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Rams with poor feed efficiency are highly responsive to an exogenous adrenocorticotropin hormone (ACTH) challenge.

Running title: Feed efficiency and stress in sheep

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Abstract

An animal's response to a stressor is to increase metabolic rate, and thus energy consumption through the activation of the hypothalamic-pituitary-adrenal axis. Changes to energy use by an animal are likely to influence the efficiency with which it is utilised. In this study, we tested the hypothesis that less efficient sheep are more responsive to exogenous administration of adrenocorticotropin hormone. This was done by firstly determining the appropriate dose (0.4, 1.6 or 6.4 μ g/kg LW) and peak serum cortisol response time (45 min) to exogenous administration of adrenocorticotropin hormone in a pilot study (n=3 sheep). Following this, adrenocorticotropin hormone (2.0 μ g/kg LW) stimulated cortisol levels were measured in a larger group of sheep (n=50) of known feed efficiency (feed conversion ratio and residual feed intake values). Less efficient sheep (more positive residual feed intake values) were found to have a greater ($P<0.001$) increase in cortisol concentration in comparison to more efficient animals. Those sheep which had higher levels of cortisol also had a greater proportion ($P<0.001$) of fat tissue. These data clearly demonstrated that efficiency of energy use, when measured as residual feed

intake, is significantly related to an animal's stress response. These findings have important implications for understanding the physiological mechanisms underpinning efficiency of energy use, and may be useful in successfully identifying animals which are superior in terms of feed efficiency.

Keywords: stress, sheep, feed efficiency, residual feed intake, cortisol

1. Introduction

Stress can result from either physical or physiological stressors. When an animal is exposed to a stressor, the biological response includes alterations in the animal's behaviour, neuroendocrine system, autonomic nervous system and immune system [1]. One of the key biological responses is to increase metabolic rate and energy consumption and utilisation through altering the function of the hypothalamic-pituitary-adrenal (HPA) axis [2, 3]. Metabolic rate increases because of increased catabolic processes such as increased lipolysis [4] and protein degradation [5]. Other responses to stress include behavioural changes, such as increased activity and frustration behaviour [6].

The efficiency in which an animal utilises feed for growth can be measured as residual feed intake (RFI). Residual feed intake is the difference between an animal's actual intake and its expected intake based on its live weight and growth rate over a specified period of time [7]. Luiting *et al.* [6] found that in chickens divergently selected for RFI, less efficient hens spent more time food pecking, and displaying escape or aggressive behaviour, suggesting that they are more susceptible to stress or have less adaptive mechanisms to cope with, and adapt to, a stressor. In beef cattle

divergently selected for RFI, Richardson and Herd [8] found that less efficient animals were likely to have higher basal blood cortisol levels.

Sheep, in both extensive and intensive (feedlot) production systems, are subjected to a variety of stressors including social (e.g. social hierarchy), environmental (e.g. weather, space allocation, transportation) and physiological (e.g. disease, parasite burden) stressors. An animal's total body response to stress can be measured through decreased rates of growth, decreased efficiency of nutrient utilisation for growth and a subsequent increase in the energetic cost of the animal maintaining homeostasis. It is possible that less efficient (high RFI) animals, being more sensitive to stress, or having less effective mechanisms to cope with and/or adapt to stressors, display responses more associated with chronic stress. During longer-term stress, growth is inhibited due to the chronic activation of the HPA axis and subsequent suppression of the secretion of growth hormone [25] and increased catabolism. The combination of behavioural changes and the catabolic responses to stress that result in energy mobilisation, thereby provide a mechanism by which the stress response contributes to the lower efficiency of energy utilisation by less efficient animals [9].

The objectives of this study were to firstly, determine the appropriate dose and peak serum cortisol response time for exogenous administration of ACTH and secondly, to determine whether less efficient sheep have a greater response to administration of exogenous ACTH.

2. Materials and Methods

All procedures in the two studies were conducted in accordance with the guidelines set out in the “Australian Code of Practice for the Care and Use of Animals for Scientific Purposes” [10] and were approved by the Department of Primary Industries Hamilton Animal Ethics Committee.

2.1 Study 1

The aim of this study was to determine the most appropriate dose and practical timing to reliably determine serum cortisol response to exogenous administration of ACTH.

