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## BRIEF COMMUNICATION: Development of ultrasound methodology to measure cow udder cistern storage capacity in the New Zealand pasture-fed context

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### Introduction

Studies have demonstrated that, for a given amount of secretory tissue, cows with a proportionally larger cistern produce more milk and are more resistant to the inhibitory effects on milk synthesis of longer milking intervals (Knight & Dewhurst 1994; Stelwagen & Knight 1997; Davis et al. 1998). This may be because they can store more milk before milk accumulation begins to inhibit further milk synthesis. Ayadi et al. (2003) demonstrated in a feedlot situation that cisternal volumes can be measured non-invasively using ultrasound technology. Dairy systems based on pasture, such as those in New Zealand, exhibit different challenges to the production of useful data using this technology compared to feed lot systems and the confounding effect of the variability in milking order and the subsequent variations of the intervals between subsequent milking (Molenaar et al. 2012).

The aim of this study was to develop a new technique to measure udder cistern characteristics using ultrasonic technology in the dairying-on-pasture environment and its initial application to identifying residual milk in the udder as cows leave the milking shed.

### Materials and methods

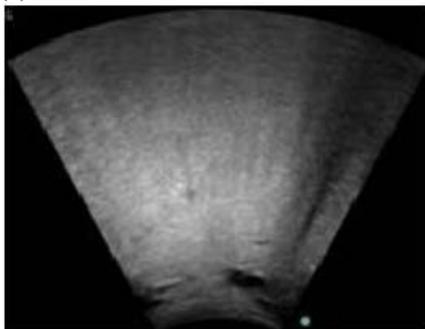
Ninety nine dairy cows were selected from a commercial research herd grazed at the AgResearch

Tokanui Dairy Research Farm. The selection comprised Friesian, Jersey and New Zealand crossbreed cows aged between two and seven years, with at least one season of milking, plus 30 randomly selected post-partum mixed breed heifers. The cows were grazed and milked with the farm herds except for when they were being scanned.

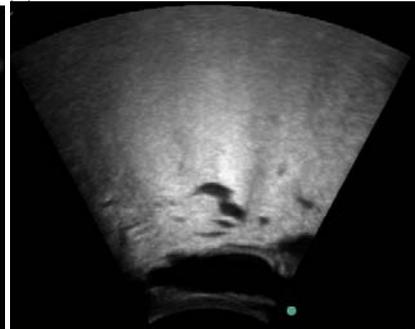
Ultrasonography was carried out in sets of 15 cows with similar days-in-milk, irrespective of their selection groups. Groups were milked eight to ten hours prior to being scanned (Ayadi et al. 2003) and taken to a holding paddock. In order to inhibit early milk let down, cows were walked from the holding paddock by a route such that the milking shed was not visible to the cows. They were held in a crush, injected intravenously in the epigastric vein with the 18-minute half-life oxytocin receptor blocker, Atosiban (Tractocile<sup>TM</sup> Pharmaco, Auckland, New Zealand). Most cows received 5 mg, while noticeably larger cows received 6 mg, administered without touching the udder, and walked to an adjacent milking shed. Unfortunately occasional and unavoidable sounds from the milking sheds such as backing gate alarms were audible. Milk leakage was observed in only five cows. Within 10 minutes of injection, the left and right back quarters of each cow's udder were scanned using an ultrasonic imager (Sonosite M-Turbo Ultrasound, Bothell, Washington, USA: 5MHz, 15 cm scan depth) with orthogonal vertical views captured beside the teat

**Figure 1** Ultrasound images of residual milk remaining in the udder after milking obtained using a Sonosite M-Turbo ultrasound scanner (5MHz, 15 cm scan depth) graded on the visual assessment score. Milk appears black in the image. (a) Score 1: One very small cistern containing less than 300 mL of residual milk, (b) Score 3: Some medium size cisterns containing 2.5 L of residual milk, (c) Score 5: Several large cisterns containing 5 L of residual milk.

