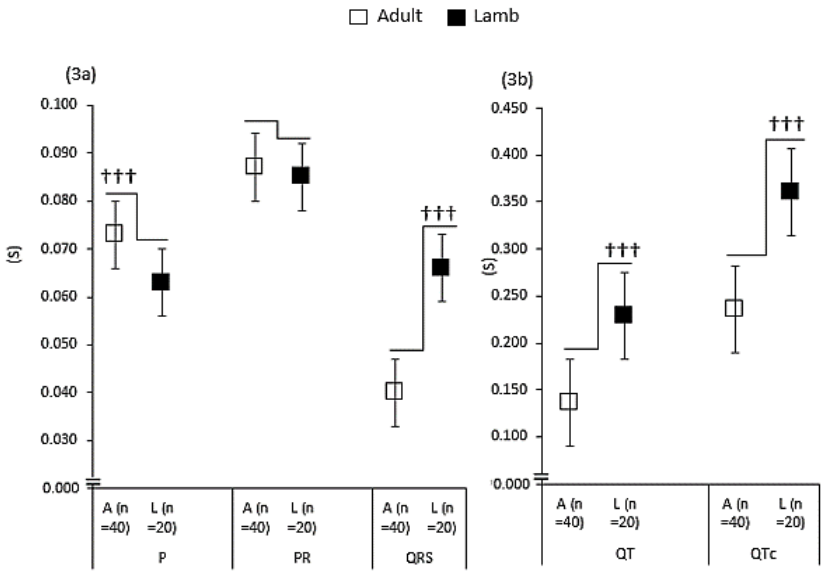

Figure 3: Comparison of the ECG parameters between adult and lamb
Key: adult (A), lamb (L)
P < 0.000 (†††)

Discussion

The present study showed that in sheep ECG, the predominant wave morphologies were positive as reported in other animal species. Higher body weight and physical activity levels may bear a corresponding increase in the intervals of ECG parameters. In this study, it is reported that Ouda being heavier in weight

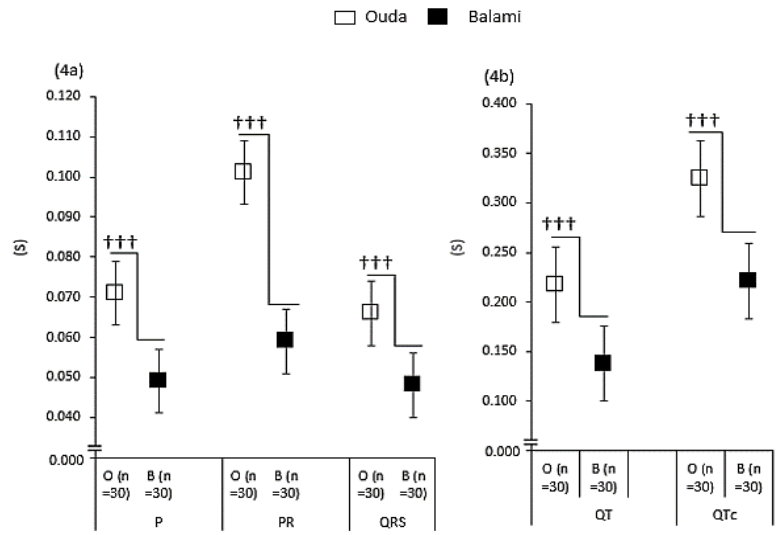

Figure 4: ECG parameters between Ouda and Balami breeds
Key: ouda (O), balami (B)
P < 0.000 (†††)