2.1.1 Experimental Design

Three cross-bred rams from a pedigree research flock (Poll Dorset in origin) (mean \pm s.d liveweight $63.5 \pm 10.9\text{kg}$; mean \pm s.d age $403 \pm 0.58\text{d}$) prepared with indwelling polyethylene catheters (Dural Plastics, Sydney, i.d. 1.0mm, o.d. 1.5mm) were used in a 3 x 3 Latin square design and assigned one of three dose rates on each of three consecutive days. Rams were housed in individual pens within sight and sound of each other and had undergone a 2-week adaptation period to the facilities and feed. Rams were given *ad libitum* access to a concentrate (cereal grain) based pellet (12 MJ/kg DM, 16% CP) which was formulated to meet dietary requirements, and had free access to water troughs in each pen. These sheep had previously been housed in this facility and were accustomed to being handled on a regular basis. The catheters were inserted a day before the commencement of the study which was conducted over three consecutive days in late October.

Rams received 0.4, 1.6 or 6.4 μ g/kg LW of ACTH purified corticotropin (Virbac® Australia Pty Ltd, Peakhurst, N.S.W. 2210) 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 (approximately 1 mL). ACTH was administered intramuscularly into the rump of the animal. All sheep were injected at individual time points between 0945 and 1002. These dose rates were selected based on previous work in sheep [11, 12], cattle [13] and dogs [14].

On each bleed day serial blood samples were collected at -60, -45, -30, -15 and 0 min before and at 30, 45, 60, 75 and 90 min after ACTH administration. Blood samples were collected into tubes containing SST gel and clot activator additives (BD Vacutainer™, Preanalytical Solutions, Franklin Lakes, NJ 07417). 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 ensure maximum serum yield. Whole clotted blood was centrifuged at 1,800 W g for 15 min at 4°C (Technospin R, Sorvall Instruments, DuPont), serum decanted and stored at -80°C until assayed for cortisol.

2.2 Study 2

The aim of this study was to measure the ACTH-induced cortisol responses in animals of known feed conversion efficiency.

2.2.1 Experimental Design

Fifty-two crossbred rams from a pedigreed research flock (Poll Dorset in origin) (mean \pm s.d liveweight, 84.5 \pm 5.3 kg; mean \pm s.d age, 439 \pm 1.3d) were housed in individual pens within sight and sound of each other. Rams had been adapted to the

facilities for a 2-week period prior to the study. Feed intake and weight gain were measured for a subsequent 62 days post-adaptation in order to calculate gross feed conversion ratio (FCR) and RFI values. Rams were given *ad libitum* access to a concentrate-based pellet (12 MJ/kg DM, 16% CP) and had free access to water. Whole animal body composition was measured using dual energy X-ray absorptiometry (DXA) at the start and the end of the 62-day measurement period in order to determine lean tissue mass (LTM) and fat tissue mass (FTM) and the change in composition over time [15, 16].

The liveweight was modelled over time separately for each sheep using a random coefficient regression including a cubic spline for time [17]. The model fitted was:

$$\text{Liveweight} = \mu + \text{day} + \text{sheep} + \text{sheep.day} + \text{spline}(\text{day}) + \text{sheep.spline}(\text{day}).$$

The term ‘day’ was fitted as a fixed covariate and all other terms were fitted as random effects, with a covariance between the sheep intercept (sheep) and slope (sheep.day). The likelihood ratio test was used to assess any spline effects after the previously mentioned terms (day, sheep and sheep.day) had been fitted. The fitted liveweight values were then used to calculate FCR (kg feed eaten : kg weight gained). Feed intake was adjusted for mean metabolic liveweight ($\text{MWT}^{0.73}$) and average daily gain (ADG) calculated from the fitted liveweight values, with the residual portion, or deviation from the expected feed intake value, as the measure of efficiency. The residual portion was defined as RFI [18].

2.3 Adrenocorticotropin Hormone Challenges and Blood Sample Collection

Adrenocorticotropin hormone challenges were administered in late November, 46 days after the end of the adaptation period (approximately 1 month after the

conclusion of Study 1). Based on the data from Study 1 and previous work by Turner *et al.* [11], all sheep received 2 µg/kg LW of ACTH (ACTH₁₋₂₄, Synacthen, Novartis Pharmaceuticals Australia Pty Ltd, North Ryde, NSW, Australia) (equivalent to 0.2IU/kg LW) 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 (approximately 1 mL). Synacthen contains a synthetic polypeptide, tetracosactrin, with the first twenty four amino acids in the sequence exactly the same as endogenous ACTH and therefore displays similar activity and efficacy as purified ACTH [14]. A dose 25% above the lowest dose that was found to maximise the cortisol response in Study 1 (1.6µg/kg LW) was used in Study 2 (ie. 2.0 µg/kg LW).

