

# final report

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## Animal Welfare Objective Measures Research Program -Validation of body condition score as a practical indicator of welfare in cattle.

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## Abstract

This project examined whether body condition score (BCS) could be applied as a practical indicator of welfare in lactating beef cows and was one of three projects within the Animal Welfare Objective Measures (AWOM) Research Program. Three experiments were successfully completed. The first focused on the development of a novel methodology to determine motivation for food in cattle and therefore provided an indication of hunger. Two subsequent experiments were conducted to examine the physiological and behavioural differences in lactating beef cows differing in BCS. BCS was moderately to highly correlated with objective predictors of body composition and endocrinal regulators of hunger and energy metabolism. There was a consistent trend which varied in significance between the studies whereby the low BCS cows were more motivated to work for a food suggestive that they were hungrier. It was concluded that BCS is a practical and reliable indicator of body composition and there is some evidence that it can be applied to assess welfare in lactating cows during under-nutrition. Regular assessments of BCS are central to the effective management of welfare risks during periods of drought or reduced pasture availability in beef herds.

## **Executive Summary**

Cattle in extensive systems are often subject to periods of reduced feed availability due to the normal seasonal cycles in pasture growth or more extreme conditions, such as drought. When nutrient intake fails to meet metabolic requirements in the animal, an adaptive response is initiated resulting in catabolism of fat and muscle tissue and subsequent reductions in liveweight and body condition. During prolonged periods of under-nutrition, the losses in body mass can be quite significant and this in turn, negatively affects productivity (eg reduced reproductive function). Whilst the productivity losses have been characterised, very little is known about the impacts on animal welfare during moderate and sustained under-nutrition in livestock.

Body condition score (BCS) has been used as a practical and effective means to monitor changes in body mass and composition in cattle on-farm. It potentially may also provide an indication of welfare status. In order to use BCS as a scientifically defensible measure of animal welfare, there is a need to firstly, determine the welfare state of animals differing in BCS and secondly, to identify the key thresholds of these measures in terms of acceptable and at-risk animal welfare.

The primary objective of this joint Australian and New Zealand project was to determine whether BCS could be applied as a practical measure of welfare in cattle during periods of moderate under-nutrition. A secondary objective was to identify the minimum BCS and maximum rate of change in BCS and live weight for application within industry welfare standards and/or assurance systems. The class of animal targeted in the research was the lactating beef cow since she is likely to experience the largest fluctuations in BCS during each pregnancy/lactation cycle. In New Zealand, the focus was the gestational ewe and this joint research formed part of the Animal Welfare Objective Measures (AWOM) Research Program.

Three cattle experiments were conducted in this project. The aim of the first experiment was to validate a methodology to objectively quantify an animal's motivation for food and therefore provide an indication of hunger. The second experiment evaluated the behavioural and physiological responses in lactating cows differing in fat score (FS). In the third and final experiment, the behavioural and physiological responses in lactating beef cows were examined in response to different rates of condition loss and at stable differences in BCS.

The primary conclusions from the research were:

• Fat scores, based on estimates/measurements of subcutaneous fat, are inadequate in the determination of body condition, or more specifically, condition loss in lactating beef cows. A more appropriate alternative is body condition scoring which is based on both visual and manual assessments of the flesh (muscle + fat) covering the skeleton. However, currently there is no national standard for body condition score is to be used as an indicator of the welfare status in cattle, there is a need to develop a national standard system that is appropriate for Australian beef cattle breeds and classes. Alternatively, given that the AUS-MEAT Fat Score System is nationally recognised, examine whether the system can be modified or augmented to

better reflect both fat and muscle loss particularly around the at welfare risk category of FS 1.

- Assessments of BCS were moderately to highly correlated with objective indicators of fatness and muscle mass (ie. body composition). Similarly, BCS was moderately associated with endocrinal regulators of appetite and energy homeostasis (eg. leptin). These results add to the evidence that BCS can be a reliable and practical indicator of body composition and can be applied to monitor cattle when they are in catabolic states.
- In Experiment 2, a significant difference in feeding motivation was found between FS1-1.5 cows and the combined FS 2 and FS 3 cows but the differences between BCS treatment groups in experiment 3 were not significant. Nevertheless, there was a similar trend to that observed in the second experiment where the low condition score cows showed higher motivation for food than the higher condition score groups. This aligns with the findings of the complementary sheep BCS research undertaken as part of the AWOM Research Program. Collectively, these data provide the first quantitative evidence of the relative hunger experienced by ruminants during under-nutrition. Although there were inconsistencies in the cattle results, there is sufficient evidence to suggest that BCS can be applied to assess the welfare of ruminants during moderate under-nutrition. However, since this is the first attempt to explore the impact of condition loss in the context of hunger and animal welfare in cattle, further research is warranted.
- Whilst BCS appears to provide an indication of welfare status in lactating cows during moderate and sustained under-nutrition, the actual BCS threshold that delineates acceptable and unacceptable welfare is much harder, and perhaps impossible to define. Alternatively, we propose that it is more appropriate to consider BCS in the context of welfare risk where there is an optimal BCS range and outside this, the welfare risks increase with either increasing or decreasing BCS. Industry guidelines recommend the optimal BCS range for heifers and cows at the time of joining and calving is 2.5 3.5 (1-5 BCS scale). This is largely predicated on maximising reproduction performance but it has relevance in terms of animal welfare. Based on the present research, at a BCS < 2.5 there appears to be an increased risk that cows will experience hunger. It must be emphasised that a cow with BCS outside the optimal range does not automatically have compromised welfare, rather it provides an indication that she may be at risk of an adverse welfare outcome.</p>
- It is recommended that regular assessments of BCS are central to the effective management of welfare risks during periods of drought or reduced pasture availability in beef herds.

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## 1 Background

Cattle in extensive systems are often subject to periods of reduced feed availability due to the normal seasonal cycles in pasture growth or more extreme conditions, such as drought. When nutrient intake fails to meet metabolic requirements in the animal, an adaptive response is initiated resulting in catabolism of fat and muscle tissue and subsequent reductions in liveweight and body condition.

Cattle have evolved to adapt to some fluctuation in body condition, especially females during lactation. From an animal welfare perspective, these normal fluctuations are probably of minimal concern. However, during moderate and sustained under-nutrition where significant weight and condition loss occurs, there is an increased risk of reduced animal welfare. Unfortunately, our understanding about the nature and magnitude of the welfare impacts of moderate and sustained undernutrition in beef cattle is somewhat limited. However, some insight can be gained from dairy cattle research focused on the perennial issue of maintaining BCS during lactation. In their recent review of the association between BCS and dairy cow productivity, health and welfare, Roche et al (2009) concluded that low BCS was associated with productivity losses (eq. milk production and impaired reproduction), reduced immune function and an increased risk of discomfort in cold environments and metabolic disorders. These authors also highlighted that in the context of welfare assessment, little was known about what dairy cows at low BCS feel (ie. their affective state) and that this warranted more attention.

It is known that animals respond peripherally to challenges with a wide variety of responses that result in activation of some or all of the following biological processes: immune system, hypothalamic-pituitary-adrenal axis, motor and other behavioural activities. Interacting with these peripheral responses is a range of neurophysiological changes, which influence both peripheral physiological responses and cognitive processes. Cognitive elements include the animal's perception of its own state (e.g. the aversiveness of the events/feelings). Much of the research on animal welfare has focused on understanding the physiological (peripheral, and to a lesser degree, central) impacts of stressors, with relatively little attention given to the impacts on the perceptions and feelings of the animals. There has been little research seeking to understand the functional linkages between physiological and cognitive processes, nor objective measurement of the resultant animal perceptions. Furthermore, there is a growing view within the scientific community, that understanding how and what animals feel is paramount in the assessment of their welfare (Dawkins 2006; Duncan 2006). One of the key aims of this project was to apply novel methodologies to characterise affective state particularly with respect to hunger during condition loss in cattle.

Body condition score (BCS) has been used as a practical and effective means to monitor changes in body mass and composition in cattle on-farm. It potentially may also provide an indication of welfare status. In order to use BCS as a scientifically defensible measure of animal welfare, there is a need to firstly, determine the welfare state of animals differing in BCS and secondly, to identify the key thresholds of these measures in terms of acceptable and at-risk animal welfare. The primary objective of this project was therefore, to validate whether BCS was a practical indicator of the welfare status of cattle during moderate under-nutrition.

Finally, this project was undertaken in collaboration with Dr Lindsay Matthews (AgResearch) and Dr Trish Fleming and Dr Catherine Stockman (Murdoch University) as part of the Animal Welfare Objective Measures (AWOM) Research Program.

## 2 **Project Objectives**

To validate body condition score (BCS) as a practical measure of welfare in cattle during periods of moderate under-nutrition.

## 3 Methodology

Three cattle experiments were conducted in this project. The aim of the first experiment was to develop and test a methodology to objectively quantify an animal's motivation for food and therefore, provide an indication of hunger (Appendix 9.1). The second experiment evaluated the behavioural and physiological responses in lactating cows differing in fat score (FS) (Appendix 9.2). In the third and final experiment, the behavioural and physiological responses in lactating beef cows were examined in response to different rates of condition loss and at stable differences in BCS (Appendix 9.3).

The detailed description of the methodology applied in the three experiments is provided in Appendices 9.1, 9.2 and 9.3.

### 4 Results and Discussion

# 4.1 Experiment 1 – Construct and test a facility for the determination of motivation for food in cattle

Several novel methodologies have been developed to assess affective states in animals (refer Kirkden and Pajor 2006; Matthews 2008; Mendl et al 2009). One approach is based on the measurement of an animal's motivation for a particular resource such as food, rest or space. This methodology is derived from the theory of behavioural economics and is reviewed in detail by Kirkden and Pajor (2006) and Matthews (2008). In practice, animals are asked to perform an operant task (eg. lever press) or work to gain access to a desired resource. The amount of work required or price is increased and the amount of the resource obtained is measured. The elasticity of the relationship between the quantity of resource obtained and price provides insight into how important the resource is to the animal.

The primary objective of this experiment was to develop and construct a simple, automated system to measure feeding motivation in cattle based on behavioural economics and using a natural (walking) operant response. This was successfully achieved and the details of the methodology and test results are presented in Appendix 9.1.

Briefly, a feed delivery system and feeder were constructed with two feed access bays. Presence at one bay (detected by infra red beams) activated feed delivery in the second bay. Access to the feed required the animal to walk around a fence panel extending out from the feeder. Continued access to feed required the animal to cycle back and forth between the bays, with the distance walked controlled by the length of the barrier. A software interface automatically recorded each feeding event and controlled the amount of feed delivered.

The system was tested and used to examine the sensitivity of cattle to feed reward size (30 and 45 g) and walking distance up to 24 m. After a period of training and adjustment to a concentrate ration, feeding motivation of six yearling Angus steers was determined. Each animal was tested at each of five distances (costs) and the number of feed rewards over a 23 h period was measured. There was a 48 h recovery period between tests. Three animals received rewards of 30 g at all five distances (randomised) before being tested at the 45 g reward amount, with the other animals rewarded in reverse order. Demand function analyses of the data showed that  $O_{max}$ , the maximum distances walked, were  $4.5 \pm 0.5$  and  $13.2 \pm 25.7$  km for the 30 g and 45 g reward sizes, respectively. However, at 45 g the curve was relatively inelastic and the estimate of  $O_{max}$  was unreliable. Nevertheless, the results showed that the cattle could clearly detect a small difference in reward size and this was reflected in their overall motivation to feed.

