final report

Project code: SHGEN.037

Prepared by: Stephanie Knott

Primary Industries Research Victoria (PIRVic) Department of Primary Industries, Victoria

Date published: June 2005

ISBN: 1 74036 790 1

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Feed Efficiency in Composite Maternal Rams

Physiological responses of animals identified to be different in feed conversion efficiency

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

ABSTRACT

Feed costs represent the major component of livestock production costs. By increasing our understanding of why some animals utilise feed more efficiently than others, and then selecting for more efficient animals, it is possible to not only reduce feed costs but also increase productivity on the same area of land. This project identified animals, which were more susceptible to stress and then measured feed efficiency, body composition, respiration and other physiological responses in those animals. More efficient animals were found to be less susceptible to stress, utilised greater quantities of oxygen to maintain the lean tissue, and displayed different basal and hormone/metabolite stimulated metabolism in comparison to less efficient animals. By increasing our understanding of the physiological mechanisms, it may be easier to incorporate these measures into selection tools and thereby increase productivity whilst minimising any detrimental impact that selection for improved efficiency may have on other traits.

EXECUTIVE SUMMARY

Feed costs are the major cost in most animal production systems. Profitability and productivity are a function of both the inputs and the outputs, and typically ways to decrease the cost of feed or the cost of gain have focussed on genetic improvements aimed at improving the outputs eg. carcass traits, fertility and liveweight. However, it is also possible to look at ways of reducing the inputs into a livestock production system. The efficiency with which an animal utilises feed for both maintenance and gain is one such trait that can reduce the inputs into a system. Improved feed conversion efficiency (FCE) could potentially contribute to the profitability of the sheep industry by reducing the amount of feed required for lambs to reach slaughter weight or to maintain adult flocks. However, the high cost involved in setting up facilities for screening for FCE in large numbers of sheep is unlikely to be commercially applicable. Therefore it is necessary to understand the underlying differences in physiology that lead to some animals displaying greater levels of efficiency compared to others. From this knowledge, it may then be possible to identify suitable indicators (either genetic or physiological markers) associated with FCE or residual feed intake (RFI) which can then be used for selection in breeding programs at a reasonable cost.

Currently, there is very little information on why feed conversion efficiency varies between animals, and whether the variation is influenced by maturity, gender, nutrition and other environmental influences. Earlier work, conducted outside this project, but within the PhD program of the principal investigator, has shown that there is a significant relationship between RFI and an animal's response to a known stressor (adrenocorticotropin hormone, ACTH) as well as animals of known efficiency showing differences in body composition, metabolism and respiration. Following on from this earlier work, the objectives of the current work were to identify animals, which were differing in serum cortisol response following administration of a known stressor. The animals identified as extremes (High Cortisol, HC or Low Cortisol, LC) were then used in more intensive studies. The objectives of the more intensive studies were to measure feed intake and weight gain to enable feed efficiency (RFI) to be calculated; explore the relationship between stress response, feed efficiency, and respiration following the administration of thyroxine to stimulate oxygen consumption; and finally to increase our understanding of the differences that exist between animals of known feed conversion efficiency and both basal and hormone or metabolite stimulated metabolism through the use of insulin, adrenaline, ACTH and glucose infusions. As part of the final objective, tissue samples were taken for gene expression work, however this has not been undertaken.

The key achievements for this work are:

- Successfully identifying rams within an unselected population of animals that are significantly different in their serum cortisol response following the administration of a known stressor. The rams selected as extremes had significantly different basal serum cortisol levels and post-ACTH serum cortisol levels. The test used was a simple challenge, involving the use of two blood samples, and administration of a specific dose of adrenocorticotropin hormone.

- Confirmation of results found in earlier work, in that there is a significant relationship between an animal's stress response to a known stressor and its feed conversion efficiency or residual feed intake. These results show that less efficient animals (more positive RFI) are more responsive to the application of a known stressor.

- Demonstrating that there are differences in oxygen consumption, and therefore heat production between animals of different stress susceptibility (either HC or LC) and animals of different feed conversion efficiency, both basally and after administration of thyroxine.

- Demonstrating that rams of different feed conversion efficiency display differences in both basal and hormone or metabolite stimulated metabolism. This could have important implications for the development of future work in this area.

These key achievements indicate that there are a number of physiological mechanisms which are possibly driving the differences between animals in terms of how well an animal utilises the energy it consumes. Increasing our understanding of the physiological mechanisms behind why some animals are more efficient than others has clearly shown that there is a strong relationship between stress susceptibility and feed conversion efficiency. Other mechanisms such as respiration (oxygen consumption/heat production) and response to various hormones and metabolites have also been shown to be of importance, although the reason for this is currently unknown and more analyses are required of the data.

These results have important implications for the meat and livestock industry in determining alternative methods of identifying animals, which are more or less efficient than others in a particular population group. Although the practical impact on the meat and livestock industry in the immediate future is not large, due to the commercial development required to extend the results of this research, in five years time it is hoped that improvements in feed efficiency in Australia's sheep flock (both meat and fibre producing animals) are starting to have a significant economic and productive impact, through enabling producers to identify those animals which are more efficient, using these animals in selective breeding programs and thereby increasing their productivity.

It is important to understand that selection for feed conversion efficiency is likely to impact on all the mechanisms (body composition, metabolism, stress plus others not studied in this work) behind this economically significant trait. Based on this relationship, the key recommendation is that further work needs to be undertaken on both the physiological basis behind feed conversion efficiency plus expression of genes, which may be important in determining why some animals are more efficient than others in the utilisation of energy. In particular, feed efficiency and the physiological basis for this trait needs to be explored in greater numbers of sheep, and comparisons need to be made between different genotypes.

CONTENTS

1. BACKGROUND

1.1 General Background

Feed costs represent the major component of livestock production costs. As productivity and profitability are a function of both the inputs and outputs, it is necessary to consider ways to reduce the inputs and or increase the outputs. Feed conversion efficiency, which can be defined simply as gross feed conversion ratio (FCR) or feed to gain is a basic measure of feed utilisation. An alternative measure is residual feed intake (RFI). RFI is a measure of feed efficiency calculated as the difference between an animal's actual intake and its expected intake given requirements for maintenance and growth. The model adopted by the Australian beef cattle industry is where an animal's feed intake is adjusted for mean metabolic liveweight ($W^{0.75}$) and average daily gain (ADG) as measured over a specified period of time. The residual portion, which is the deviation from the expected value based on the regression, is the measure of efficiency (Koch *et al.* 1963; Archer *et al.* 1999).

An animal's utilisation of energy is affected by a number of parameters including body composition, stress, deposition and maintenance of tissues, disease status, and lactation status, stage of maturity, nutrition, genotype, thermoregulatory mechanisms and nutrient absorption. Currently, there is very little information on why feed conversion efficiency varies between animals, and how this variation is influenced by parameters such as those given. The aim of this work was to gain further understanding of the physiological differences between animals which are of differing feed efficiency and body composition through the measurement of gas exchange (oxygen O_2 in / carbon dioxide CO_2 out); stress susceptibility; glucose / insulin metabolism and tissue expression of genes associated with cellular respiration to help explain the metabolic basis for differences in feed conversion efficiency in ruminants. By increasing our understanding of why some animals utilise feed more efficiently than others, and then selecting for more efficient animals, it is possible to not only reduce feed costs but also increase productivity on the same area of land.

1.2 Stress

One of the key biological responses of stress is to increase metabolic rate and therefore energy consumption and utilisation through altering the function of the hypothalamic-pituitary-adrenal (HPA) axis (Alados *et al.* 1996; Gupta *et al.* 2004). The catabolic response to stress results in energy mobilisation, thereby providing a mechanism for stress to contribute to the lower efficiency of energy utilisation observed in less efficient animals (Elsasser *et al* 2000). Previous work has shown that rams with poor feed efficiency have a greater serum cortisol response to a known stressor such as adrenocorticotropin hormone (ACTH) than those that are more efficient (Knott *et al.* 2004). Although there is a relationship between serum cortisol levels and residual feed intake, it was unclear whether stress susceptibility or stress response alone could be used to identify animals, which are more efficient than others.

1.3 Respiration and Heat Production

Energy intake in an animal can conceptually be divided into that which is required for production, and that which is required for maintenance. Maintenance energy can be broken down into a number of functions in particular, basal metabolic rate which is the energy required in order to maintain cells within an animal's body in a functional state, together with minimal activity such as respiration and circulation enabling the animal to survive. However not all energy which goes into an animal is used, with losses of energy in faeces, urine, methane, heat increment of feeding, respiration etc. In order to study the extent to which the energy content of the food is utilised by the animal it is necessary to measure the animal's heat production. This can be achieved by the use of indirect calorimetry to measure gas exchange (respiration) and then to estimate heat production.

