Chapter 1

Literature Review

1.Introduction

Livestock producers in Australia face a great challenge to produce red meat for the consumer across a wide range of market specifications. The 1990's have seen a continuance of the sedentary lifestyle of the previous decade in this country with even greater concern about less animal fats in the diet. This has probably been a factor in the consumption of red meat in this country declining from 86.9kg of sheep meats and beef per head per annum in 1961 to 63.8kg/head in 1990 (Anon.,1991). The present consumption rate has been fairly static over the last three to four years.

Contrary to the above, beef producers in Australia have a demand from Japan for highly marbled product (marble score 5+). Expansion of this trade is only likely if our producers can meet the Japanese specifications. Another North Asian export market for beef is Korea. Their demands for specified product is intermediate to the domestic and Japanese market (Anon.,1992).

At present, producers can rely on genetic diversity within and between breeds of animals for selection of early and late maturing animals in order to meet a wide range of market specifications. Although the adoption of this quantitative approach is inexpensive, quantitative selection programs result in slow gains in any particular production trait. Pharmaceutical agents are available which are capable of decreasing carcase fat and increasing skeletal muscle mass (Thornton et al.,1985). Vaccines capable of stimulating the production of antibodies specific to hormones (Spencer and Garssen,1983;Flint,1992) and to adipocyte plasma membranes (Malony and Allen,1989;Nassar and Hu,1989) are capable of repartitioning energy within the body, thereby manipulating carcase characteristics. If proven successful under commercial conditions, these alternative techniques have the advantage of being rapid in their actions and could play a role in manipulating carcase composition for various market specifications. The negative side to such technologies, is the increase in production costs to treat individual animals and the adverse public perception that animals had been treated with chemicals to achieve the results.

The aim of this review is to assess the biological factors affecting the development of fat depots in the two major species used in the grazing industry in Australia.

2. Fat Deposition

In mammals, the lipids of the body co nprise those which are structural within the cell membrane, mainly phospholipids and those which serve as reserves of energy in the form of depot fat. Triacylglycerol, the majo· lipid of adipose tissue is present in small amounts in the neonate. At day 60 of gestation, the lamb foetus comprises only 0.8% stored fat, compared to 4.0% at birth (Thompson et al.,1985).

Brown adipose tissue is present in neonatal mammals of many species, being of greatest significance in hibernating animals an l utilized during the arousal process proceeding hibernation (Joel,1965). It is generally accepted that the major function of brown adipose fat is to provide energy substrate for n on-shivering thermogenesis (Rothwell,1889). The same study indicated that the control c f lipogenesis and lipolysis in this tissue emanates from the ventromedial hypothalamus.

While Joel (1965) identified that most brown fat was located in the thoracic region of hibernating mammals, in lambs most is found around the kidneys and extending along the dorsal wall of the abdomen. For the nc wborn lamb it is the major source of energy for heat production, apart from the ingestion of maternal milk.

The rates of fatty acid synthesis have been observed to be 5 times greater in brown fat tissue than for white adipose tissue (M cCormack,1982) with glucose acting as the major substrate. Insulin stimulation of fatty acid synthesis can be blocked by anti-insulin sera in rats while the major physiological stimulus for thermogenesis in the tissue is believed to be noradrenaline. For non-hibernating mammalian species, the importance of brown adipose tissue early in life is superseded by white adipose tissue as the animal matures.

2.1 Morphology

Brown adipocytes are characterised by their large and numerous mitochondria (Garton,1974) and derive much of the r colour from erythrocytes in their extensive vascular network. These factors may well account for its more labile nature of stored lipid as compared to the later maturing whi e adipose tissue. Structural differences exist as well as the number of mitochondria betwee n brown and white adipocytes, which provide brown fat with its characteristic granu ar appearance. The brown adipocytes are smaller, have numerous small lipid inclusions and are more closely associated with both vascular and neural elements (Napolitano,1965).

The single nucleated adipocyte varies in the amount of lipid stored in droplet form and appear far from homogeneous in size. Many small lipid droplets appear in the cytoplasm before fusing to form a single large or e, which is characteristic of the mature cell actively storing triacylglycerol. Observed micr oscopically, the cells have a very thin rim of

cytoplasm in which mitochondria appear and the cell surface membrane shows micro-invaginations. The nucleus is eccentrically displaced to the side of the cell by large lipid droplets and is in close proximity to capillaries (Sheldon,1965).

2.2.1 Lipogenesis and Growth

For ruminants, the major source of energy is volatile fatty acids (VFA's) which are the products of fermentation in the rumen (Leng,1970). Acetate, propionate and butyrate constitute the main VFA's derived from dietary carbohydrate absorbed from the ruminant gut into the blood stream, although much of the butyrate is metabolized by the epithelial cells lining the rumen (Van Houtert, 1991). Acetate provides the major energy source for adipose tissue (Smith and Crouse,1984) which undergoes oxidative phosphorylation in the citric acid cycle to satisfy the cellular metabolic requirements for adenosine triphosphate (ATP). The excess acetate may be stored as fat in adipose tissue. Principal pathways involved in the synthesis and hydrolysis of triacylglycerol are described in figure 1.1

A source of fatty acid and glycerol 3-phosphate is required for the synthesis of triacylglycerol. Propionate is the responsible precursor for the latter substrate, after being extracted by the liver and used as a precursor for gluconeogenesis. Glycerol is also derived from triacylglycerol catabolism. Hence in fat synthesis, glucose is required to provide most of the reduced co-enzyme in the form of nicotinamide adenine dinucleotide phosphate (NADPH) (Van Houtert,1991).

The relative importance of propionate availability in the utilization of acetate as an energy substrate has been the subject of some conjecture as other sources of substrate are available via different energetic pathways.

Energy yielding nutrients in excess of the maintenance requirements of the animal are converted to long chain fatty acids, which undergo esterification and hydrolysis to form triacylglycerols that are deposited as lipid in adipose tissue. Long chain fatty acids are also the products of lipolysis or dietary lipid of which green herbage contains 5-10% (Annison,1993). Fatty acids which are the products of lipolysis, enter the circulation in the form of lipoproteins via lymph. Their transfer into adipose tissue occurs after their complete hydrolysis which is catalysed by the enzyme lipoprotein lipase (Frayn et al.,1992). This enzyme is synthesized in the adipocyte and is sequestered in the endothelial cells lining the capillaries adjacent to adipocytes (Vernon,1992a). Fatty acids and monoacylglycerols released are thought to be transported into the adipocyte via the action of fatty acid translocase. Despite the low amounts of fat in the diet of ruminants,

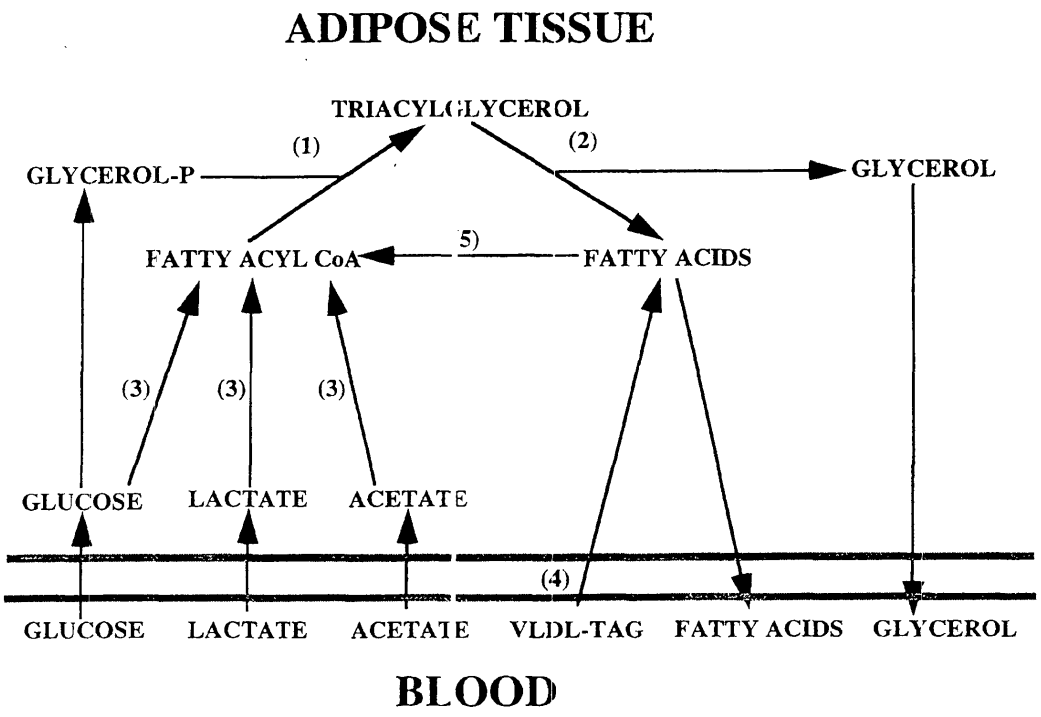


Figure 1.1 Schematic representation of lipid metabolism in ruminant adipocytes (adapted from Vernon and Flint, 1984). Pathway (1) Esterification; (2) Lipolysis; (3) Fatty acid synthesis; (4) Lipoprotein lipase activity resulting in release of fatty acids from very low density lipoproteins; (5) Re-esterification.

lipoprotein lipase activity can supply in excess of 50% of fatty acids deposited (Vernon,1980).

The hydrolysis of triacylglycerol by lipase is hormone sensitive. Two molecules of fatty acids are cleaved, leaving monoacylglycerol to be hydrolysed to glycerol and fatty acid. Within the adipocyte, esterification and lipolysis are occurring simultaneously and on a continuous basis so that triacylglycerol is in a state of flux. The relative rates of these processes determines whether lipid within adipose tissue is increasing or decreasing (Vernon,1992b).

The enzyme acetyl Co A carboxylase (260 kDa) catalyses the first step in fatty acid production, the conversion of acetyl Co A to malonyl Co A. This reaction is under hormonal control (Perdereau et al.,1982). Insulin activates the enzyme and increases net lipogenesis (Salans et al.,1972), while catecholamines decrease the activity of this enzyme (Vernon,1992). Insulin administration to rats can increase lipid content and size of adipocytes without having an effect on hyperplasia.

