A SIMPLE, PRACTICAL METHOD FOR MEASUREMENT OF FAT IN MILK, APPLIED TO SAMPLES FROM MID- TO LATE-LACTATING WORKING ELEPHANTS IN A MATERNITY CAMP IN MYANMAR

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Introduction

During late pregnancy and throughout lactation, logging elephants owned by Myanmar Timber Enterprises are managed separately from the working herd in select maternity camps, affording training, observation, and research opportunities that target improved management, health, and calf survival (http://myanmar-timber-elephant.group.shef.ac.uk/). This project was undertaken as part of a larger investigation to examine relationships among nutrient composition of milk, forages consumed by the cows, and growth/health response of elephant calves. Although elephants have been trained for manual milk collection, potential challenges to standardized laboratory analysis for milk in Myanmar include sample size, transportation, limitations of reagents, equipment, and electricity, as well as applicability of methodologies developed for dairy livestock species to wildlife. Here we describe a modification of a rapid, economic method that has previously been used for estimating fat and energy content in livestock (Fleet and Linzell, 1964) and human (Lucas et al., 1978) milks, tested on elephant milk samples, for ultimate field application.

Methods

To test the methodology, fresh milk samples from local dairy cows in Myanmar, and purchased whipping cream were thoroughly mixed and ~75 µl aliquots (n=8) were drawn by capillary action into standard glass capillary tubes (75 X 15 mm outside diameter) that were sealed by clay. Tubes were centrifuged at 12,000 rpm (approximately 14,500 g) for 15 min using a hematocrit centrifuge (Clay Adams Autocrit model CT-2905, New York, NY USA), then immediately the fat layer(s) at the top were measured to the nearest 0.01 mm using digital calipers (Powerfix model Z22855, Paget Trading Ltd., London, UK), and expressed as a percentage of the total milk column, following the details in Lucas et al. (1978). Measurement of the solid fat layer meniscus was measured at the top, rather than bottom due to opacity. If clear, liquid fat was present at the top of the cream layer, that fraction was also included with the fat measurement. Measured values compared favorably with known crude fat content of dairy products in Myanmar (3.39 to 4.25% for milk; 56.67% for whipping cream).

Milk samples (5 to 20 ml) from 6 Asian elephant (*Elephas maximus*) cows were obtained on 6 separate dates between the wet season (Jul through Sep 2016; n=3) and dry season (Oct 2016 through Mar 2017; n=3) from mature cows in mid- (16 mo) to late lactation (38 mo) (see Table 1 for animal details). Aliquots were blended by inversion, and measured in duplicate to obtain "creamaotcrit" and liquid fat percentages as per the protocol described above. Percent solids, crude protein, ash, and vitamin E were also determined on the elephant milk samples using published methods (AOAC, 1990); values are reported elsewhere (Yadana, 2017).

Results

One sample was determined to be an outlier, and not used in further analyses. Centrifuged elephant milk samples separated into 3 distinct layers: the bottom aqueous fraction, presumably containing proteins, carbohydrates, and soluble minerals, a dense white fat layer, and a clear liquid lipid layer (Figure 1). Percentage lipid fractions in Myanmar elephant milk samples, using the simplified "creamatocrit" method, are found in Table 2 (15.35 mean ± 4.44 SD %). The liquid fat layer accounted for (78.88±12.52%, range 30.99 to 97.14 %) of the total fat, as opposed to the denser cream layer (21.11±12.52%; range 2.86 to 69.01%). The large liquid fat layer in elephant milk differs substantially from results using this method with human or bovine milk samples, where the liquid layer was non-existent or comprised a minor proportion (Lucas et al., 1978; this study). Total fat content ranged from 7.20 to 26.47% in milk samples over the 9 months of this sampling period. Significant effects of individual animal, associated with stage of lactation, were found (Table 3); cow age and/or parity effects were not statistically significant. Seasonality was also not significant in this dataset; the mean wet season fat values (15.57 ± 1.47%; n= 17 samples from 6 cows) were identical to values measured through the dry season (13.52 ± 3.29%, n=18 samples from 6 cows). However, if lag effects due to diet difference are considered, fat content of milk samples from the beginning of the dry season (end of wet season, October; 17.37 ± 2.05%) differed significantly from samples collected at the end of the dry season 12.77 ± 3.05%; $p < 0.01$ (Figure 2).