Manipulating maintenance energy requirements through continuous feeding and the administration of a metabolic challenge (thyroid hormones) whilst measuring gas exchange will also assist in understanding the variability that exists between animals of known different efficiency and body composition. Thyroid hormones, in particular thyroxine, have a broad action on an animal through elevating oxygen consumption. In this proposal, thyroxine will be used as the metabolic challenge.

1.4 Metabolite and Hormone Responses

Insulin, glucose and adrenaline are of interest due to preliminary results which suggest that more efficient animals have slightly different metabolite pathways and responses to these substances either endogenously and exogenously administered.

Glucose metabolism is an essential physiological function, which is controlled by the secretion of insulin and glucagon from the pancreas. The standard challenge for the ability of the pancreas to secrete insulin is a glucose tolerance test, where an excessive amount of glucose is given orally or intravenously. The clearance rate is directly proportional to the secretion of insulin and hence reflects the ability of the pancreas to respond to hyperglycaemia. Administration of insulin intravenously or subcutaneously causes blood glucose concentration to decrease and is a good measure of the sensitivity of insulin-dependent tissues to an insulin challenge. Previous results suggest that more efficient animals exhibit differences in glucose and insulin metabolism.

In addition to glucose and insulin, and animal's response to adrenaline and adrenocorticotropin hormone (ACTH) are also of interest.

Adrenaline (a combination of the catecholamines epinephrine and norepinephrine) is released into the blood stream during periods of stress, leading to increased heart rate and cardiac contractility, a dilation of the skeletal muscle blood vessels, an increase in blood pressure, increased glycogenolysis, and a dilation of the pupils and airways in readiness for the animal's flight or fight response to the stressor. Catecholamines also promote mobilisation of fuel stores to meet short-term energy demands such as those to do with the stress response.

2. PROJECT OBJECTIVES

By the 30th June, 2005, the Research Organisation will achieve the following to the satisfaction of MLA:

1. Identify animals differing in stress susceptibility and then use animals with extreme values in terms of stress response, in more intensive measurements.

2. Measure gross feed conversion efficiency and residual feed intake in selected animals.

3. Explore the relationship between animals of known feed conversion efficiency and oxygen consumption (heat production) through undertaking indirect calorimetry work with the administration of a metabolic challenge (thyroxine).

4. Increase our understanding of the differences that exist between animals of known feed conversion efficiency and glucose / insulin metabolism and the expression of the GLUT4 glucose transporter gene.

3. METHODOLOGY

3.1 Timeline of Activities

The experiment comprised of five different periods covering a time period of twenty weeks in total (Appendix 1 – Experimental Timeline). Period 1 was undertaken on a commercial producer's property and involved one hundred animals. Periods 2 – 6 were undertaken in the Animal House located at DPI Hamilton and involved twenty-four animals selected on results collected during Period 1. One of the selected animals died part way through the project, so measurements were collected on twenty-three animals only.

3.2 Objective 1 – Identifying animals

3.2.1 Animals

One hundred (100) maternal sire cross-bred rams from a pedigreed stud flock (initial age 415 ± 17 days; initial weight 52.9 ± 4 kg) were used in a screening test to identify animals which are more or less susceptible to stress. Full pedigree data is available on these animals (LAMBPLAN indices and estimated breeding values, EBVs).

3.2.2 Adrenocorticotropin Hormone (ACTH) Induced Stress Response Selection Test

An adrenocorticotropin hormone (ACTH) induced stress response was used as a selection test to identify animals with extreme responses. Selected extreme animals were then used in more intensive measurements.

One hundred rams were weighed at the start of the week. Two days later, ACTH induced stress responses were measured in all animals. All sheep received 2 µg/kg LW of ACTH (Synacthen) drawn into a 1mL syringe. The remaining volume of the syringe was filled with sterile saline solution so that the total volume injected remained equal for each animal. Rams were randomly allocated into five groups of twenty, with groups sampled as pairs simultaneously, and the last group sampled as a single group. ACTH was injected intramuscularly into the rump of the ram between 0942 and 1410 and the time recorded for each animal. Blood samples were collected immediately pre- and 45 minutes post-injection and the time of collection recorded. Blood samples were taken by jugular venipuncture and collected into tubes containing SST gel and clot activator additives (Vacutainer®).

3.2.3 Cortisol Assay

Commercially available RIA kits (Orion Spectria Cortisol RIA) that had been modified slightly (an additional low standard of 2 nmol/L was included, incubation time reduced to 30 min) were used to determine serum cortisol concentrations. For the following levels (nM/L) of cortisol, 90 nM; 424 nM; 723 nM, the respective between assay coefficients of variation (CV) were 7.4%, 8.8% and 11.1%. The corresponding within assay CVs were 6.5%; 8.5% and 10.1% respectively.

3.2.4 Selection of Rams

Rams were ranked on serum cortisol levels after the administration of ACTH. Rams which weighed less than 45kg or greater than 60kg were excluded from the selection process, as were rams born either before the 25th August or after the 25th September. Those animals with either the highest or lowest post-ACTH serum cortisol levels were selected to be used in Objectives 2, 3 and 4.

3.3 Objective 2 – Measuring feed efficiency

3.3.1 Intake Measurements

Rams were fed a formulated ration on a daily basis for the duration of the experiment. Initially a concentrate-based pellet was used, but problems relating to storage of these pellets (development of mould), lead to the feeding of a concentrate based dry mash mixture, identical to the pellet formulation, however not pelletised. The metabolisable energy (ME) content of the mash and pellets was 12.5 MJ/kg DM, crude protein (CP) content of 14.5% DM and a neutral detergent fibre (NDF) content of 23% DM. A mixture of dry mash and pellets was fed for six days (Day 23 – Day 28) with the ratio of dry mash to pellets increasing, until the diet consisted solely of the dry mash. Dry mash was fed as the sole ration until further problems developed with the feed, following the diagnosis of obstructive urolithiasis in one ram due to a mineral imbalance in the both the previously feed pellets and the dry mash. For the remaining 19 days (Day 31 – Day 49) rams were fed a mixture of one-third dry mash to two-thirds lucerne chaff to reduce the mineral intake. The lucerne chaff had a ME content of 9.6 MJ/kg DM, CP content of 19.7%DM and a NDF content of 41.3%DM.

During Period 1, pellets were fed out to the sheep while they were in the paddock. The amount fed during this period was approximately 13kg every second day. During Period 2, in the animal house, rams were fed pellets at an increasing level until *ad libitum* levels were reached by each of the animals. The *ad libitum* level was maintained until the end of Period 3, although the ration constituents changed as described above.

From the conclusion of Period 3 for the remainder of the experiment, animals were fed a lucerne / grain pellet with no mineral or vitamin additives (9.4MJ/kg DM ME, 9.9% DM CP and 38.3% DM NDF). This pellet was fed at maintenance plus 10% ($ME_{m+10\%}$) based on the individual live weights of each animal and calculated through the use of the following equation (SCA 1990).

ME_m (MJ/d) = {K.S.M. ($0.28W^{0.75}$exp(-0.03A))} / k_m

Where K = 1.0 for sheep and goats; S = 1.15 for entire males; M = 1; W = live weight in kg; A = age in years with a maximum value of 6; and k_m = net efficiency of use of ME for maintenance.

Due to problems with weather causing animals to go off feed (extreme humidity and high temperatures for four days in a row in early December), FCR and RFI was calculated on 40 days worth of data rather than the preferred 49 days.

3.3.2 Weight Measurements

All sheep (n=100) were weighed during the selection process in Period 1, to establish appropriate doses of ACTH. Selected sheep were weighed on entering the Animal House at the start of Period 2, and then weekly for the duration of Period 2. At the start and end of Period 3, rams were weighed on two consecutive days to give an average start and end weights, and then throughout this period were weighed every four days.

3.3.3 Dual Energy X-ray Absorptiometry Scanning

Sheep were scanned using dual energy x-ray absorptiometry (DXA) technology during Period 2 and at the end of Period 3. Sheep were weighed on the morning of the day prior to scanning to obtain accurate liveweights for anaesthesia. Rams were separated into two groups of twelve, with each group scanned on consecutive days. Feed was removed from animals between 1500 and 1600 on the day prior to scanning. On the day that sheep were scanned, animals were transported to the DXA facility at PIRVic – Werribee.