The behavioural demand facility and recommended test protocols were then utilised in subsequent experiments to quantify motivation for food in lactating cows differing in BCS which in turn would provide an indication of differences in overall hunger.

#### 4.2 Experiments 2 & 3 – Effects of short and long term condition loss on the physiological and behavioural responses in lactating beef cows

Two large cattle experiments were undertaken to examine the effects of condition loss in lactating beef cows and therefore assess the suitability of BCS as a practical welfare indicator in cattle.

Full details of the methodology and results for both experiments are presented in Appendices 9.2 and 9.3 and only a brief summary is presented in the following.

#### 4.2.1 Experiment 2

Twenty-four pregnant Hereford cows (approx. 4-7 years) were used in the experiment. Immediately after calving, the cows were randomly allocated (after stratification for calving date, liveweight and P8 fat depth (fat score) to one of three condition loss treatments. These were designed so that the cows achieved a fat score (FS) of 3 (i.e. no loss), 2 and 1-1.5 (on the 6-point AUSMEAT scale), over a 4-month period of lactation. The AUS-MEAT Fat Score System (see Appendix 9.2) was used in the experiment. Although body condition score and fat score are not completely synonymous, the AUS-Meat system was chosen since it is the national reference system. The condition loss was achieved by reducing pasture availability through increased stocking density. The cows were weighed, condition scored and ultrasound scanned fortnightly during condition loss. Ultrasound images were recorded at the P8 or rump site (fat depth) and over the 12/13<sup>th</sup> ribs (fat depth and *m.longissimus* area). P8 fat depth was used as the objective determinant of fat score. The cows were also assessed for body condition score (BCS) on a 9 point scale.

Once the cows were stable at the desired FS, their motivation for food was determined. After a habituation period including training in the facility, the cows' behavioural demand for food was measured over a 24-h period at five work or price

levels (1.5, 7.7, 17.6 and 40 m). The increase in price was achieved by increasing the distance the animals walked to access the 30 g food reward. Blood samples (9 ml EDTA vacutainer) were collected via jugular venepuncture at the start and finish of each BD test for the measurement of insulin, ghrelin and leptin.

#### **Results Summary**

Significant differences in liveweight, fat depth, *m.longissimus* or eye muscle area (EMA) and BCS were observed between the FS treatment groups particularly for the contrast between FS 3 and FS 1-1.5. Although higher in FS 2 relative to FS 1-1.5, the differences in fat depth were not significantly different.

There were concomitant reductions in fat depth and EMA but the temporal responses differed. For the FS 1-1.5 treatment group, there was a 55% reduction in P8 fat depth in the first half of the condition loss period but there was only a further 1.5% reduction in the latter half. For EMA, there was 16.3% and 13% reduction during the first and second halves of condition loss.

Significant correlations were found between the various measures and the only non-significant correlations (P>0.05) were observed between BCS and fat depth (P8 and  $12/13^{th}$  rib). EMA was the most highly correlated measurement with liveweight (r = 0.78).

The analysis of motivation for food initially revealed no significant differences between the FS treatments. However, when the FS 1-1.5 and FS 2 treatments groups were pooled and contrasted against FS 3, a significant difference was observed (P<0.05). The pooling of the FS 1-1.5 and FS 2 groups was justified given the fact that FS treatments were predicted on P8 fat depth and there was no significant difference in fatness after condition loss.

The  $P_{max}$  (price or cost at maximal work) values for the FS 3 and pooled FS 2 and FS 1-1.5 groups were 21.4 and 26.0 m, respectively. The corresponding  $O_{max}$  (total work output at  $P_{max}$ ) values were 1368 and 2857 m. These pooled differences were significant (P<0.05). These results provide objective evidence that animals with lower fat scores (ie. poorer condition) will work harder for a small food reward than better conditioned animals. By inference, this indicates that their affective state (i.e. hunger or motivation to feed) has been influenced by their condition.

Ghrelin, leptin and insulin are known regulators of appetite and energy homeostasis. There were no significant differences in insulin or ghrelin concentration between the FS groups prior to or after testing motivation for food. However, significant differences in leptin concentration were observed prior to testing (FS1-1.5 0.60 ng/ml, FS 2 0.60 ng/ml and FS 3 0.9 ng/ml).

The correlation analysis between the blood measures and the number of feed rewards during food motivation testing only revealed a significant association between the log change in leptin concentration (ie. post – pre-motivation testing) and average number of feed rewards (r=0.64; P<0.05). As the difference in leptin concentration increased, there was a commensurate increase in the number of feed rewards.

The results reinforced the view that measures of fatness, and therefore, fat scores, were not the best criteria to apply when assessing the condition of cattle during periods of under-nutrition.

#### 4.2.2 Experiment 3

Twenty-four Angus cows approximately 4 - 4.5 years of age were used in this experiment. After calving (March-April 2009), the cows were randomly allocated, after stratification for liveweight, P8 fat depth and calving date to one of three condition loss treatments: no condition loss (BCS 3), a loss of 1 BCS (BCS 2) or a loss in 1.5 - 2 BCSs (BCS 1-1.5). The cows were subjected to their condition loss treatments for approximately 5 months. BCS rather than fat score was used given the findings from Experiment 2.

The cows and calves were moved into large pens and held in their BCS treatment groups (8/pen). After a period of adaptation, the cows received group feed allowances (pelleted ration - 16.2% CP, 9.0 MJ ME/kg DM) that were designed to achieve the desired rate of condition loss. The feed allowances were determined after calculating their maintenance requirements (based on SCA Feeding Standards for Australian Livestock – Ruminants). These allowances were subsequently adjusted during the condition loss phase when the rate of condition loss deviated from the desired trajectory. Once the cattle reached the desired BCS, the ration was adjusted to maintain the cows at this BCS.

The cows were weighed, condition scored and ultrasound scanned at approximately fortnightly intervals during the condition loss treatment phase. Ultrasound images were recorded at the P8 or rump site (fat depth) and over the 12/13th ribs (fat depth and *m.longissimus* area). Blood samples were collected fortnightly for analysis of leptin, IGF-1 and insulin concentrations.

One month after imposition of the condition loss treatments (i.e. at different rates), the motivation for food was measured. After this, the condition loss treatments continued until the cows stabilised at the desired BCS, approximately 3.5 months later. Their motivation for food was then re-tested.

#### **Results Summary**

The linear rates of loss over the initial 76 days were -0.08, -0.12 and -0.16 BCS unit/day for the BCS 3, BCS 2 and BCS 1-1.5 treatment groups, respectively. The different rates of condition loss over the period did not result in significant differences in the motivation for food between the BCS groups. The  $P_{max}$  values (price at maximal work) were 31.3 ± 11.8, 21.7 ± 3.9 and 24.5 ± 5.1 m for the BCS3, BCS2 and BCS1-1.5 groups, respectively.

The IGF-1, insulin or leptin concentrations were not different between the BCS treatments over this initial treatment period. The time of sampling during the period was a significant effect for all three plasma constituents and a significant treatment x sampling time interaction was found for plasma leptin concentration (P<0.001).

The mean loss in liveweight, fat depth and EMA over the entire treatment phase (condition loss + stable BCS period) for the BCS 1-1.5 and BCS 2 groups was 68.7 kg, 5.5 mm and 7.3 cm<sup>2</sup> and 47.1 kg, 6.5 mm and 1.9 cm<sup>2</sup>, respectively. In the BCS1-1.5 group, there was a 60.7% and 10.3% reduction in P8 fat depth and EMA, respectively. For the BCS 3 group, some loss in BCS, liveweight and fat depth

occurred during the initial period of lactation but these losses were restored over the treatment phase with the exception of liveweight.

Based on the average of the last 2 (EMA only) or 3 measurements over the stable BCS period, significant differences in liveweight, fat depth, EMA, leptin and insulin concentrations were observed particularly for the contrast between the BCS 3 and BCS 1-1.5 groups.

Moderately high correlations (r = 0.6 - 0.8) were found between the body compositional measures. Of the plasma measures, leptin concentration was moderately correlated with live animal measurements (r = 0.4 - 0.6).

There were no significant differences in feeding motivation between the BCS groups at the conclusion of the condition loss phase. Although there was a trend for increasing  $P_{max}$  (BCS 3 28.9 ± 5.9 m, BCS 2 40.8 ± 13.6 m, BCS 1 – 1.5 46.6 ± 18.8 m) and  $O_{max}$  (BCS 3 6.9 ± 0.7 km, BCS 2 8.8 ± 1.3 km, BCS 1-1.5 10.1 ± 1.9 km) with declining BCS, the differences were not significant. Notwithstanding the lack of a significant effect, the results showed that poor conditioned cows (BCS 1-1.5) were prepared to walk up to 10 km, some 3 km more than BCS3 cows, to continuously access a small 30 g feed reward.

#### 4.2.3 General discussion

The primary objective of this project was to determine whether body condition score (BCS) could be applied as a practical measure of welfare in cattle during periods of moderate under-nutrition. The class of animal targeted in the research was the lactating beef cow since she is likely to experience the largest fluctuations in BCS during each pregnancy/lactation cycle. The emphasis on under-nutrition was predicated on the perceived welfare risks and the fact that in Australia, this can be a common scenario in some production regions.

From the results (particularly from Expt. 3), it can be concluded that assessments of BCS were moderately to highly correlated with objective indicators of fatness and muscle mass (ie. body composition). Similarly, BCS was moderately associated with endocrinal regulators of appetite and energy homeostasis (eg. leptin). These results reinforce the evidence that BCS is a reliable and practical indicator of body composition and can be applied to monitor cattle when they are in catabolic states. From an animal welfare perspective, metabolic disorders (eg. ketosis, mineral/vitamin deficiency) that arise as a consequence of acute and chronic catabolic states are a primary concern. Similarly, when the mobility and physical capacity of the animal has been affected due to the significant loss in muscle, this is clearly an unacceptable welfare outcome. However, it can be argued that these are representative of extreme cases of compromised animal welfare. In the two experiments conducted here, there was no evidence of metabolic disorders, lethargy or reduced locomotive capacity in the low condition score cows. Therefore, what can be concluded in terms of animal welfare based on the results of the current research?

The affective state of an animal is a central element of its welfare (Dawkins 2006; Duncan 2006) and during periods of under-nutrition, animals will experience hunger. According to D'Eath et al (2009), hunger is defined as negative subjective state when animals are chronically undernourished. Being subjective, hunger can only be indirectly measured and in these experiments, the animal's motivation to obtain a small feed reward was determined as an indicator of hunger. In Experiment 2, a

significant difference in feeding motivation was found between FS1-1.5 cows (BCS 2.2  $\pm$  0.08) and the combined FS 2 and FS 3 cows (BCS 2.41  $\pm$  0.08) but the differences between BCS treatment groups in experiment 3 were not significant. Nevertheless, there was a similar trend to that observed in second experiment where the low condition score cows showed higher motivation for food than the higher condition score groups. For example, when contrasting the O<sub>max</sub> values (total work output at P<sub>max</sub>), the BCS 1-1.5 cows were prepared to walk a further 3.2 km more than the BCS 3 cows to obtain a 30 g feed reward. It is difficult to explain the lack of significance in the third experiment. Paradoxically, the actual difference in BCS between the lowest and highest treatment groups was greater in Experiment 3 (1.17 BCS units) compared to that in Experiment 2 (0.28 BCS units). Furthermore, the cows used in Experiment 3 were more tractable and habituated to the motivation testing far better than those in the second experiment.