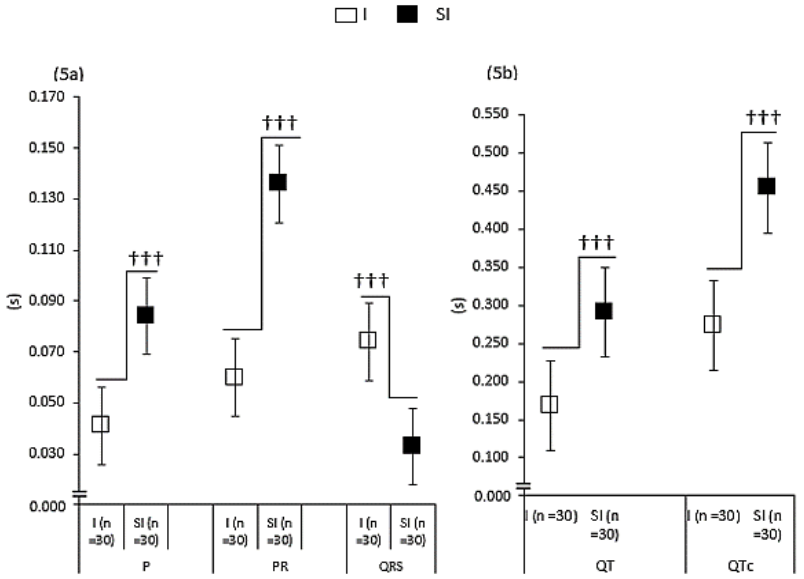

Figure 5: ECG parameters between sheep reared under intensive system against those reared semi-intensively
Key: intensive (I), semi-intensive (SI)
P < 0.000 (†††)

than the Balami breed (41.7 ±12.7Kg v 33.5 ±13.8Kg), demonstrated higher parameters than the latter. Similarly, the ewe weighs more than the ram, and their ECG parameters were higher, just as the adult sheep had compared to the lamb. It was also reported that the physical activity level of the semi-intensively reared sheep, which may be comparatively higher than the intensively managed one, showed longer intervals of ECG waves. These findings were on background normal ranges of the serum electrolytes level (K^+, Ca^{++}), an important factor known to influence ECG parameters. The EUCr of the two sheep management systems did not follow any predictable pattern that was concurrent with their body size. The mean weight of the ewes was insignificantly higher than that of the rams, the rams demonstrated more time scaling of the PR and QT intervals than the ewes. The PR and QTc intervals are the more clinically important parameters of ECG and have been reported to be scaled up with higher body mass (Meijler & Meijler, 2011). The rams are more active physically than the ewes. This may explain the higher ECG parameter intervals in the rams compared with the ewes. It has been found by Azizi *et al*., (2020) and Sokunbi *et al*., (2021) that physical activity is a well-recognized factor that can result in increased timing of ECG waves in human subjects.
Moreover, activity levels have an

Table 1: Slopes of the relationships between the weight and some electrolytes with the ECG indices of the ram compared with ewe

	ECG parameter	β-coefficient	R^2		ECG parameter	β-coefficient	R^2
Weight: Ram	v PR Ram	0.0002	0.0043	*Weight: Ewe*	v PR Ewe	0.0002	0.0199
	v QTc Ram	0.0014	0.0483		v QTc Ewe	-0.0003	0.0064
K^+: Ram	v PR Ram	0.0006	0.0021	*K^+: Ewe*	v PR Ewe	0.0007	0.0066
	v QTc Ram	0.0103	0.1374		v QTc Ewe	0.0010	0.0029
Ca^{++}: Ram	v PR Ram	0.0010	0.0006	*Ca^{++}: Ewe*	v PR Ewe	0.0053	0.0370
	v QTc Ram	0.0268	0.0860		v QTc Ewe	-0.0299	0.2590

Table 2: Slopes of the relationships between the weight and some electrolytes with the ECG indices of the adult sheep compared with lambs of the study population

