The effect of physiological state, milk production traits and environmental conditions on milk fat globule size in cow's milk.

Leonie Walter[1,2]*, Sue Finch[3], Brendan Cullen[1], Richard Fry[1], Amy Logan[2], Brian J Leury[1]

Supplementary File

Supplementary Methods

*Metabolisable energy (**ME**) value of the feed*

Silage (9.4 MJ ME per kg dry matter (**DM**), 14.1 % crude protein per kg DM, 56 % neutral detergent fiber (**NDF**) per kg DM is fed predominately on the feed pad and cows are offered three breaks of pasture each day. Pasture feed quality varies throughout the year, with the lowest quality in summer (January; 9.3 MJ ME per kg DM, 18.8 % crude protein per kg DM, 55 % NDF per kg DM) and the highest quality in winter (August; ME 11.8 MJ ME per kg DM, 25.3 % crude protein per kg DM, 37.9 % NDF per kg DM). Feed composition of silage was previously tested by a feed testing laboratory (Feed Quality Service at the Wagga Wagga Agricultural Institute, Wagga Wagga, NSW, Australia) and pasture composition for each month was obtained through the Perennial Pastures Database provided by the Department of Primary Industries Victoria (2018). The concentrate (12.5 MJ ME per kg DM, 16 % crude protein; Optimilk Lacta Max, Rivalea Australia - Stockfeeds, Corowa NSW, Australia) is fed during milking in the robot (Lely Astronaut) and cows can finish their individually allocated feed in a feeding station (Lely Cosmix) after milking.

Calculations for the concentrate allowance of individual animals

Daily concentrate allowance for the individual animal is calculated based on milk production as follows: animals > 28 days in milk (**DIM**) were fed up to 5 kg concentrate per day for cows producing less than 20 kg of milk; 7 kg for cows producing 20 to 25 kg; 8 kg for cows producing 25 to 30 kg; 9 kg for cows producing 30 to 35 kg; 10 kg for cows

producing 35 to 40 kg and 11 kg for cows producing more than 40 kg of milk per day. Concentrate intake for cows in the first 28 DIM is sequentially increased from 5 kg on the first day, 7 kg per day between day 1 to day 14 and then 9 kg per day up to day 28.

Calculations for the daily average feed composition of the whole herd

The cows' average concentrate intake was recorded by the T4C software and silage intake was estimated based on the amount offered in the feed pad and estimated wastage rates. Pasture intake was estimated by a back-calculation technique. The energy from pasture intake was estimated by first calculating the animals' total energy requirements and then subtracting the energy supplied from concentrate and silage. The animals' total energy requirements were calculated according to the procedures of Primary Industries Standing Committee (2007), using daily milk production and milk composition, body weight and gestation.

Limitations of our study

Measurements of fat percentage in our study are based on in-line measurements using near infrared spectroscopy, and the data obtained gives a good indication of the fat content in raw milk. However, we observed reduced accuracy for milk with very low-fat percentages (less than 3 %), when compared to measurements made using laboratory-based FTIR techniques (data not shown). Therefore, the results from our study only allow us to draw limited conclusions regarding the relationship between MFG size and fat yield.

Supplementary Results

Table S1. Test statistics for variables included in the linear mixed models for single variable and multiple variable effects

Explanatory variable	Single variable effects			Multiple variable effects		
	F statistic	df	*P*-value	F statistic	df	*P*-value
Primary variables (used in multiple variable model)						
Milk yield (× 10 kg)	34.89	202.6	< 0.001	4.08	81.4	0.047
DIM (× 100 d)	75.31	179.8	< 0.001	43.71	183.9	< 0.001
Concentrate intake (kg)	12.74	199.4	< 0.001	2.79	211.6	0.096
Number of milkings	19.49	202.0	< 0.001	1.39	230.1	0.240
Fat yield (kg)	21.23	206.4	< 0.001	2.28	107.6	0.134
Parity	23.69	133.9	< 0.001	3.50	223.5	0.009
Other variables						
Fat indication (%)	21.3	196.6	<0.001	-	-	-
Protein indication (%)	1.2	249.6	0.266	-	-	-
Fat:Protein ratio	21.5	214.7	<0.001	-	-	-
Days pregnant	32.9	170.6	<0.001	-	-	-
Rumination minutes	0.5	260.4	0.480	-	-	-
SCC indication (× 10^3 cells per mL)	0.6	218.2	0.445	-	-	-
Weight (kg)	0.8	205.9	0.359	-	-	-
Age	4.9	113.0	0.029	-	-	-
Concentrate (kg) per 100 kg milk	2.0	166.2	0.156	-	-	-

Cow number and sampling day are included as random effects.
SCC = somatic cell count.