Notwithstanding the experimental differences in statistical significance, there was a consistent trend where poorer conditioned or leaner cows displayed higher motivation for food. This aligns with the findings of the complementary sheep BCS research undertaken as part of the AWOM Research Program. Matthews (2010) reported a significant inverse relationship between feeding motivation in gestational ewes with increasing BCS. In addition to the behavioural data in the current study, the plasma leptin concentrations were significantly lower in the low FS (Expt. 2) and BCS (expt. 3) groups. Leptin is directly associated with the level of adiposity in the animal and the regulation of feed intake (Wynne et al 2005). It is therefore likely that the low FS and BCS cows would have been chronically stimulated to increase their feed intake. Collectively, these data provide the first quantitative evidence of the relative hunger experienced by ruminants during under-nutrition. Returning to the objective, the results, although not completely conclusive, provide support that BCS can be applied to assess the welfare of ruminants during moderate or moderate and sustained under-nutrition. Given that this is the first attempt to explore the impact of condition loss in the context of hunger and animal welfare in cattle, further research is warranted to validate these initial findings and to explore whether BCS influences the animals capacity to cope with additional production challenges.

Interestingly, the effect of short term condition loss at different rates (Expt. 3) did not influence feeding motivation in lactating cows. This was also established in sheep by Matthews (2010). These results suggest that significant condition loss through moderate under-nutrition has a greater effect on the animal's affective state than acute condition loss even at different rates.

Whilst BCS appears to provide an indication of welfare status in lactating cows during catabolic states, the actual BCS threshold that delineates acceptable and unacceptable welfare is much harder, and perhaps impossible to define. Alternatively, we propose that it is more appropriate to consider BCS in the context of welfare risk where there is an optimal BCS range and outside this, the welfare risks increase with either increasing or decreasing BCS. There are numerous industry guidelines (eg. MLA More Beef from Pastures 2004) that specify that the optimal BCS range for heifers and cows at the time of joining and calving is 2.5 - 3.5 (1-5 BCS scale). This recommendation is based on science and is largely predicated on maximising reproductive or production outcomes. However, it can be argued that this is also the optimal range in terms of animal welfare. For example, at BCS < 2.5, there is an increased risk that cows will experience hunger based on the present research. Furthermore form the dairy literature, cows with a low BCS are likely to

experience discomfort during cold environments (Roche et al 2010) and calving difficulty (Gearhart et al 1990), although the latter association is somewhat equivocal (Berry et al 2007). The capacity to cope with further production or stress challenges (e.g. transport) may also be compromised when animals are in poorer condition. Matthews (2010) has demonstrated that gestational ewes at low BCS have a reduced capacity to respond to cold challenges. Above the optimal range, it is well known that when cows calve at high BCS (3.5), there is an increased risk of periparturient metabolic disorders such as ketosis (Roche et al 2010). It must be emphasised that a cow with BCS outside the optimal range does not automatically have compromised welfare, rather it provides an indication that she may be at risk of an adverse welfare outcome (eg. metabolic disorders or the inability to respond appropriately to additional challenges).

It is recommended that regular assessments of BCS are central to the effective management of welfare risks during periods of drought or reduced pasture availability in beef herds.

## **5** Success in Achieving Objectives

The objective as outlined in Section 2 has been achieved through the successful completion of the three experiments. The research focused on development of a methodology to assess feeding motivation in cattle and applying this and other body compositional and endocrinal measures to validate the utility of BCS as a practical indicator of welfare in lactating beef cows. The outcomes of this research provide scientifically defensible evidence of the impact of under-nutrition on the affective state in cattle and the validity of BCS as an indicator of welfare status. This research will also have relevance to the current process of redrafting of the Model Codes of Practice for the Welfare of Cattle into Standards and Guidelines.

In addition, three journal manuscripts arising from the 3 experiments are currently being drafted for submission to Applied Animal Behaviour Science (Experiment 1) and Physiology and Behaviour (Experiments 2 & 3) subject to the normal MLA approval processes.

# 6 Impact on Meat and Livestock Industry – now & in five years time

This research has provided new understanding of the welfare impacts of moderate and sustained under-nutrition in lactating beef cows. Further it has demonstrated that BCS is a reliable and practical indicator of body composition and therefore should be applied to monitor cattle particularly when they are in catabolic states. Regular assessments of BCS during variable nutritional conditions will enable producers to make more informed and timely decisions about when to supplement, sell or slaughter. The project has also shown evidence that BCS has relevance in the context of animal welfare and the outcomes immediately reinforce why it is important to maintain breeding females at the optimal BCS range of 2.5 - 3.5, especially the optimal minima of 2.5.

In five years' time, the peer-reviewed, internationally-published information generated by this project will continue to place the industry in good position by demonstrating its willingness to research the impacts of different animal production issues with the view to improving livestock welfare.

## 7 Conclusions and Recommendations

The key conclusions and recommendations arising from the research are:

Fat scores, based on estimates/measurements of subcutaneous fat, are inadequate in the determination of body condition, or more specifically, condition loss in lactating beef cows. A more appropriate alternative is body condition scoring which is based on both visual and manual assessments of the flesh (muscle + fat) covering the skeleton. However, currently there is no national standard for body condition scoring in beef cattle. It is therefore recommended that if body condition score is to be used as an indicator of the welfare status in cattle, there is a need to develop a national standard system that is appropriate for Australian beef cattle breeds and classes. Alternatively, given that the AUS-MEAT Fat Score System is nationally recognised, examine whether the system can be modified or augmented to better reflect both fat and muscle loss particularly around the at welfare risk category of Fat Score 1.

Assessments of BCS were moderately to highly correlated with objective indicators of fatness and muscle mass (ie. body composition). Similarly, BCS was moderately associated with endocrinal regulators of appetite and energy homeostasis (eg. leptin). It was concluded that BCS is a reliable and practical indicator of body composition and can be applied to monitor cattle particularly when they are in catabolic states.

Although there were experimental differences in significance, a consistent trend was observed where poorer conditioned or leaner cows displayed higher motivation for food, indicative of increased hunger. This is the first objective evidence of the impact of condition loss in the context of hunger and animal welfare in cattle. However, given that there were inconsistencies in statistical significance, further research is warranted to validate these initial findings and to explore whether BCS influences the animals capacity to cope with additional production challenges.

Acute condition loss at different rates did not significantly influence feeding motivation or endocrinal regulators of appetite and energy metabolism.

Body condition scoring can be practically used to assess the welfare risks associated with under-nutrition in lactating cows. There has been a long standing recommendation that cows and heifers should be maintained at a BCS of 2.5 - 3.5 in order to maximise their productivity. The evidence presented here provides additional support for the minimal threshold of 2.5 on animal welfare grounds.

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### 9 Appendices

# 9.1 Appendix 1 - Milestone 3.2 Report - Build and test cattle behavioural demand for food facility.

#### Background

It is recognised internationally that there is growing need for methodologies that can provide some objective indication of animal perceptions and feelings. The assessment of behavioural demand (BD), which is underpinned by the theory of behavioural economics, is one methodology that has proved useful in this context (Matthews 2008).

#### Aims

The aims were to construct a behavioural demand for food facility for cattle and to determine key operational conditions, specifically, food reward size and cost levels.

#### **Materials and Methods**

#### (i) Construction of BD facility

The facility was constructed close to cattle handling facilities on the CSIRO Chiswick site. The facility comprised two 50 x 6 m pens, each with a feeder and water station located at one end (Fig. 1). The external fences (1.3 m high) were constructed from plain wire and ringlock netting. Hessian was used to cover the central 1.8 m fence between the two pens and this acted as a visual barrier between the two animals during behavioural demand testing.

The feeders and feed delivery systems were designed and constructed by the Science and Engineering Workshop at the University of New England. Each feeder had two feeding bays with a wire mesh partition between them (Fig. 2ab). This enabled the animal to see through to other bay. A near infrared sensor was located in each feeding bay to detect when an animal had placed its head in the bay to feed. A feed hopper (capacity 25 kg pellets) was attached above the feeding bays and delivered pellets via 100 mm PVC tubes into each bay. A small motor attached to the base of the hopper drove an internal screw to deliver feed into the tubes. Activation of the motor was controlled by a purpose built software interface. The software had one input control to regulate the duration the motor operates for each feeding event. In addition, the software recorded when a feeding event had occurred including the date and time.

The feed delivery mechanism was initiated when an animal placed its head into a feeding bay. On breaking the near infrared beam, the motor was activated for the set duration to deliver feed into the opposite feeding bay. The animal observes this and walks around the feeder to the other bay which activates the cycle again. Multiple entries into the same feeding bay do not initiate feed delivery.

Increasing the cost in this instance was achieved by increasing the distance the animal had to walk to get to the opposite feeding bay. This was facilitated via the placement of a steel panel from the feeder which extended down the central axis of each pen (Fig 3). The distance was increased through the addition of panels.

The BD facility was designed with simplicity in mind relying on the animal's natural behaviour rather than operant control. To date, it has been shown to be robust and effective.



Fig 2a Feeder and hopper

Fig2b Feed bays separated by mesh



Fig 3 Central panel (24 m cost)

#### (ii) Feed delivery calibration

In order to test the facility, the first task was to determine the association between the motor duration and the amount of food delivered for each feeder. This was achieved by repeatedly weighing the food amount for a set duration over a range in time from 0.5 - 2 s. The time was incremented by 0.2 - 0.3 s and 30 replicates were recorded at each duration. Regression analysis was then performed to derive the algorithms to predict the feed amount for a set duration.

#### (iii) BD testing and validation

Apart from evaluating the functionality of the BD facility, the aims of this experiment were to determine the sensitivity of cattle to feed reward amount and cost levels. This would then enable the identification of the appropriate test parameters (feed reward amount and cost levels) that will be applied in the subsequent BD experiment in cows which differ in body condition.

Whilst BD has been used in animal motivation studies since the early 1980s, there has been limited application in cattle. Most of the recent research has been conducted by the AgResearch behaviour and welfare team testing motivation for food in dairy cattle (Champion and Matthews, 2006; Matthews et al 2006; Schutz et al 2006). Given the paucity of background research and the fact that our BD facility differed in design and operation from previous work, the task of identifying the initial BD test parameters was based on judgement after several discussions between the AgResearch and CLI teams. It was decided that we would test the motivation for food at two feed rewards amounts (30 and 45 g) over five cost levels (1.5, 2.2, 4.9, 10.8 and 24 m). The cost distances are linear when transformed to a log10 scale.

