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Detection of subclinical mastitis in camels (*Camelus dromedarius)* using somatic cell count, N-acetyl-β-D-glucosaminidase and lactate dehydrogenase activity

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ABSTRACT

Clinical and subclinical mastitis (SCM), mostly related to intramammary infection (IMI), is prevalent in pastoralist camel herds. An IMI has implications for public and animal health as well as for household economy. As bacterial culturing is expensive, time consuming and impractical in a pastoralist setting, other early detection methods for SCM in camels need to be investigated. Somatic cell count (SCC) is the standard for detecting SCM in cattle. The udder health indicators of N-acetyl-β-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH) activity are useful as diagnostic markers in cow, sheep and goat milk; they could be of potential use in camel milk production. The aim of this study was to improve the understanding of SCM in camels, and specifically to assess SCC, and NAGase- and LDH activity in camel milk. In addition, potential associations between SCM (defined by a California Mastitis Test (CMT) score ≥3 and no signs of clinical mastitis) and SCC, NAGase- and LDH activity were investigated.

In total, 40 healthy camels without clinical mastitis were sampled in four herds in Kenya. Quarter milk samples were collected aseptically and screened using CMT. SCC was analysed using a direct cell counter (DCC, DeLaval), and NAGase and LDH activity was analysed using kinetic fluorometric measures.

In total, 116 milk samples were tested with CMT and analysed for SCC. Of these, 88 were analysed further for NAGase and LDH. The median SCC was 151,000 cells/mL (IQR: 49,500–709,000 cells/mL), and median NAGase and LDH were 18.5 U/l (IQR:14.8–24.0 U/l) and 12.0 U/l (IQR: 8.5–16.2 U/l) respectively. All inflammatory markers (SCC, NAGase, LDH) were significantly associated with SCM ($P < 0.001$). In conclusion, SCC, NAGase and LDH are potential inflammatory indicators in camel milk that can be used for detection of udder quarters with SCM.

1. Introduction

Camel milk is a crucial source of nutrition for the inhabitants of the arid and semi-arid regions of the Horn of Africa. With regard to ongoing climate change, leading to increased droughts and inconsistent rainfall in the region, the camel milk sector is becoming increasingly important to food security as camels can survive and produce milk under harsh conditions with limited access to feed and water (Faye and Konuspayeva, 2012). The Horn of Africa has the highest density of one-humped camels (*Camelus dromedarius)* in the world (FAOSTAT, 2020); most of them are being managed by pastoralists who adhere to traditional husbandry practices (Hjort af Ornäs and Eng, 1993). Mastitis is a common and costly disease in dairy camels, significantly affecting milk yield, quality and hygiene, as well as household economy (Matofari et al., 2007; Saleh et al., 2013; Wahinya et al., 2014). Mastitis can be either clinical, with symptoms easily recognised by animal owners, or subclinical with a complete absence of clinical signs. Detection of subclinical mastitis thus warrants further diagnostic testing in order to

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identify affected camels and to prevent the disease from spreading within the herd. Mastitis is usually the consequence of an intramammary bacterial infection (IMI), and causes pathological changes in the mammary epithelium resulting in alterations to milk composition. These changes include an increase in the somatic cell content and leakage of cellular enzymes from damaged epithelial cells (Sandholm, 2008). As bacterial culturing is expensive, time-consuming, and most importantly, practically impossible to perform in pastoral settings, other indirect methods to determine the presence of IMI should be investigated.

