

**Associations among body energy status, feeding duration and activity in dairy cows on diets with different energy and protein contents**

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**SUPPLEMENTARY FILE**

**Materials and methods**

**Cow management and recording**

Strict management protocols were operated both within and between systems. Cows were housed in the same building and managed by the same staff. Within a system, one complete diet was offered to all cows irrespective of milk yield and stage of lactation. The complete diet was offered at 1.05% of daily requirement and refusals removed daily.

Animal activity was monitored through use of accelerometers (IceQube Sensors®, Icerobotics Ltd, UK) that were fitted on each cow. Each IceQube had a unique identification number which was linked to the cow identification number and was attached on either the left or right rear leg above the fetlock joint. The downloaded data were from 15-minute block summaries of the overall activity (motion index) number of steps, lying bouts, standing and lying durations making a total of 96 entries for each variable per day. The 15 minutes summaries were later aggregated into daily summations. Overall activity was measured by the motion index which was determined by measurement of acceleration against gravity on each of the three body axes (de Mol, 2013). A high motion

index meant a lot of movement and this is highly correlated with the number of steps that an animal takes (Rushen & de Paselle, 2012).

The individual feed and water feed intake were recorded on 3 days out of six using Hoko gates (Insentec BV, Marknesse, The Netherlands). Feeding duration data included daily summaries of total time spent eating from the individual feed intake bins. Samples of complete diet fed and refusals were taken daily for determination of oven dry matter. Samples of complete diets and individual feeds were taken weekly and bulked into monthly samples for determination of chemical compositions. All feed samples were analysed at SRUC Analytical Services, Edinburgh. Table 1 shows the typical chemical composition of the feeds. The target crude protein (CP) and metabolisable energy (ME) content in the BP diet was 185 g/kg DM and 12.3 MJ/kg DM while it was 180 g/kg DM, and 11.5 MJ/kg DM for the HG diet, respectively.

Digestibility of dry matter (DM) of the feeds was determined by the in vitro technique of Alexander (1969). The grass silage was analysed by near infrared reflectance spectroscopy (NIRS) to estimate metabolisable energy (ME) content (Barber et al., 1989). The ME content of the concentrate feeds was determined by the equation of Thomas et al. (1988):

$$\text{ME (MJ/kg DM)} = (0.14 \text{ NCGD}) + (0.25 \text{ AHEE})$$

where

NCGD = neutral cellulose gaminase digestibility

AHEE = acid hydrolysis ether extract (g kg/DM) (MAFF,1993).

Weekly body energy content (BEC) was calculated using weights and body condition score according to formulae described by Banos et al. (2006) as summarized below:

$$\text{BEC (MJ)} = [(9.4 \times \text{body lipid weight}) + (5.7 \times \text{body protein weight})] \times 4.1868$$

Where:

$$\text{Body lipid weight (kg)} = (0.037683 \times \text{BCS}) \times (\text{empty body weight})$$

$$\text{Body protein weight (kg)} = [(0.200886 - 0.0066762 \times \text{BCS})] \times \text{empty body weight}$$

BCS were expressed on a scale of 1 to 9 (BCS9). The BCS in the database were on the scale of 1 to 5 (BCS5) and were converted to BCS9 using the formula:

$$\text{BCS9} = (\text{BCS5} - 1) \times 2 + 1$$

$$\text{Empty body weight (kg)} = \text{live weight (kg)} \times 0.96 \times 0.85$$

BEC is one of the body energy measures used in other literature (Banos et al., 2006; Coffey & Pollot, 2008). The trait indicates the absolute level of energy in the body per day regardless of previous day's energy use and intake (Banos et al., 2006). Changes in BEC from calving to nadir BEC and service were also calculated. Nadir BEC was defined as the lowest BEC in the lactation. Energy balance was determined according to Banos et al. (2006) where body lipid and protein weight changes during lactation were used. Weekly energy balance was calculated using cumulative lipid and protein weight changes and based on these calculations cows were further subdivided into those in positive and negative energy balance.

The generalized linear mixed model was as follows:

$$Y_{ij} = a + \sum b_i x_i + \lambda_j + \varepsilon_{ij}$$

Where:  $Y_{ij}$  = trait outcome

$a$  = intercept

$b_i$  =  $i$ th fixed effect ( $i=1,2,3 \dots$  feeding system, genotype, lactation)

$x_i$  = value of  $i$ th fixed effect

$\lambda_j$  = random effect of cow  $j$

$\varepsilon_{ij}$  = Error

## RESULTS

Table S1: Feed chemical composition of rations and milk production in 2012 for the Langhill herd

Variable	Type of ration (Mean $\pm$ SD)			
	BPS	BPC	HGS	HGC
Crude protein (g/kg)	185 $\pm$ 7	185 $\pm$ 7	180 $\pm$ 7	180 $\pm$ 7
Metabolisable energy (MJ/ kg DM)	12.3 $\pm$ 0.3	12.3 $\pm$ 0.3	11.5 $\pm$ 0.3	11.5 $\pm$ 0.3
Dry matter (%)	50	50	38.4	38.4

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Estimated intake (kg	22.1	22.1	15.8	15.8
DM/cow/day				
Milk production	11677	9712	7440	6686
(litres/cow/year)				

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Source: Roberts & March 2013