(a) Score 1



(b) Score 3



(c) Score 5



for a natural size cisternal scan. The cows were milked to estimate the cisternal volume, milk samples obtained and 20 iu of Oxytocin (Ethical Agents, Auckland, New Zealand) administered intramuscularly in the rump to displace and override the Atosiban and promote milk let down. Following a ten-minute waiting period, the scanning and milking procedure was repeated to obtain the distended cistern scans, alveolar milk volume and alveolar sample. After the milking machine had automatically removed the cups at the nominal completion of milking with a flow rate of 0.2 L per minute, a further scan and milk volume were obtained of the residual volume.

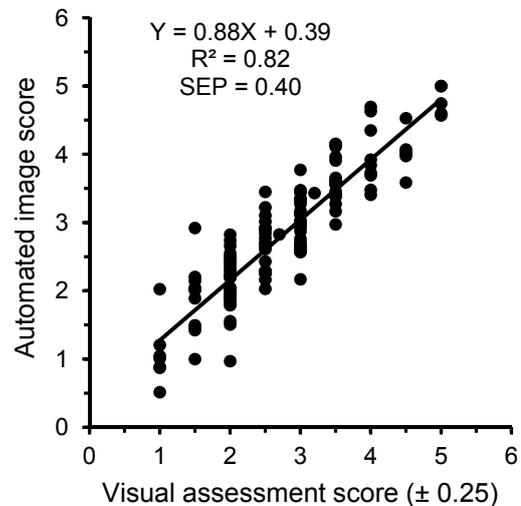
A representative set of 150 ultrasound images were visually scored on a 0 for no cisterns observed to 6 for numerous large cisterns observed (Figure 1). No Score 0 or Score 6 were observed in this study. The scores were used as the reference set for developing an automated image-scoring program in Matlab™ (Mathworks Inc, Natick, Massachusetts, USA). During automated image processing, each image was divided into four radial sectors, the pixels in each sector were given an “Is Cistern” score by a “top hat” threshold, and an overall image score was assigned based on the presence and relative area of cisterns detected in each sector. The automated scores were compared to the manual scores and then used to process 3,000 further images.

## Results and discussion

Automated image processing demonstrated a linear correlation to the reference manual scores (Figure 1) of  $Y = 0.88X + 0.39$ ,  $R^2 = 0.82$ , standard error of prediction = 0.40 (deviation of the predicted value from the observed value) with a reference data set standard deviation = 0.25. Thus 95% of the values in the data set were within twice the standard error of prediction of the line of best fit for the calibration data set. If 95% of the data for a calibration set are within twice the standard error of prediction the calibration may be considered robust. A further set of 3,000 images was processed with no processing failures. For a validation sub-set of 1,429 images, 96.4% of the automatic scores were within  $\pm 0.8$  (twice the standard error of prediction) of their manual scores. As >95% of the data in the calibration validation set are within twice the standard error of prediction then the calibration may be considered robust. These results suggest that the image processor is robust for the range of images processed to date. The processor-generated score was subsequently compared to the manual score in a random selection of images and found to be consistent.

Almost all cows in the trial left the milking platform with between 5% and 15% of their milk remaining in the udder (Figure 1). This residual milk can contribute to an early reduction in the rate of milk synthesis by reducing the total udder capacity for newly synthesised milk (Ouweltjes 1998) than would occur if the udder were completely emptied and

**Figure 2** Comparison of 150 visual assessment scores of ultrasound images of udder cisterns and automated image scores obtained by using Matlab™. SEP = Standard error of prediction.



therefore affecting both total milk production and lactation persistency. If this finding extends to the national herd, then farmers could obtain an increase in milk volumes with milking machines that removed this residual milk, such as by using square milking cup liners (M Prendergast, Personal communication) and by selecting cows that milk out completely.

In summary we have developed a system for obtaining and automatically scoring images of udder cisterns in pasture fed cows. We are now attempting to develop this methodology to a point where drug interventions are not required enabling an additional service to be offered to New Zealand dairy farmers so that they can use the information derived from ultrasonic images to assist in their herd-management decisions.

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