The minimum reward size (30 g) was calculated (using published information) so that the test animals could obtain at least their maintenance food allowance under the most stringent cost conditions (40 m). Cattle have been reported to walk between 4 and 11 km under temperate grazing conditions (Bailey et al 2008; Hessle et al 2008) and it was assumed that steers would walk up to 6 km/d in a test. A total walking distance of 6 km a day is equivalent to 250 visits of 24 m each. The maintenance requirements of a steer with a 470 kg body weight is 7.4 Mcal (31.2 MJ) per day (NRC, 2007). The energy requirements for walking horizontally are 2.6 kJ/kg bw/km,

which means that a 470 kg steer making 250 visits of 24 m needs 7.3 mJ per day for walking. The total energy requirements are then 38.5 mJ per day. The energy requirements will be equally divided over 250 rewards so that the average reward size for a 470 kg steer per visit is 154 kJ or around 19.1 g for a cattle pellet with a dietary energy content of 8.04 MJ/kg.

As can be seen the estimated feed reward size is approximately two thirds that which was used in the validation study (ie 30 g). Moreover, the results in the following conclusively show that a feed reward of 30 g was appropriate for these animals. This demonstrates that attempting to calculate feed reward size based on estimated maintenance requirements is not always accurate. Essentially it provides a guide for the estimate of feed reward but maintenance is not hard wired in the animal and the animals' motivation to seek the feed reward is not just driven by its maintenance requirements.

Six yearling Angus steers were used in the study. The cattle were maintained on pasture throughout the experiment apart from each 24 h period of BD testing. Over a two-week period prior to the commencement of the BD testing, the cattle were offered small supplements of the pellets they would receive during testing. The pellets were a commercial cattle starter ration (10.5 MJ ME/kg, 13.5 % crude protein DM). The cattle also underwent a period of training in the BD facility. It was not known how long each animal might take to learn the process of feeding in the facility, but it quickly became apparent that the cattle adapted and learnt very quickly. Training was undertaken in four stages. Initially, 2 animals were placed in each pen for 1 h without food to simply allow them to habituate to the pen environment. The following day, individual animals were placed in a pen for 1 h and pellets were manually placed in both feeder bays. This was repeated on the third day except the animals remained in the pen for 24 h. On the fourth and final stage, individual cattle spent either 3-4 h during the day or 14 h overnight in the feeders with the feeding system in operation. The feed reward was set at 40 g at minimal cost (i.e. the animal had to walk from one side of the feeder to the other a distance of 1.5 m). As noted, it was guite apparent during the final stage that the cattle had learnt the process of acquiring feed.

For the main experiment, the sequence of cost levels was randomised for each animal and half the animals received one feed reward amount at all five cost levels before being tested at the alternate feed reward amount. The other half received the feed reward amounts in reverse order.

BD was assessed over a 23-h period and there was a gap of 48 h prior to each animal being retested at a different cost level. The number of feeding events was recorded, which enabled the estimation of the total feed intake and the total distance walked during feeding.

#### **Results and Discussion**

#### Feed delivery calibration

The association between feed motor duration (s) and feed reward (g) for each feeder are shown in Fig 4.





A slight difference in the intercept and slope was found between the two feeder algorithms (Fig. 4) which can be attributed to small structural or operational differences between the feeders. The residual error was found to be constant over the range in predicted feed reward.

In the unlikely event there is a drift in the prediction equation, a safeguard has been built into the standard operating proecedure where the residual feed left in the hopper is weighed at the conclusion of each BD test. The total amount of feed eaten is determined by the difference in the amount of feed placed in the hopper (start of the test) minus the residual feed left at the conclusion of the test. This should reconcile with the estimated feed eaten based on the feed reward x no. of feed rewards.

#### BD testing and validation

Overall, the feeders and computer system functioned very well during the course of the experiment. All cattle started feeding within minutes of entry in the feeder. A sequence of photos taken during BD testing is shown in Fig 5 abcd.

A minor technical problem was encountered on two occasions requiring the specific animals to be retested. The problem was attributed to a build-up of dust etc over the infra-red sensors and reflectors which led to a malfunction in the delivery of feed. As a result, the sensors are checked and cleaned on a regular basis as part of a standard operating procedure.



Fig 5 Sequence of photos during BD testing (2.2 m cost).

The results showing the association between the log of the number of food rewards and log cost for the two feed reward amounts are shown in Figure 6a and 6b, respectively. Similarly, the association between the log total distance walked and log cost at the two feed reward amounts is shown in Figure 7a and 7b, respectively.





Fig. 7 Association between log total distance walked and log cost at a feed reward of a. 30 g and b. 45 g

The results indicate that the cattle were able to identify the small difference in feed reward based on the differences in the shape and slopes of the curves. An analysis of covariance statistically confirmed this as the interaction between feed reward x log cost (covariate) was significant (P<0.05) for both dependent measures (log number of rewards and log total distance walked).

A difference of 15 g may not seem very much but clearly the cattle could detect this. The flatter (no of rewards) and more linear (total distance walked) associations at 45 g indicate that the cattle were more motivated to work for the higher feed reward even at higher costs. To put this into perspective, at the highest cost of 24 m, the average total distance walked at 30 g and 45 g was 4.8 km and 6.5 km, respectively. It is likely that higher costs would need to be imposed at 45 g before significant reductions in reward number were evident. Given this and our desire to identify costs that are not too excessive for some animals (ie. prohibits meaningful assessment of BD), we determined that the feed reward level ought to be set at 30 g for subsequent BD testing

At a reward of 30 g there was clear effect of cost. However, whilst there was a reduction in motivation as evidenced by the increasingly negative slope of the association between total distance walked and cost, the expected classical steep negative slope at the higher costs was not apparent. It was decided to retest the animals at an additional cost of 53 m to establish whether this could be achieved. The results are presented in Figure 8ab and indicate a very pronounced decline in motivation to feed at the higher cost of 53 m.



**Fig. 8** Associations between a. number of rewards and cost, and b. distance walked and cost for a 30 g rewards and a maximum cost of 53 m.

In the results above, simple quadratic functions were fitted to the data. These are useful in that they provide a good approximation of the shape of the relationship. However, the preferred function that has been applied in animal behavioural demand studies (Hursh and Winger 1995) is as follows:

 $\ln Q = \ln(L) + b[\ln(P)] - a(P)$ 

where;

Q is the measured consumption of the resource eg. number of rewards,

P is the price (or cost) for a unit of the resource, and

L, b and a are parameters characterising the initial level of the curve at minimal price, the corresponding slope at minimal price, and the acceleration or increase in slope with increases in price, respectively.

According to Matthews (2008), elasticity (b - a(P)) is the point slope of this function and is a linear function of price. The price or cost at maximal work  $(P_{max})$  is calculated as (1+b)/a, occurs when elasticity takes the value -1.  $O_{max}$ , the total work output at  $P_{max}$  is considered the best indicator of animal motivational strength.

The data were sent to Drs Lindsay Matthews and David Duganzich (AgResearch) specifically to fit the function to determine  $P_{max}$  and  $O_{max}$ . The results based on the five original cost levels for the two feed reward amounts are presented in Figure 9.



**Fig.9** behavioural demand functions fitted to the associations between number of rewards and cost for the 30 and 45 g feed rewards.

The P<sub>max</sub> values for the 30 and 45 g feed rewards (5 cost levels) were 20.8 ± 5.8 m and 114.2 ± 287.6 m, respectively. The corresponding O<sub>max</sub> values are 4.5 ± 0.5 and 13.2 ± 25.7 km. As the function relating rewards to cost was near linear with a flat slope, the P<sub>max</sub> and O<sub>max</sub> values for the 45 g reward are unreliable. Importantly, when the 30 g feed reward data was re-analysed with the additional cost level (53 m), the P<sub>max</sub> was very similar (17.4 ± 1.0 m) to the initial estimate based on only five costs. This result provides confidence in the use of the generic demand equation and associated parameters to describe the data (when a sufficiently wide range of costs have been utilised). Moreover, this result confirms that the initial range in cost (1.5 – 24 m) was appropriate for the cattle used in the study.

#### Conclusions

Having successfully constructed a BD facility, the results of this study confirm that behavioural demand for food in cattle can be reliably and practically measured. From the validation study, it was concluded that the cattle were able to distinguish between the feed reward amounts (30 versus 45 g) as reflected in the measures of motivation. Furthermore, the initial cost levels 1.5 - 24 m were

appropriate for the class of cattle used in the study and that the addition of an extra cost did not necessarily improve the estimates of  $P_{max}$  and  $O_{max}$ .

#### Recommendations

The underlying goal of this study was to identify the operating BD protocols (feed reward and cost levels) that will be applied during subsequent BD testing of the lactating cows that differ in fat score (i.e. body condition). It is recognised that whilst the initial test protocols were appropriate, the results are nevertheless specific to young growing cattle. This raises the question how applicable are these protocols for lactating cows?

After considering this issue, our best judgement is that the BD protocols would be applicable but it is recommended that the highest cost level be increased in distance (from 24 to 40 m). Obviously, it is not known at this stage but in the event that the motivation for feed is higher in these beef cows, this adjustment will ensure that BD can still be reliably determined. It is recommended that the cost levels of 1.5, 7.7, 17.6 and 40 m be applied and the feed reward be set at 30 g. The reduction from five to four cost levels was necessary to allow completion of the BD testing within a reasonable time frame.

In a another study investigating the maximum distance lactating and non-lactating dairy cows would walk for a fixed reward of 25 g of silage, Schutz et al (2006) reported a distance of 30.7m and 70 m, respectively. The results for the lactating cows (30.7 m) is obviously more relevant in the context of the forthcoming experiment so the recommended increase of the maximal cost to 40 m seems appropriate.

These protocols will be applied for BD testing of the lactating cows which is due to commence in late April as the treatment groups have now reached the desired fat/condition score (see Appendix 1).

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Appendix 1 – Liveweight and ultrasound P8 fat depth profiles for the three fat score (FS) treatment groups.





Fat Score	P8 fat depth range (mm)
1	0 – 2
2	3 – 6
3	7 - 12

(Source: AUSMEAT)

# 9.2 Appendix 2 – Milestone 4 Report – Behavioural and physiological responses in lactating beef cows differing in fat score.

Milestone 4 Report – Submit report on the outcomes of the cattle BCS experiment 2.1.

#### Background

Australian and New Zealand livestock are subjected to periods of variable nutrition as a consequence of natural seasonal and climatic cycles. During periods of food restriction, significant losses in liveweight and body condition can occur. Whilst these losses are clearly evident, it is not known what the impacts are in terms of animal welfare.

Body condition score (BCS) and bodyweight can be practically and reliably assessed/measured onfarm and so the question remains can they also be used to provide an indication of animal welfare. To determine whether these variables can be used as scientifically defensible measures of animal welfare will require 1) assessment of the welfare state of animals differing in body condition score; and 2) the identification of key thresholds of BCS or the change in BCS that are indicative of acceptable and at-risk animal welfare.

A joint Australian and New Zealand research project was initiated with the primary goal to validate the utility of BCS as an indicator of animal welfare in two livestock classes – the lactating beef cow (Australia) and gestational ewe (New Zealand).

This report details the results of the first of two major cattle studies where the behavioural and physiological responses were evaluated in lactating cows differing in BCS. In this particular study, fat score was used to assess body condition as this is the national reference system currently used in Australia.