Table S2. Effects of selected variables on the average milk fat globule (MFG) size fitted using single variable linear mixed models

Explanatory variable	Single effect on MFG size (µm)	Confidence interval	*P* - value
Milk yield (× 10 kg)	0.18	0.12, 0.24	< 0.001
DIM (× 100 d)	-0.16	-0.19, -0.12	< 0.001
Concentrate intake (kg)	0.04	0.02, 0.06	< 0.001
Number of milkings	0.25	0.14, 0.35	< 0.001
Fat yield (kg)	0.47	0.27, 0.67	< 0.001
Parity			< 0.001
Stepwise comparison between factor levels for parity			
Factor levels (number of lactations)	Mean difference for single effect (µm)	Confidence interval	*P* - value
2 - 1	0.05	-0.22, 0.31	0.735
3 - 2	0.30	0.15, 0.45	< 0.001
4 - 3	0.12	-0.04, 0.28	0.144
5 - 4	0.04	-0.24, 0.31	0.801
Other variables			
Fat indication (%)	-0.18	-0.26, -0.10	< 0.001
Protein indication (%)	-0.13	-0.36, 0.1	0.266
Fat:Protein ratio	-0.75	-1.06, -0.43	< 0.001
Days pregnant	-0.00	-0.00, -0.00	< 0.001
Rumination minutes	0.00	0.00, 0.00	0.480
SCC indication (× 10^3 cells per mL)	0.00	0.00, 0.00	0.445
Weight (kg)	0.00	-0.00, 0.00	0.359
Age	0.06	0.01, 0.11	0.029
Concentrate (kg) per 100 kg milk	-0.01	-0.01, 0.00	0.156

Cow number and sampling day are included as random effects.
DIM = days in milk.
SCC = somatic cell count.

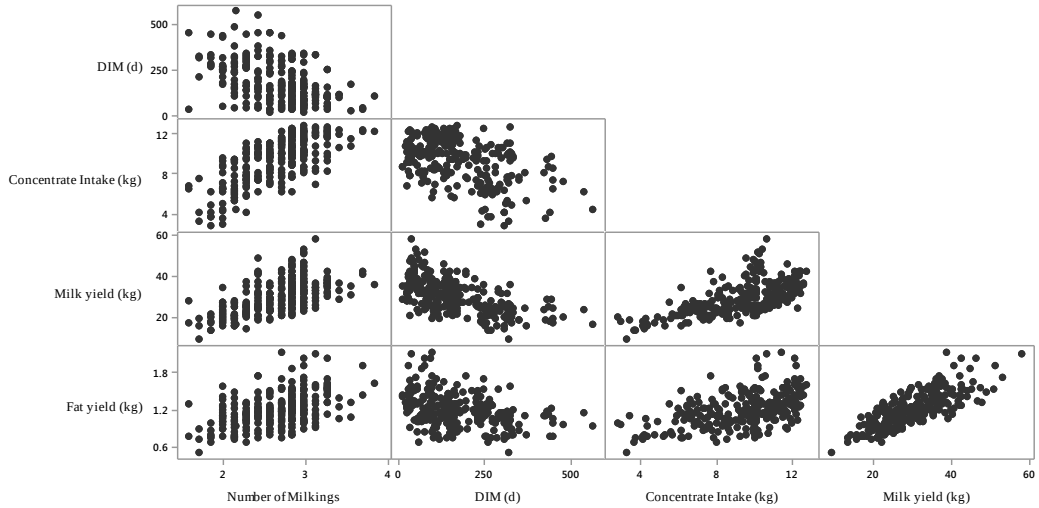

Figure S1. Relationship between selected explanatory variables used to fit the multiple variable model.DIM = days in milk.

References for the Supplementary File

Primary Industries Standing Committee 2007 Nutrient Requirements of Domesticated Ruminants. Collingwood, Australia: CSIRO Publishing