All animals were fed between 0830 and 0900 on the morning of the challenge. Adrenocorticotropin hormone was injected intramuscularly into the rump of the animal between 1215 and 1345 and the time recorded for each animal. Two blood samples were collected, one immediately before and one 45 min after the injection and the time of collection recorded. Post injection sample time was based on the peak response time determined in Study 1. The two blood samples were taken by jugular venipuncture and collected into tubes containing SST gel and clot activator additives (Vacutainer ®). Blood samples were then processed as described in Study 1 above.

2.4 Assay Procedures

Commercially available RIA kits (Orion Spectria Cortisol RIA) that had been modified slightly were used to determine serum cortisol concentrations. The modifications were to double the sample volume to 40µL; include an additional low standard of 10nmol/L; reduce the tracer volume by half to 250 µL; and to reduce the

incubation time to 30 min. For the following levels (nmol/L) of cortisol, 104 nmol/L and 344 nmol/L, the respective between assay coefficients of variation (CV) were 6.7% and 5.6%. The corresponding within assay CVs were 5.5% and 4.8% respectively.

2.5 Statistical Analyses

For Study 1 the peak serum cortisol response was obtained by determining the serum cortisol levels before and after administration of ACTH. A linear mixed model, using cubic smoothing splines [17], was fitted to test for the effects of the different cortisol concentrations, and allowing for random animal effects. This model was chosen in order to look at the response over time (i.e a continuous measure) rather than the response at discrete points in time.

For Study 2, two animals were removed from the analysis as basal serum cortisol values for these animals were missing. The remaining data were analysed by linear regression. The R^2 values reported are adjusted R^2 values from GenStat and are presented in percentage form. Spearman's rank correlation was used to examine the relationship between individual animal ranks for serum cortisol concentrations before and after the ACTH injections with both RFI and FCR respectively.

All statistical analyses were performed using Genstat 7.1 [19].

3. Results

3.1 Study 1

The effects of dose of ACTH on serum cortisol concentrations over time were plotted with fitted trendlines (Figure 1). The fixed and random spline terms for the model used to generate the fitted trendlines are shown in Table 1. The administration of 1.6µg or 6.4µg of ACTH/kg LW resulted in a greater increase in serum cortisol concentrations over time compared to the lowest dose, but not to each other. The response to each of the three different doses of ACTH was maximised at 45 min. The mean (\pm s.d) cortisol concentrations at 0 min were 21.6 (\pm 15.15) nmol/L, 15.2 (\pm 6.84) nmol/L and 30.8 (\pm 34.17) nmol/L for the dose rates of 0.4µg ACTH/kg LW, 1.6µg ACTH/kg LW and 6.4µg/kg LW respectively. At 45 min, the mean (\pm s.d) cortisol concentrations for the dose rates of 0.4µg ACTH/kg LW, 1.6µg ACTH/kg LW and 6.4µg/kg LW were 61.7 (\pm 23.41) nmol/L, 93.4 (\pm 8.07) nmol/L and 89.9 (\pm 12.10) nmol/L respectively.

{Insert Figure 1 around here}

{Insert Table 1 around here}

3.2 Study 2

Pre-ACTH basal serum cortisol concentrations ranged from 6.22 to 65.5 nmol/L with a mean (\pm s.d) value of 23.3 (\pm 14.09) nmol/L. Serum cortisol concentrations 45 min after administration of 2.0µg ACTH/kg LW, rose by a mean (\pm s.d) of 129.3 (\pm 27.87) nmol/L to give a mean (\pm s.d) post ACTH serum cortisol concentration of 153.0 (\pm 29.11) nmol/L.

Following the 62-day measurement period, the mean (\pm s.d) DMI was 2.4 (\pm 0.19) kg DM/day and the mean (\pm s.d.) ADG was 418.5 (\pm 55.86) g/day. For the two measures of efficiency, the mean (\pm s.d.) RFI value was 0.00 (\pm 1.03) and the mean (\pm s.d.) FCR value was 5.9 (\pm 0.64).

When RFI was modelled as a function of serum cortisol concentration, there were significant relationships with both the post ACTH serum cortisol concentrations and the incremental change in serum cortisol concentrations (Figure 2). Spearman's rank correlation coefficients between RFI and both post ACTH serum cortisol and incremental serum cortisol concentrations were highly significant ($P < 0.001$). In terms of FCR, the only significant rank correlation was with the incremental serum cortisol concentration (Table 2, Figure 3a, 3b and 3c).