Anaesthesia was induced by an appropriate intravenous dose of thiopentone sodium at 10-15 mg/kg LW. Depth of anaesthesia was initially monitored by eye reflex following induction. Animals were then scanned using DXA to accurately quantitatively measure whole body composition in the live animal.

Following scanning, animals were returned to holding pens where they were allowed to recover from the anaesthetic. Animals were closely monitored at regular intervals throughout the day and for an hour after the last animal had been scanned, before being transported back to PIRVic – Hamilton, where on arrival they were returned to pens in the animal house.

3.4 Objective 3 – Measuring respiration

3.4.1 Selection of sheep

Only twelve sheep were measured for oxygen consumption. These sheep were selected based on their initial cortisol group (ie. 6 high cortisol, 6 low cortisol) and their FCR value calculated from intakes and weight gains achieved during Period 3.

3.4.2 Indirect Calorimetry

Carbon dioxide production, oxygen consumption and respiration rate were measured using an open circuit indirect calorimetry system with data recorded automatically using a PowerLab real time data acquisition unit (ML870B80 Exercise Physiology System) and Chart™ 5 software with the additional MLS240 Metabolic module (ADInstruments Pty Ltd. Castle Hill, NSW, 2154 Australia). During this period, all animals were fed at maintenance plus 10% ($ME_{m+10\%}$) ration, which was equally divided between a morning feed fed at approximately 7am (AM), and an afternoon feed, fed at approximately 5pm (PM). Measurements were made on twelve rams only, as these animals were identified as the extremes in terms of feed conversion efficiency from data collected during Period 3. Two rams were measured each day. L-Thyroxine was administered during the course of the respiration measurements in order to increase oxygen consumption.

Rams were trained to wearing facemasks to facilitate respiration measurements, and were restrained using collars and chains during measurements. Masks were worn for the duration of each measurement run (25 minutes) and then removed, enabling sheep access to water.

On the first day of measurements, rams were not given their AM feed. Six twenty-five minute respiration measurement runs were undertaken with the start of each one an hour apart. Each run consisted of a five-minute adaptation period and a twenty-minute measurement period. At the conclusion of the last run, animals received 300µg/kg LW L-thyroxine (L-Thyroxine sodium salt pentahydrate, T2501, Sigma-Aldrich, Castle Hill, NSW, Australia, 2154) dissolved in 3ml of 0.0135M sodium hydroxide/saline, given as an intramuscular injection into the rump (Chandrasekhar *et al,* 1986; O'Callaghan *et al,* 1993). They were then fed their PM feed.

The second day of measurements, no respiration measurements were made, instead, rams were fed their AM feed, given an injection of 300µg/kg LW L-Thyroxine at 10am and then given their PM feed in the evening. On the third day of measurements, rams were not given their AM feed. Three twenty-five minutes respiration measurement runs were undertaken with the start of each run an hour apart. At the conclusion of the third run, rams received their final 300µg/kg LW L-thyroxine injection. Following the injection, six twenty-five minute runs were undertaken and then rams received their PM feed.

Analysis of the data gave the total volume of expired air (VE_T), oxygen consumption (VO2), carbon dioxide output (VCO2) and respiratory exchange ratio means for each ten-second interval over the twenty-minute measurement period.

3.5 Objective 4 – Glucose/insulin metabolism and gene expression

3.5.1 Catheter Preparation

Rams were prepared with two indwelling polyethylene jugular catheters (Tyco Electronics / BIOCORP Aust. Pty. Ltd., Huntingdale, VIC. 3166, i.d. 1.0mm, o.d. 1.5mm). The catheters were inserted the day before the commencement of the metabolite/hormonal challenges. Catheters were checked on a daily basis and flushed with a solution of heparin saline to maintain patency.

3.5.2 Glucose Challenge

For the glucose tolerance test, basal blood glucose levels were determined in animals through taking small samples (approx. 5mL) every 15 minutes for 1 hour (-60, -45, -30, -15, -1min) prior to administration of glucose. A bolus of glucose administration intravenously at a dose rate of 0.4g/kg LW of a 50% glucose solution (dextrose) causes an increase in blood glucose. Repeated blood samples (5mL) were taken at 3, 6, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 105 and 120min post glucose administration. Repeated blood samples taken after the challenge enable the clearance rate to be established, and this is directly proportional to the secretion of insulin and hence reflects the ability of the pancreas to respond to hyperglycaemia and produce insulin.

3.5.3 Adrenaline Challenge

To look at the impact of adrenaline on various parameters, epinephrine was given at two different dose rates (0.1µg/kg LW and 2.0µg/kg LW) to all sheep on the same day (Sechen *et al.* 1990). Epinephrine was injected via the jugular cannula, immediately followed by 4ml of sterile saline. Blood samples (5ml) were taken every 15 minutes for 1 hour prior to the administration of the lower dose of epinephrine and then at 3, 6, 10, 15, 20, 30, 45, 60, 90, 120, 150, 165, 170 and 179 minutes relative to the infusion. The last three samples taken following the administration of the lower dose were used as the basal measurements for the higher dose. The higher dose was administered intravenously 180 minutes post the infusion of the lower dose and a similar post-infusion sampling regime was undertaken. The changes in glucose, lactate, glycerol and NEFA over time enable the determination of the sensitivity of the animal to increased epinephrine levels and thus the ability at which an animal mobilises energy stores.

3.5.4 Insulin Challenge

For the insulin tolerance test, basal blood glucose levels were determined in animals through taking small samples every 15 minutes for 1 hour prior to administration of insulin. Insulin ('ActRapid') was administered intravenously at a rate of 0.5IU followed by 4mL saline. Repeated blood samples were taken at 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240 and 300min post insulin administration. The rate of decline in glucose following insulin administration reflects the combination of suppression of hepatic glucose output and stimulate of peripheral glucose uptake by insulin.

3.5.5 Adrenocorticotropin hormone (ACTH) Challenge

To determine whether individual ram's response to a known stressor had changed during the course of the experiment, a second stress challenge was imposed on the selected animals (n=23).

Following the other hormonal and metabolite challenges, rams received 2.0μg/kg LW of ACTH ($ACTH_{1-24}$, Synacthen, Novartis Pharmaceuticals Australia Pty Ltd, North Ryde, NSW, Australia), equivalent to 2.0μg/kg LW, drawn into a syringe. The remaining volume of the syringe filled with sterile saline solution, so that the total volume injected remained equal for each animal. The ACTH was injected intramuscularly into the rump of the animal and the time recorded for each animal. Serial blood samples were collected at every 15 minutes for an hour prior to ACTH administration and at 30, 45, 60, 75 and 90 minutes after ACTH administration. Blood samples were collected into tubes containing SST gel and clot activator additives (Vacuette™). Tubes were left at room temperature for an hour for the clot to form properly and then stored at 4°C overnight for the clot to retract to enable maximum serum yield. Whole blood was centrifuged at 1800 x g for 15 min at 4°C (Technospin R, Sorvall Instruments, DuPont), serum decanted and stored at -80°C until assayed for cortisol.

3.5.6 Metabolite and Hormone Assays

Muscle Biopsies

Muscle samples were taken from all sheep (n=23) seven days after the second DXA scan. An area 20cm^2 was clipped over the right hind leg over the groove between the *semitendinosus* (ST) and *semimembranosus* (SM) muscles. Rams were given an intramuscular dose of xylazine at 0.1mg/kg LW to cause mild sedation and were restrained against a pen wall. The clipped area was scrubbed using an iodine solution and wiped with gauze soaked in 70% ethanol. Local anaesthetic (Lignocaine 20, 2-3ml) was infiltrated under the skin over the incision area. A single 'stab' incision was made through the skin and the biopsy needle passed into the SM muscle, with a simultaneous vacuum applied to hold the muscle sample within the biopsy drill. The biopsy drill uses an 18 gauge x 38mm needle to take a sample. The drill is similar to a captive bolt stunning device, but instead it has a cutting cannula for the sample. Following biopsy, the wound was sprayed with a topical antibiotic and all rams received a 4ml intramuscular injection of a broad spectrum, long acting antibiotic (Illium Oxytet – 200 LA).

Once samples were collected, they were placed in labelled RNA-free sterile microtubes and submerged in liquid nitrogen for immediate freezing. Once frozen, they were transferred to a -80°C freezer for storage for future analysis. Gene expression analysis for the GLUT4 glucose transporter gene has not been undertaken to date, due to the time constraints placed on the PhD candidature.