2.2.2 The Influence of Physiological State on Lipogenesis

High affinity insulin receptors in the plasma membranes of omental adipose tissue increase in early pregnancy when lipogenesis is dominant and decrease during lactation in sheep when fat depots are being mobilised (Guesnet et al.,1991). In this study, acetate incorporation into adipose tissue was insulin dependent up to high concentrations, when it plateaued, whereas at supraphysiological levels of insulin incorporation declined. In the same study the trend appears to be reversed in late pregnancy when adipocytes apparently develop resistance to the lipogenic effects of insulin. Several hormones which increase late in the gestation period may be responsible for this but as yet the precise mechanism is not clearly understood. A substantial fall in fatty acid synthesis in sheep adipose tissue occurs during lactation (Vernon and Finley,1988). It appears that in order to repartition nutrients for milk production, adipose tissue becomes refractory to insulin in sheep. At the same time a marked increase in growth hormone secretion occurs, which antagonizes insulin induced lipogenesis (Etherton et al.,1987). The metabolic consequence of GH action in adipose tissue is to redirect nutrients away from these depots to other tissues such as muscle and mammary tissue.

Glucocorticoids can inhibit lipogenesis in the absence of insulin, but at higher concentrations synergize with insulin to induce fatty acid synthesis (Vernon and Taylor,1988; Walton et al.,1986). The insulin induced fatty acid synthesis in adipose tissue is modulated by a number of hormones which independently or together increase or decrease the rate of lipogenesis.

Suckling animals have a change in die at weaning which leads to an increase in fatty acid synthase and acetyl Co A carboxylase while phosphoenolpyruvate carboxykinase mRNA decreases in rats (Perdereau et al.,199?).

As in ruminants and monogastric anim als, weaning removes a substantial proportion of dietary lipid from growing animals, thereby changing the available substrates for lipogenesis and the need for specific e nzymes to process the substrates. As well, changes in endocrine status and availability of ipogenic substrates during pregnancy and lactation complicate the regulatory mechanisms controlling lipogenesis.

2.3 Lipolysis

The breakdown of triacylglycerol into its original components of fatty acids and glycerol is controlled by the rate limiting lipase reaction which is hormone sensitive (Frayn et al.,1992). In ruminants, catecholamines are potent stimulators of lipolysis whereas glucagon is far less effective (Vernon,1992), but both work through the same cAMP/protein kinase pathway after interacting with their specific receptor in the plasma membrane.

The ß-adrenergic agonists (isoproterenol,clenbuterol, cimatrol and ractopamine) are potent analogues of catecholamines and can reduce carcass fatness (Etherton and Louveau,1992; O'Connor et al.,1991; Thornton et al.,1985; Ricks et al.,1984; Shackelford et al.,1992; Thornton et al.,1987) by as much as 30% while simultaneously increasing lean tissue accretion in growing rumin ants and in rodents (Mills and Orcutt,1988). The cross-sectional area of M.longissimus (eye muscle) has been reported to increase by as much as 42% in sheep treated with ß- agonists (Thornton et al.,1987). Clenbuterol has been shown to stimulate lipolysis 7 fold and reduce acetate incorporation 5 times when compared with controls in isolated ad pocytes (Thornton et al.,1985). The magnitude of carcase fat reduction by ß-adrenergic agonists can vary between carcase and non-carcase depots (Maloney et al.,1991) and between species (Etherton and Louveau,1992) with more marked responses occurring in ruminants and pigs.

There is evidence to suggest that ß-agonists are capable of down-regulating their own receptors by reducing receptor number (Kim et al.,1992; Linsay et al.,1992) which may help account for the differing responses reported within and between species. To date none of these compounds have been registered for agricultural use in spite of their efficacy due to perceived problems with residues in the meat of the animals and with possible side effects. Personnel inhaling the pharmaceuticals when handling treated feedstuffs can suffer from pulmonary and cardiac problems.

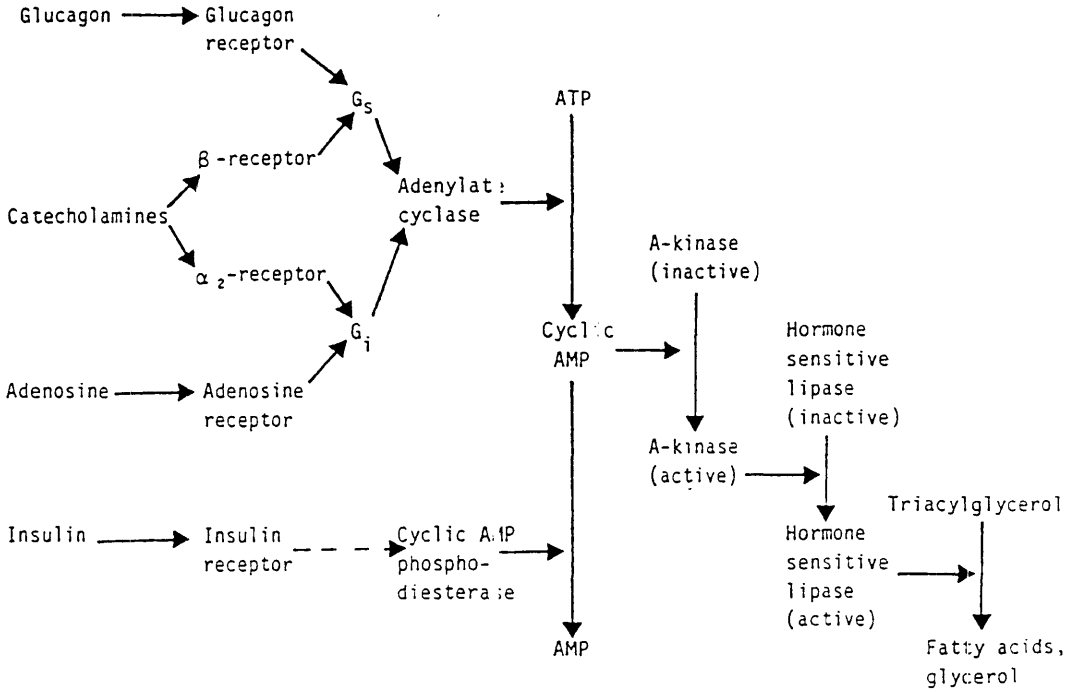


Figure 1.2 Lipolytic signal transduction cascade in the adipocyte (adapted from Vernon,1992).

Site and sex differences in the lipolytic activity of white adipocytes have been reported (Lacasa et al.,1991), with the lipolytic response to catecholamines being related to ß-adrenoceptor density on the cell membrane.
High concentrations of fatty acids are associated with an inhibition of lipolysis presumably via feed-back mechanisms involving endocrine signals designed to prevent the potentially fatal build-up of ketone bodies in the circulation.
Conflicting results exist on the effect of growth hormone on lipolysis. Vernon (1992) demonstrated enhanced lipolytic responses to catecholamines with growth hormone treatment in cattle, whereas Thornton et al. (1986) showed no such synergy in isolated ovine adipocytes. In the latter study, GH decreased lipogenesis but had no effect on lipolysis. GH could be acting by either antagonising the anabolic actions of insulin or by potentiating the effects of lipolytic hormones such as adrenaline or glucagon. Further studies need to be conducted to gain a better understanding of the interactions of GH with the other lipolytic hormones.
Stimuli for either net lipogenesis or lipolysis is the culmination of a complex set of interactions between hormones and their receptor signal transduction pathways on one hand, and the concentrations of lipogenic substrates and products on the other.

2.4 Cellularity

White fat depots increase in size as the animal grows due to hyperplasia and hypertrophy (Miller et al.,1991; Thornton et al.,1979; Hood and Thornton.,1980; Rule et al.,1987; Field et al.,1985; Hood,1982; Hood and Thornton,1979). Adipocytes in the subcutaneous, perirenal and omental depots, undergo cellular hypertrophy during positive growth in sheep. The volume of subcutaneous adipocytes is linearly related to the weight of fat in the boned out carcase. Lewis and Siebert (1985) demonstrated that not only does total intramuscular fat increase with age (1% to 7.5% over 26 months), but the ratio of polyunsaturated to saturated fats decrease with age. However the amount of unsaturated fat is generally low in ruminants, since all dietary fats are hydrogenated in the rumen (Van Houtert,1991).
Differences in adipocyte volume have been demonstrated between mature rams and wethers (Thompson and Butterfield,1988). The castrates had larger adipocytes in all the major carcase and non-carcase fat depots than their entire counterparts, with the greatest difference occurring in the subcutaneous and omental fat partitions. This trend was reversed for adipocyte number in this study, with greater cell numbers found in each of the ram fat depots.

Cell size appears to be the major determinant of lipid synthesis per cell (Rule et al.,1987) as surface area of adipocytes increases with greater cell size, a larger area is available for substrate uptake. However when expressed on a surface area basis, lipid synthesis within adipocytes from different depots differs.

Fat cells are incapable of depositing lipid indefinitely and due to physiological controls reach a maximum volume of around 2nl (Vernon,1992b). An equilibrium is reached at this size with similar rates of fat synthesis and hydrolysis.

When adipocytes attain this mature size it would seem that excess energy is redirected to smaller adipocytes within the same depot or elsewhere, giving rise to the notion of an optimum adipocyte diameter being required to support peak rates of lipogenesis.

2.4.1 The Role Of Phospholipids In Hormonal Signal Transduction.

In foetal development, the phospholipids by their nature, are central to mitosis and the actions of polypeptide hormones since they comprise part of the signal transduction pathways for many of them. Removal of oestrogens by ovariectomizing rats can modulate the membrane transduction system which controls lipolysis (Lacsa et al.,1981). This removal of ovarian steroids resulted in decreased cAMP in parametrial adipocytes leading to decreased lipolysis, whereas the same treatment had no effect on subcutaneous adipocytes.

The transfer of extracellular stimuli to intracellular second messengers is relayed via a range of receptor-effector mechanisms sited at the cell membrane (Hollywood,1991). These include transmembrane receptors that act as protein tyrosine kinases, membrane ion channels and membrane associated guanine nucleotide binding proteins. The stimulation of cell-surface receptors also initiates hydrolysis of membrane-bound inositol lipid which produces the second messengers diacylglycerol (DAG) and inositol triphosphate (Berridge and Irvine,1989). The latter acts by stimulating the release of calcium ions from the endoplasmic reticulum as well as an influx via membrane calcium channels.