{Insert Figure 2a, 2b and 2c around here}

{Insert Table 2 around here}

{Insert Figure 3a, 3b and 3c around here}

Serum cortisol concentrations before and after ACTH administration, and the change in serum cortisol concentration were modelled as functions of dry matter intake (DMI), ADG and measures of body composition. Significant relationships were observed for post-ACTH serum cortisol concentration and the change in serum cortisol concentration (Tables 3 and 4) with specific body composition and intake parameters ($P < 0.05$). All other relationships were not significant ($P > 0.05$) and have not been reported.

{Insert Table 3 around here}

{Insert Table 4 around here}

4. Discussion

The change in serum cortisol concentration induced by administration of exogenous ACTH was highly related to energetic efficiency estimated by RFI. Between 35- 40% of the variation in RFI was attributed to changes in serum cortisol concentration following administration of ACTH. This is the first time that this relationship has been demonstrated in a group of animals that had not undergone any selection pressure for RFI, and clearly indicates that variation in RFI is underpinned by measurable differences in physiological mechanisms in sheep, thus confirming results in other species [8, 20-22].

A number of different factors influence the energy utilisation of an animal including body composition, reproductive status, metabolic rate, disease status and stress [23]. Increased cortisol levels due to an animal's stress response appear to facilitate the energy mobilising effects of both glucagon and the catecholamines [4] thereby increasing gluconeogenesis and providing more energy to support the animal. The variation that exists in the serum cortisol levels in sheep unselected for RFI, following administration of a dose of ACTH which was chosen to elicit a maximum response from the adrenal cortex, is likely to be related to the animal's responsiveness to ACTH. The use of the 2.0µg/kg LW dose to stimulate an immediate and maximal response however may also limit the detection of differences amongst sheep. For instance the use of a range of sub-maximal doses might be useful to detect differences in sensitivity to ACTH however that was not the main focus of this study. Similarly,

exploring the variation in endogenous basal ACTH might further clarify the relationship between basal ACTH concentration, basal cortisol concentration and cortisol concentration following exogenous administration of ACTH.

The data obtained in Study 2, indicate that animals that are more responsive to an ACTH challenge are likely to be more susceptible to stress, suggesting that the stress response in these animals, places increased demands on nutrient utilisation, diverting nutrients away from growth in order to mount the response. These animals are therefore less efficient in their energy utilisation, and are also likely to be slower growing, as tissue accretion will only occur if the metabolic needs of the immune system and the stress response are met [9].

Similar responses have been observed in other species. Luiting *et al.* [24] found that following nine generations of selection for RFI in chickens, more efficient hens displayed a significantly lower maximum corticosterone response 30 minutes after an ACTH challenge, yet the maximum response was also maintained for a longer period of time, up to 60 minutes, in comparison to the less efficient hens. Earlier work in cattle selected for RFI, demonstrated some trends ($P>0.05$, $P<0.1$) between RFI and serum cortisol concentrations [22]. However a limitation of that particular work was that a known stressor was not applied to the animals to enable quantification of the cortisol response. Nonetheless, the authors postulated that stress response, when combined with measures of protein turnover and tissue metabolism accounted for approximately 37% of the variation in RFI [8]. In combination with the previous work, the current data enable the assumption to be made that an animal's stress

response has a significant effect on energy utilisation and thus provides a mechanism to explain some of the variability that exists in animals of known feed efficiency.

However, the relationship between efficiency of energy use and cortisol concentration is not as strong when related to gross feed conversion efficiency. Although Hennessy and Jackson [26] found that following an ACTH challenge in pigs, those animals with lower cortisol responses displayed significantly ($P < 0.01$) improved growth and feed conversion efficiency than those in the higher range of responsiveness, the relationships between FCR and cortisol presented here are only weak. Feed conversion ratio is dependent on DMI and ADG. Significant positive relationships were found between average DMI and the serum cortisol concentration after ACTH administration, and the change in serum cortisol concentration. Sheep with lower cortisol levels following ACTH administration typically ate significantly less than others within the cohort. However, in the current work, no significant relationships were observed with ADG, yet the relationship with DMI indicates that there are positive benefits with animals that have a low potential to respond to stress, due to reduced intake and as such reduced feed costs [26].