4. RESULTS AND DISCUSSION

4.1 Objective 1 - Identifying animals

4.1.1 Results

Table 1: Mean serum cortisol levels (± s.d) pre and post ACTH administration for all sheep (n=100), and selected extreme sheep (n=12 for both groups). Different subscripts in columns denote significant differences between HC and LC groups only.

Group	Pre ACTH serum cortisol (nM)	Post ACTH serum cortisol (nM)	Incremental change in serum cortisol (nM)
All sheep	67.1 ± 40.6	165.3 ± 44.1	98.1 ± 48.8
High cortisol	85.8 ± 56.0[a]	215.6 ± 18.0[a]	129.8 ± 53.8[a]
Low cortisol	59.0 ± 16.1[a]	113.0 ± 15.1[b]	54.0 ± 20.2[b]

[a,b] denotes $P<0.001$

4.1.2 Discussion

These results show that it is possible for an unselected population of animals to have a large range in their cortisol response to a known stressor and that based on these results it is possible to select out extreme animals based on a simple stress challenge and for these animals to be significantly different in both their basal serum cortisol concentration, but also their post ACTH serum cortisol concentration.

4.2 Objective 2 - Measuring feed efficiency

4.2.1 Results

Significant differences were observed between the high (HC) and low (LC) cortisol groups for RFI (Table 1). There was a significant correlation between RFI and post ACTH serum cortisol levels (r = 0.424, P < 0.05).

Table 2: Mean ± s.d. values for High Cortisol (HC, n=11), Low Cortisol (LC, n=11) and all selected sheep (n=22) for RFI, FCR, basal cortisol, post ACTH cortisol and incremental cortisol response. Different superscripts in rows denote significance between HC & LC only

	High cortisol (HC)	Low Cortisol (LC)	All sheep
ADG (g/day)	360 ± 80[a]	310 ± 120[a]	330 ± 100
DMI (kg DM/day)	2.07 ± 0.12[a]	1.75 ± 0.43[a]	1.91 ± 0.35
FCR (kg feed:kg gain)	6.44 ± 1.5[a]	5.64 ± 1.4[a]	6.04 ± 1.5

	High cortisol (HC)	Low Cortisol (LC)	All sheep
RFI	0.45 ± 0.8[a]	-0.35 ± 1.0[c]	-0.05 ± 1.0
Basal cortisol (nM)	79.5 ± 54[a]	59.5 ± 17[a]	69.48 ± 40
Post ACTH cortisol (nM)	215.6 ± 18[a]	113.3 ± 16[b]	164.5 ± 55
Incremental cortisol (nM)	136.1 ± 52[a]	53.9 ± 21[b]	95.0 ± 57

[a,b] $P<0.001$; [a,c] $P=0.057$

Body composition measurements, lean tissue mass (LTM), fat tissue mass (FTM) and ash (ASH) were not significantly different between the two cortisol groups, except for the final FTM, and the total change in FTM. The HC rams had a significantly higher level of FTM at the end of the experiment in comparison to the LC rams (HC, 11.22kg; LC, 9.00kg; l.s.d 1.79, $P<0.05$). RFI was significantly correlated to the change in FTM (r=0.410, $P<0.05$). There were no significant correlations between RFI and LTM at either the start of the end.

4.2.2 Discussion

These results clearly show that there is a significant relationship between stress susceptibility or stress response and feed conversion efficiency, and that screening animals based on stress response may be an option for identifying animals which are likely to be more efficient. In regards to the body composition measurements, these results confirm earlier work, in that there is no relationship between RFI and lean tissue mass. Further analysis and discussion of these results will be undertaken in the PhD thesis.

4.3 Objective 3 - Measuring respiration

4.3.1 Results

Preliminary analysis of the VO_2 consumption data has shown some interesting differences between groups of animals as shown in Figures 1 and 2. The full analysis of this data, which will be included in the PhD thesis is yet to be undertaken.

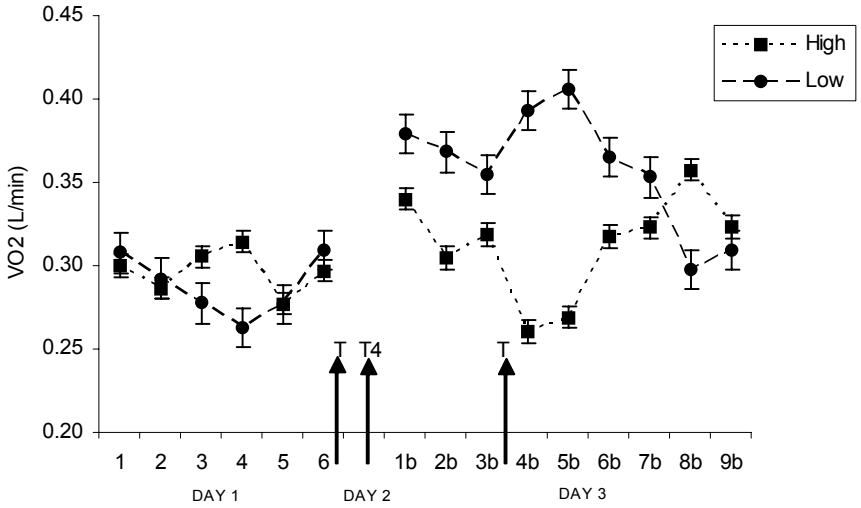


Figure 1: Mean ± s.e. oxygen consumption (VO2, L/min) for high cortisol (High), low cortisol (Low) and all sheep before and after three injections of thyroxine (T4).

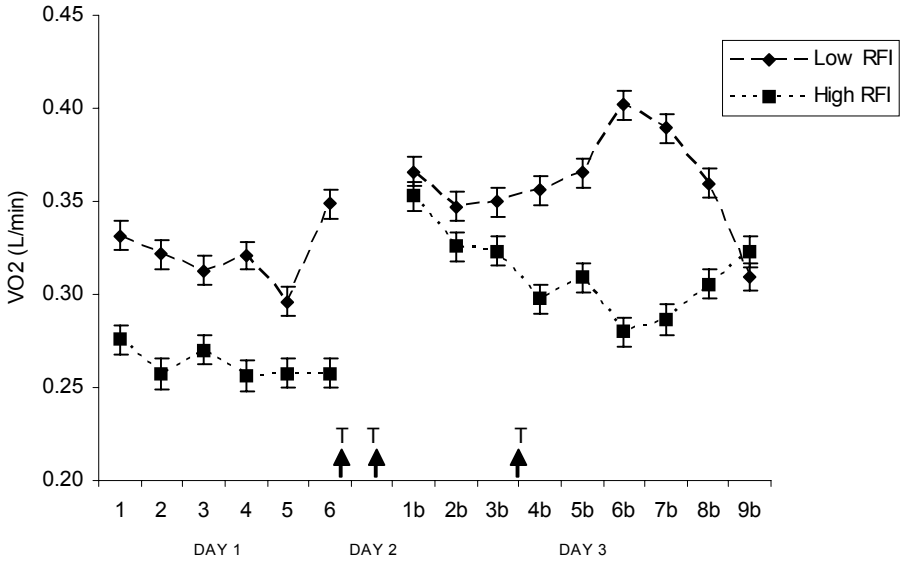


Figure 2: Mean ± s.e. oxygen consumption (VO2, L/min) for low RFI (ie. more efficient) rams (n = 6), high RFI (n=6) and all sheep before and after three injections of thyroxine (T4).

4.3.2 Discussion

Preliminary results indicate that there are significant differences between animals in terms of oxygen consumption both before and after successive thyroxine injections. More efficient animals, which also tend to have lower cortisol levels have increased oxygen consumption following administration of thyroxine. This may be due to increased sensitivity of these animals to thyroxine, but it is also possible that the low RFI animals actually have a greater proportion of lean tissue, which is energetically more expensive to maintain, and thus requires higher levels of oxygen to be consumed. A thorough analysis and discussion of the results will be included in the PhD thesis.

4.4 Objective 4 – Glucose/insulin metabolism and gene expression

4.4.1 Results

Preliminary analysis of the results from the metabolite and hormone challenges indicates that there are significant differences between animals in regards to their glucose, insulin, adrenaline and cortisol metabolism. Key results are shown in the figures below (Figure 3 – 6).