Somatic cell count (SCC) is the standard method for detecting subclinical mastitis in cows. In camel milk, SCC has been investigated at quarter level (Abdurahman et al., 1995; Guliye et al., 2002; Saleh and Faye, 2011) with inconclusive results. In Europe, the legal hygienic standard for bovine milk is SCC of <400,000 cells/mL (EU, 2004) in bulk tank milk, and the threshold value for a healthy udder is estimated to be around 200,000 cells/mL (Schepers et al., 1997). Similar efforts to develop threshold levels have been made for goat (Contreras et al., 1996; Persson and Olofsson, 2011), sheep (González-Rodríguez et al., 1995) and buffalo milk (Moroni et al., 2006). For camel milk, however, there is a knowledge gap regarding SCC in healthy quarters and in quarters with subclinical mastitis (SCM). As SCC directly enumerates the number of somatic cells in milk, this method offers a more precise determination of the udder health status compared with California Mastitis Test (CMT), which is an indirect subjective assessment of somatic cells, commonly used in all dairy species. For camels, the sensitivity and specificity for correctly identifying quarters with IMI when using a CMT score of 3 [Scandinavian scale 1–5; (Klastrup and Madsen, 1974)] as a cut-off point has been shown to be 68.0–95.4 % and 30.4–92.0 % respectively (Abdurahman et al., 1995; Younan et al., 2001; Seligsohn et al., 2020). A comparison of paired opposite CMT-negative and CMT-positive udder quarters indicates that a high CMT score is associated with a decrease in camel milk yield of up to 44.7 % (Tinggren, 2019). Other potential indicators of mastitis, such as the inflammatory enzymes N-acetyl-β-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH), have been suggested for mastitis detection. The NAGase activity in camel milk was first determined by Abdurahman (1995), who found it to be a reliable indicator of mastitis. However, the usefulness of NAGase was challenged in later studies (Chaffer et al., 2000; Guliye et al., 2002). In goat and sheep milk, NAGase activity increases when infection is present (Leitner et al., 2004a; Maisi, 1990), and for bovine milk NAGase can be used as an indirect measure to identify quarters positive for intramammary infections (IMI) (Hovinen et al., 2016; Nyman et al., 2016). Similar relationships were explored for LDH in cow milk (Larsen, 2005; Nyman et al., 2016) as well as goat and sheep milk (Katsoulos et al., 2010). To the best of the authors' knowledge, LDH activity has not previously been studied in camel milk.

The aims of this study were, therefore, (1) to measure SCC and NAGase and LDH activity in milk from traditionally managed camels in Kenya and (2) to study the association between SCC, NAGase and LDH and SCM, measured as a CMT score of ≥3, in order to assess their potential use as biomarkers for detection of SCM.

2. Materials and methods

2.1. Study area

The study was conducted in Laikipia County, Kenya. Laikipia is classified as semi-arid, with an average annual rainfall of 639 mm and great biodiversity (Georgiadis et al., 2007). The total camel population is 2,300 and this number is steadily growing due to the increasing frequency of drought (Musinga et al., 2008). Sampling took place during the dry season in January - February 2018.

2.2. Sampling

Four camel herds (A–D) were selected by applying a convenience sampling strategy with the inclusion criteria that the herds were kept extensively and were being milked. Geographical accessibility and cooperation of the owners were additional important factors in the choice of herds. Management data and sample sizes are presented in Table 1. Sampling was conducted in two ranch herds, i.e. stationary herds belonging to private land owners with employed workers tending to their animals, and in two commercial pastoralist herds, defined as traditional mobile herds producing milk predominantly for sale to urban consumers. The final sample size was based on financial constraints and time limitations. In each herd all lactating camels were selected for sampling if they were clinically healthy with no signs of swelling, redness or lesions on the udder, and producing apparently normal milk on visual inspection. Herds were visited once for collection of milk samples.

2.3. Sample collection and analysis

Sampling was carried out during the morning milking. Milk let-down was stimulated by the suckling calf. The CMT was used to assess subclinical mastitis at udder quarter level (Schalm and Noorlander, 1957) and CMT scoring was carried out by the same observer in all herds. After the first streaks of milk were discarded, approximately 3−4 mL of milk was directed into the CMT paddle and mixed with an equal amount of CMT fluid. Grading of the viscosity of the solution was done according to the Scandinavian scoring system (1–5), where a score of 1 represents a completely liquid state and a score of 5 represents the highest viscosity with a distinct peak formation of the gel. Subclinical mastitis was defined as a test score of ≥3 and the absence of clinical signs of mastitis. For herd D, the CMT was not performed immediately; instead, milk was collected in 10 mL plastic vials and placed in a cooler. The milk samples were then transported back to the research facility where CMT was performed by the same investigator as for herd A–C, approximately 2 h after collection according to the guidelines of the National Mastitis Council (Adkins et al., 2017). Delaying the CMT procedure have no significant impact on CMT scores (Tucker and Paape, 1966; Malinowski et al., 2008). In camels with no positive CMT reaction (CMT 1) in any of the udder quarters, milk samples were collected from all quarters. For camels with a CMT score ≥2 in one quarter, a milk sample was collected from the CMT-positive quarter and from the paired opposite CMT-negative quarter. In herd A, all CMT-positive quarters had matching opposite CMT-negative quarters. In herds B, C and D, not all CMT-positive quarters had a matching opposite CMT negative quarter, however, only CMT-positive quarters with a matching opposite CMT-negative quarter were included in the analysis.