#### Methodology

#### Animals

A total of twenty-four pregnant Hereford cows (approx. 4-7 years) were used in the experiment. These were sourced from a local property in the New England Tablelands. After arrival at Chiswick (August 2007), the cows were maintained on improved temperate pastures right through calving. The pasture conditions ensured they maintained their average body condition score of 3. The AUS-MEAT Fat Score (FS) System (Appendix 1) was used in the experiment. Although body condition score and fat score are not completely synonymous, the AUS-Meat system was chosen since it is the national reference system. (NB: To the best of our knowledge NZ does not have a body condition scoring system for beef cattle. In lieu of this, video footage of each animal was collected monthly during the condition loss phase to enable a retrospective assessment of condition score using an alternative body scoring system).

The cows calved between 26 Sept. – 11 Dec. 2007 and calving rounds were performed twice daily to tag calves and identify their dams.

Immediately after calving, the cows were randomly allocated (after stratification for calving date, liveweight and P8 fat depth (fat score) to one of three condition loss treatments. These were designed so that the cows achieved a FS of 3 (i.e. no loss), 2 and 1-1.5 (on the 6-point AUSMEAT scale), over a 4-month period of lactation. The condition loss was achieved by reducing pasture availability through increased stocking density. For the FS 3 treatment, the cows had unrestricted access to improved pasture. To achieve a decline in FS to 2 and 1-1.5, the cows were managed at increased stocking densities.

The actual stocking densities depended on pasture growth and availability at the time, and on the fortnightly assessment of liveweight and FS of the animals. Additional supplement in the form of meadow hay was also strategically provided to ensure that weight/condition loss was achieved at the desired rate and endpoint.

#### Measurements

The cows were weighed, condition scored and ultrasound scanned at fortnightly intervals during the condition loss treatment phase. Ultrasound images were recorded at the P8 or rump site (fat depth) and over the 12/13<sup>th</sup> ribs (fat depth and *m.longissimus* area). The P8 fat depth was used as the objective determinant of fat score. The cows were assessed for body condition score (BCS) on a 9 point scale based on the Lowman (1976) system (http://www.aragriculture.org/livestock/beef/nutrition/visual\_learning\_center/body\_condition/default.ht m). This involved both visual assessment and manual palpation.

Video footage of the cattle (on exit from crush into open yard) during and at the conclusion of their condition loss treatments was recorded for qualitative behavioural assessment by Dr Trish Fleming and her team at Murdoch University.

The pasture conditions (DM% and herbage mass kg DM/ha) of the three nutritional treatments were assessed at three time-points during the 4-month treatment period.

#### Behavioural demand

Once the cows were stable at the desired FS, their motivation for food was determined using the purpose-built behavioural demand (BD) facility (refer Milestone Report 3.2). The measurement of behavioural demand has been applied in cattle to assess their motivation for a given resource such as food (Schutz et al 2006). This methodology facilitates some interpretation of the how animals perceive their state based on their overall motivation. The animal's demand for the reward, in this case, food, is obtained by measuring the change (elasticity) in the amount of food obtained as it becomes increasingly costly to access. Cost in this instance is the energy exerted as the distance the animal has to walk to gain access to the food is increased.

Groups of 8 cows and calves (approximately balanced for treatment) were moved from their paddocks into the BD facility. Each cow and calf was placed in an individual pen (3 x 6 m). The calves were allowed to freely exit and enter these pens via calf gates. The calves had access to a paddock and hay supplement adjacent to the pens. The cows were then introduced to the pelleted ration (16.2% CP, 9.0 MJ ME/kg DM) over 2 weeks and this was fed at a level estimated to achieve maintenance based on the SCA Feeding Standards for Australian Livestock – Ruminants (1990).

During the 2-week habituation phase, the cows also underwent training in the BD facility. The training was undertaken in three stages. Initially the cows were trained to simply eat out of each side of the feeders where feed was manually put in both sides. Once this had been achieved, feed was placed in the feed hopper and the feed delivery mechanism was activated (30 g feed reward). Once the cows had successfully demonstrated that they were feeding from the facility they went onto to the third and final stage. This involved imposing a small cost (1.2 m) during feeding. Initially, the cows were left in the facility for approximately 6 - 12 h during each training bout, but this was reduced to 1.5 h in the final stage once it was clear the cows were actively feeding.

BD training took longer to complete than originally anticipated and this was largely due to the poor temperament of some of the cows and their failure to habituate to the conditions in the facility. Four cows (group 2) could not be trained and were removed from the study. Another cow became lame prior to behavioural demand testing and a decision was made to also remove this animal from the study to facilitate treatment.

After the training period, the cows' behavioural demand for food was measured over a 24-h period for each energy cost level. As described previously, the energy cost was achieved by increasing the distance the animals walked to access the 30 g food reward. The energy cost levels (1.5, 7.7, 17.6 and 40 m) were randomised for each animal. At the conclusion of each behavioural demand period, the cows were returned to their pens for 4 days before returning for the next 24-h assessment of behavioural demand.

Blood samples (9 ml EDTA vacutainer) were collected via jugular venepuncture at the start and finish of each BD test for the measurement of insulin, ghrelin and leptin. Details of the methods used to quantify the concentrations of insulin, ghrelin and leptin in plasma are provided by Blache et al (2000), Miller et al (1995) and Miller et al (2009), respectively.

For the first group, the calves remained with their mothers during BD testing. The calves had access to hay within a creep area that they could access via a calf gate at one end of the BD pen. For groups 2 and 3, it was necessary to secure the calf within this creep area during BD testing as it became evident that some of the larger calves could access the food reward in the BD feeder.

At the conclusion of the BD testing, the calves were weaned and the cows were given access to high quality pasture to restore body condition.

#### Statistical analysis

The data were analysed using ANOVA and regression analysis in GENSTAT. The following function described by Hursh and Winger (1995) was fitted to the behavioural demand data:

 $\ln Q = \ln(L) + b[\ln(P)] - a(P)$ 

where;

Q is the measured consumption of the resource eg. number of rewards,

P is the price (or cost) for a unit of the resource, and

L, b and a are parameters characterising the initial level of the curve at minimal price, the corresponding slope at minimal price, and the acceleration or increase in slope with increases in price, respectively.

According to Matthews (2008), elasticity (b – a(P)) is the point slope of this function and is a linear function of price. The price or cost at maximal work ( $P_{max}$ ) is calculated as (1+b)/a, occurs when elasticity takes the value -1.  $O_{max}$ , the total work output at  $P_{max}$  is considered the best indicator of animal motivational strength.

#### **Results and Discussion**

(i) Liveweight, fat depth (fat score), muscle area and BCS

The changes in liveweight, P8 fat depth and 12/13<sup>th</sup> rib eye muscle (*m. longissimus*) area (EMA) for the three treatments are presented in Figure 1a,b and c.

There were pronounced losses in all three traits in the two condition loss groups, particularly the FS 1-1.5 group. In contrast, these traits remained relatively stable in the FS 3 group despite the increased metabolic demands of lactation. The mean loss in liveweight, P8 fat depth, EMA and BCS for the FS 1-1.5 group was 74.8 kg, 4.6 mm, 11.0 cm<sup>2</sup> and 0.44 BCS score. Similarly, for the FS 2 group the average loss was 37.3 kg, 4.0 mm, 5.8 cm<sup>2</sup> and 0.1 BCS score.

At the conclusion of the condition loss phase, the treatment differences in liveweight, ultrasound measures and BCS are shown in Table 1. Fat scores were not presented as they can be directly inferred from P8 fat depth.

Trait	FS 1 – 1.5	FS 2	FS 3
Liveweight (kg)	376.7 ± 12.8 <sup>a</sup>	415.0 ± 12.0 <sup>b</sup>	467.8 ± 12.0 <sup>c</sup>
P8 fat depth (mm)	3.4 ± 1.0 <sup>a</sup>	5.0 ± 1.0 <sup>a</sup>	11.5 ± 1.0 <sup>b</sup>
12/13 rib fat depth (mm)	$2.2 \pm 0.3^{a}$	$2.5 \pm 0.3^{a}$	$4.2 \pm 0.3^{b}$
12/13 <sup>th</sup> rib EMA (cm <sup>2</sup> )	36.5 ± 1.7 <sup>ª</sup>	$43.8 \pm 1.6^{b}$	48.5 ± 1.6 <sup>c</sup>
BCS (1-9)*	3.96 ± 0.14 <sup>a</sup> (2.20)	$4.22 \pm 0.13^{ab}$ (2.34)	4.47 ± 0.13 <sup>b</sup> (2.48)

**Table 1**Liveweight, ultrasound measurement and BCS means ( $\pm$  sem) for the three FS groups atthe conclusion of the condition loss phase

a, b, c means with different superscripts are significantly different (P<0.05)

\*Values shown in parentheses are the estimated equivalent BCS based on a 5-point scale

The differences between the FS groups for liveweight and fat score were all significant (P<0.05). However, for the fat depth measures and BCS, significant differences were only observed for the contrast between FS 1-1.5 and FS 3 treatment groups.

These results align with the well documented evidence (Seebeck and Tulloh 1968, Butler-Hogg 1984, Aziz et al 1992) that during moderate weight/condition loss in ruminants, there is a loss in both fat and muscle tissue. The relative losses of both tissues are dependent on the age or stage of maturity at the beginning of the condition loss (Butler-Hogg 1984). Intuitively, the physiological state of the animal will also be an important factor but this has not received much attention. In a study involving Merino wether lambs undergoing severe weight loss (133 g/day over 75 days), protein catabolism and therefore, loss in muscle mass, was much higher in the latter period (62%) of weight loss compared to the initial period (15%) (Aziz et al 1992). The rate of fat mobilisation was highest in the middle and latter stages of weight loss and combined it represented 95% of the total fat loss.



**Figure 1** Changes in a. liveweight, b. P8 fat depth and c. 12/13<sup>th</sup> rib EMA means (±se) for the three FS treatments over the condition loss phase.

Although the data presented in Figure 1 shows concomitant reductions in fat depth and EMA, the temporal responses differ. For the FS 1-1.5 treatment group, there was 55% reduction in P8 fat depth in the first half of the condition loss period but there was only a further 1.5% reduction in the latter half. For EMA, there was 16.3% and 13% reduction during the first and second halves of condition loss. With regard to fatness, the ultrasound measures of fat depth provide an indication of the change in one fat depot within the body, namely the subcutaneous depot. Whilst changes in other fat depots can be inferred, it is important to recognise that the rate of mobilisation of fat from each depot is not constant and internal depots such as kidney and channel fat may be mobilised faster than the carcass depots (subcutaneous and intermuscular) during weight loss (Seebeck and Tulloh 1968). The minimal change in fat depth (FS 1 – 1.5 group) during the final stage of condition loss is most likely due to depletion of fat reserves and therefore, protein catabolism is the dominant pathway for the supply of nitrogen and energy.

The correlations between the various measures at the conclusion of the condition loss phase are shown in Table 2. The only non-significant correlations (P>0.05) were observed between BCS and the two ultrasound fat depth measures. EMA was the most highly correlated measurement with liveweight.