However, steroid hormones bypass these membrane initiated signal transduction components, since they pass directly into the cytosol, where they interact with specific receptors prior to translocation and interaction with hormonal response elements in the nucleus.

By-products of lipolysis, can modulate signal transduction within the cell membrane or the cytosol.

2.5 Metabolic Differences Between Depots

As ruminants pass from a growing phase to a fattening phase, an order of development exists between fat depots from the early developing omental and perirenal depots to the subcutaneous, intermuscular and finally intramuscular depot (Vernon,1992). The respective differences in average fat cell sizes correlate with differences in the rate of lipogenesis between depots. This hierarchy is not consistent across all mammalian species however (Vezinhet and Prud'hon,1975). Although the physiological mechanisms are not clearly understood, several explanations are plausible. Increased blood flow to intra-abdominal fat depots in non-fasted versus fasted sheep have been reported (Gregory et al.,1986), suggesting that blood supply may be rate limiting for lipogenesis. This would result in variations in total substrate for potential lipogenesis reaching the different adipocytes and various concentrations of lipogenic and lipolytic hormones reaching their respective receptors on the adipocyte surface. Steroid hormones (androgens) have been shown to modulate fat cell receptors, and in the case of testosterone can increase ß-agonist receptors on specific abdominal fat depots in men (Bjorntorp et al.,1990). Glucocorticoids induce lipoprotein lipase (LPL) activity in specific abdominal fat depots in the male whilst progesterone synergises with cortisol to induce LPL activity in the gluteal-femoral region in women (Bjorntorp et al.,1990). The specific regional effect of cortisol on abdominal regions in humans is associated with a greater density of glucocorticoid receptors in these fat depots and their interaction with LPL, suggesting these depots are possible target tissue for glucocorticoids.

Transplanting adipocytes from genetically obese to lean rodents and vice versa significantly changed fat cell weights (Ashwell,1992), indicating that that the phenotypic environment of adipocytes may somehow impact on their metabolic activity. Site-specific properties of adipose tissue may well be an adaptive process of evolution to cope with changing environmental and nutritional conditions and physiological state. Scientific evidence for such a theory is however scant.

3. Factors Influencing Adiposity

3.1 Age

Under ideal nutritional conditions the growth of an animal follows a sigmoidal liveweight curve to maturity (Butterfield,1988). Thompson et al.(1985) define maturity for ad libitum feeding as an animal reaching at least 0.85 of its asymptote for the exponential relationship between body weight and the cumulative food consumed, and the weekly liveweight change for a period of 10 weeks being not significantly different from zero.

However, over a restricted period, measurements of the growth curve prior to maturity can display linear growth (Atkins and Thompson,1979) when liveweight is plotted against age. This linear relationship is only possible for a short period of the overall growth curve.

Strong and fine wool Merino rams achieved maturity at 120 weeks of age and at 117kg and 91kg respectively (Butterfield et al.,1983). Muscle, fat and bone all increased over the growing period but their relative contribution to the total tissue changed over the period of measurement. Body organs grew at vastly different rates, resulting in a range of maturity coefficients for muscle, fat and bone. Individual muscles within the body develop at differing rates (Butterfield et al.,1983) depending on their function. The major muscle groups responsible for locomotion and for suckling are the earliest maturing. The sex related neck and crest muscles of rams and bulls and the early maturing abdominal muscles of females prior to pregnancy are secondary sexual characteristics which are androgen and oestrogen dependent respectively. This would suggest development occurs in muscles according to the physiological needs of the animal.

Fat is not partitioned at the same rate between depots throughout the growth phase in sheep (Thompson and Butterfield,1987; Butterfield et al.,1985;Butterfield and Thompson,1983) and in cattle (Ciannzo et al.,1982) as the animal develops in size. The various fat depots can be early or late maturing but tend to have growth coefficients not significantly different from 1.0.

Due to variations in the quantity and quality of feed available and to the multiplicity of the environmental stressors to which animals at pasture are exposed, not a lot of emphasis is placed on the age of an animal in gauging its level of maturity and body composition.

3.2 Liveweight

Liveweight of an animal provides a good indicator of the stage of maturity an individual has attained (Butterfield,1988). Growth rate generally peaks at 0.2-0.3 stage of maturity and then declines to maturity. Likewise the food efficiency of sheep decreases from a peak at a similar stage of maturity. In the same study, a mature Merino ram at approximately 100kg body weight, comprised 22.5% carcase muscle, 5.5% bone and 21.8% carcase fat. The balance (50%) is comprised of non-carcase tissue. Muscle to fat ratio in the body is highest soon after birth (9:1) and decreases subsequently as liveweight increases to a ratio of close to 1:1 (Butterfield,1988).

It has been shown that both fat and protein content of the body increased as empty body weight increased in both lambs (Searle et al.,1979) and in cattle (Sully and Morgan,1982).

A convenient way of expressing the development of fat and muscle within the body, is to express it as a proportion of total tissue at maturity. The maturity coefficients derived from a quadratic equation describing the growth pattern of various tissues facilitate the classification of tissues on the basis of the stage of growth at which their incremental gains constitute most to liveweight increase. As stated in the previous section, tissues may be classified as being either early medium or late maturing.

Protein gain for sheep, expressed in grams per day, has been shown to decrease after weaning (Searle and Graham,1987) and therefore the reason for the increase in carcase fatness post-puberty was a decrease in protein accumulation per se and not due to an acceleration in lipogenesis.

Butterfield's (1988) results were similar to those of Searle, with muscle weight increasing to maturity but with the rate of gain decreasing after 0.4 of the mature liveweight, which corresponds to a liveweight that is slightly later than puberty. In the same study, carcase fat increased over the entire period up to maturity with the increments increasing in the latter stages.

Selection for high and low weaning weights has no impact on the proportion of dissected or chemical fat and muscle in the body as proportions of the total carcase (Thompson et al.,1985). Animals selected for greater weaning weight had significantly more bone while later maturing patterns for fat were found in animals selected for low weaning weight between the selection lines while no difference was evident in the pattern of muscle development. Differences in the energetic efficiency of growth were highlighted. The faster growing sheep displayed a greater thermochemical efficiency (TCE) after weaning with maximum TCE occurring at an earlier stage of maturity (Thompson and Parks,1985). TCE is calculated as the gain in body energy per unit of metabolizable energy intake.

In general, as age and liveweight increase, the sum of all fat depots increases, which corresponds to a decline in the rate of nitrogen accretion or protein deposition in ruminants (Black and Griffiths,1975) hereby reflecting the normal allometric pattern of tissue growth. As an animal approaches mature weight, nutrient requirements for lean tissue decline and a greater proportion of nutrients consumed is deposited as fat. This phenomenon occurs at around puberty under good nutrition, when the epiphyseal plates of the long bones fuse, limiting further skeletal development. This point in time, incidently correlates with a decrease in growth hormone secretion (Vander et al.,1980). The demand for nutrients for muscle development is diminished, leading to a nett surplus of energy for storage as triacylglycero .

3.3 Breed

3.3.1 Cattle

Within species, differences exist between breeds in the stage of maturity at any given liveweight and the partitioning of fat depots. Early maturing breeds of cattle have a greater percentage body fat at 6, 13, 20 months of age (Hereford v's Friesian) than later maturing breeds (Truscott et al.,1983). In this study Friesian steers were heavier than their Hereford counterparts and were leaner. Herefords deposited more dissectible fat subcutaneously than Friesians, whereas the Friesians deposited more in the non-carcase depots.

A more extensive study in dairy cattle (Butler-Hogg and Wood.,1982) demonstrated Friesians to be significantly heavier than Jersey's at all ages and to have less body fat from day 89 until the conclusion of the experiment. A comparison of the steers slaughtered at the same age, showed that Friesians comprised of more lean, greater subcutaneous fat and intermuscular fat at most ages, while Jerseys contained more kidney and channel fat and intra-abdominal fat. There is a suggestion from these data, that there is a possible physiological link between the capacity for milk-fat production in a breed and the relative partitioning of fat within the body. The tendency is for breeds with greater milk-fat to deposit proportionally more fat intra-abdominally. The data of Thompson and Barlow (1981) support breed differences in fat partitioning in their study of a range of breeds and their respective crosses. In addition to sire breed effects, the partitioning of fat also changed with carcase fatness. Subcutaneous fat increased and intermuscular fat decreased with increasing maturity.

Studies within a range of crossbred cattle (Cianzio et al.,1982) divided into those with large and small frames, demonstrated that carcases from the smaller frame group contained 3% more fat than than the larger framed animals; however the fact that the comparison was made at the same age confounded this experiment since it was most likely that the two groups were at a different stage of maturity at slaughter. Therefore caution must be exercised in interpreting results of experiments of this nature.

3.3.2 Sheep

Searle and Graham (1971) found that Border Leicester x Merino wethers when compared to wethers of Peppin Merino origin, grew faster and were heavier at all stages to 10 months of age. Between the liveweights of 17kg to 45kg, Merinos were fatter than the crossbreds, indicating that an animal of smaller mature size is fatter, on a relative weight basis than an animal of large mature size.

The data of Reid et al. (1968) also show differences in body composition between sheep of various breeds. Corriedales contained more fat at any given body weight than Suffolks which are known to be larger animals. However, Butler-Hogg (1984) found no differences in the relative growth of total body fat, muscle or bone between the faster growing Clun sheep when compared to Southdowns. The studies of McClelland and Russel (1972) comparing Scottish Blackface with the larger Finnish Landrace lambs support these findings.

In conclusion, body mass is the major determinant of body composition. Reid (1972) demonstrated in a regression analysis of compositional data for sheep of a given breed and sex that body weight accounts for 88-95% of the variance in total body water, fat and protein, while age had only a minor impact on carcase composition.