Both serum cortisol concentration after injection of ACTH and the incremental change in serum cortisol concentration were significantly related to DXA measures of lean tissue mass (LTM) and fat tissue mass (FTM), either as an absolute weight, or as a proportion of total LW. Of particular interest are the positive relationships between measures of FTM and the exogenous ACTH induced serum cortisol concentrations. These relationships support earlier work reported by Veenvliet *et al.* [27] where in sheep divergently selected for (FAT) or against (LEAN) fatness, the maximal cortisol

response following an ACTH challenge was greater in the FAT genotype. They postulated that the sensitivity of the adrenal gland may play a role in determining body composition, due to the interactions of cortisol with insulin and growth hormone and thus changes in nutrient partitioning. As a consequence of this, those rams with higher serum cortisol responses to ACTH tend to have higher FI, FCR and RFI values and have significantly greater levels of fat tissue on both an absolute and proportional basis. It is also possible that these animals which are less efficient are more susceptible and/or more responsive to various stressors which may act more as an underlying chronic stressor rather than an acute stressor. Chronic stress can exert a powerful effect on body composition due to the effect that cortisol can have on increasing catabolic processes and thus metabolic rate [4, 5, 28]. Increased cortisol concentrations, especially during chronic stress or in diseases causing hypercortisolism such as Cushing's disease, can decrease growth rate, and are associated with adiposity and insulin resistance [25, 29, 30]. Intuitively, it would be expected, given the relationship between chronic stress and both changes in body composition and growth rate, that there would be a significant relationship with the basal cortisol concentrations and the efficiency, growth, intake and body composition measures. However this was not observed in the current work. The lack of association may have been partly due to the sampling regime, as only two samples were taken in Study 2. The sampling regime used may have precluded the detection of any relationship between growth parameters and basal cortisol, as the single basal measurement would not have taken into account the presumed diurnal variation in cortisol concentration in sheep.

From an animal production point of view, given the relationship identified here between stress susceptibility and feed conversion efficiency measured as RFI, identifying animals which are less susceptible to stress, would have significant production benefits through enabling the reduction of the effects of acute or chronic exposure to stressful stimuli on an animal. This would lead to an increase in production through better growth, improved reproductive performance and reduced incidence of disease [31].

These data demonstrate that there is a strong, significant relationship between RFI and serum cortisol concentrations following the administration of ACTH. It was initially hypothesised that less efficient sheep would be more responsive to the administration of exogenous ACTH, and the data reported clearly supports this hypothesis. The relationship between ACTH-induced cortisol response and measures of body composition is also of interest, as these results indicate that sheep, which are more responsive to a known stressor, are in fact fatter. Nevertheless, the observed variation in cortisol concentration and cortisol-induced response to an ACTH challenge also indicates that regulation of RFI in sheep, is multi-faceted and no one physiological measure, such as body composition or response to a stressor can account for all the variation in RFI that is demonstrated within a group of animals.

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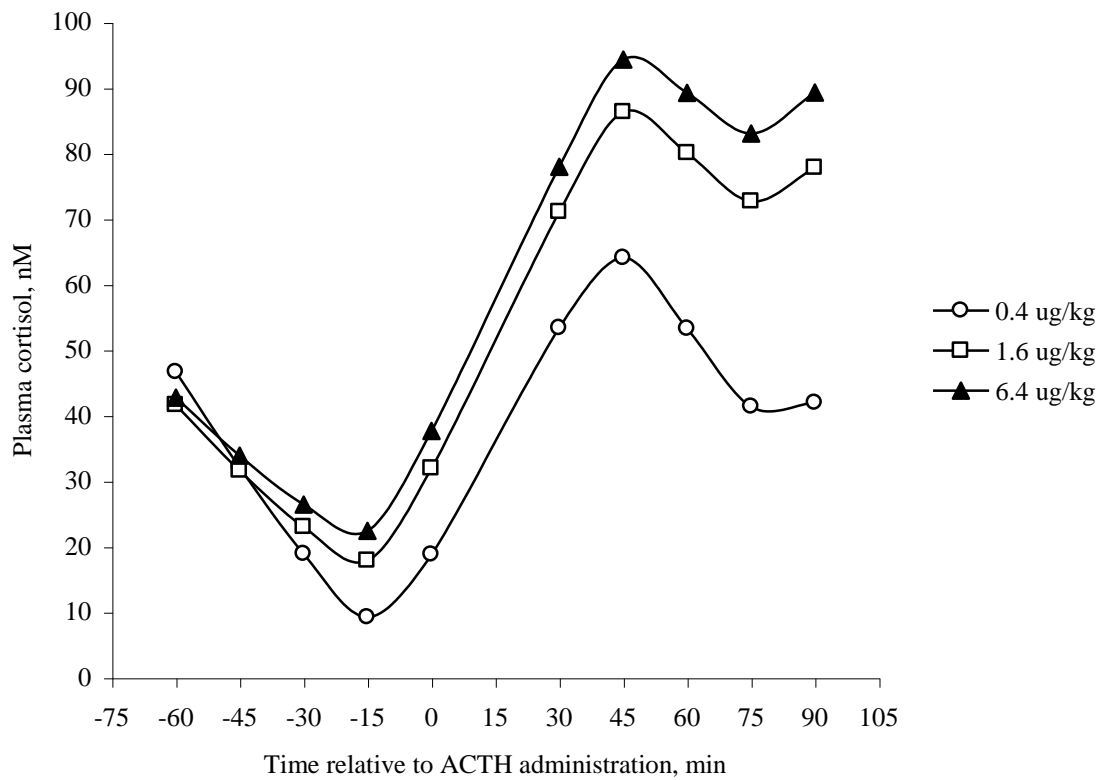