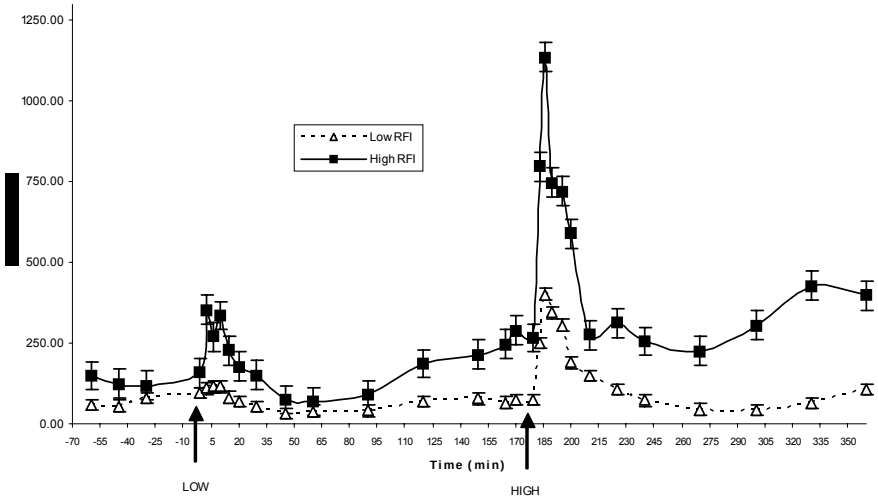


Figure 3: Mean ± s.e non esterified fatty acid (NEFA) plasma concentrations in selected extreme high RFI (n=5) and low RFI (n=5) following the administration of either a low dose (LOW) or high dose (HIGH) of adrenaline.

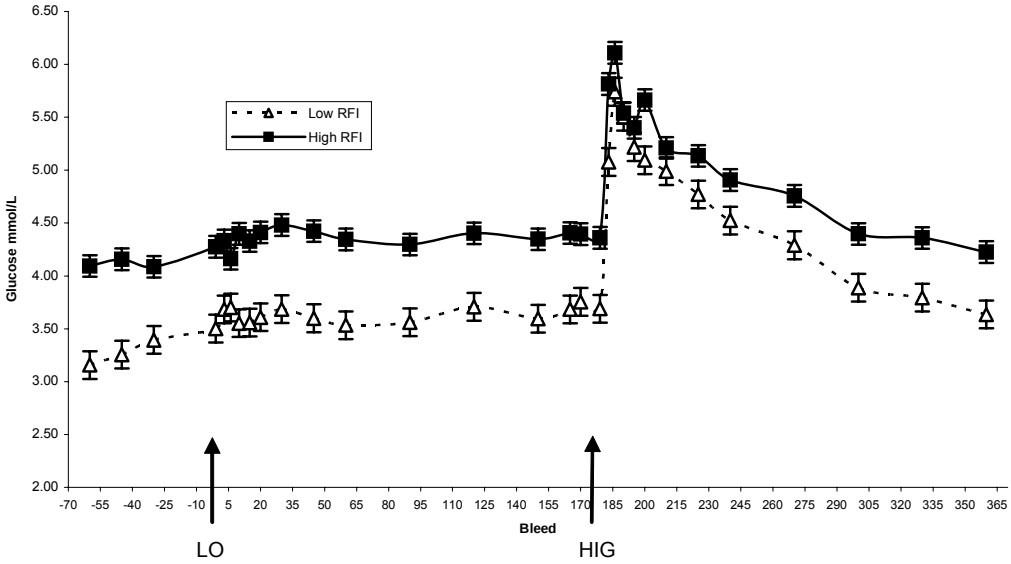


Figure 4: Mean ± s.e. plasma glucose concentrations in selected extreme high RFI (n=5) and low RFI (n=5) following the administration of either a low dose (LOW) or high dose (HIGH) of adrenaline.

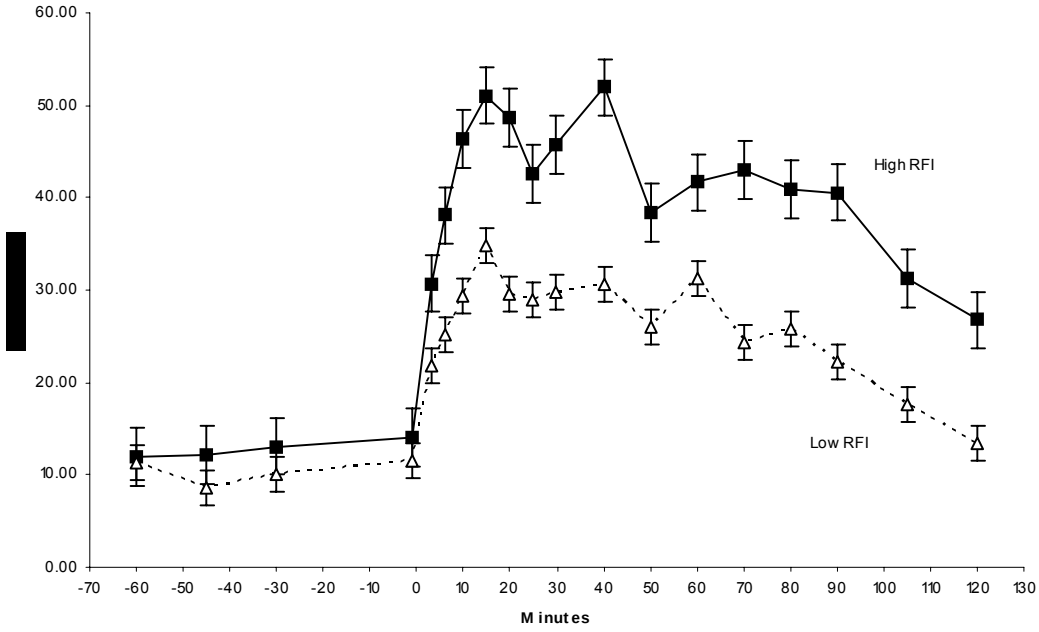


Figure 5: Mean ± s.e plasma insulin concentrations in selected extreme high RFI (n=5) and low RFI (n=5) following the administration of glucose.

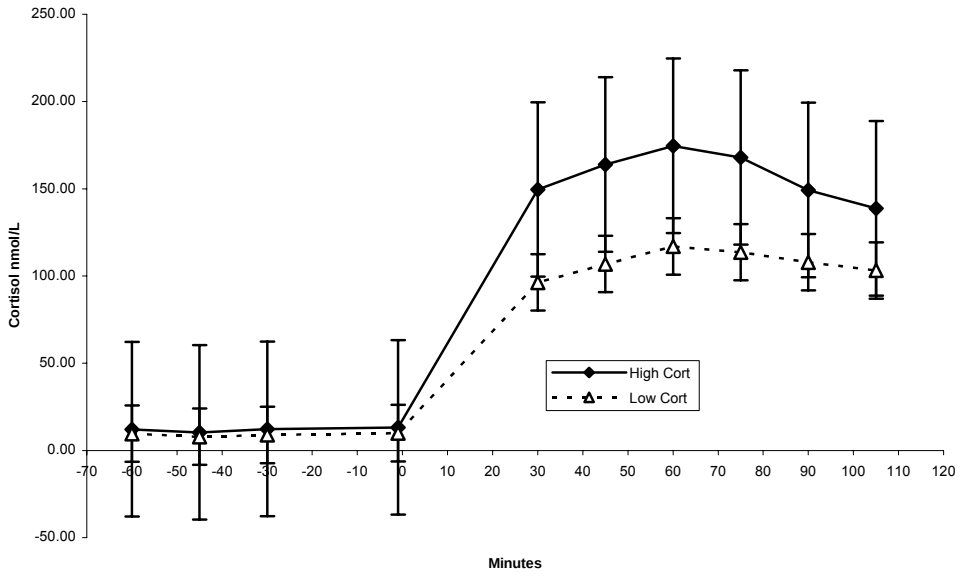


Figure 6: Mean ± s.e plasma glucose concentrations in selected extreme high RFI (n=5) and low RFI (n=5) rams following administration of insulin.