3.4 Sex

The debate over the effect of sex on carcase fatness has continued for many years. Comparisons have been made between females, males and castrates at either a constant age or a constant weight (Vezinhet and Prud'hon,1975). Beyond 100 days of age, total fat is higher in female sheep than in rams The sex difference found in Merinos by these workers was firstly evident in the perirenal and pelvic fat and later in the omental fat. Differences in subcutaneous fat depth were demonstrated between wethers and ewes (Atkins and Thompson,1979). Mature wethers have 3% more body fat than rams at maturity (Butterfield et al.,1985). Wethers partitioned more fat in the subcutaneous depot and less in the intermuscular depot while rams tended to have a greater proportion of total body fat in the mesentry compared to wethers.

At the same liveweight rams have higher protein levels than wethers, which in turn are higher than ewes (Morgan and Owen, 973). Rams have a greater efficiency of feed conversion into liveweight relative to wethers and ewes at the same liveweight. The superior growth rates of rams and their ultimate heavier mature bodyweight is mainly accounted for by testes weight and larger head and neck (Butterfield et al.,1984) resulting in lower dressing percentages.

In the studies of Thompson et al. (1985) the relative proportions of bone, muscle and fat differed between sexes up to maturity. Muscle to fat ratio of rams was higher than in ewes at maturity (1.5 v's 1.1), while ewes have been shown to have a higher weight of fat per kilogram of muscle (Butterfield,1988).

The development of musculature in rams tends to be superior for muscles connecting the forelimb to the neck and the neck and thorax region. This has functional significance and thus is a contributing factor to the hierarchy of flock rams and has probably evolved from

natural selection over many generations. Ewes tend to develop the muscles of the proximal hind limb and abdominal wall (Perry et al.,1987) in preparation for pregnancy . In cattle Berg et al.(1979) demonstrated that Shorthorn sired XB heifers are fatter than steers, which in turn are fatter than bulls when compared at a common muscle weight. Significantly greater accumulation rates of lipid in subcutaneous, intermuscular and body cavity fat depots relative to total fat exist in heifers when compared with bulls (Jones et al.,1981).
The sex differences observed, can be accounted for in the different stages of maturity reached at the same liveweight and by the anabolic impact of gonadal steroids.

4. Nutrition

4.1 Diet

Under conditions of continuous positive growth, the higher the growth rate of an animal, the fatter it will be at any given weight (Morgan and Owen,1972,1973; Sully and Morgan,1982). To meet the above criteria an animal's diet needs to be balanced in both satisfying the protein needs and energy requirements of the animal. The dietary requirements change throughout life from birth until maturity with greater demands for such physiological states as pregnancy and lactation.

4.1.1 Protein

Type of diet can have a significant effect on digestion and the accretion of fat and lean. Treating high protein rations (Leng,1986) and silage (Thompson et al.,1981) with formaldehyde, substantially increases protein supply in the duodenum and leads to an enhanced nett protein deposition. Protected protein, otherwise referred to as by-pass protein, is being utilised in feed rations to a greater extent. A concomitant reduction in net fat deposition takes place in cattle under the same feeding conditions, due to less microbial breakdown of dietary protein in the rumen leading to lower levels of VFA's. High protein supplements that are protected from rumen degradation such as fishmeal, substantially increase growth by more than 30% and protein gain by 50% with negligible effects on fat gain (Beever et al.,1992). High protein diets that are unprotected (eg. lupins) are substantially degraded by rumen microflora, liberating VFA's to meet the animals energy requirements (Leng,1986). A correct balance of protein and energy is required in the diet to allow an animal to attain a mature bodyweight as quickly as possible.

4.1.2 Energy

A positive relationship exists between the energy content of the diet and the rate of muscle and fat accretion in the body (Oddy,1993). The principal energy-yielding components of fresh forages are water soluble carbohydrates and fibre, giving relatively high yields of acetate and butyrate from the rumen (Van Houtert,1991). In contrast yields of propionate and therefore glucose synthesis and lipid absorbed from the small intestine, are typically low. Most forage diets tend to promote the synthesis of body fat, derived mainly from the synthesis of acetate and ß-hydroxy butyrate (Smith and Crouse,1984). This phenomenon may well be due to environmental adaption of ruminants to the cyclic nature of diet quality and quantity, peaking in spring and early summer when animals typically deposit fat reserves. Diet quality drops off generally to its lowest level in late autumn and winter, placing demands on the animal's fat reserves for survival through to the following spring.

4.2 Growth Path

A reduced frequency of milk intake for young lambs can impact on growth rate, thereby determining the stage of maturity that a growing animal will reach at any given age. Reducing the frequency of feeding of artificially reared lambs decreased growth temporarily and was possibly due to the small volume of the reticulo-rumen in young animals and therefore the inability of the lamb to consume the appropriate volume of milk. Thus more frequent feeding of milk in smaller volumes improves the efficiency of growth. Decreased growth rate prior to weaning, however, had no long term impact on body composition of the mature sheep maintained on ad libitum feeding of a high quality ration after weaning (Morgan and Owen,1972). However, lambs maintained with restricted access to milk, ate greater amounts of concentrate prior to weaning than lambs with unlimited access to reconstituted milk. This substitution effect probably occurred to meet the energetic requirements of the animal, irrespective of the feed source. Butler-Hogg and Johnsson (1986) have studied carcase compositional changes in animals restricted to grow at two defined growth rates, (220g/day v's 118g/day). In the study, the faster growing lambs contained significantly heavier fat depots in their carcases at 20 weeks of age, while there were no differences in the weight of lean tissue or bone. Changing from a low growth rate to a high growth path leads to greater carcase fat accumulation than the reverse situation, which is probably due to the lower energy levels being available for lipogenesis in low growth rate animals up to slaughter.

4.3 Weight Loss and Compensatory Gain

A great deal of attention has been placed on the impact of the nutritional history of animals on their growth and carcase composition of animals (Butler-Hogg,1986;Graham and Searle,1975;Searle et al.,1979;Thornton et al.,1979). The early stages of weight loss or stasis in weaner sheep are characterized by a large loss of water and protein from the tissues, and after prolonged weight loss, body fat loss contributes significantly. Composition of body components during compensatory growth is similar to that of uninterrupted growth. Thornton et al. (1979) reported similar findings but contrasted weight loss at two stages: weight loss in mature animals contained a greater proportion of fat to protein, resulted in substantially more fat being lost than protein, but in immature animals protein and fat depots were mobilized at the same rate. Fat mobilization was greatest in non carcase fat depots. Loss of lipid reserves from mature sheep was brought about by cellular atrophy rather than by a reduction in adipocyte numbers, while in young sheep both atrophy and hypoplasia contributed to the decrease in fat content. This study highlights the mobile nature of intra-abdominal fat depots.

4.4 Maintenance

Animals maintained at constant liveweights for prolonged periods tend to adapt to the situation by reducing basal metabolic rate (Graham and Searle,1975). In their study, after four or six months of weight stasis the carcases of wethers contained less protein, more water and the same amount of fat when compared to control sheep which were fed to maintain a normal rate of growth. In a more detailed study Ryan and Williams (1990) showed that feeding sheep at a maintenance level for 12 and 17 weeks significantly reduced the weight of liver, digestive tract and hide after 12 weeks. This may be explained by the fact that the liver and mucosal lining of the gastro-intestinal tract are considered to be more metabolically active and therefore the composition of an animal at weight stasis results in a proportionately greater effect on these tissues. In contrast, after 17 weeks at constant bodyweight, both protein and fat in the carcase had increased as a result of possible adaptation and lowered metabolism of vital organs.

5. Hypothalamic-Pituitary-Adrenal Axis

Enviromental stress and external stimuli can lead to changes in the physiology of an animal in order to maintain homeostasis and adapt to changing situations. A consequence of chronic stress is the activation of the hypothalamic-pituitary-adrenal axis (HPA). The axis is under hormonal control, with three regulatory levels controlling synthesis and secretion of hormones within distinct glands. Several peptide hormones are responsible

for initiating the pathway to adrenal cortex stimulation. As well as controlling the HPA axis, these hormones may also have physiological functions within muscle and adipose tissues.

5.1 Neuropeptide Regulation of Pituitary Function

Until recently it was thought that the hypothalamic neuropeptide, corticotrophin releasing hormone (CRH) was the major regulator of adrenocorticotrophic hormone (ACTH) release from the pituitary in rodents and humans (Jessop et al.,1989; Harbuz and Lightman,1992; Seplier et al.,1992; Hu and Lightman,1992; Antoni et al.,1992). Most of the early research was conducted using rats. More recent investigations have demonstrated the importance of arginine vasopressin (AVP) as a stimulator of ACTH release in ruminant species in response to stress, whereas the pattern of CRH release seems more involved in the regulation of basal secretory mechanisms (Familari et al.,1989; Engler et al.,1993; Hu et al.,1992). Both CRH and AVP are synthesised and secreted from neurones which are localized in the hypothalamus. The cell bodies synthesising CRH form part of the parvocellular division of the paraventricular nucleus, while AVP cell bodies emanate from the magnocellular region of the same nucleus. Synaptic and hormonal inputs play important roles in the expression of CRH and AVP including innervation by noradrenergic and adrenergic axons (Engler et al.,1993). Previous studies (Engler et al.,1988) support a unidirectional hypothalamic stimulation of ACTH release.

The presence of a CRH was recognised over 40 years ago (Guillemin and Rosenberg,1955) but the characterizat on of the 41 amino acid hypothalamic peptide that stimulated the secretion of corticotropin and ß-endorphin in sheep was not achieved until 1981 (Vale et al.,1981).

Axons from the CRH producing neurones terminate in the median eminence, where CRH-41 is released into the hypophysal portal blood stream supplying the pituitary. A variety of stressors enhance the release of CRH into portal blood (Plotsky and Vale,1984) which can be blocked by CRH antagonists competitively binding to the receptor and by passive immunoneutralization with anti-CRH antibodies (Rivier et al.,1982; Rivier et al.,1984) resulting in a fall in ACTH.

