protein determination (as the sum of whey protein and casein) in comparison to the direct measurement on the basis of a true protein calibration. The advantage of independent calibrations for each protein fraction is the flexibility in application and quality control over time: each calibration can be independently designed, improved, controlled, corrected and applied according to the specific needs of the analyst or the requests of the customer, or in accordance with regulatory agreements between the milk producers and the dairy industry. The present study also implies that reliable estimates of whey protein contents of milk can be calculated as the difference of the infrared results of true protein and casein with no loss of accuracy when these calibrations are under statistical control and maintained correctly.

Acknowledgements
The authors wish to thank the numerous farmers and cheese-makers for their conscientious support in the collection of the milk samples used in the study.

5. References
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The effect of heat treatment, pasteurization and different storage temperatures on insulin concentrations in camel milk

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The effect of heat treatment, pasteurization and different storage temperatures on the insulin concentration of 19 dromedary milk samples was tested. Insulin concentrations between the milk samples obtained from individual camels varied widely, and the overall mean insulin value of fresh camel milk taken 3 times within one month was 41.9 ± 7.38 μU/ml (mean ± SEM). There was a significant difference in insulin concentration between the 3 milk samples collected on three different occasions within a one month period. Pasteurization, freeze drying or storage of camel milk at 4°C for 4 days as well as freezing at -20°C resulted in a statistically significant reduction in insulin concentrations; however, this was minimal. Contrary to other researchers, our study demonstrated that the mean insulin concentration in camel milk does not significantly exceed the values found in bovine milk. However, the fact that camel milk, in comparison to bovine milk, does not coagulate in acid environment most probably is the main factor for its therapeutic effect on insulin-dependent diabetes mellitus patients.

Wirkung der Wärmebehandlung, Pasteurisierung und unterschiedlicher Lagerungstemperaturen auf die Insulininkonzentrationen von Kamelmilch

Es wurde die Wirkung einer Wärmebehandlung (Kochen), der Pasteurisierung und unterschiedlicher Lagerungstemperaturen auf die Insulinkonzentration von 19 Dromedarmilch-Proben untersucht. Die Insulinkonzentrationen der einzeln Kamelen unterschieden sich sehr, und der Gesamtinsulinwert der innerhalb eines Monats dreimal genommenen frischen Kamelmilch lag bei 41.9 ± 7.38 μU/ml (Durchschnitt ± SEM). Ein signifikanter Unterschied der Insulinkonzentration bestand zwischen den 3 Milchproben, die innerhalb eines Monats zu unterschiedlichen Zeiten gekocht, getrocknet oder bei 4°C gelagert wurden. Allerdings war die Pasteurisierung, die noch geringere Insulinkonzentration führte, als die Lagerung bei -20°C. Dennoch lag die mittlere Insulinkonzentration in Kamelmilch unterhalb der in Rindermilch. Die Fähigkeit der Kamelmilch, in saurem Milieu nicht zu klumpen, ist wohl der Hauptgrund für ihr therapeutisches Effekt bei insulinabhängiger Diabetes mellitus.
1. Introduction

Camel milk components differ considerably from those of ruminants, and possess greater similarities to human milk (1). Camel milk is very important for human survival in many developing countries. In Somalia, for example, approximately 5 million dromedaries are almost exclusively kept for their milk. Over the last years camel milk is also experiencing a novel awareness in the western world after it has been consumed for centuries by nomadic people for its nutritional and medicinal properties. Medicinal properties in camel milk were reported by several researchers (2), and according to Agarwal et al. (3), it contains high levels of insulin and can therefore be used to treat Diabetes mellitus type I.

This paper shows the effect of heat treatment, pasteurization and different storage temperatures on insulin concentrations in camel milk.