	Liveweight	P8 fat	12/13 <sup>th</sup> fat	EMA
P8 fat	0.60			
12/13th fat	0.57	0.89		
EMA	0.78	0.58	0.46	
BCS	0.60	0.37	0.27	0.48

**Table 2** Pearson correlation coefficients between animal measures

Correlation coefficients shown in bold are significant P<0.05

Overall, these results reinforce the view that measures of fatness are not the best criteria to apply when assessing the condition of cattle during periods of under-nutrition. This was also highlighted in a review of condition scoring systems by Gaden (2005). Systems based on body condition scores which take into account the depth of flesh (muscle and fat) over the skeleton and overall fatness are clearly more useful. However, there is no national standard of body condition scoring for beef cattle (particularly for mature females) in Australia or New Zealand. It is therefore recommended that if body condition score is to be used as an indicator of the welfare status in cattle, there is a need to develop national standard systems that are appropriate for the beef cattle breeds and classes relevant in each country. As an example, a condition scoring system for cows (pregnant and lactating) was recently established in the United Kingdom (DEFRA 2001; www.defra.gov.uk/corporate/publications/pubfrm.htm).

#### (ii) Behavioural demand

The analysis of the number of feed rewards revealed significant differences due to intake group where group 2 was significantly different from groups 1 and 3. This was probably due to the smaller size of group 2. Importantly, the differences in calf management that were necessitated during behavioural demand testing did not appear to have had an effect. Unlike intake group 1, the calves from the remaining two groups had to be secured in the creep area at the end of the facility to prevent them from accessing feed from the BD feeder.
The effect of FS treatment was initially not found to be significantly different (Figure 2a). However, one of the animals was found to exert high influence in the analysis and it was subsequently removed from the analysis. When the data was re-analysed a significant effect due to FS treatment was observed (P<0.05) but only when the FS 3 treatment was compared against the pooled FS1 and FS 2 treatments (Figure 2b). This particular contrast was not *a priori* foreshadowed in the analysis, however, it is appropriate given the fact that FS treatments were predicted on P8 fat depth and there was no significant difference in fatness after condition loss (Table 1). These results indicate that the FS 3.0 animals work at a consistently lower fraction of the FS 1 and 2 animals (pooled), regardless of the cost (Figure 2b).

The  $P_{max}$  (price or cost at maximal work) values for the FS 3 and pooled FS 2 and FS 1 groups were 21.4 and 26.0 m, respectively. The corresponding  $O_{max}$  (total work output at  $P_{max}$ ) values were 1368 and 2857 m which were significantly different(P<0.05).

These results provide objective evidence that animals in poorer condition (FS 1 and FS 2) will work harder for a small food reward than better conditioned animals. By inference, this indicates that their affective state (i.e. hunger or motivation to feed) has been influenced by their condition. This is further reinforced when their feed intake during BD testing is expressed as a proportion of their normal daily feed intake (Table 3). The interaction between FS treatment x cost was not significant (P>0.05) but the main effects of FS treatment and cost were significant (P<0.01). The mean ( $\pm$ sem) feed intake ratios were 0.86  $\pm$ 0.09, 0.72  $\pm$ 0.09 and 0.44  $\pm$ 0.1 for the FS 1-1.5, FS 2 and FS 3 treatments, respectively. Relative to their daily feed intake, the FS 3 group consumed significantly less during behavioural demand than either the FS 1-1.5 or FS 2 treatments.

Overall, these results align with the preliminary behavioural demand results in pregnant ewes at different body condition scores (Matthews *personal communication*). Following on from the review by Matthews (2008), this study provides further support of the value of behavioural economics in understanding animal affective state.



**Figure 2** Association between log number of food rewards and log cost for a. all FS treatment groups and b. FS 3 and pooled FS 1-1.5 & FS 2 treatment groups.

Cost (m)	FS 1-1.5	FS 2	FS 3
1.5	1.24 ± 0.17	1.13 ± 0.16	0.78 ± 0.17
7.7	1.01 ± 0.17	0.86 ± 0.17	0.52 ± 0.22
17.6	0.81 ± 0.17	0.58 ± 0.16	0.36 ± 0.17
40	0.38 ± 0.17	0.30 ± 0.16	0.11 ± 0.17

(iii) Changes in blood ghrelin, leptin and insulin concentrations

In this study, the changes in ghrelin, leptin and insulin concentrations during BD testing were targeted because of their known regulatory roles in appetite and body energy homeostasis (see reviews by Cummings 2006, Smith and Ferguson 2008).

The concentrations of ghrelin, leptin and insulin immediately prior to and after BD testing are shown in Figure 3. The differences in insulin concentration due to intake group, FS treatment and BD cost were not significantly different at either time point. Significant differences in leptin concentration were observed pre-BD. Notable here was the significantly higher leptin concentration in the fatter FS3 group compared with the mean of the FS 1-1.5 and FS 2 groups (P<0.05). This is consistent with the literature, as leptin concentration is proportional to adipose tissue mass (Smith and Ferguson 2008).



**Figure 3** Plasma concentrations of insulin, leptin and ghrelin for the FS treatment groups pre- and post-behavioural demand testing.

The trends were similar post-BD but the differences were not significant. Ghrelin concentration was not affected by FS treatment or BD cost but a significant effect (P<0.05) due to intake group was observed. Intake group 2 had the lowest concentration followed by intake groups 1 and 3 for preand post-BD measurements.

Significant correlations between the blood measures were observed. Pre-BD insulin concentration was significantly correlated with pre-BD leptin (r=0.74; P<0.01) and post-BD leptin (r=0.59; P<0.05) concentrations. This association was expected as insulin is a known regulator of leptin secretion. The pre- and post-BD leptin concentrations were also highly correlated (r=0.7; P<0.5).

The correlation analysis between the blood measures and the number of feed rewards during BD testing only revealed one significant association. The log change in leptin concentration (ie. post-BD – pre-BD concentration) was significantly correlated with average number of feed rewards (r=0.64; P<0.05). As the difference in leptin concentration increased, there was a commensurate increase in the number of feed rewards.

Apart from this association, the results from the blood measures were not very informative with regard to understanding differences in feeding motivation. However, it should be stressed that the extension of the BD testing due to the delays associated with habituation could have influenced the results. For example, the delays meant that the intake groups differed in their stage of lactation.

# Conclusions

A main conclusion arising from this study is that fat score based on measurements of subcutaneous fat depth is inadequate in the determination of body condition, or more specifically condition loss in lactating beef cows. A more appropriate alternative is body condition scoring which is based on both visual and manual assessments of the flesh covering the skeleton and overall degree of fatness. It is therefore recommended that if body condition score is to be used as an indicator of the welfare status in cattle, there is a need to develop national standard systems that are appropriate for the beef cattle breeds (both *Bos indicus* and *Bos Taurus*) relevant in both Australia and New Zealand.

It can also be concluded that leaner cows (i.e. poorer condition) were more motivated to obtain a small feed reward compared with fatter cows. From this it can be interpreted that their overall condition or body composition is directly influencing their affective state. Intuitively, this makes sense but the real value of these results is that it now provides some objectivity about our understanding of the impact of condition loss in the context of hunger and animal welfare. It is recognised that this is only one study, and problems and delays were encountered habituating some animals to the behavioural demand facility. Therefore, some caution has to be exercised in the interpretation of the results. The results from the second planned study will therefore provide validation of these initial results as well as provide further understanding of the impact of both acute and moderate condition loss in lactating cows.

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Fat Score/ Depth mm	Picture	Manual Assessment	Visual Assessment
<b>1</b> 0-2mm		No fat around tailead. Short ribs sharp and easily distinguished. Hip bone and ribs clearly visible and hard to touch	Skeleton clearly distinguishable, spine prominent and sharp. Muscle wastage in thighs and stifle.
<b>2</b> 3-6mm		No fat around tailhead. Short ribs sharp and easily distinguished. Hip bone and ribs visible and hard to touch	Backbone clearly defined. Short ribs can be seen separately. Ribs are distinguishable.
<b>3</b> 7-12mm		Short ribs can be individually felt but feel increasingly rounded. Ribs clearly felt. Hip bone still quite hard, and only light deposit of flank fat and around tail head.	Fore ribs not noticeable but last 2 ribs can be seen. Short ribs and hips rounded. Tailhead still prominent.
<b>4</b> 13-22mm		Short ribs only felt with firm pressure. Moderate fat cover around tail head. Hip bone carrying some fat cover.	Short ribs rounded and cannot be seen separately. Area either side of tail head filling but not rounded.
<b>5</b> 23-32mm		Short ribs cannot be felt or need very firm pressure. Ribs and hip well covered. Tail head fat as slight mounds, soft to touch.	Ribs fully covered and not noticeable. Hindquarters plump and full. Abundant fat cover either side of tail head.
<b>6</b> over 32mm			Heavily covered with fat. Lumpy deposits easily detected around hips and tail head.

# Appendix 1 – AUS-MEAT Fat Score System for Cattle Table 4: AUS-MEAT 1-6 Fat scores for cattle

Source: McKiernan et al 2000. NSW Department of Primary Industries Agfact A.2.7.16

# 9.3 Appendix 2 – Milestone 7 Report – Behavioural and physiological changes in lactating beef cows following different rates of condition loss and at different BCSs



#### Milestone report

MLA project code:

B.AWW.0135

 

 MLA project title:
 BCS - Animal Welfare Objective Measures Research Program - Validation of body condition score as a practical indicator of welfare in cattle

 Project leader:
 Drewe Ferguson

 MLA project manager/coordinator:
 Keith Walker

7

# Milestone

Milestone number:

Milestone 7 - Submit report to MLA on the outcomes of different rates of condition loss in lactating cattle.

#### Abstract

This milestone reports details the results from the second major experiment of this project examining the effects of different rates of condition loss and different body condition scores in lactating beef cows. Highly significant differences in body composition and endocrinal measures relevant to metabolism and growth but not feeding motivation were observed between different BCS treatment groups. In contrast, the different rates of short term condition loss had minimal effects on both endocrinal measures and feeding motivation.

#### **Project objectives**

To validate BCS as a practical measure of welfare in cattle during periods of moderate undernutrition.

#### Success in achieving milestone

This milestone has been successfully achieved.

#### Background

Australian and New Zealand livestock are subjected to periods of variable nutrition as a consequence of natural seasonal and climatic cycles. During periods of food restriction, significant

losses in liveweight and body condition can occur. Whilst these losses are clearly evident, it is not known what the impacts are in terms of animal welfare.

Body condition score (BCS) and bodyweight can be practically and reliably assessed/measured onfarm and so the question remains can they also be used to provide an indication of animal welfare. To determine whether these variables can be used as scientifically defensible measures of animal welfare will require 1) assessment of the welfare state of animals differing in body condition score; and 2) the identification of key thresholds of BCS or the change in BCS that are indicative of acceptable and at-risk animal welfare.

A joint Australian and New Zealand research project was initiated with the primary goal to validate the utility of BCS as an indicator of animal welfare in two livestock classes – the lactating beef cow (Australia) and gestational ewe (New Zealand).

This report details the results of the second of two major cattle studies where the behavioural and physiological responses were evaluated in lactating cows differing in BCS. In this particular study, fat score was used to assess body condition as this is the national reference system currently used in Australia.