Milk samples were collected at udder quarter level in plastic test tubes with colourless bronopol added as preservative for subsequent cell

Table 1
Demographic data for four camel herds visited for collection of milk samples in Laikipia county, Kenya, during the dry season in January-February 2018.

Herd ID	Management System	Milking herd size[1]	Number of sampled camels	Milking frequency	Watering frequency
A	Ranch	25	7	Once a day	Every second day
B	Commercial pastoralists	50	18	Once a day	Every second day
C	Commercial pastoralists	20	6	Once a day	Once a week
D	Ranch	40	9	Once a day	Every second day

[1]Total herd size, was regarded as confidential by the owners and the figures presented are an estimate based on observations by the field team.

counting and analysis of enzyme activity. The samples were cooled and transported to the Mpala Research Centre (Mpala Research Centre, Nanyuki, Kenya) where analyses for SCC (cells/mL) were performed on the day of sampling using a portable direct cell counter (DCC, DeLaval International AB, Tumba, Sweden; (Berry and Broughan, 2007; Gonzalo et al., 2004)). After performing the SCC analysis, 28 samples were excluded from further analyses due to insufficient milk volumes. The remaining 88 milk samples were kept frozen at −20 °C. At the end of the study period, the frozen samples were transported on dry ice to the Department of Animal Science at Aarhus University in Denmark. Kinetic fluorometric measurements were used to analyse enzyme activity. Analysis of NAGase activity (U/L) was performed according to Larsen et al. (2010) and LDH activity (U/L) according to Larsen (2005).

2.4. Statistical analysis

Descriptive statistics to illustrate the distribution of SCM, SCC, NAGase and LDH were performed using Stata (Release 13.1; College Station, TX, USA: StataCorp LP). Associations between SCM (yes/no; independant variable) and SCC, NAGase and LDH (dependent variables) were investigated using univariable linear mixed-effect regression analysis. Herd and camel were included as random effects, taking into account that camels within a herd are more similar than camels between herds, and that quarters within camels are more similar than quarters of different camels, using an independent covariance structure. Before analysis, continuous independent variables (SCC, NAGase and LDH) were assessed by visual examination to see if they were linearly related to the outcome, using the lowess command in Stata. SCC, NAGase and LDH were not normally distributed and were transformed using the lnskew0 command in Stata (which is applied to obtain zero skewness using the natural logarithm) to obtain normal distribution. In addition, univariable analyses of associations between the inflammatory markers (two models for each marker with the other markers as independent variables in respective analysis) were performed using a univariable linear mixed-effect regression analysis. Scatterplots and the Pearson product-moment correlation coefficient were used to assess the correlation between the inflammatory markers.

3. Results

3.1. Study herds

In total, 40 camels were sampled: seven (7/25; 28 %) from herd A, 18 (18/50; 36 %) from herd B, six (6/20; 30 %) from herd C and nine (9/40; 23 %) from herd D.

Data for parity and length of lactation were recorded for all sampled camels, except for two individuals. The median and mean parity was 3.0 (IQR: 2–4) and 3.2 (SD:1.7), respectively, and the median and mean stage of lactation was 7.0 months (IQR:3−8 months) and 6.0 months (SD: 2.5 months), respectively. The majority of the camels were of pure Somali breed; but some were of the Pakistani-Somali and Turkana-Somali cross-breeds.

3.2. Subclinical mastitis, somatic cell count and inflammatory markers

The number of milk samples used for analysis was limited due to difficulties in obtaining sufficient volumes of milk for all analyses to be performed. In total, 158 udder quarters were investigated using CMT. Milk samples were collected from 116 of these quarters. The remaining 42 quarters did not meet the inclusion criteria for sampling and were excluded from further analysis. The median CMT score for the 116 quarters included in the study was 1 (IQR:1−3). In total, 26 of the 40 camels (65 %) had at least one quarter with an indication of SCM (CMT ≥ 3) and there was at least one camel in each herd with SCM. At quarter level, 36 out of 116 (31 %) tested quarters had SCM.

The SCC was analysed in all milk samples; the median SCC was 151,000 cells/mL (IQR: 49,500–709,000 cells/mL).

The NAGase and LDH activity was analysed in 88 of the milk samples from 34 camels. The median and mean NAGase activity was 18.5 U/L (IQR:14.8–24.0 U/L) and 23.5 U/L (SD:18.6 U/L), respectively. The median and mean LDH activity was 12.0 U/L (IQR: 8.5–16.2 U/L) and 15.6 U/L (SD: 14.0 U/L), respectively. Distribution of SCC, NAGase and LDH for udder quarters that were positive/negative for subclinical mastitis are shown in Table 2.