Figure 1. Fitted values for plasma cortisol concentrations for sheep for 60 min before to 90 min after intramuscular injection of one of three doses of ACTH; $n = 3$ for all treatment groups. Significant changes over time and between dose rates are described in the Results.

Table 1. A summary of the fixed and random spline terms of the model fitted to serum cortisol concentrations.

Parameter	Wald statistic	d.f.	P-value
Fixed terms			
Dose	5.8	2	>0.05
time	75.0	1	<0.001
Dose x time	13.5	2	<0.001
Random spline terms			
Spline (time)	34.1 ^a		<0.001
Dose x spline (time)	0.1 ^a		>0.05

The Wald statistic is an approximate *F*-test [17].

^aChange in REML log likelihood

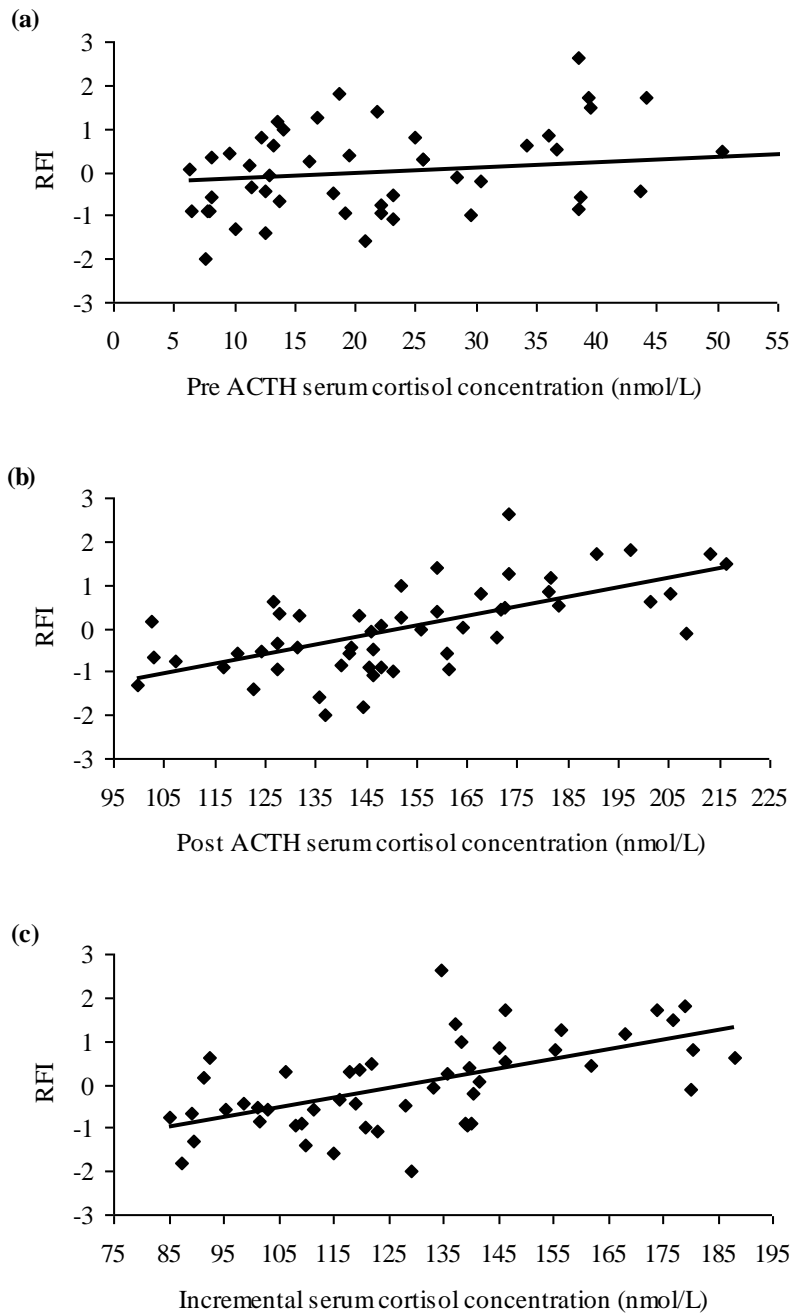


Figure 2. Linear regression models $y = a + bx$ that predict residual feed intake (RFI)