2. Research design and methods

Milk samples were aseptically collected from 19 individual dromedaries on 3 occasions within a one month period and tested for insulin concentration by radioimmunoassay (RIA). The camels originated from two different herds. The first 11 camels (age range 8–15 years) belonged to a small camel dairy unit stationed at CVRL, and were in different stages of lactation. They all received the same feed consisting of pelleted concentrate (4 kg/d), fresh alfalfa (4 kg/d) and hay ad libitum. The 2nd group of dromedaries consisted of 8 animals which roamed freely in the desert and after milking in the evening were fed with good quality hay ad libitum and alfalfa. This group was also in different stages of lactation. The 19 dromedaries were milked by hand and the milk pooled from all 4 teats into sterile plastic containers. The first set of camel milk samples were separated into 3 aliquots, each of which was either untreated, pasteurized (72°C for 5 min) or boiled (98°C for 5 min) prior to analysis. Ten days later milk samples were again collected from the same dromedaries and separated into 2 aliquots, each of which was either stored for 24 and 96 h at 4°C or 24 h at –20°C prior to analysis. Additionally, the same samples were also tested for insulin content following freeze drying. Again 20 d later 18 milk samples were collected and tested raw. Camel 2 ceased to give milk. Camel milk insulin concentrations were measured by radioimmunoassay (RIA) using a commercially available human insulin kit (INS-Imra, KIP 1251 - KIP 2154, Biosource Europe S.A., Belgium), following the company’s recommendations. Human serum was used as control. The kit has previously been used to test bovine milk insulin concentrations (4).

Statistical analysis was performed by analysis of variance with day of collection, treatment and animal as factors in the models.

3. Results

Nineteen dromedary milk samples were tested with the RIA fresh, pasteurized, boiled, freeze dried and after keeping them at different temperatures. The results are shown in Table 1 and Figures 1, 2 and 3.

![Fig. 1: Insulin content of fresh camel milk at different collection times (C13 excluded)](image1)

![Fig. 2: Effect of different treatments on insulin content of camel milk](image2)

There was a significant difference in insulin concentration between dromedaries (P<0.001, Table 1), and...
Table 1: Insulin content of 19 dromedaries in fresh raw milk, after treatment and after different storage temperatures (µU/ml)

<table>
<thead>
<tr>
<th>Camel</th>
<th>14/07/04</th>
<th>24/07/04</th>
<th>14/08/04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh raw</td>
<td>Pasteurized 72°C - 5 m</td>
<td>Boiled 98°C - 5 m</td>
</tr>
<tr>
<td>1</td>
<td>24.3</td>
<td>21.2</td>
<td>14.2</td>
</tr>
<tr>
<td>2</td>
<td>103</td>
<td>92</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>45</td>
<td>22.2</td>
</tr>
<tr>
<td>4</td>
<td>14.6</td>
<td>14</td>
<td>11.5</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>22.5</td>
<td>18.4</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>27.5</td>
<td>15.5</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>35.3</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>9.5</td>
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<td>6.2</td>
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<tr>
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<td>20</td>
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<td>10</td>
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<td>11</td>
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<tr>
<td>19</td>
<td>25</td>
<td>24.4</td>
<td>22</td>
</tr>
</tbody>
</table>

also between collection times (Fig. 1). The mean insulin concentration in fresh camel milk on three different days was 41.9 ± 7.38 µU/ml (mean ± SEM).

In comparison to untreated camel milk, the different storage conditions resulted in a minimal, yet significant reduction in milk insulin concentrations. The magnitude of the reduction was dependant on the particular conditions (Figs. 2 and 3).

![Effect of different treatments on Insulin content of camel milk II](image)

4. Conclusions
It has previously been shown that camel milk, as an adjunct to insulin therapy, can improve long-term glycaemic control, and can result in a significant reduction in the required parenterally administered insulin dose in type 1 diabetic patients for maintenance of glycaemic control (3, 5). Very little information relating to the concentration of insulin in camel milk can be found in the scientific literature. AGRAWAL et al. (3) mentioned that camel milk has a greater concentration than cow milk (52 µU/ml and 16.32 ± 5.98 µU/ml, respectively) and a similar concentration to human milk (60.23 ± 41.05 µU/ml) without citing any references. However, a limited number of milk analyses, using the RIA insulin tests, demonstrated fresh camel milk to have an insulin concentration of 32 µU/ml compared with a concentration of 23 µU/ml in cows milk (WIDDDEL, 2004 unpublished).