3.3. Associations between SCM and SCC, NAGase and LDH

The univariable linear mixed-effect regression analysis showed a significant association between SCM and lnSCC ($P < 0.001$), SCM and lnNAGase ($P < 0.001$) and SCM and lnLDH ($P < 0.001$), revealing increasing levels of SCC, NAGase and LDH activity in milk samples from udder quarters with SCM.

The results of the statistical analysis investigating the relationship between SCM and the inflammatory markers are presented in Table 3. The correlation coefficients for the relationship between lnSCC and lnNAGase, lnSCC and lnLDH and lnNAGase and lnLDH were 0.54; 0.66 and 0.46 respectively and are visualised using scatter plots (Fig. 1**a-c**).

4. Discussion

This study investigated SCM and the associations between milk SCC, NAGase and LDH in clinically healthy dromedary camels.

No bacteriological analysis of the milk samples was performed in the present study due to practical constraints, but CMT was used to detect IMI (Seligsohn et al., 2020). The sensitivity and specificity of CMT for correctly identifying quarters with IMI in camels were previously shown to be 82 % and 92 %, respectively, when using a CMT score of 3 as a cut-off point and thus CMT results were used as an approximation of IMI status in the present study. The overall mean and median SCC at quarter level for quarters negative for SCM were found to be in accordance with previous results for camel milk. Saleh and Faye (2011) determined the mean SCC in non-infected quarters to be 125,000 cells/mL and Guliye et al. (2002) found a mean SCC in infected and non-infected quarters of 414,954 cells/mL and 215,774 cells/mL respectively. Aljumaah et al. (2020) suggested a cut-off point for healthy quarters at 472,500 cells/mL. This is comparable to established SCC values for dairy cows, where a SCC of <100,000 cells/mL is considered normal whereas a SCC of > 200,000 cells/mL is classified as pathological (Bradley and Green, 2005). In contrast, lower overall levels of SCC were found compared with values reported for goat (Persson and Olofsson, 2011) and buffalo milk (Patil et al., 2015). Nevertheless, there was a wide range of values for SCC, which was not normally distributed, as illustrated by the discrepancies between median and mean SCC. This is similar to the situation in other dairy species (Paape et al., 2007). Although we did not obtain data on the last time of watering prior to sampling, this is not likely to have influenced the SCC results. While camel milk osmolality varies in relation to water intake, milk lactose content does not; camels maintain their milk volume during the first week of water deprivation (Bekele et al., 2011).

The NAGase activity in the present study was found to be lower than previously reported for camel milk (Abdurahman, 1995; Guliye et al., 2002), but similar to values reported for sheep and goat milk from both healthy and infected udder halves (Leitner et al., 2004a, 2004b) and higher than for cow milk (Hovinen et al., 2016; Nyman et al., 2016). The main sources of NAGase content in cow milk are damaged mammary epithelial cells and inflammatory cells (Kitchen et al., 1978). Camel milk contains a large proportion of anucleated cytoplasmic particles (Abdurahman et al., 1992). Abdurahman (1995) suggested that these cell fragments might contribute to the higher levels of NAGase activity seen in healthy camel milk in comparison with levels reported for bovine milk.

The measurements of LDH activity in camel milk revealed

Table 2
Distribution of the median (Interquartile range (IQR)) and mean (standard deviation (SD)) values of the somatic cell count (SCC, n = 116 quarters from 40 camels), N-acetyl-β-D-glucosaminidase (NAGase, n = 88 quarters from 34 camels) and lactate dehydrogenase (LDH, n = 88 quarters from 34 camels) for quarters positive or negative for subclinical mastitis defined as a CMT-score ≥3 and no clinical symptoms in quarter milk samples from camels in four herds in Laikipia County, Kenya, (2018).