(y) based on serum cortisol concentrations in $n = 50$ sheep.

(a) Serum cortisol levels for pre ACTH with the coefficient values (\pm s.e) for $a = -0.28$ (0.28) and $b = 0.012$ (0.010) and the adjusted R^2 (%) = 0.7 ($P > 0.05$);

(b) Serum cortisol levels for post ACTH, with the coefficient values (\pm s.e) for a = -3.38 (0.59) and b= 0.02 (0.003) and the adjusted R^2 (%) = 39.5 ($P<0.001$);

(c) Incremental change in serum cortisol levels, with the coefficient values (\pm s.e) for a = -2.85 (0.57) and b= 0.02 (0.004) and the adjusted R^2 (%) = 34.2 ($P<0.001$).

Table 2. Spearman's rank correlation coefficients between pre ACTH cortisol, post ACTH cortisol and the incremental change in cortisol with both RFI and FCR respectively

Parameter	FCR	RFI
Pre ACTH Cortisol, nmol/L	-0.015 ^a	0.231 ^a
Post ACTH Cortisol, nmol/L	0.266 ^a	0.648 ^c
Change in Cortisol, nmol/L	0.313 ^b	0.601 ^c

^a $P > 0.05$; ^b $P < 0.05$; ^c $P < 0.001$

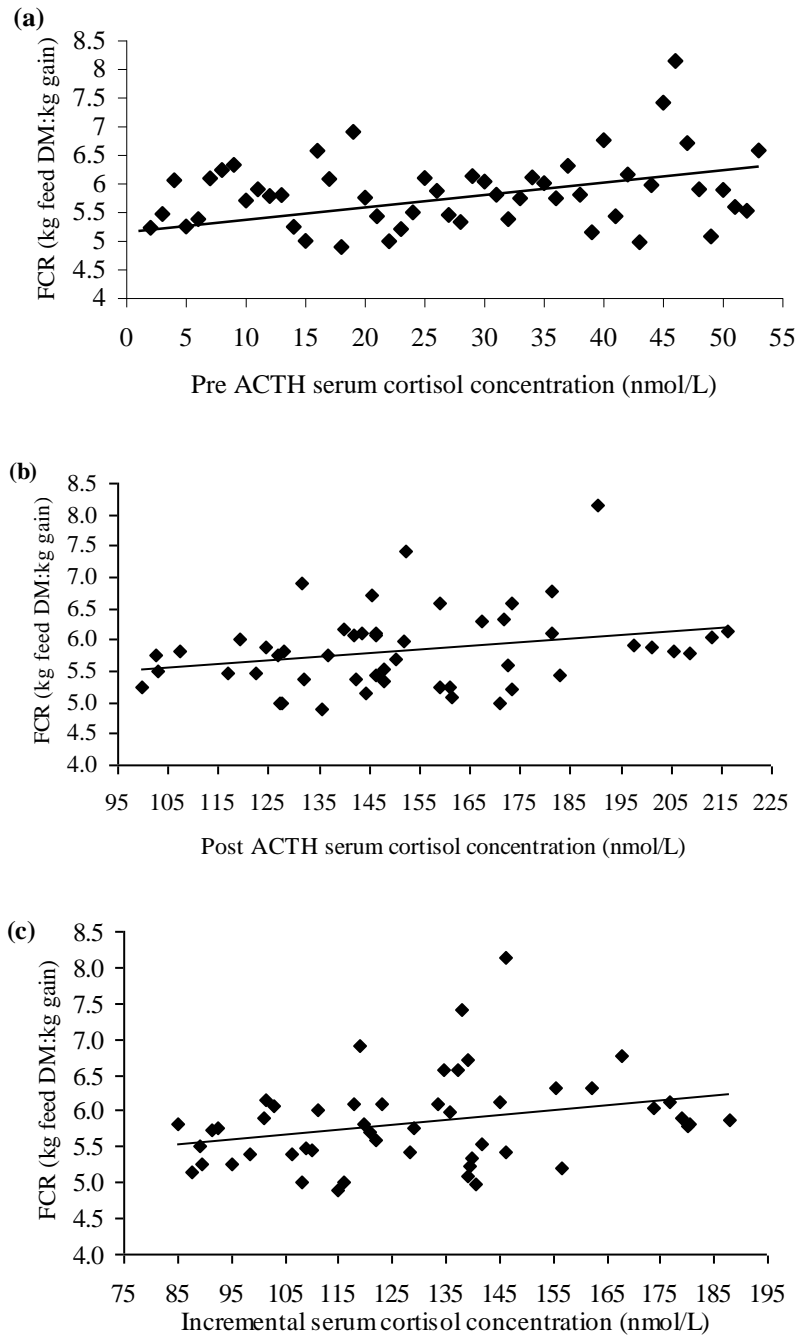


Figure 3. Linear regression models $y = a + bx$ that predict feed conversion ratio (FCR) (y) based on serum cortisol concentrations in $n = 50$ sheep.

(a) Serum cortisol levels for pre ACTH with the coefficient values (\pm s.e) for $a = 5.85$ (0.177) and $b = -0.001$ (0.0065) and the adjusted R^2 (%) = the variance accounted for is 0.0 ($P > 0.05$);

(b) Serum cortisol levels for post ACTH, with the coefficient values (\pm s.e) for $a= 4.96$ (0.465) and $b= 0.006$ (0.0030) and the adjusted R^2 (%) = the variance accounted for is 5.2 ($P>0.05$);

(c) Incremental change in serum cortisol levels, with the coefficient values (\pm s.e) for $a= 4.98$ (0.417) and $b= 0.007$ (0.0032) and the adjusted R^2 (%) = the variance accounted for is 6.6 ($P<0.05$).