Discussion

Despite the use of standardized methodologies for proximate analysis of milk samples, high fat content of the elephant milk samples resulted in a non-quantifiable liquid fat residue regardless of time in the drying oven to determine water content/dry matter, hence proximate fractions quantified do not necessarily correspond with total solids measured. Due to limitations of sample sizes and in-country laboratory capabilities, we evaluated total fat content using this low-technology, practical method that could also be transferred to the field for immediate monitoring of milk quality, and potential nutritional interventions if necessary.

While overall mean total fat values (15.35± 4.44%) in our study average considerably higher than those reported by Mainka et al. of 7.6 ± 2.6% (1994; n=1 cow) during the first 9 mo of lactation, Simons (1959) and Peters et al. (1972) reported levels varying from 0.95 to 19.0% during the first 18 mo of lactation. More recently, Abbondanza et al. (2013) demonstrated distinct changes in milk fat content throughout lactation in the same individuals (n=3 cows), following the same pattern as reported for African elephants by McCullagh and Widdowson (1970). For captive-fed Asian elephants, milk samples taken at ≤ 9 mo of lactation contained < ~13% fat, whereas samples taken from 18 to 30 mo of lactation contained 15-20% fat. As the Myanmar samples were all from mid- to late-lactation cows, our data fit well with these described patterns and confirm high fat content in late lactation milks from Asian elephants. In general, the protein and fat contents of elephants' milk increase over lactation time.

Although stage of lactation impacted milk fat content in logging camp elephants, there was no significant effect of seasonality in this data set if one defines seasons strictly by calendar months. Nonetheless, milk fat decreased significantly from the beginning to the end of the dry season, with individual cows (4 of 6) displaying end of season fat values ~60 to 70% those seen 5 months earlier, likely due to nutritive changes in available vegetation (data not shown here), and/or hydration status of the animals. In the case of the latter, however, one might expect milk fat to increase or not change, since higher milk fat has been considered a physiologic mechanism for water conservation in elephants (Abbondanza et al., 2013). Consistent seasonal (environmental) effects and potential impacts on nursing calves need to be examined through continued long-term monitoring programs of the elephant herds.

McCullagh and Widdowson (1970) previously reported that elephant (African) milk lipid globules are half the size of bovine milk fats, with a unique fatty acid signature; fatty acid details of Asian elephant milk have not been published. Osthoff et al. (2007) confirmed a high content (~70%) of short-chain saturated fatty acids (FAs), particularly capric and lauric acids, low levels of polyunsaturated FAs, and omega-3: omega-6 FA ratios of approximately 1:1 in mid-lactation African elephant milk, with increasing degree of short chain FA as lactation progresses. Regulatory mechanisms for this process are not yet defined, nor have they been examined in Asian elephant milks. While smaller and shorter chain lipid molecules may be more liquid at room temperatures, the observation of widely ranging proportions of both liquid and solid fat fractions in the centrifuged elephant milk samples need to be investigated further. It is tempting to speculate that the highest proportion of liquid lipid fractions, found in samples from the 2 cows with the oldest calves, may represent this pattern of increased short chain FA in later lactation milks, but no clear pattern of variation (by individual, stage of lactation, month, or season) was discernable in this limited data set.

Most importantly, however, this study documents that reliable fat values for milk samples can be quantified rapidly and economically using this methodology. In the field, it can be very difficult to collect both the samples, as well as substantial quantities for analysis. By using the small volume necessary for this technique, we can quickly assess dam milk fat quality and calculate energy values, while minimizing removal of essential nutrients for the growing calf.

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Table 1. Description of Asian elephant cows (n=6) in a Myanmar maternity camp from which milk samples were obtained for nutritional analysis between 18 and 30 mo of lactation.