	SCC (×1000 cells/mL)			NAGase (U/L)			LDH (U/L)		
	n	Median (IQR)	Mean (SD)	n	Median (IQR)	Mean (SD)	n	Median (IQR)	Mean (SD)
Subclinical mastitis									
Yes	36	1,035 (449–2,949)	1,888.3 (1,801)	23	21.7 (17.4–39.2)	35.1 (31.3)	23	21.2 (13.3–30.4)	27.6 (22.0)
No	80	99 (27.5–186)	282.7 (782)	65	17.4 (14.1–21.9)	19.4 (8.2)	65	9.6 (7.5–13.5)	11.4 (6.0)

Table 3
Univariable linear mixed-effect regression analysis of associations between SCM and somatic cell count (lnSCC, n = 116 quarters from 40 camels), N-acetyl-β-D-glucosaminidase (lnNAGase, n = 88 quarters from 34 camels) and lactate dehydrogenase (lnLDH, n = 88 quarters from 34 camels) in quarter milk samples from camels in four camel herds in Laikipia County, Kenya (2018).

SCM	Negative	Positive
SCC, ×1,000 cells/mL[1]	100.1	857.9
95 % CI (LSM)	47.3 – 211.7	379.6 – 1,938.7
P-value[3]	Ref.[2]	<0.001
NAGase, U/L[1]	8.8	16.8
95 % CI (LSM)	6.9 – 11.2	12.1 – 23.3
P-value	Ref.[2]	<0.001
LDH, U/L[1]	5.7	16.5
95 % CI (LSM)	4.4 – 7.2	11.7 – 23.2
P-value	Ref.[2]	<0.001

[1] Representing least square means (LSM) of lnSCC, lnNAGase or lnLDH transformed back from the natural logarithm.
[2] Reference level.
[3] *P*-value from the univariable mixed-effect linear regression analysis.

considerably lower levels than previously described for ovine and bubaline milk from both healthy udders and udders with SCM (Batavani et al., 2003; Guha et al., 2012; Katsoulos et al., 2010) but higher than the levels in bovine and caprine milk (Nyman et al., 2014; Persson et al., 2014; Stuhr et al., 2013). Lactate dehydrogenase is a non-lysosymal cytoplasmic enzyme; increased levels of activity in bovine milk have been demonstrated to be the result of cellular necrosis or damage to cell membranes (Bogin et al., 1977). The present results showed that increased LDH activity was associated with SCM in camels which is in line with studies on goat (Stuhr et al., 2013) and cow milk (Babaei et al., 2007; Chagunda et al., 2006). In bovine milk, LDH activity is a sensitive mastitis biomarker and increased levels can be seen in early onset mastitis (Symons and Wright, 1974). Although the present observations were based on limited study material and should be interpreted with caution, it is believed that the usefulness of LDH as a frontline mastitis indicator and its physiological variations in dairy camels should be further explored using wider study material and in parallel with bacterial culturing, including longitudinal data, to evaluate individual differences at camel level.

Subclinical mastitis is common among dairy animals worldwide, causing economic losses due to both pre-harvest and post-harvest losses (Kashongwe et al., 2017.; Nielsen et al., 2010). In cows, the most common cause of SCM is an IMI commonly due to bacteria invading the udder tissue (Nyman et al., 2014; Sandholm, 2008). The relationship between SCM and IMI has also been investigated in camels (Seligsohn et al., 2020). The prevalence of SCM (31 %) in the present study, as defined as a CMT-score ≥3 and no clinical symptoms of the udder, corresponded well with previous findings under similar conditions. In a cross-sectional study in Ethiopia, Regassa et al. (2013) found a quarter prevalence of SCM of 22.3 %, using the CMT score trace as a cut-off point, and in a cross-sectional study of pastoralist camels in Kenya, the quarter prevalence was 26 %, using CMT 3 as a cut-off value (Seligsohn et al., 2020).

In the present study, an association was found between SCC and NAGase activity and a similar relationship was identified between SCC and LDH activity, as well as between NAGase and LDH activity. When evaluating the diagnostic test performance of a variety of inflammatory markers for the detection of IMI-positive quarters in dairy cows, Nyman et al. (2016) found that SCC was more reliable than both NAGase and LDH. Obara and Komatsu (1984) found NAGase activity to be a better indicator of the severity of inflammation in cow milk than a reliable predictor of SCC.

As the sample population was limited to 40 camels distributed across four herds, the results should not be interpreted as being representative for any larger camel population. Nonetheless, the results contribute to a more comprehensive evidence-based understanding of SCM in camels. To evaluate the practical use of these inflammatory markers and determine a cut-off point for healthy and unhealthy udders in dairy camels, bacterial culturing should be carried out in parallel with measurements of inflammatory markers in milk. Subclinical mastitis has been shown to reduce milk yield and result in milk post-harvest losses in pastoralist camel herds (Kashongwe et al., 2017), with negative implications for household income. The vulnerable economic situation of most pastoralist camel dairy herds further emphasises the urgent need to maximise the gain and avoid losses in milk production. Early detection of the frequent subclinical mastitis cases observed in this and other studies in the area could initiate swifter action, which would reduce disease progression and transmission and result in improved herd udder health. Given that SCC offers a more precise determination of udder health status than CMT and can be undertaken with an easy-to-use portable device, this inflammatory marker could be of considerable benefit in improving camel udder health.