	ECG parameter	β- coefficient	R^2		ECG parameter	β- coefficient	R^2
Weight: Adult	v PR Adult	-0.0001	0.0048	Weight: Lamb	v PR Lamb	0.0015	0.1121
	v QTc Adult	-0.0001	0.0013		v QTc Lamb	-0.0006	0.0052
K^+: Adult	v PR Adult	0.0005	0.0014	K^+: Lamb	v PR Lamb	0.0010	0.0023
	v QTc Adult	-0.0009	0.0021		v QTc Lamb	0.0215	0.2870
Ca^{++}: Adult	v PR Adult	-0.0004	0.0001	Ca^{++}: Lamb	v PR Lamb	0.9986	0.0232
	v QTc Adult	0.0143	0.0415		v QTc Lamb	-0.0106	0.0094

Table 3: Slopes of the relationships between the weight and some electrolytes with the ECG indices of the Ouda breed in comparison with the Balami breed of the study population

	ECG parameter	β- coefficient	R^2		ECG parameter	β- coefficient	R^2
Weight: Ouda	v PR Ouda	0.0062	0.0880	Weight: Balami	v PR Balami	0.0001	0.0324
	v QTc Ouda	-0.0008	0.0466		v QTc Balami	0.0001	0.0011
K^+: Ouda	v PR Ouda	0.0277	0.0167	K^+: Balami	v PR Balami	-0.0007	0.0228
	v QTc Ouda	0.0060	0.0248		v QTc Balami	-0.0064	0.0685
Ca^{++}: Ouda	v PR Ouda	0.2527	0.0412	Ca^{++}: Balami	v PR Balami	0.0033	0.0074
	v QTc Ouda	0.0264	0.0144		v QTc Balami	0.0030	0.0002

Table 4: Slopes of the relationships between the weight and some electrolytes with the ECG indices of the Sheep managed intensively compared with that managed semi-intensively

	ECG parameter	β- coefficient	R^2		ECG parameter	β- coefficient	R^2
Weight: Intensive	v PR Intensive	0.0001	0.0071	Weight: S - intensive	v PR S-intensive	-0.0002	0.0068
	v QTc Intensive	0.0001	0.0006		v QTc S-intensive	-0.0006	0.0309
K^+: Intensive	v PR Intensive	0.0001	0.0001	K^+: S-intensive	v PR S-intensive	-0.0030	0.0353
	v QTc Intensive	0.0001	0.0001		v QTc S-intensive	0.0043	0.0438
Ca^{++}: Intensive	v PR Intensive	0.0123	0.1879	Ca^{++}: S-intensive	v PR S-intensive	0.0071	0.0122
	v QTc Intensive	0.0108	O.0344		v QTc S-intensive	-0.0197	0.0566

Key: Semi-intensive (S-intensive)

implication on the geometry of the ventricles, especially the left and indeed the functions of the heart (Azizi *et al*., 2020; Sokunbi *et al*., 2021). However, the study by Koether *et al*., (2016) and Mirabad & Rezakhani (2019) did not find any difference in ECG wave timing or other parameters between rams and ewes. Although, the study by Koether *et al*., (2016) is punctuated by being conducted just among lambs. Furthermore, the robust intergenerational Framingham study (Kornej *et al*., 2022), did not find evidence of an association between physical activity level and ECG traits. But the Framingham study was purely a human study, hence may not be correct to draw a straightaway extrapolation on sheep ECG. In contrast, the QT interval in females was found to be longer than in males in the study by Rabkin (2015).

The preceding data that compared the weights of the adult sheep to that of the lamb in the present study, showed a significant weight in favour of the former (42.0 ±10.5Kg v 13.8 ±2.2Kg), the lamb generally showed better tendencies to changes in the ECG parameters with every change in the weight or serum Ca^{++} or K^+. Again, this may not be unrelated to the comparatively more active nature of the lamb, beyond the level of activity seen in the adult sheep. The lamb is more likely to wander around compared to the adult sheep of the same or others breeds

(Soulsbury & Halsey, 2018). Furthermore, the study by Pradhan *et al*., (2017), which was conducted among black Bengal goats of varying ages showed a significant difference in ECG parameters. Likewise, the study by Mirabad & Rezakhani (2019) revealed conspicuous higher ECG parameters, when young and older sheep were compared. This finding is, however, contrary to the report by Mohapatra *et al*., (2015), where no differences were observed in the QT interval when older and younger Ganjam sheep were compared. However, this contrasting finding of the study by Mohapatra *et al*., (2015), may be due to the fact that they used only one breed of sheep and that the sample size was just twelve sheep. Therefore, the study by Ma *et al*., (2019), corroborated our data in this study that there is QTc prolongation with ageing in human subjects.