5.2 Pituitary Corticotrophs

CRH and AVP stimulate the release of ACTH and induce transcription of pro-opiomelanocortin (POMC) mRNA, the ACTH precursor (Tanaka and Kurosumi,1992). CRH is also present in the neurointermediate lobe (NIL) of the pituitary in amounts

between 10-20% of those found in the median eminence (Jessop et al.,1989). The presence of CRH in the NIL system and its function there are not clearly understood. CRH injection to both sheep and human subjects, leads to a rapid rise in ACTH in peripheral blood within 5 minutes (Saphier et al.,1992). The peak primary ACTH response to CRH occurred more quickly in sheep than in the human and was of a biphasic nature (peaks 100 minutes apart). Overall, the half-life of hCRF in man is considerably shorter than that of ovine CRH in sheep (oCRH). This cannot be easily explained, since sheep have lower levels of binding globulins which should increase the clearance rate of hormone from the body. CRH concentrations in the hypothalamic-pituitary portal system of 1μg/litre contrast with levels of only 15ng/litre in the peripheral circulation (Linton et al.,1987). The greatly diluted hormone is considered insufficient to stimulate ACTH release from this latter source since the concentration is significantly lower than the dissociation constant for the CRH receptor (Wynn et al.,1985). Therefore is likely to be functionally insignificant.

A 2-fold increase in CRH in portal circulation resulting from the stress of haemorrhage can result in a 10- fold elevation of circulating ACTH (Plotsky and Vale,1984), suggesting specificity and amplification of the responsiveness of corticotrophs to CRH stimuli.

Other control mechanisms exist, including the neuropeptide hormone atrial natriuretic peptide (ANP), which has been demonstrated to modulate ACTH release (Fink et al.,1991; Fink et al.,1992), possibly by suppressing POMC mRNA and thereby limiting the synthesis of the precursor molecule (Tan et al.,1993).

The fetal sheep anterior pituitary contains both immature corticotrophs and adult type cells, the former maturing as parturition approaches. By day 140 of gestation 90% of corticotrophs have matured (McDonald et al.,1992). Hypothalamic paraventricular nuclear lesions delay the development of fetal pituitary corticotrophs but do not ablate maturation entirely, suggesting that other factors which are yet to be identified are involved as well as CRH. The prolonged exposure to excess CRH at this stage of development, however, results in an increased number of corticotrophs in the sheep pituitary (Asa et al.,1992).

Receptors exist for this hypothalamic peptide in the plasma membrane of corticotrophs. The primary effect of CRH bound to its receptor is the activation of adenyl cyclase and an increase in the second messenger cyclic AMP (Antoni et al.,1992) which correlates with increased accumulation of intracellular free calcium ions. Activated adenylate cyclase catalyses the transformation of ATP to cyclic 3',5'-adenosine monophosphate (cAMP),

which in turn activates the enzyme protein kinase A (Vander et al.,1980). This chain of events ultimately leads to the processing of POMC into its constituent peptide fragments. Proteolytic processing of POMC gives rise to ACTH and ß-lipotrophin in the anterior pituitary, whereas in the intermediate lobe the latter fragment is cleaved to form ß-endorphin, α-melanocyte stimulating hormone (α-MSH) along with corticotropin like intermediate peptide (CLIP) and gamma lipotropin. Differences in the specific peptides found in the anterior and intermediate lobe corticotrophs reflect differences in the processing enzymes present in these two pituitary lobes, resulting in further processing of the peptides in the NIL.

Other hormones can potentiate the act on of CRH in vitro. The two nonapeptides vasopressin (AVP) and oxytocin together with angiotensin can potentiate the biological activity of CRH to increase ACTH secretion from corticotrophs (Vale et al.,1983). The catecholamines, adrenaline and noradrenaline can also play a similar role . This potentiation of ACTH releasing activity of CRH is likely to occur, when animals are exposed to any stressor and has been demonstrated for haemorrhage and hypoglycaemia (Engler et al.,1988). Other researchers have demonstrated that AVP and CRH act synergistically in the release of ACTH, with total release rates of ACTH being far greater than the sum of individual responses (Gillies et al.,1982). Increased portal blood concentrations of AVP following stress (Gibbs,1985) and the identification of AVP receptors in membranes of the anterior pituitary (Gaillard et al.,1984) further support the role for this hormone in regulating release of ACTH in response to stressful stimuli. A great deal of research is focussed on a better understanding of the controlling mechanisms of the HPA axis and its relationship with stress with a view to understanding the impact of stress on human health, however, far less research has been conducted to determine the impact of this axis on animal productivity.

Figure 1.3 schematically depicts the HPA axis.

5.3 Circadian Rhythm

A feature of the sheep pituitary-adrenal axis is the circadian rhythm of POMC derived peptides and glucocorticoids (Jones,1990) which is also evident in rats (Atkinson and Waddell,1993). Circulating ACTH, ß-endorphin, a-MSH and cortisol levels peak during darkness and decline during daylight hours, apparently under the regulatory influence of suprachiasmatic neurones of the hypothalamus. The rise in circulating hormones is characterised by increased amplitude of the secretory pulses without any alteration in pulse frequency. The length of the diurnal secretory zenith is dependent on the length of the dark phase. ACTH receptor numbers in the adrenal are doubled in early morning

REGULATION OF GLUCOCORTICOID PRODUCTION

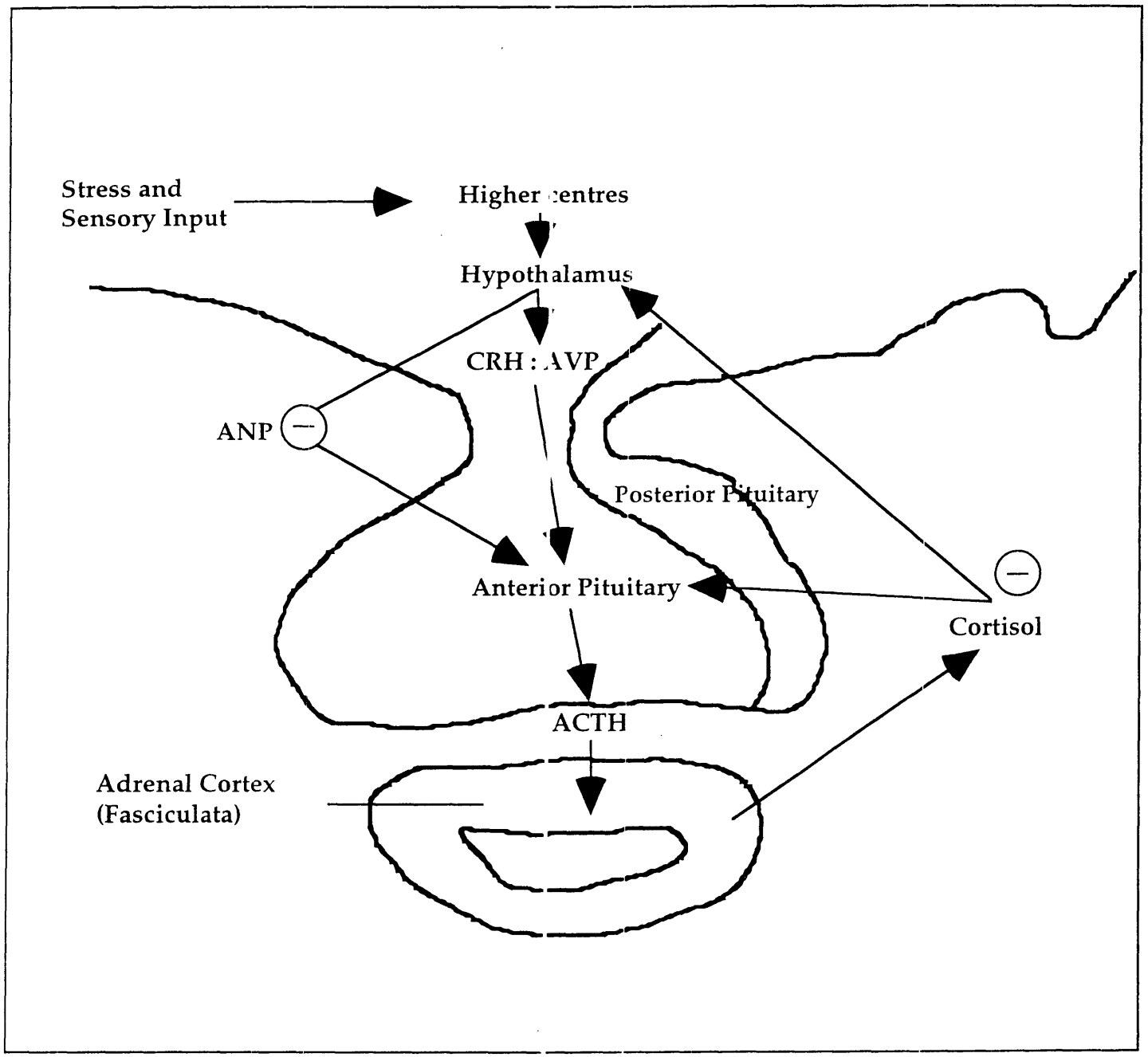


Figure 1.3 Schematic representation of the HPA axis and the regulation of glucocorticoid production.

compared to the afternoon (Jones,1990) suggesting that ACTH may regulate its own target tissue receptor and therefore responsiveness. Gonadectomy ablates the circadian rhythm, indicating that these steroid hormones have a role in maintaining this rhythmicity. Consistent with these findings is the fact that prepubertal lambs do not exhibit diurnal variation in POMC peptide secretion, when steroidogenesis would be very low (Jones et al.,1988). The role of these interactions in controlling the circadian rhythm is not well understood, however.

5.4 The Regulation of Adrenal Steroidogenesis

Physical and psychosocial stimuli result in elevated levels of ACTH in peripheral blood reaching the major target tissue, the adrenal cortex (Baxter and Rousseau,1979). The adrenal glands are situated near the left and right kidney and derive blood from the aorta and renal arteries via small arterial branches. Afferent adrenal vessels drain into either the vena cava or renal vein. The adrenal cortex comprises three distinct zones, the peripheral glomerulosa, the fasciculata and the inner reticularis. ACTH binds to specific high-affinity receptors on adrenocortical cell membranes, which are saturable and which bind ACTH in a temperature and time dependent fashion (Jones,1990).

This peptide hormone acts via adenylate cyclase and cAMP, to stimulate steroidogenesis within the cells of the zona fasciculata. The major steroids produced are collectively termed glucocorticoids due to their carbohydrate regulating properties. Cortisol, cortisone and corticosterone are all synthesized from cholesterol (Baxter and Rousseau,1979). The series of enzymatic reactions is thought to be rate limiting at the level of the conversion of cholesterol to pregnenolone. This is then converted to progesterone and subsequently to cortisol through three successive hydroxylations. They all have the classical carbon ring structure with similarities to the sex steroids produced in the gonads. Cortisol is the major glucocorticoid in man, whereas corticosterone is predominant in the rat (Baxter and Rousseau,1979; Sillence and Rodway,1987a).