No.	**Name of elephant**	**Age (year)**	**Parity No.**	**Body Wt. (kg)**	**Date of Calf Birth**	**Sex of baby**
1	Than Bo Mi	40	2	2964	23.3.2015	Female
2	Swe Htay Oo	41	4	2358	2.1.2015	Female
3	Htoo Thin Kyi	35	2	2407	27.2.2015	Male
4	Thin Aye Moe	26	3	2582	16.1.2014	Male
5	Shu Zarni	24	2	2404	30.6.2014	Male
6	Win Moe Thwe	31	2	2706	22.9.2014	Male

Table 2. Percentage of total milk fat in Asian elephant milk samples, measured using the "creamatocrit" methodology; both solid and liquid lipid fractions were apparent in all samples.

Description	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Total Fat %	35	7.20	26.47	15.35	4.44
Cream % Total Fat	35	2.86	69.01	21.11	12.52
Liquid % Total Fat	35	30.99	97.14	78.88	12.52

Table 3. Variation of milk fat composition from individual Asian elephants in Myanmar, sampled between 16 and 38 months of lactation.

Animal	**1**	**2**	**3**	**4**	**5**	**6**	**P value**
Mo of Lacation	**16 - 24**	**18-25**	**17-25**	**30-38**	**25-33**	**22-30**	
Milk fat, %	14.56 ±4.78	16.14 ± 2.55	15.63 ± 7.08	12.73 ± 3.44	16.23 ± 2.90	15.36 ± 4.96	0.001
% Cream fat	15.93^{d}	33.97^{a}	29.52^{b}	17.93^{d}	22.54^{c}	14.9^{d}	0.001
% Liquid fat	85.07^{a}	71.36^{b}	72.48^{b}	79.40^{ab}	85.90^{a}	87.93^{a}	0.001

Figure 1. Measuring milk fat in elephant milk samples (as a percentage of total sample) using a simplified centrifugation technique and calipers, suitable for field sampling.

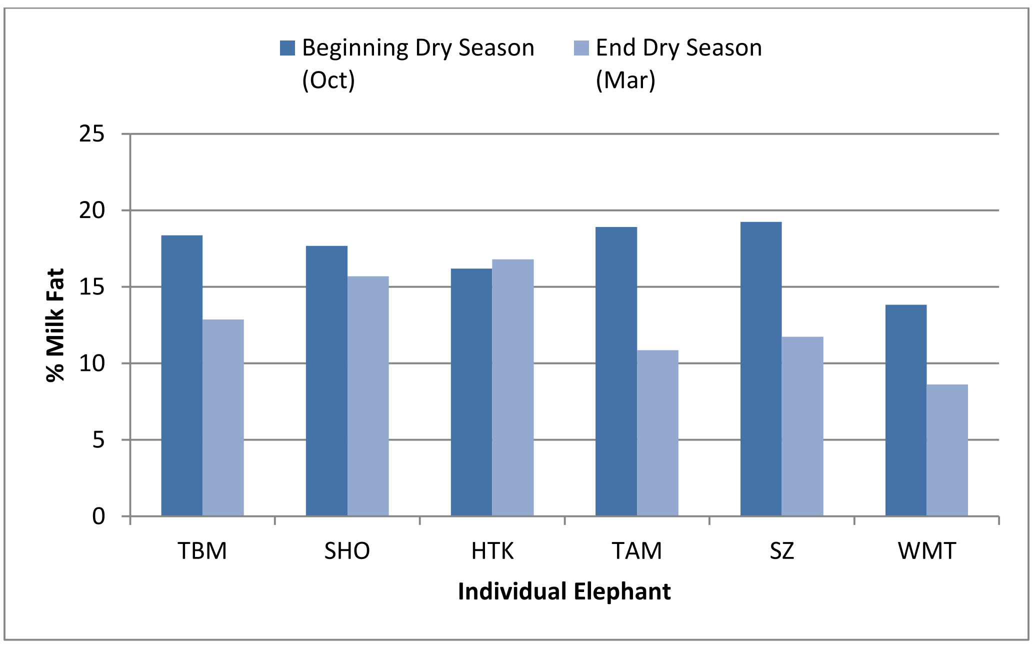

Figure 2. Total milk fat percentages from semi-free ranging Asian elephants (n=6) in Myanmar sampled at beginning (Oct 2016) (Sep/Oct 2016) and end of dry season (Mar 2017), between 19 and 38 months of lactation.