The Ouda breed simply showed better correlations, between the independent variables (weight and electrolytes) and the ECG parameters, beyond the Balami breed, in the present study. Though, the Ouda breed are significantly heavier in weight, compared to the Balami , which has a morphometry that entails less physical activity compared to the Ouda in the present study (Fayeye & Adewale, 2017; Oke & Ogbonnaya, 2011). Indeed, both genetic and environmental factors interact at various levels to determine the level of growth in sheep and as well influence their activity levels (Assan, 2020; Lavvaf *et al*., 2007). In this regard, Ouda sheep are assumed to be more active than other breeds of sheep commonly found in the Sokoto environment (Oke & Ogbonnaya, 2011). Our result further confirms the work of Xing *et al*. (2009) and Ivonin *et al*., (2020), both of which suggest that genetic and environmental factors have a role in determining ECG phenotypes in sheep.

The semi-intensively reared sheep in the present study showed superiority in time scale of the ECG morphologies, when compared to the sheep managed intensively. This may not be unconnected to the effect of the management methods of both sub-groups. While the intensive method confined the sheep to a pen at all times, the semi-intensive management system allows the sheep to wonder around to search for feed the whole day, but in addition they are tethered or confined to a pen during the night, where they are then fed hay and other food supplements. Indeed, increased physical activities is a well-recognized factor for sheep muscle development, not only in the skeletal system, but the cardiac muscle morphometry and functions, hence the electrical activities of the heart (Drezner *et al*., 2017; Azizi *et al*., 2020; Sokunbi *et al*., 2021). And this translates to variation in timing of depolarization and repolarization of the cardiac electrical cycles (Drezner *et al*., 2017; Kviesulaitis *et al*., 2018).

The present study, although conducted in sheep, is in consonance with the report of Drezner *et al*., (2017), where the ECG of athletes were agreed upon to result in the scaling up the timing of ECG waves durations. This however, negate the Framingham studies of Kornej *et al*. (2022) and Mutikainen *et al*. (2009) where no significant effect of physical activities on ECG was seen, but in a rather human study, not ECG of sheep. Furthermore, the study reported more on ECG voltage, not on the timing of the morphologies. And our findings run contrary to the one by Michishita *et al*. (2015), where longer QTc were recorded with increased inactivity and a sedentary lifestyle.

Although the sample size in the present study was small, we ensured that each sub-group evaluated in the study was fair. The study was also a cross-sectional descriptive type, instead of a longitudinal one, which makes inferential statements difficult. However, the sampling method was a random one and data collection followed very meticulous techniques, to obviate both biasness and observer errors (Storlund *et al*., 2021).

The habitual physical activity levels of the sheep were assumed to be what was anecdotally known of sheep generally based on their gender, age and management system, not objectively measured either by distance covered or odometry. However, we considered in addition to the foregoing, the heart rate of the sub-groups, to adjudge them as comparatively more active.

In conclusion, the result of this research showed that both body weight and the habitual physical activity level of sheep may have an upward influence on ECG durations which was correlational. This may stem from the known fact that habitual physical activities result in not only the development of the skeletal muscles, but in remodeling the ventricular geometries. However, when two groups of sheep are being compared, and one group has higher weight against the other, which on the other hand has higher activities level than the former, the activities level may dominate the ECG wave timing phenotype obtained.

We therefore, recommend that a more sound study, with massive sample size and possibly prospective in nature, as well as more statistically empowered, that will evaluate in further details which of the two factors (body weight or activities levels) influences better, the morphology of ECG waves in healthy sheep.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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