Adrenal innervation via the splanchnic nerves is capable of promoting the steroidogenic action of ACTH (Edwards et al.,1986), since bilateral splanchnic nerve section halved the dose response of plasma cortisol to ACTH stimulation. Either reduced adrenal blood flow to the gland or reduced neural adrenal medullary activity could account for such observations. Injection of ACTH can evoke a dose-dependent rise in plasma cortisol (Crowley et al.,1991) with peak plasma concentations occurring 15 minutes after intravenous injection. The dose-response curve plateaus at higher ACTH concentrations indicating saturation of the receptor effector mechanism.

Differences in steroid transport in blood exists between species. In humans and rats nearly 95% of cortisol present in the circulation is bound to plasma proteins, mostly to a specific corticosteroid-binding globulin (CBG) and to a lesser extent albumin (Gala and Westphal,1965). In the rat, CBG activity is greater in females than males. Castration increases binding in males to a level closer to that of females.

The situation is quite different in sheep. A greater amount of glucocorticoid is not bound to protein (22% free), with the concentration of albumin in the plasma of sheep less than in man and the fraction of plasma cortisol bound by albumin less in sheep (Paterson and Hills,1966). The study by Ali et al.(1992) demonstrated that only small amounts of CBG exist in adult sheep, which contrasted to high levels observed in the first and third trimester of pregnancy in the developing fetus. This pattern was similar to changes in fetal cortisol levels. The differences in CBG levels need to be further studied to get a better understanding of their dynamics

The kinetics of glucocorticoid response or metabolism may well be influenced by plasma binding. In humans cortisol has a half life of around 60 minutes (Baxter and Rousseau,1979) and is extensively metabolized in a number of tissues, but mainly in the liver. Glucocorticoid analogs are more resistant to metabolism with a lower affinity for CBG (Blecher,1966). This results in their greater biological potency.

5.5 Negative Feedback

The glucocorticoids have a negative feedback influence at the levels of both the hypothalamus and pituitary (Antoni et al.,1992; Hu et al.,1992) thereby dampening the responsiveness of the HPA axis. Elevated levels of steroid hormone inhibit CRH release at the hypothalamic level (Vale et al.,1983; Plotsky and Vale,1984) and decrease mRNA for the ACTH precursor protein POMC (de Kloet et al.,1991). Receptors for cortisol have been identified in parvocellular neurones of the paraventricular nucleus (De Wied and Croiset,1991) and in pituitary corticotrophs (de Kloet et al.,1991). Negative feedback prevents the HPA axis from over-responding which could be detrimental to body tissues and physiological function.

5.6 Glucocorticoid Actions

Adipose tissue has been shown to be a target organ for glucocorticoid hormones as Feldman and Loose (1977) identified glucocorticoid receptors in adipocytes from rats. However, the ubiquitous nature of receptors around the body for this group of hormones (Ballard et al.,1974), is testimony for the importance of these steroids in relation to cellular function in most tissues.

5.6.1 Metabolic Actions

Glucocorticoids appear to be site specific in their ability to inhibit the uptake of glucose into adipocytes (Hauner and Pfeiffer,1989; Mendes et al.,1985) and can reduce the total number of insulin receptors on adipocytes. This can lead to reduced basal and insulin stimulated rates of lipogenesis. Hauner and Pfeiffer (1989) demonstrated glucocorticoids to have a low capacity to stimulate fatty acid synthesis in epididymal and perirenal fat depots, and they have no effect on subcutaneous fat cells. The combined actions of ß-agonist and corticosterone in these studies did not enhance the lipolytic effect of the agonist alone.

Exposure of adipocytes to glucocorticoid hormones inhibits glucose transport and metabolism (Roth and Livingston,1976). The promotion of insulin resistance and lowered glucose transport into peripheral tissues ultimately conserves glucose for essential organs including the brain, liver and kidneys. Glucose is the major energy substrate for neural tissue maintenance and metabolism.

Blood glucose is elevated progressively with increasing circulating glucocorticoid levels as is urinary nitrogen (Bassett,1963). This latter response is indicative of the catabolism of protein and the deamination of amino acids undergoing gluconeogenesis in the liver. Only small changes in blood ketones and plasma free fatty acid levels occur, suggesting no substantial increase in lipolysis.

5.6.2 Glucocorticoids and Growth

Excess glucocorticoids can significantly reduce growth in laboratory rats and man (Wehrenberg et al.,1990; Mendes et al.,1985). The inhibitory effects of pharmacological levels of dexamethasome treatment can be partially reversed by the passive immunization of animals against somatostatin (Wehrenberg et al.,1990), an inhibitory neuropeptide of GH. However, results were not conclusive, as the presence of antibodies to somatostatin were not capable of fully restoring the growth rates in the absence of dexamethasome which may be due to the actions of somatostatin in other parts of the body.

Glucocorticoids can stimulate growth hormone secretion at low levels or inhibit secretion at high levels (Seifert et al.,1985). Somatotroph receptors to growth hormone releasing hormone (GHRH) are decreased after adrenalectomy, which can be reversed with low levels of glucorticoid hormone replacement therapy. Human subjects with ACTH deficiency have lowered growth hormone responses to GHRH (Giustina et al.,1989) while replacement therapy with glucocorticoids restores the GH response (Wehrenberg et al.,1983). It would appear that a physiological window exists for adrenocortical stress hormones to optimize GH response in vivo.

Treatment with dexamethasone is capable of increasing feed intake in sheep when stressed due to unfamiliar penned situations (Adams and Sanders,1992). High doses have the opposite result, thereby suppressing intake and growth.
Spurlock and Clegg (1962) increased feed intake and growth of lambs using cortisone acetate at low and moderate levels, while the response plateaued at the highest dose level. Fat in the carcase was significantly increased and protein decreased with increasing levels of steroid. Similar results have been obtained with cortisol administration to non pregnant ewes (Bassett,1963) . Another study examining ACTH treatment twice daily, led to sheep refusing feed rations (Doney and Smith,1969). In summarizing, it would appear that extreme under or over-secretion of glucocorticoids is deleterious to the growth of an animal.

5.6.3 Glucocorticoid Receptors

Although the actions of glucocorticoids may appear similar within various fat depots on the basis of similar affinity to dexamethasone but when corrected for differences in cytosolic protein concentrations, receptor numbers vary (Feldman and Loose,1977). The potent analogue dexamethasone has greatest affinity for the receptor, followed by corticosterone, aldosterone, testosterone and finally oestradiol. Work by Roth and Livingston (1976) identified an age effect on glucocorticoid receptors. Concentrations and absolute numbers of receptors per adipocyte were reduced during maturation and aging of cells. Gradual loss of glucocorticoid receptors over time could lead to decreased glucocorticoid responsiveness.

5.6.4 Glucocorticoid Induced Hyperplasia of Adipocytes

In the presence of insulin, cortisol can significantly promote hyperplasia of adipocytes (Hauner et al.,1987). Differentiation of preadipocytes into mature cells was increased 30 to 70 fold in human subcutaneous tissue compared to the absence of cortisol. Neither cortisol nor insulin alone were effective in promoting adipocyte differentiation, although dexamethasone was more potent than cortisol in stimulating the differentiation of adipocyte precursor cells. The gonadal steroids testosterone, oestradiol and progesterone had no adipogenic activity. The onset of differentiation is accelerated by glucocorticoids by activating the transcription of genes that are expressed in the mature adipocyte (Knight et al.,1987). The conversion of the cell from an inactive preadipocyte to a state in which lipid accumulates in the mature adipocyte undoubtedly results from the activation of enzymes responsible for lipogenesis. The impact of glucocorticoid induced hyperplasia of

adipocytes has not been quantified in terms of its possible contribution to overall lipid accretion in the various fat depots during the fattening phase of animals.

5.7 Glucocorticoid Induced Immuno-suppression

This group of steroids are well-known immunosuppressive agents when present at high concentrations. They are capable of dampening the immune response at several points. Glucocorticoids inhibit macrophages, the secretion of interleukin 1 and 2 and macrophage activating factor (Jasmine,1991) and thereby inhibit all immune activity.

Studies in calves, have shown a significant decrease in B lymphocyte cells in response to dexamethasome treatment, while T cell lymphocyte numbers decreased for the sub-populations expressing the gamma and delta form of the T cell receptor (Oldham and Howard,1991).

The immuno-suppression induced by elevated glucocorticoids in animals has a somewhat selective action on various functions of the immune system.

The compromise of the immune system will be discussed in more detail in chapter 4 as well as experimentation to negate the possible immuno-suppressive effects of elevated glucocorticoids.

Chapter 2

Literature Review

Techniques for the measurement of body and carcase composition

1.Introduction

A need exists for reliable methods to assess carcase and or body composition. The three major compartments within the carcase, muscle, fat and bone can be further subdivided into specific site and physiological functions and can impact on the value of sections of the carcase to the processor. Most methods of measurement have limitations either on their accuracy, their significant labor input, their destructive nature and their lack of repeatability, thereby creating the need to conduct serial slaughters to measure animals over the growing and fattening phases

Techniques capable of repeated and precise estimates of body composition on the live animal have marked advantages over serial slaughter techniques, giving the researcher the opportunity to design experiments examining changes within the same animal or animals over time. Statistically, it reduces the between animal variation and hence is a more powerful tool for detecting small but significant differences. Recent technology (Simm,1987) has overcome the need for destructive measurements with several accompanying benefits, but have limitations on their overall use, as will be discussed in this chapter. The meat industry would be advantaged enormously if simple and precise methods were available to describe precisely the live animal in terms of musculature, fat deposition and distribution in order to comply with specific market specifications and to monitor experimental treatments aimed at partitioning body fat.