5. Conclusions

The results of this study indicate that SCC, NAGase and LDH are significant inflammatory markers in camel milk similar to those of other dairy species. Specifically, SCC and LDH showed a strong association with SCM and could be valuable as biomarkers. In a setting with poor economic resources and limited access to laboratory analyses, CMT remains a reliable and affordable camel-side tool for monitoring SCM. However, as CMT is subjective and indirect, SCC is a more precise way of determining the udder health status of individual camels as well as of the herd. The findings of SCM-positive camels in all the examined herds underlines the importance of herd monitoring. Regular scanning of camel herds to identify affected individuals is a cornerstone for improving herd udder health.

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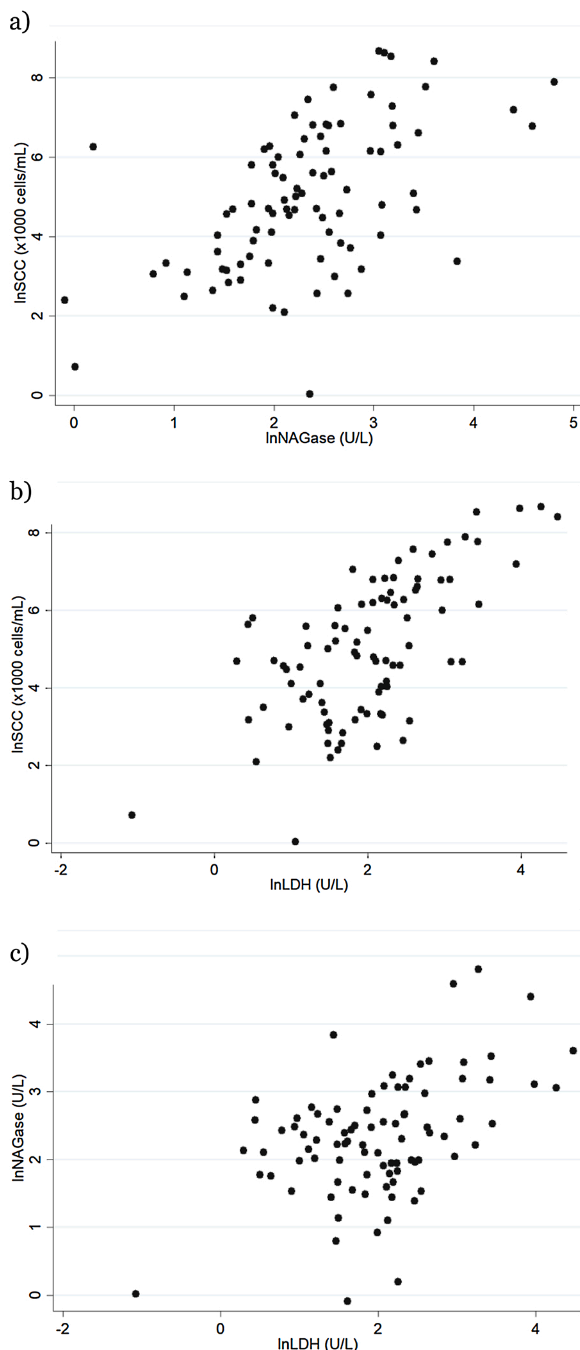

Fig. 1. a-c. Scatterplots visualising the relationship between the inflammatory markers (logarithmically transformed using the natural logarithm) somatic cell count (lnSCC, n = 116 quarters from 40 camels), N-acetyl-β-D-glucosaminidase (lnNAGase, n = 88 quarters from 34 camels) and lactate dehydrogenase (lnLDH, n = 88 quarters from 34 camels) measured in quarter milk from camels in four herds in Laikipia County, Kenya (2018).

were not involved in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication

Ethical statement

This study was approved by the National Commission for Science, Technology and Innovation, Nairobi, Kenya (Permit number: NACOSTI/P/19/84,995/13,088). Camel owners included in the study gave their oral permission to participate.

Declaration of Competing Interest

The authors declare no competing interests.

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