Table 3 Coefficients (\pm s.e) and the total variance accounted for using the linear model $y = a + bx$ that predicts serum cortisol concentrations following exogenous administration of ACTH (Post ACTH cortisol) based on dry matter intake (DMI), and dual energy X-ray absorptiometry (DXA) values for lean tissue mass (LTM) and fat tissue mass (FTM) as either absolute masses or proportions (%) of total liveweight at either the start or end (2) of the measurement period for $n = 50$ sheep.

Parameter (x_1)	a (\pm s.e)	b_1 (\pm s.e)	Adjusted R^2 (%)	Significance of F ratio
DMI	-12.8 (46.50)	70.1 (19.60)	18.8	<0.001
FTM_2	81.5 (22.40)	4.4 (1.35)	15.7	0.002
FTM Change	82.4 (18.20)	7.1 (1.80)	22.3	<0.001
FTM_2 %	80.0 (23.70)	396 (127.0)	14.6	0.003
FTM Change %	101.1 (17.00)	131.4 (41.90)	14.7	0.003
LTM_2 %	498 (118.0)	-489 (167.0)	12.9	0.005
LTM Change %	239.7 (29.70)	-161.3 (54.70)	13.1	0.005
LTM_2 : FTM_2 Ratio	210.8 (18.80)	-14.6 (4.66)	14.7	0.003

Table 4 Coefficients (\pm s.e) and the total variance accounted for using the linear model $y = a + bx$ that predicts the incremental change in serum cortisol concentrations following exogenous administration of ACTH based on dry matter intake (DMI), and dual energy X-ray absorptiometry (DXA) values for lean tissue mass (LTM) and fat tissue mass (FTM) as either absolute masses or proportions (%) of total liveweight at either the start or end (2) of the measurement period for $n = 50$ sheep.

Parameter (x_1)	a (\pm s.e)	b_1 (\pm s.e)	Adjusted R^2 (%)	Significance of F ratio
DMI	-1.7 (47.10)	55.3 (19.8)	12.2	0.007
FTM_2	62.0 (21.50)	4.1 (1.29)	15.6	0.003
FTM Change	65.3 (17.70)	6.5 (1.75)	20.5	<0.001
FTM_2 %	57.2 (22.50)	292 (121.0)	16.4	0.002
FTM Change %	77.8 (16.10)	130.7 (39.80)	16.7	0.002
LTM_2 %	481 (112.0)	-497 (158.0)	15.4	0.003
LTM Change %	218.6 (28.00)	-165.8 (51.60)	16.0	0.002
LTM_2 : FTM_2 Ratio	187.1 (17.90)	-14.56 (4.41)	16.8	0.002