#### Aims

The specific aims of this study were to quantify the behavioural and physiological changes in cows:

- (i) undergoing different rates of condition loss
- (ii) that differ in their stable BCS.

#### Methodology

Animal ethics approval for the experiment outlined below was obtained through the CSIRO FD McMaster Laboratory Animal Ethics Committee (Authority No. 08/27).

#### Animals

Thirty-one Angus cows approximately 4 - 4.5 years of age were purchased from a property in Victoria and transported to CSIRO Chiswick in Oct 2008. The cows were purchased as being pregnant (PTIC) and this was subsequently confirmed at Chiswick. The cows were maintained on pasture and managed so that they were at a similar body condition score (BCS 3 - 4) prior to calving. For the majority of the cows this required them to lose some weight and condition as they were in very good condition on arrival (Mean P8 fat depth 17.1± 6.7 mm).

In Dec 2008 – Jan 2009, the cows were trained to use the behavioural demand (BD) facility. This particular group learnt to use the feeding system very rapidly and all cows were successfully trained.

The cows calved between 2 March and 7 April 2009 and in mid-April a subset of 24 cows were selected and randomly allocated, after stratification for liveweight, P8 fat depth and calving date to one of three condition loss treatments: no condition loss (BCS 3), a loss of 1 BCS (BCS 2) or a loss in 1.5 - 2 BCSs (BCS 1-1.5). The cows were subjected to their condition loss treatments for approximately 5 months. In this experiment, BCS rather than fat score was used given the findings from the first experiment (refer 06AR-CS05 Milestone 4 Report). BCS was assessed on a 9 point scale based on the Lowman (1976) system

http://www.aragriculture.org/livestock/beef/nutrition/visual\_learning\_center/body\_condition/default.ht m.

#### Condition loss treatments

The cows and calves were moved into large pens (27 April) and held in their BCS treatment groups (8/pen). Each group pen had two purpose-built feed troughs (2.8 m long x 0.68 m wide) which provided every animal high trough space allowance (0.7 m/cow). Calf gates were installed in each pen to allow calves to freely move to and from their pens into an adjacent paddock. The cows were gradually introduced onto the pelleted ration (16.2% CP, 9.0 MJ ME/kg DM) over two weeks and they were also given access to roughage (sorghum hay) during this period. After this period, the cows received group feed allowances that were designed to achieve the desired rate of condition loss. The feed allowances were determined after calculating their maintenance requirements (based on SCA Feeding Standards for Australian Livestock – Ruminants). These allowances were subsequently adjusted during the condition loss phase when the rate of condition loss deviated from the desired trajectory. Once the cattle reached the desired BCS, the ration was adjusted to maintain the cows at this BCS.

All cows were still lactating at the end of the condition loss phase.

#### Measurements

The cows were weighed, condition scored and ultrasound scanned at approximately fortnightly intervals during the condition loss treatment phase. Ultrasound images were recorded at the P8 or rump site (fat depth) and over the 12/13th ribs (fat depth and *m.longissimus* area). The schedule of measurements and blood sampling is shown in Table 1.

I able 1 Measuremen	t and blood sampling schedule
Date	Measurement/sampling
12 March	LWT, FD, BCS, EMA
14 April	LWT, FD, BCS, EMA
1 May	Blood sampling (chemistry)
7 May	Blood sampling (leucocyte RNA)
13 May	LWT, FD, BCS
27 May	LWT, FD, BCS, Blood sampling (chemistry)
10 June	LWT, FD, BCS, Blood sampling (chemistry)
24 June	LWT, FD, BCS, EMA, Blood sampling (chemistry)
8 July	LWT, FD, BCS, Blood sampling (chemistry)
22 July	LWT, FD, BCS, Blood sampling (chemistry)
31 July	LWT, FD, BCS,
7 August	Blood sampling (leucocyte RNA)
12 August	LWT, FD, BCS, EMA
14 August	Blood sampling (chemistry)
26 August	LWT, FD, BCS, EMA, Blood sampling (chemistry)
31 August	Blood sampling (leucocyte RNA)
9 September	LWT, FD, BCS, Blood sampling (chemistry)
24 September	LWT, FD, BCS, EMA, Blood sampling (chemistry)

 Table 1
 Measurement and blood sampling schedule

LWT – liveweight; FD – ultrasound fat depth at P8 and 12/13<sup>th</sup> rib sites; BCS – Body condition score; EMA – 12-13<sup>th</sup> rib eye muscle area

A blood sample (9 ml EDTA vacutainer) was collected approximately fortnightly for analysis of leptin, IGF-1 and insulin concentrations. Details of the methods used to quantify the concentrations of leptin, IGF-1 and Insulin in plasma are provided by Miller et al (1995) and Blache et al (2000). In addition to this, 6 x 9ml EDTA vacutainers were collected at three points over the condition loss phase for leucocyte gene expression analysis as part of a separate Beef CRC Project.

# Behavioural demand

One month after imposition of the condition loss treatments (i.e. at different rates), the motivation for food was measured in the BD facility. Three sub-groups of eight cows balanced for treatment were tested sequentially through the facility. The sub-groups were selected based on calving date with the first group comprising cows that calved earliest.

The measurement of behavioural demand has been applied in cattle to assess their motivation for a given resource such as rest (Jensen et al 2004, 2005) and food (Schutz et al 2006). This methodology facilitates some interpretation of the how animals perceive their state based on their overall motivation (Matthews 2008). The animal's demand for the reward, in this case, food, is obtained by measuring the change (elasticity) in the amount of food obtained as it becomes increasingly costly to access. Cost in this instance was the distance walked to gain access to the food. The distances to receive each 30 g food reward were the same as those used in experiment 1 (1.5, 7.7, 17.6 and 40 m). The cost levels were randomised for each animal.

Two cows and their calves were moved from their group pens and placed in individual pens adjacent to the BD facility for 24 h prior to BD measurement. This ensured they received their full feed allocation prior to testing. After this, BD was measured over a 23 h period at each cost level. At the conclusion of this, the cows and calves were returned to their group pens for 4 days before given another opportunity to work for food (at a different cost).

During the behavioural demand testing periods, the calves remained with the cows but had access to a creep-feeding area within the test facility.

After the initial BD assessment (59 days), the condition loss treatments continued until the cows reached the desired BCS (a further 35 days). After 7 days, a second BD assessment was made according to the protocol described above. The only difference was that the calves were maintained in a pen at the front the BD facility as they had reached a size where they could access the feeder in the BD facility.

Video footage of the cows during each BD assessment was recorded for qualitative behavioural assessment of the animals by Dr Trish Fleming and her team at Murdoch University. Additional video footage to enable any retrospective assessment of BCS was collected at various time points along the condition loss phase.

At the completion of the second BD assessment, the calves were weaned and the cows were returned to improved pastured paddocks to regain their condition.

#### Statistical analysis

The data were analysed using ANOVA and regression analysis in GENSTAT.

The following function described by Hursh and Winger (1995) was fitted to the behavioural demand data:

 $\ln Q = \ln(L) + b[\ln(P)] - a(P)$ 

where;

Q is the measured consumption of the resource eg. number of rewards,

P is the price (or cost) for a unit of the resource, and

L, b and a are parameters characterising the initial level of the curve at minimal price, the corresponding slope at minimal price, and the acceleration or increase in slope with increases in price, respectively.

According to Matthews (2008), elasticity (b - a(P)) is the point slope of this function and is a linear function of price. The price or cost at maximal work  $(P_{max})$  is calculated as (1+b)/a, occurs when elasticity takes the value -1.  $O_{max}$ , the total work output at  $P_{max}$  is considered the best indicator of animal motivational strength.

# **Results and Discussion**

1. Overall change in compositional measures (BCS, liveweight, fat depth, EMA)

The condition loss groups (BCS 2 and BCS 1-1.5) progressively lost BCS, liveweight and subcutaneous fat over the treatment phase (Figure 1, 2a and b, respectively). The BCS3 group which were fed to maintain condition lost some weight during the introductory stage to the new diet. It is important to note that this also coincided with the estimated timing of peak lactation. Roche et al (2009) concluded that some condition loss in inevitable during early lactation even in cows fed energy rich diets. It can be seen, however, that the cows in this group started to recover their condition loss groups, the loss in 12/13<sup>th</sup> eye muscle area (Figure 2c) proceeded more slowly with most occurring in the last third of the condition loss phase particularly for the BCS 1-1.5 group. This would suggest that cows had considerable fat reserves in other body depots at the commencement of the condition loss phase.

The mean loss in liveweight, fat depth and EMA over the entire treatment phase (condition loss + stable BCS period) for the BCS 1-1.5 and BCS 2 groups was 68.7 kg, 5.5 mm and 7.3 cm<sup>2</sup> and 47.1 kg, 6.5 mm and 1.9 cm<sup>2</sup>, respectively.

These results align with the well documented evidence (Seebeck and Tulloh 1968, Butler-Hogg 1984, Aziz et al 1992) that during moderate weight/condition loss in ruminants, there is a loss in both fat and muscle tissue. The relative losses of both tissues are dependent on the age or stage of maturity at the beginning of the condition loss (Butler-Hogg 1984). In a study involving Merino wether lambs undergoing severe weight loss (133 g/day over 75 days), protein catabolism and therefore, loss in muscle mass, was much higher in the latter period (62%) of weight loss compared to the initial period (15%) (Aziz et al 1992). The rate of fat mobilisation was highest in the middle and latter stages of weight loss and combined it represented 95% of the total fat loss.



**Figure 1** Effect of condition loss treatments on the change in BCS. Also shows phases of BS testing.



**Figure 2** Effect of condition loss treatments on a) liveweight, b) P8 fat depth and c) 12/13<sup>th</sup> rib eye muscle area (EMA)

The minimal loss in muscle mass (ie. based on  $12/13^{th}$  rib EMA) in this experiment suggests that the cattle had higher internal fat reserves prior to the commencement of the condition loss or they preferentially conserved muscle at the expense of fat tissue during condition loss. In the BCS1-1.5 group, there was a 60.7% and 10.3% reduction in P8 fat depth and EMA, respectively. By way of contrast, there was a 56.5% and 29.3% reduction in fat depth and EMA in the severe condition loss treatment group (FS1-1.5) from the first experiment (refer Project 06AR-CS01 Milestone 4 report). The mean P8 fat depth for the two experimental groups at the commencement of the condition loss were very similar (Experiment 1 - 9.0 ± 1.9 mm; Experiment 2 - 11.6 ± 0.9 mm). It's important to note that the ultrasound measures of fat depth provide an indication of the change in one fat depot, namely the subcutaneous depot. The proportions of fat in other body depots can only be inferred based on these measures and these relationships are at best only moderate. Furthermore, the rate of mobilisation of fat from each depot is not constant and internal depots such as kidney and channel fat may be mobilised faster than the carcass depots (subcutaneous and intermuscular) during weight loss (Seebeck and Tulloh 1968). Presumably, there are also differential rates of accretion between the depots.