In animals there are large discrepancies in reported insulin concentrations. For example, reported concentrations in bovine colostrum vary from less than 1 to 50 µU/ml (6, 7, 8). Different milk constituents vary considerably depending on the stage of lactation (colostrums and mature milk), both in animals and humans (9). Although in humans and animals, the milk insulin concentrations depend on various factors, in bovines, pigs and humans it has been shown to be partly dependant on the stage of lactation. The concentration is highest around parturition and declines rapidly within 14 days to reach a steady level. This process is very rapid in cows, more gradual in women and most prolonged in sows (10).

In bovines, 327 µU/ml of insulin were found in the first milking, declining within the first 24 h postpartum to 50% of its initial value. Seven days following parturition it reached 46 µU/ml, 14% of its initial value. At this stage of lactation, it remained constant for several months (11). A similar pattern of declining insulin concentration was also detected in porcine milk with high concentrations (411 ± 214 µU/ml) detected in porcine Colostrums, decreasing to 26 ± 17 µU/ml (12) following 72 h of lactation. Similar changes in milk insulin levels during lactogenesis were also reported from humans. In women, however, the level falls more gradually until reaching a low and constant value (13). It is currently not known whether a similar stage of lactation-related pattern of milk insulin concentration is also present in camels.
Other factors which have an influence on reported milk insulin concentrations include breed, quantity of milk produced, diet and methodology of testing. The origin of milk insulin is not known but probably originates from the systemic circulation. Values are considerably greater in milk compared with serum (14). For example, Weström et al. (12) reported 25- to 100-fold greater insulin concentrations in porcine mammary secretions compared to serum of farrowing sows.

Our results also demonstrated both marked camel to camel and temporal variations with respect to milk insulin concentration. Taking all three different sampling days into consideration, camel 2 had the greatest milk insulin concentration (420 μU/ml) compared to camel 8, which had the lowest milk insulin concentration (9.5 μU/ml). None of the 19 dromedaries were in the early lactation stage; however, camel 2 was in the late lactation stage. The relatively high insulin concentration detected in the milk obtained from this camel may have reflected the reduced amount of milk produced at this stage of lactation, thus resulting in a relatively lower degree of dilution. This dromedary dried out 10 days after the second sample was collected. In light of the aforementioned variations in camel milk insulin concentration, consideration should therefore be given to the measurement of camel milk insulin prior to its use as an adjunct to the treatment of type 1 diabetes.

The mean value of insulin in fresh camel milk collected on three separate days was 41.9 ± 7.38 μU/ml (mean ± SEM) including camel 2 with the relatively high concentration. Removal of this camel from analyses as a statistical outlier, resulted in an overall mean camel milk insulin concentration of 33.8 ± 2.5 μU/ml, a concentration within the range of milk insulin in bovines. This finding is therefore in contrast to the findings of other studies which report a greater milk insulin concentration in camels compared with bovines (15).

It has been previously demonstrated that consumption of fresh camel milk can reduce blood sugar levels in type-1 diabetic patients. However, it is believed that this property is not only attributable to the insulin content of the camel milk, but also to the fact that camel milk does not coagulate in acid environments. This may therefore lead to a more rapid rate of passage through the stomach and into the small intestine where it is more readily available for absorption (5).

We demonstrated that different treatment and storage conditions resulted in reductions of different magnitudes in milk insulin concentration. Pasteurization and storage at 4°C for 24 h both resulted in a slight decrease (approx. 7%); more prolonged storage (96 h) at 4°C and freezing at −20°C resulted in a slightly greater reduction (app. 13%); whereas freeze-drying and boiling (5 min) both resulted in a more marked reduction (approx. 19 and 26%, respectively).

It should be highlighted that our study employed the use of a test kit for the detection of insulin in human serum or plasma. According to the manufacturer, strong cross reactivity with porcine and bovine insulin (100%) has been observed with this kit. It is obvious from the study that such cross reactivity also exists with camel insulin, a finding which is perhaps not surprising as the kit relies on monoclonal antibody recognition of a single human insulin epitope. It would appear that such an epitope may be partly, of fully conserved across certain species. Studies are currently underway to compare the structure of camel insulin with the human, bovine and porcine insulin.

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5. References