2. Slaughter

2.1 Dissection

Historically, half carcase dissection of a slaughtered animal and weighing the components has been the most reliable estimate of body composition to the anatomist. The laborious dissection of individual muscles, the thorough cleaning of bones and the demarcation of various fat depots is open to substantial errors unless performed by the most skilled technician. Dissection losses can be proportionately less than 1% of the total animal when extreme care is taken (Thompson et al.,1985). Total dissection time for lambs is

commonly in the order of 18 hours per carcase (Timon and Bichard,1965). The expense in labour costs for the 1990's would be hard to justify with ever diminishing research budgets. In the same study, fat measurements were much more variable than muscle measurements, which in turn show greater variation than skeletal bone measurements. When analysing animals at a constant carcase weight, the only measurement which strongly correlated with carcass muscle percentage was eye muscle area (EMA). This work highlighted the need to increase the number of animals per treatment, to minimize the sampling error when studying homogeneous groups of animals. In order to examine small differences between animals statistically, the need for larger groups is accentuated. In contrast, earlier studies by Palsson (1939) indicated various bone and carcase measurements were highly correlated with carcass bone and muscle. The wide range in the age and genetic diversity of the animals sampled in the latter study may account for the variance in results of the two studies.

2.2 Sample Joint

The dissection of sample joints from lambs and their relationships between the fat, muscle and bone percentages of the carcase has been examined (Timon and Bichard,1965; Palsson,1939). Moisture losses have been discussed as a problem during extended dissection periods (Palsson,1939) which can be partially remedied by half carcase sections or as a last option, by increasing the number of personnel performing the dissections. The latter has the disadvantage of increasing operator error. Dissection times varied widely from 1.5 hours for the neck carcase joint to 4 hours for the leg joint. None of the seven sample joints were consistent in predicting either carcase fat, muscle or bone. As the number of sample joints were increased for use in the regression equation for the prediction of total carcase components, the better the correlation that was achieved. This of course has the disadvantage of markedly increasing dissection times for reliable estimates and defeats the purpose of sample joint estimation. The greater percentage of a particular variable in an individual joint, did not indicate that its prediction would be better for the total tissue within the carcase. These findings supported by Kempster et al. (1976) using a number of sheep breeds, indicate variable dissection costs, across the full range of sample joints. The main problems encounted are the definition of an adequate sample size as a proportion of the total size of the carcase, the definition of cutting boundaries between joints and the losses of moisture from exposed surfaces.

Although multiple carcase measurements increase the accuracy of prediction equations of total tissue present and are useful experimentally, they are not acceptable commercially for cost reasons.

2.3 Chemical Determination of Composition

Solvent extraction of lipid from meat samples using cellulose thimbles in soxhlet apparatus (Field et al.,1985) has been a reliable method of estimating percentage fat in muscle samples or percentage fat of the total carcase or body. Stringent mixing of ground material and non biased sub-sampling is most important when only 10g of sample is being analysed as a proportion of large animals which can be in excess of 50kg bodyweight. Other methods for the estimation of fat in tissue samples include the Foss-Let density apparatus, utilizing the solvent tetrachoroethylene (Usher et al.,1973). This method has the advantage of analysing larger sub-samples (up to 50g) of the tissue of interest and hence reducing possible sampling error. The relationship between density and percentage fat in the solvent is linear over an adequate range for meat samples. The results from this method deviate from those obtained using chloroform and other similar solvent extraction methods when samples with a high phospholipid content such as dried egg and neural tissue are evaluated. Estimates from the Foss-Let method are lower for these tissue types and probably underestimate other animal tissues to a lesser extent.

3. In Vivo Techniques

A great deal of attention has been placed on methods to measure body composition in vivo. Sufficiently accurate methods would overcome serial slaughter experiments and the need for the destruction of the animal to obtain carcase measurements.

Until recently, most in vivo methods relied on simple anatomical measures to give the best prediction and correlation to the total tissue present. Measurements include such objective measures as hip height, heart girth and liveweight in cattle and subjective measures such as condition scoring (Bullock et al.,1991;Faulkner et al.,1990).

Advanced equipment, which until recently has been used solely for diagnostic purposes for human medicine, is now becoming available for experimental purposes to assess body components and form. This equipment has been extremely expensive, but as technology advances, the relative unit cost is dropping to within the relms of the budgets of animal scientists as it becomes old technology in a relative short space of time. The technology relies greatly on the co-operation of the patient for good resolution of images, which is a problem with animal experimentation

3.1 Ultrasound Equipment

Ultrasound travels at 1500 metres per second (Wells,1991) through the soft body tissues, rebounding off boundaries between tissues of different densities. These echoes are detected by the transducer held parallel to the point of examination. Most ultrasound

equipment has been used in subcutaneous fat estimates in cattle to predict carcass fat and M.longissimus area to predict total muscle (Smith et al.,1992;Bullock et al.,1991).The earlier ultrasonic measurement devices used, of which the "scanogram" is an example, produced a printed image and was capable of measuring subcutaneous fat at several positions on the body and for measuring muscle area of the M.longissimus in cattle (Truscott et al.,1980) and in sheep (Moody et al.,1965). This equipment was not particularly accurate for measurements of EMA on consecutive occasions.
Ultrasound equipment has been used to assess marbling (intramuscular fat) in cattle with limited success (Stouffer et al.,1989). Qualitative measurements of this parameter are reasonably accurate but not so for quantitative assessment.
In a study conducted by Smith et al (1992), subcutaneous fat thickness over the eye muscle was measured with good repeatability, with a strong correlation to fat thickness measured on the carcase. Results from trials conducted by Bullock et al. (1991) are at variance with those of this worker. In assessing fat thickness at two sites, the measurement at the rump (P8 position) was a better estimate than the one taken at the twelfth rib. Both positions were variable, depending on the body condition of the animal. The greater range in age and liveweights in the latter study could account for such differences when compared to the homogeneous group of lot fed steers in the former study.
Both scanograms and real-time ultrasound equipment can suffer in the precision of measurements and repeatability due to the make and model of the equipment used, the level of operator skill, the thickness of the haircoat and the fat level of the animal. Other sources of error may be due to differences in the speed of ultrasound travelling through fat and muscle (Wells,1991). Depending on the calibration of the machine, either for fat or muscle or a compromise of the two, over or under estimates of these body tissue measurements can arise.
On the contrary, Ferguson (1994) has demonstrated this non-invasive technique to be as comparable in accuracy as cut-and-measure techniques when commercial hide pullers are used to prepare beef carcases due to the variability of fat residues left on the hides.
With continuous improvements in the technology, greater reliance on this non-invasive technique is inevitable.

3.2 CAT Scanning

Computerized tomography offers the scientist the opportunity to study the body form in greater detail in vivo. The attenuation of X-rays from a rotating source around the body to

a fixed array of detectors can lead to excellent anatomical details and striking discrimination between soft tissue, bone and air.
However, the standard software for CAT-Scanners is set up to measure distances between two points and simple areas directly from the screen for medical diagnosis. When first adopted for animal experimentation, it originally lead to the utilization of the technology to predict body composition from anatomical measurements taken from one or two scans of the animal (Skjervold et al.,1981).
As discussed earlier, these types of relationships do not always correlate highly with total body or carcase composition. More precise estimates of body composition have been obtained with several scans of the same animal (Sehested,1984) for all tissues. Software and methodology developed by Thompson and Kinghorn (1991) allows the scanning of the whole body to avoid the need for complex prediction models. Based on Cavalieri's Principle (Gundersen et al.,1988) the direct estimation of the volume of tissue can be calculated from a series of parallel sections taken equi-distantly without bias. The CATMAN software in combination with a CAT-Scanner provides an efficient and accurate means for predicting muscle, fat and bone in the body of live animals.
The equipment is limited to sheep, goats and deer or smaller animals due to the aperture size of the X-ray tube. Animals have to be kept immobilized and still for up to 30 minutes in order to take the required number of scans along the body. Pharmaceutical sedatives are useful, but they can reduce food intake for a couple of days after treatment. Combined with the physical restraint, fasting and the stress and trauma associated with scanning, all these factors could have a deleterious effect on the growth of a young animal. This may compromise the experimental protocol.
Discrimination between internal fat depots can present a problem if the focus of research is on partitioning of body fat. The technique is therefore not suited for such investigations.
The time taken to manipulate files in preparation for image analysis can detract from the efficiency of delivering results quickly. Despite these minor drawbacks, the technology offers tremendous opportunities to scientists for precise and accurate estimates of body composition which were not available previously (Allen,1990).

3.3 Magnetic Resonance Imaging

Nuclear Magnetic Resonance (NMR) is an extension of an analytical laboratory technique into a piece of equipment capable of imaging the structure of body form (Wells,1991). The NMR scanner has been developed for humans with a narrow opening in the electromagnet approximately 600mm in diameter and 2 metres long. The technology is

based on the alignment of atomic nuclei that have an odd number of protons or neutrons in the body when exposed to a magnetic field (Simm,1987). The hydrogen atom forms by far the most numerous source of such nuclei in the body. Images discriminate between fat, muscle and bone equally as well as the CAT-scanner (Allen,1990). Other applications for the equipment is in the area of "spectroscopy", which offers greater advantages over X-rays alone. NMR imaging has all the limitations of CAT-scanning, plus the added cost associated with this more recent technological advance.

3.4 PET Scanning

The Positron-Emission Tomography technique is similar to CAT scanning in the image produced. Rather than relying on an external radiation source (X-rays), PET scanning requires the injection or dosing of a biological compound which has been labelled with a radioactive isotope (Allen,1990) prior to scanning. The decay of the radioactive label emits gamma rays which are recorded by an annular array of detectors after passing through the body tissues. This technique has possibilities for determining tissue lipid content, by labelling esters with radioactive carbon and administering them to the subject. Although this technique is currently being utilized for medical diagnosis, it is unlikely to be used as an experimental device for determining body composition in animals in the forseeable future.

3.5 Total Body Water Assessment

Animals are chemically composed of fat, protein, water and ash in known and various proportions (Ryan and Williams,1990). From a knowledge of the body weight and the determination of the total body water, the weight of fat and protein can be estimated from prediction equations (Graham,1982). Reliable methods for determining the volume of total body water using tritiated (Searle and Graham,1975) or deuterated water injected intravenously rather than given orally into the animal are available (Till and Downes,1961). A source of error in this method for sheep, is estimating the fleece weight present on the animal, which has to be assessed without shearing for serial studies. Other errors include gut fill variation throughout the day, changes in physiological state (pregnancy and lactation), repeatability errors in the order of 5% and water losses due to respiration. Respiratory water loss fluctuates with ambient temperature over the 5-6 hour equilibration time.