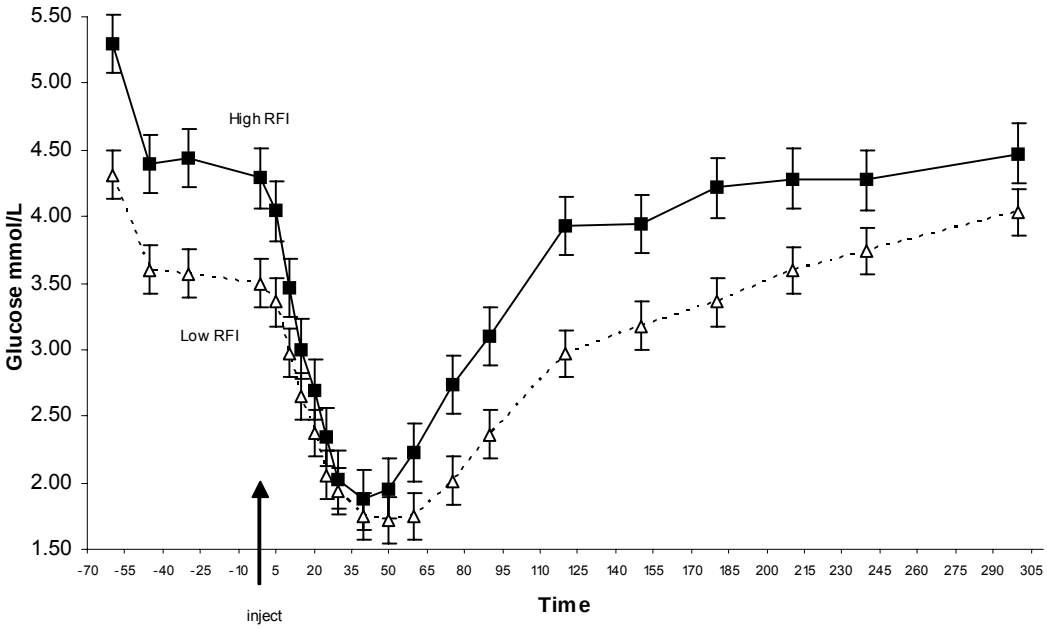


Figure 7: Mean (± se) serum cortisol concentrations in selected extreme high RFI (n=5) and low RFI (n=5) rams following administration of adrenocorticoptropin hormone at time 0min. .

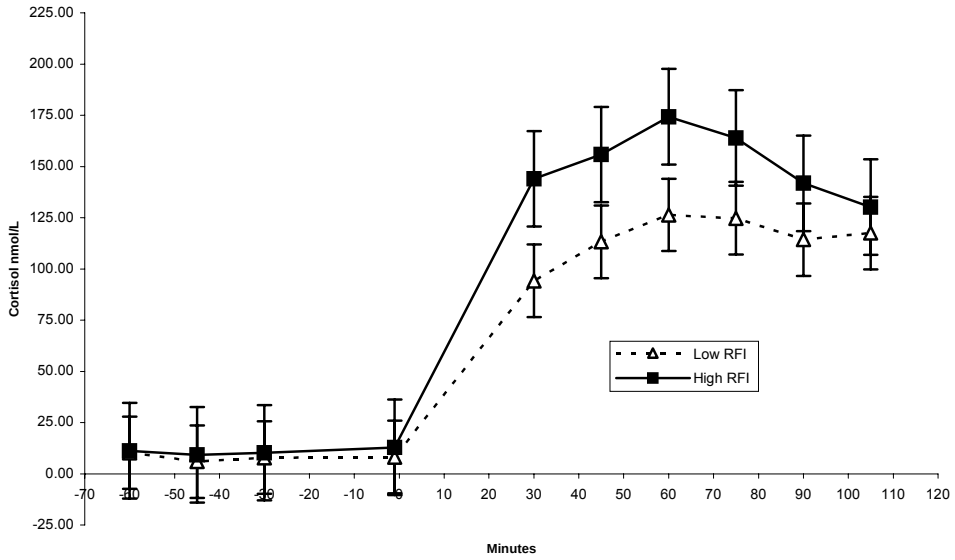


Figure 8: Mean (± se) serum cortisol concentrations in the high cortisol (High Cort, n=11) and low cortisol (Low Cort, n=11) rams following administration of adrenocorticotropin hormone.

4.4.2 Discussion

More efficient animals are less responsive to exogenous insulin, glucose, adrenaline and adrenocorticotropin hormone administration in comparison to less efficient animals. Basal plasma glucose concentrations between extreme RFI animals are significantly different, which confirms trends found in earlier work suggesting that more efficient animals have slight differences in their glucose metabolism. It is interesting to note, that although the differences are not significant, animals which were identified as either low or high cortisol in the initial screening process, retain the same relative group at the end of the experiment (Figure 8). Although some results are presented in this final report, the data collected as part of this objective needs further analysis in order to fully determine the significance and implications of the results.

5. SUCCESS IN ACHIEVING OBJECTIVES

The objectives have been achieved, however Objective 4 has only been partially achieved due to time constraints within the PhD candidature of the project leader. Full details and results of all activities relating to this project plus other work, which has been undertaken in conjunction with this project, are currently being analysed, discussed and collated in Stephanie's thesis. MLA will receive a copy of this thesis as Stephanie is in receipt of a MLA postgraduate studentship. MLA has acknowledged that the results presented in this document represent a high level summary of the data analysis to date (H. Oddy, *pers. comm.*)

Background details of the project and the results from the first two objectives have been presented at the annual MLA postgraduate workshop, and will be presented at the Recent

Advances in Animal Nutrition conference to be held in Armidale in July. Abstracts have been written for these two events (Appendix 3). Preliminary results from all four objectives were presented at the Sheep Genomics Muscle and Energy Utilisation Sub-Program Scientific Advisory Committee meeting held in Brisbane in late June.

6. IMPACT ON MEAT AND LIVESTOCK INDUSTRY – NOW & IN FIVE YEARS TIME

Increasing our understanding of the physiological mechanisms behind why some animals are more efficient than others has clearly shown that there is a strong relationship between stress susceptibility and feed conversion efficiency. This has important implications for the meat and livestock industry in determining alternative methods of identifying animals, which are more or less efficient than others in a particular population group.

Although the practical impact on the meat and livestock industry in the immediate future is not large, due to the commercial development required to extend the results of this research, in five years time it is hoped that improvements in feed efficiency in Australia's sheep flock (both meat and fibre producing animals) are starting to have a significant economic and productive impact, through enabling producers to identify those animals which are more efficient, using these animals in selective breeding programs and thereby increasing their productivity.

7. CONCLUSIONS AND RECOMMENDATIONS

Although this final report, is a high-level summary of the results to date, and further work is required in analysing the results and discussing the implications of them, it is possible to conclude that feed efficiency is driven by a number of different traits, and not one individual trait can be classed as the main driver of why some animals are more efficient than others. It is important to understand that selection for feed conversion efficiency is likely to impact on all the mechanisms (body composition, metabolism, stress plus others not studied in this work) behind this economically significant trait. Based on this relationship, the key recommendation is that further work needs to be undertaken on both the physiological basis behind feed conversion efficiency plus expression of genes, which may be important in determining why some animals are more efficient than others in the utilisation of energy. In particular, feed efficiency and the physiological basis for this trait needs to be explored in greater numbers of sheep, and comparisons need to be made between different genotypes.

BIBLIOGRAPHY

Alados CL, Escos JM, Emlen JM (1996) Fractal structure of sequential behaviour patterns: an indicator of stress. *Animal Behaviour* **51**, 437-443.

Archer JA, Richardson EC, Herd RM, Arthur PF (1999) Potential for selection to improve efficiency of feed use in beef cattle: a review. *Australian Journal of Agricultural Research* **50**, 147-161.

Chandrasekhar, Y, D'Occhio MJ, Setchell BP (1986) Reproductive hormone secretion and spermatogenic function in thyroidectomized rams receiving graded doses of exogenous thyroxine. *Journal of Endocrinology* **111**, 245-253.

Elsasser T, Klasing K, Filipov N, Thompson F (2000) The metabolic consequences of stress: targets for stress and priorities of nutrient use. In 'The biology of animal stress: basic principles and implications for animal welfare.' (Eds G Moberg and J Mench) pp. 77-110. (CABI Publishing: Wallingford)

Gupta S, Earley B, Ting STL, Leonard N, Crowe MA (2004) Technical note: Effect of corticotropin-releasing hormone on adrenocorticotropic hormone and cortisol in steers. *Journal of Animal Science* **82**, 1952-1956.