#### 2. Effect of different rates of condition loss

One of the aims of this experiment was to examine the effect of different rates of condition loss over the initial treatment period. From Figure 1 it can be seen that differences in the rate of condition loss were achieved but the differences between the treatment groups were slightly less than expected. The rates of loss over the initial 76 days were estimated and the linear slopes for the BCS 3, BCS 2 and BCS 1-1.5 treatment groups were -0.08, -0.12 and -0.16, respectively (Figure 3). Obviously, it was the intended to maintain the slope for the BCS 3 group as close to zero however, as indicated earlier, this group lost weight and condition over the initial period despite increasing their ration.



Figure 3 Rate of condition loss over the initial 76 days of condition loss treatments.

# (i) Feeding motivation

No difference was observed in the demand profiles (Figure 4) or Pmax (price or cost at maximal work) between the treatment groups. The  $P_{max}$  values were  $31.3 \pm 11.8$ ,  $21.7 \pm 3.9$  and  $24.5 \pm 5.1$  for the BCS3, BCS2 and BCS1-1.5 groups, respectively. This indicates that differences in rates of condition loss, similar to those observed here, have not influenced motivation for feed in lactating cows. A similar lack of difference in demand for food was observed in the complementary study in gestational ewes where the differences in the rates of loss between treatments were much greater (Matthews *personal communication*).



**Figure 4** Log-log plots of number of feed rewards per cost level during feeding motivation testing in lactating cows after an initial period of condition loss at different rates.

#### (ii) Endocrine measures

The effect of the condition loss treatments on the plasma concentrations of IGF-1, insulin and leptin are shown in Figure 5abc.

Repeated measures analyses of the plasma concentrations of IGF-1, insulin and leptin over the initial 87 days of condition loss (5 blood samplings) were conducted. This period included the initial phase of condition loss and the first testing of feeding motivation. The initial (day 4) concentration was included in the model as a covariate. The results are presented in Table 2.



**Figure 5** Effect of condition loss treatments on plasma a) IGF-1, b) insulin and c) leptin concentrations.

No significant treatment differences in IGF-1, insulin or leptin concentrations were observed. Sampling time was a significant effect for all three plasma constituents and a significant treatment x sampling time interaction was found for plasma leptin concentration (P<0.001). There was no consistent trend despite the significant effect of sampling time for the three measures. The treatment x sampling time interaction for leptin revealed that on day 31, the BCS3 group had the lowest leptin concentration but by day 73, the BCS1-1.5 group had a significantly lower concentration (P<0.05) compared to the other two treatment groups. Leptin is an indicator of adiposity (Smith and Ferguson 2008) and this interaction reflects the increased loss in adipose tissue in the BCS1-1.5 treatment group relative to the other treatment groups.

concentration of IGF-1, insulin and leptin during the initial 87 days of treatme						
Effect	IGF-1 (ng/ml)	Insulin (ng/ml)	Leptin (pg/ml)			
Treatment	ns	ns	ns			
BCS 3	25.5 ± 2.5	8.71 ± 1.03	1.20 ± 0.12			
BCS 2	27.3 ± 2.2	9.46 ± 1.05	1.23 ± 0.12			
BCS 1-1.5	30.2 ± 2.3	8.71 ± 1.07	1.21 ± 0.12			
Sampling time (day)						
	P<0.05	P<0.05	P<0.05			
31	24.1 <sup>b</sup> ± 2.0	11.3 <sup>ª</sup> ± 0.9	$1.2^{ab} \pm 0.1$			
45	$30.2^{a} \pm 2.0$	10.2 <sup>ab</sup> ± 0.9	1.3 <sup>a</sup> ± 0.1			
59	30.7 <sup>a</sup> ± 2.0	$7.3^{\circ} \pm 0.9$	1.2 <sup>a</sup> ± 0.1			
73	27.1 <sup>ab</sup> ± 2.0	$8.8^{bc} \pm 0.9$	1.1 <sup>b</sup> ± 0.1			
87	26.4 <sup>ab</sup> ± 1.1	$9.2^{abc} \pm 0.9$	1.2 <sup>a</sup> ± 0.1			
Treatment x Sampling time	ns	ns	P<001			

Table 2Effect of condition loss treatment, sampling time and the interaction on the plasma<br/>concentration of IGF-1, insulin and leptin during the initial 87 days of treatment.

abc Means with a different superscript were statistically different (P<0.05).

Although the differences in plasma IGF-1 concentration were not significant, it is interesting to observe that highest and lowest levels were evident for the BCS1-1.5 and BCS3 groups, respectively. IGF-1 is a key regulator of the somatotrophic axis but this trend contrasts with the published evidence that animals on sub-optimal nutrition have lower circulating levels of IGF-1 (Steele and Elsassser 1990).

#### 3. Differences at stable BCS

#### (i) Live animal body composition and endocrine measures

The treatment group differences once the cows had stablilised at the desired BCS are presented in Table 3. The least square means presented were based on the mean of the last three measurements (day 120, 134 and 149 from the commencement of the treatment phase) with the exception of EMA where only two measurements were made during this period.

Significant differences were observed for all measures with the exception if IGF-1. This latter result was contrary to expectations given what is known about the regulatory role of IGF-1 during growth and weight loss (Steele and Elsasser 1990). Inexplicably, the plasma mean concentration of insulin

for BCS 2 was significantly higher (P<0.05) than the other two treatment groups. There was a declining trend in all other measures with the reduction in BCS (BCS3  $\rightarrow$  BCS1-1.5) with significant differences primarily being observed between the BCS3 and BCS1-1.5 groups.

Table 3	Effect of treatment on the mean of the last three measurements of liveweight, BCS,
	P8 fat depth, 12/13 EMA and plasma concentrations of IGF-1, insulin and leptin.

Measure	BCS3	BCS2	BCS1-1.5	P-value
Liveweight (kg)	$612.4^{a} \pm 10.3$	$542.2^{ab} \pm 10.3$	$539.6^{b} \pm 10.3$	<0.001
BCS*	5.1 <sup>a</sup> ± 0.2 (2.83)	$3.6^{ab} \pm 0.2$ (2.0)	3.0 <sup>b</sup> ± 0.2 (1.67)	<0.001
P8 fat depth (mm)	10.9 <sup>a</sup> ± 0.9	5.7 <sup>b</sup> ± 0.9	$4.7^{b} \pm 0.9$	<0.001
12/13 <sup>th</sup> rib EMA (cm <sup>2</sup> )*	62.4 <sup>a</sup> ± 1.9	57.8 <sup>ab</sup> ± 2.0	53.1 <sup>b</sup> ± 1.9	<0.01
IGF-1 (ng/ml)	27.9 ± 2.8	24.9 ± 2.5	24.3 ±2.6	0.66
Insulin (ng/ml)	$6.3^{a} \pm 0.9$	$9.9^{b} \pm 0.9$	7.5 <sup>ª</sup> ± 0.9	0.04
Leptin (pg/ml)	1.5 <sup>a</sup> ± 0.1	1.1 <sup>ab</sup> ± 0.1	0.9 <sup>b</sup> ± 0.1	0.05

\*Values shown in parentheses are the estimated equivalent BCS based on a 5-point scale

The correlations between the various measures (average of last 3 measurements) at stable BCS are shown in Table 4. Moderately high correlations (r = 0.6 - 0.8) were found between the body compositional measures. Of the plasma measures, leptin concentration was moderately correlated with live animal measurements.

Table 4	r cais	on coneial				ieasuies
	P8 FD	EMA	LWT	BCS	Insulin	Leptin
EMA	0.59					
LWT	0.67	0.68				
BCS	0.82	0.65	0.79			
Insulin	0.04	0.08	0.08	0.04		
Leptin	0.51	0.57	0.61	0.56	0.42	
IGF-1	0.39	0.68	0.57	0.55	0.13	0.69

Table 4	Pearso	on correlatio	on coefficie	ents betwee	en animal n	neasures

Correlation coefficients shown in bold are significant P<0.05

(ii) Feeding motivation

There was a trend suggestive of an effect of BCS on feeding motivation at higher costs (BCS1.5>BCS2>BCS3) where the BCS1-1.5 cows were more motivated than the BCS2 and BCS3 to obtain the feed reward (Figure 6). However, from the analysis, the interaction between treatment x cost was not significant (P>0.5). Similarly, there was non-significant trend for increasing  $P_{max}$  and

 $O_{max}$  with declining BCS (Table 5). Whilst there are several factors that might explain the lack of a significant effect, it is reasonable to speculate that larger differences in BCS are required before significant differences in feeding motivation might occur. However, there is a trade-off to consider here in experiments of this nature. Experimentally, it was important that to avoid extreme condition loss as this potentially may have resulted in the cessation of lactation or compromised the ability of the animal to perform the operant task, in this case, walking. Another relevant result here was that the  $P_{max}$  estimates for the BCS2 and BCS1-1.5 groups were close to the maximum cost of 40 m used in the test. This indicates that a larger cost range should have been applied in this experiment. However, it is important to stress that the costs used were the same as those used in the first experiment where the  $P_{max}$  estimates (FS3 = 21.4 m and pooled FS1-1.5+FS2 = 26 m) were well within the cost range. Given this it would appear that the overall motivation for feed was higher in the cows within the second experiment compared to those used in first study.



**Figure 6** Log-log plots of number of feed rewards per cost level during feeding motivation testing in lactating cows differing in BCS.

The trends are similar to that found in the first BCS experiment (refer Project 06AR-CS01 Milestone 4 report) where a significant difference was observed in feeding motivation when the fat score 3 was contrasted against the pooled FS2 and FS1-1.5 groups. Collectively, these results provide some insight into the inherent hunger of cows at different body condition scores. Notwithstanding the lack of a significant effect, the result of the current study that poor conditioned cows (BCS 1-1.5) were prepared to walk up to 10 km, some 3 km more than BCS3 cows, to continuously access a small 30 g feed reward, is suggestive of differences in their overall hunger. In the complementary sheep BCS

research, Verbeek et al (in preparation) reported a significant difference in feeding motivation in gestational ewes differing over a broader range in BCS (BCS 2, 3 and 4).

able 5	P <sub>max</sub> and O <sub>max</sub> values in cows differing in BCS during testing for feedin					
	Measure	BCS3	BCS2	BCS1-1.5	P-value	
	Pmax (m)	28.9 ± 5.9	40.8 ± 13.6	46.6 ± 18.8	0.50	
	Omax (km)	6.9 ± 0.7	8.8 ± 1.3	10.1 ± 1.9	0.19	

# **Table 5**P<sub>max</sub> and O<sub>max</sub> values in cows differing in BCS during testing for feeding motivation.

#### Conclusions

The results from this experiment add new objective information of the welfare impacts of undernutrition in lactating beef cows. Sustained exposure to under-nutrition resulted in highly significant differences in body composition and endocrinal measures relevant to metabolism and growth but not feeding motivation. In contrast, the different rates of short term condition loss had minimal effects on both endocrinal measures and feeding motivation.

The results from this experiment and the first experiment will now be integrated to develop recommendations with respect to the recommended minimum BCS and maximum rate of change in BCS for application within industry animal welfare standards and assurance systems.

#### Overall progress of the project

The project is on schedule for completion by the contract date in December 2010.

#### Recommendations

All recommendations arising from this AWOM project will be detailed in the final report which will be submitted in December 2010.

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