Knott SA, Leury BJ, Brien FD, Cummins LJ, Dunshea FR (2004) Less efficient sheep are more responsive to an ACTH induced stress challenge. *Asia Pacific Journal of Clinical Nutrition* **13**(supp), S89

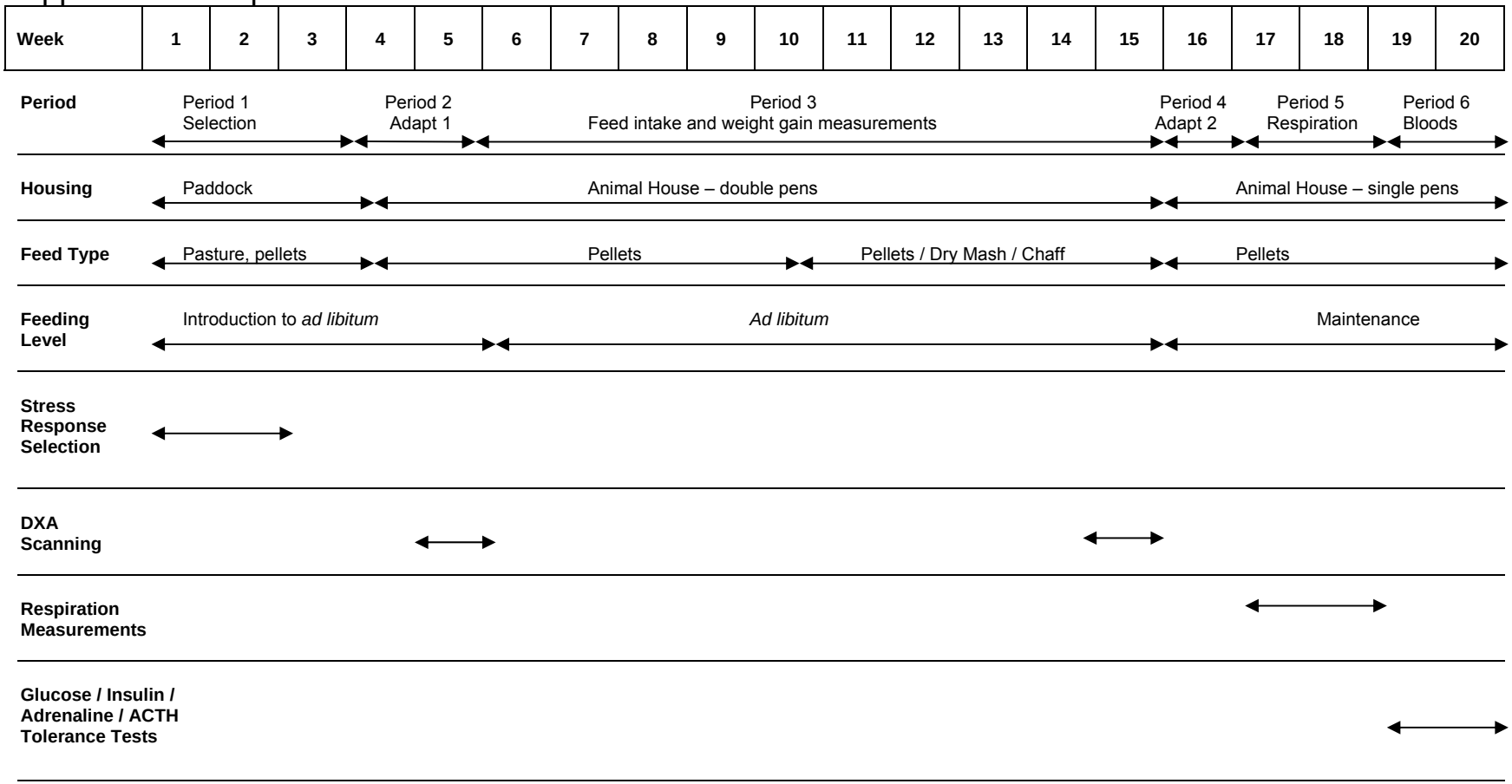
Koch RM, Swiger LA, Chambers D, Gregory KE (1963) Efficiency of feed use in beef cattle. *Journal of Animal Science* **22**, 486-494.

O'Callaghan D, Wendling A, Karsch FJ, Roche JF (1993) Effect of exogenous thyroxine on timing of seasonal reproductive transitions in ewes. *Biology of Reproduction* **49**, 311-315

Sechen SJ, Dunshea FR, Bauman DE (1990) Somatotropin in lactating cows: effect on response to epinephrine and insulin. *American Journal of Physiology - Endocrinology and Metabolism* **258**, E582-588.

APPENDICES

Appendix 1 – Experimental Timeline



Appendix 2 – Body composition and respiration photos

Image 1. Scanning anaesthetised sheep for body composition using dual energy x-ray absorptiometry (DXA)

Image 2. Computer image of a DXA scanned sheep. Analysis of scans gives total bone, lean tissue mass, and fat tissue mass. FCR & RFI values can then be adjusted for differences in body composition between animals

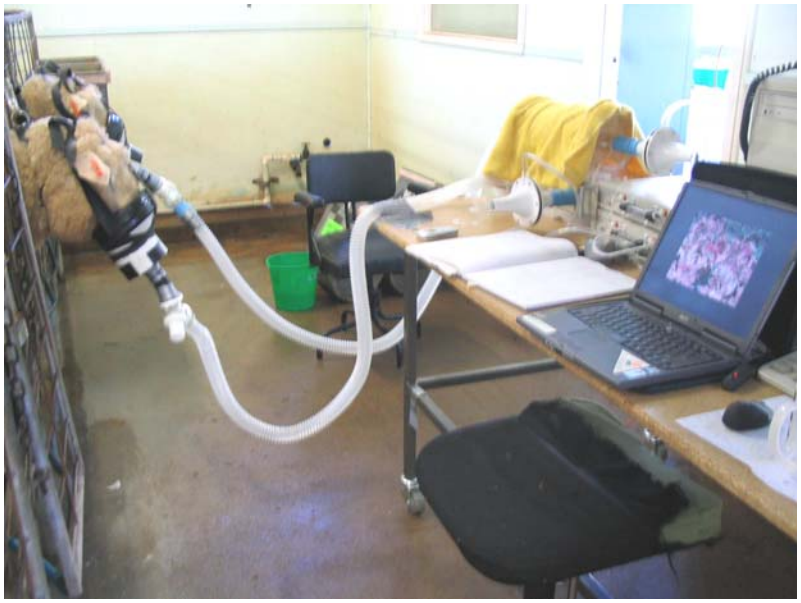
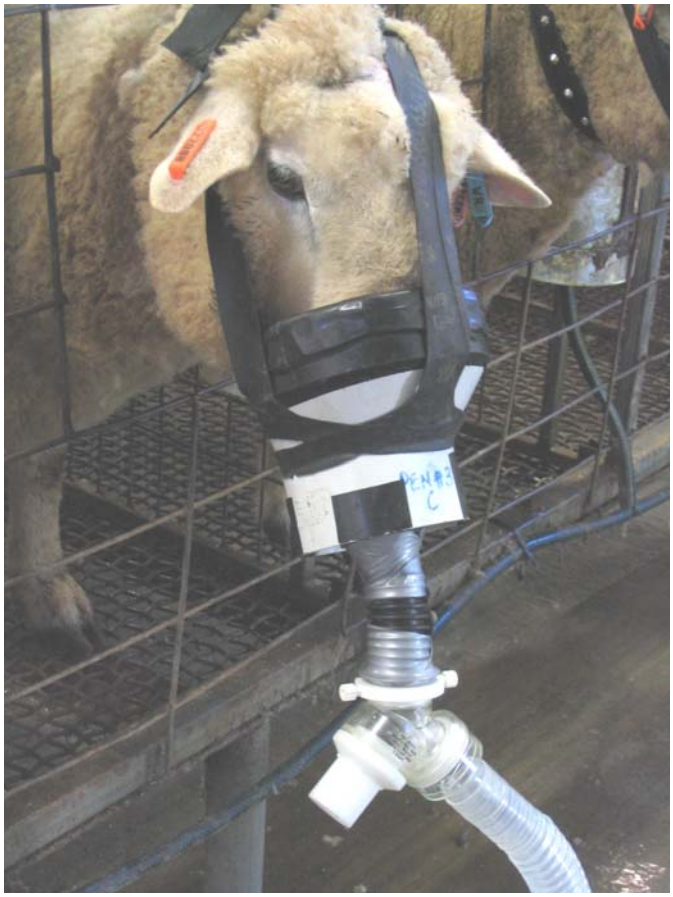


Image 3. Indirect calorimtery equipment and measuring sheep for respiration

Image 4. Individual sheep masks to enable respiration measurements.



Appendix 3 - Publications

MLA Annual Postgraduate Workshop Abstract – May 2005.

Less efficient rams are more responsive to an adrenocorticotropic hormone (ACTH) induced stress challenge.

S.A. Knott[1,2], L.J. Cummins[1], B.J. Leury[2] and F.R. Dunshea[2,3]

[1]Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Hamilton, Victoria 3300.
[2]Faculty of Land and Food Resources, The University of Melbourne, Parkville, Victoria 3010.
[3]Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Werribee, Victoria 3030.

Net feed intake (NFI) is a measure of feed efficiency calculated as the difference between an animal's actual intake and its expected intake based on its liveweight and growth rate over a specified period of time (Richardson *et al.* 2002). NFI is commonly used to identify animals that are more efficient at converting energy intake into gain, however there are a number of physiological mechanisms which influence energy utilisation including stage of maturity, reproductive status and disease or stress status. One of the key biological responses when an animal is exposed to a stressor is to alter the function of the hypothalamic-pituitary-adrenal (HPA) axis thus increasing metabolic rate and energy consumption and utilisation. Increased cortisol levels cause catabolic processes such as lipolysis and protein degradation in order to increase energy mobilisation. Work in chicken and cattle suggests that animals with more positive NFI values (less efficient) tend to display more escape or aggressive behaviour or have higher blood cortisol levels (Luiting *et al.* 1994; Richardson and Herd 2004). These types of responses thereby provide a mechanism for stress to possibly contribute to the lower efficiency of energy utilisation observed in some animals.

The objectives of this study were to firstly determine the appropriate dose and peak serum cortisol time for exogenous administration of adrenocorticotropin hormone (ACTH) and then to measure cortisol responses in sheep of known NFI. Three cross-bred rams (12 mo, 63.5kg) were used in a 3 x 3 Latin square design and assigned one of three ACTH dose rates (0.4, 1.6 or 6.4μg/kg LW) on each of three consecutive days. Serial blood samples were taken through indwelling jugular catheters at –60, -45, -30, -15 and 0 min before and at 30, 45, 60, 75 and 90 min after intramuscular ACTH administration. Serum cortisol levels were determined using a commercially available RIA kit. The administration of 1.6μg or 6.4μg of ACTH/kg LW resulted in a greater increase in cortisol response over time compared to the base dose, but not to each other. The peak cortisol concentration occurred around 45 min following ACTH administration. Based on the results from the first part of the study, cortisol responses were measured in fifty-two cross-bred rams (12 mo, 84.5kg) of known NFI, with a blood sample taken immediately prior to and 45 min post an i.m. injection of 2.0μg/kg LW ACTH. Linear regressions were generated between RFI, pre-ACTH, post-ACTH and incremental serum cortisol levels. Highly significant (P<0.001) relationships existed between NFI with both post-ACTH and incremental serum cortisol levels. The results indicated that animals more susceptible to stress, induced by an ACTH challenge, are also less efficient in their energy utilisation.

Following on from this work, an experiment was developed exploring the hypothesis that animals identified as more susceptible to an ACTH induced stress challenge would be less efficient in their energy utilisation. One hundred rams of a composite maternal sire line

(mean ± s.d., initial age 415 ± 17days; initial liveweight 53.2 ± 6kg) were used in a screening test to identify animals of either low or high susceptibility to a known stressor. Blood samples were taken immediately prior to and 45 minutes after, intramuscular administration of ACTH (Virbac®, 2μg/kg LW) and analysed for total serum cortisol concentrations. Rams were ranked on post-ACTH serum cortisol concentrations, and those with extreme values selected (mean ± s.d.; High Cortisol, HC, n=12, $\bar{x}$=215.6±18 nmol/L; Low Cortisol, LC, n=11, $\bar{x}$=113.3±15.8 nmol/L). Rams were then individually housed and fed a concentrate-based diet (11.5MJ/kg DM; 16% CP, 28% NDF) with *ad libitum* feed intakes and liveweights recorded for 40 days. Feed intake was regressed against mean metabolic liveweight ($W^{0.75}$) and average daily gain, with the residual portion defined as NFI. Analysis of variance was undertaken on a group basis, with results for one of the HC rams not used as it had an atypically low feed intake and did not gain weight over the 40 days.

NFI was higher in the HC rams than in the LC rams (HC $\bar{x}$=0.45; LC $\bar{x}$= -0.35; s.e.d. 0.398; *P*=0.057). There was a significant correlation between NFI and post ACTH serum cortisol concentration (r = 0.424, *P*<0.05) but no significant correlations with the pre ACTH serum cortisol concentration nor the incremental change. The data from the two experiments reported demonstrate that there is a biologically useful relationship between NFI and stress susceptibility and that screening animals for their cortisol responsiveness to a known stressor may be a relatively cheaper and accurate way of identifying animals, which are more efficient.

Luiting P, Urff EM, Verstegen MWA (1994) *Netherlands J. of Ag. Sci.* **42**, 59-67.
Richardson EC, Herd RM (2004). *Aust. J. of Exp. Ag* **44** 431-440.
Richardson EC, Herd RM, Colditz I, Archer JA, Arthur PF (2002) *Aust. J. of Exp. Ag.* **42** 901-908.

Recent Advances in Animal Nutrition Abstract – July 2005

An adrenocorticotropin hormone (ACTH) induced stress challenge can be used to identify rams, which are different in net feed intake.

S.A. Knott[1,2], L. Cummins[1], B.J. Leury[2], F.R. Dunshea[3]

[1] Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Hamilton, Victoria. 3300.
[2] Faculty of Land & Food Resources, The University of Melbourne, Parkville. Victoria. 3052.
[3] Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Werribee. Victoria. 3030.

Net feed intake (NFI) is a measure of feed efficiency calculated as the difference between an animal's actual intake and its expected intake given requirements for maintenance and growth. Previous work has shown that rams with poor feed efficiency have a greater serum cortisol response to a known stressor such as adrenocorticotropin hormone (ACTH) than those that are more efficient (Knott *et al.* 2004). In beef cattle divergently selected for NFI, Richardson and Herd (2004) found that less efficient animals tend to have higher basal blood cortisol levels and suggested that stress, tissue metabolism and protein turnover could contribute 37% to the variation in NFI. The catabolic response to stress results in energy mobilisation, thereby providing a mechanism for stress to contribute to the lower efficiency of energy utilisation observed in less efficient animals (Elsasser *et al.* 2000). The hypothesis for this experiment was that animals identified as more susceptible to an adrenocorticotropin hormone (ACTH) induced stress challenge will be less efficient in their energy utilisation.

One hundred rams of a composite maternal sire line (mean ± s.d., initial age 415 ± 17days; initial liveweight 53.2 ± 6kg) were used in a screening test to identify animals, which are more or less susceptible to a known stressor. Blood samples were taken immediately prior to and 45 minutes after, intramuscular administration of ACTH (Virbac®, 2μg/kg LW) and analysed for total serum cortisol concentrations. Rams were ranked on post-ACTH serum cortisol concentrations, and those with extreme values selected (mean ± s.d.; High Cortisol, HC, n=12, $\bar{x}$=215.6±18 nmol/L; Low Cortisol, LC, n=11, $\bar{x}$=113.3±15.8 nmol/L). Rams were then individually housed and fed a concentrate-based diet (11.5MJ/kg DM; 16% CP, 28% NDF) with *ad libitum* feed intakes and liveweights recorded for 40 days. Feed intake was regressed against mean metabolic liveweight ($W^{0.75}$) and average daily gain, with the residual portion defined as NFI. Analysis of variance was undertaken on a group basis, with results for one of the HC rams not used as it had an atypically low feed intake and did not gain weight over the 40 days.

NFI was higher in the HC rams than in the LC rams (HC $\bar{x}$=0.45; LC $\bar{x}$= -0.35; s.e.d. 0.398; *P*=0.057). There was a significant correlation between NFI and post ACTH serum cortisol concentration (r = 0.424, *P*<0.05) but no significant correlations with the pre ACTH serum cortisol concentration nor the incremental change. These data confirm that there is a biologically useful relationship between NFI and stress susceptibility.

Elsasser T, Klasing K, Filipov N, Thompson F (2000) The metabolic consequences of stress: targets for stress and priorities of nutrient use. In 'The biology of animal stress: basic principles and implications for animal welfare.' (Eds G Moberg & J Mench) pp. 77-110 (CABI Publishing: Wallingford)

Knott SA, Leury BJ, Brien FD, Cummins LJ, Dunshea FR (2004) Less efficient sheep are more responsive to an ACTH induced stress challenge. Asia Pacific Journal of Clinical Nutrition 13(supp), S89

Richardson EC and Herd RM (2004) Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Australian Journal of Experimental Agriculture 44, 431-440