More recently, this method combined with ultrasonic fat depth measurement reduced residual standard deviation values, suggesting that extra precision can be obtained by incorporating both techniques in the determination of body composition (Allen,1990).

The technique has been useful as an experimental tool to estimate total fat, muscle and bone during the growing and fattening phases of animals but has the limitations of providing no estimate of fat partitioning which can be of major interest to researchers. More recently this technique has been superseded by more reliable and acceptable technologies.

4. Conclusion

If industry is to benefit from the development of new experimental treatments to produce a higher protein to fat carcase ratio, reliable and sensitive in vivo techniques will be required to monitor subtle developmental changes in carcase composition. The review of techniques for the prediction of carcase or body composition by Allen (1990) highlights the accuracy of CAT scanning for the determination of carcase composition of small domestic animals. Cost and accessability of this technique, however, limits its use to restricted experimental purposes. The more transportable and cheaper technology of real-time ultrasound equipment and its wide application will have greater reliance placed upon it, especially if improved accuracy can be achieved with advances in new models and greater proficiency of operators.

No single technique will universally meet the requirements of the various research and industry needs, therefore many of the methods discussed will play a role in assessing animal composition as long as they are accurate and repeatable.

Chapter 3

Active ACTH Immunizaton of XB Ewe Lambs

1. Introduction

Persistent elevated circulating glucocorticoid levels have been shown to reduce growth rates (Wehrenberg et al.,1990) in human subjects, laboratory rats and in sheep (Adams and Sanders,1992), increase catabolism of muscle (Bassett,1963) and induce lipoprotein lipase activity in adipocytes in abdominal fat depots (Bjorntorp et al.,1990). Pharmacological doses of cortisone have significantly increased carcase fat per se in lambs (Spurlock and Clegg,1962; Clark et al.,1963) and in cattle (Carroll et al.,1963). Cushing's syndrome of humans, which is characterized by high cortisol levels due to a pituitary tumor or an adrenal tumor, is associated with differential changes to partitioning of body fat (Liddle,1981). Fat is partit oned away from the distal extremities of the body (mainly limbs) and preferentially to the upper back region, face and abdomen leading to the characteristic pear shaped body. It would appear that glucocorticoids are capable of being lipogenic in some adipose depots and lipolytic in others in humans. This differential response may be due to a modification of receptor affinity for this steroid, an interaction with other lipolytic or lipogenic hormones, or a direct modulation of ß-receptors or insulin receptors within the adipocyte cell membrane. Fat cells increase in size under the influence of glucocorticoids (Hauner et al.,1987) which may contribute to the overall effect on fat accretion in specific depots.

Stress, either physical or psychosocial, is well recognized as a stimulant of the HPA with a resulting elevation in glucocorticoids in the circulation (see Chapter1). It is generally accepted that the HPA plays a vital role in regulating metabolism and hence survival of animals in the more extreme hostile conditions in which the foraging ruminant has adapted to over many thousands of years. In more recent times, with better nutrition and improved husbandry practices resulting in improved growth efficiency, stress induced elevation of glucocorticoids may play a more dominant role in improving the efficiency of production systems.

Thus the modulation of circulating levels of these hormones by active immunization against ACTH may reduce fat status per se or modify the partitioning of fat between specific depots and reduce the impact of ante-mortem stress on glycogen mobilisation and therefore decrease the ultimate pH of muscles. Lowering glucorticoid levels may also improve the growth of lambs under psychological and physiological stress which the

lambs are continuously exposed to in the flock environment. Passive immunization against ACTH in female rats (Sillence et al.,1992) resulted in significant weight gain in the treated animals. As most experimental evidence for such an effect has been reported in laboratory monogastric animals, we have posed the question as to whether similar effects can be observed in growing lambs maintained under conventional production conditions.

2.Materials and Methods

2.1 Design

The experiment was set up in a 2x2 factorial design, with half the animals immunized against ACTH and the balance not immunized against the hormone. These two groups were split into stressed or unstressed sub groups.

2.2 Animals

Female second cross lambs (Dorset x Border Leister-Merino) were maintained at pasture on the CSIRO Chiswick property at Armidale, NSW. The animals were weaned at a group mean age of 63 days (17/1/1991) and grazed on improved temperate pastures until the completion of the trial. A decrease in pasture quality and availability in May led to supplementary feeding at pasture with Fielders lucerne pellets at the rate of 400 g/head/day (16% protein; ME 8.9 MJ\kg) for the remainder of the experiment in order to maintain the growth of the lambs.

2.3 Protocol

One hundred ewe lambs were stratified on body weight into 4 experimental groups (n=25). The four groups consisted of unstressed control (C), unstressed immune (I), stressed control (SC) and stressed immune (SI) animals. Immune groups received ACTH antigen and control groups were injected with just the carrier protein molecule of the antigen (ovalbumin), with a primary vaccination given at mean age of 63 days. Booster injections were given 38, 66, 123 and 179 days after the primary injection. Blood samples were collected from all animals from the jugular by venepuncture into heparinized vacutainers (Becton-Dickinson, Rutherford, New Jersey,U.S.A.) at the time of, and 7 days post-injection. These samples were cooled in ice directly after collection,centrifuged (1200g for 15 mins.) and plasma stored at -20°C pending analysis.

The stress paradigm:

The types of stressors imposed on animals during the experiment were mainly psychosocial and physical. Animals in the stressed groups were grazed in two equal mobs

and twice weekly herded by dogs and moved quickly to sheep yards and penned for 1h. The combined two groups were then split randomly and allocated to different paddocks probably leading to the new groups having to establish a new social hierarchy, at high stocking rates (8 sheep/acre). Imposition of stress commenced at weaning (102 days mean age). Unstressed lambs were grazed at a much lower stocking rate (2.5 sheep/acre) and only handled when the experimental protocol required. All sheep were grazed on plots with similar pasture availability and moved to new paddocks when feed quality or quantity dropped so as not to impose any specific nutritional stress. Body weights were recorded monthly from day 1 of the experiment.

Animals were dosed at day 77 with chromic oxide capsules for feed intake determination. Faecal sampling was undertaken 14 days post dosing.

On days 119 and 120 ten animals, representative of each of the stressed groups were transported 20km to the University of New England to undergo CAT scanning. Afterwards they were returned to their respective groups on the same day. The balance of the group not scanned were penned off feed for the period of transport and scanning.

From day 122 all experimental lambs were supplemented three days per week (Mon.Wed.Fri.) with lucerne pellets (50% lucerne, 10% wheat, 18% bran,20% pollard) at the equivalent rate of 400g/head/day until the end of the experiment.

A final CAT scan was completed on the same twenty lambs prior to slaughter on days 182-183.

On day 189 slaughter and carcase dissection commenced. Stressed animals were fasted 48h prior to killing. The same animals were trucked to Longford Field Station (70 km) unloaded and retrucked to the Chiswick Carcase Analysis Building to simulate commercial stressors prior to slaughter. Stressed animals were exercised for 20 min with the use of dogs the morning of slaughter. Unstressed lambs were fasted for 24h with no other stress placed upon them. Half of each treatment group was slaughtered daily over two days. A final fasted liveweight was recorded and a blood sample taken for cortisol analysis and ACTH antibody titre determination.

2.4 Feed Intake

Lambs were dosed with Captec Chrone® capsules when pastures were at a high level in nutritive value (digestibility 73%) with a predominant legume component. Release rate of chromic oxide from the capsules was 213mg/day. Feed intake was calculated by measuring the concentration of chromic oxide in faeces and estimating the inverse of the pasture digestibility with the formula :

$100 \times Cr_2O_3$ RELEASE RATE/CONCENTRATION Cr_2O_3 x (100-DIGESTIBILITY)

Measurements and results are calculated on the dry matter of faeces and pasture samples.

2.5 CAT Scan Analysis

Ten animals from the stress/control group and ten from the stress/immune group were transported to the University of New England tomography room for scanning. All animals underwent an overnight fast and were individually placed and restrained on the moving bed apparatus. The first scan commenced at the distal end of the femur and a series of scans of equi- distance taken through to the 6th cervical vertebra along the neck. On the first scan, images were obtained every 30mm and at the heavier liveweights on the second scan, 40mm apart. Approximately 20 scans per animal were completed. Body composition was determined in a manner similar to that described (Thompson and Kinghorn,1992).

2.6 Antigen

ACTH1-24(Synacthen,Ciba-Geigy) was coupled to ovalbumin(OA)(Sigma) with 1-ethyl-3-(dimethylaminopropyl)carbodiimide(ECDI)(Sigma) in a 1:1:10 molar ratio. The reaction was stirred overnight at room temperature and the products were filtered then dialysed against 0.9% NaCl for 48h. Animals received an injection of ACTH-OA (0.5mg) or OA (0.5mg) intramuscularly in an emulsion of Freund's complete adjuvant(FCA)(Sigma)(1ml) and saline (1ml). The 2ml dose was given at two sites intramuscularly and injected into each hind leg.

2.7.1 Plasma Cortisol

Cortisol concentrations were determined by the radioimmunoassay (RIA) with modifications to the method of Foster and Dunn(1974). Borate buffer(760µl,0.02M,pH7.6) with gelatin(0.001%)(300bloom,Sigma) and sodium azide(0.5%,Sigma) was heated with plasma(20µl) or standard(20µl) to 60°C for 1h in 12x75 mm glass tubes. Cortisol antisera (100µl) and tracer (6,000cpm/50µl)were added on cooling, prior to incubation for 18-24h at 4°C. A charcoal suspension (100ul)(0.025%)(activated charcoal,Ajax) with dextran T70(0.0025%)(Pharmacia) was subsequently added and incubated for 1h at 4°C. Tracer not bound to antibody was isolated after centrifugation at 1,000g at 4°C for 15 min and aspiration of the supernatant. The assay was sensitive to 0.8ng/ml and inter- and intra-assay coefficients of variation were 6.1 and 2.9 % respectively.

2.7.2 ACTH Antibody Titre

Antibodies specific for ACTH were measured by enzyme linked immunosorbent assay (ELISA) in which the titre was determined as the dilution of antiserum that bound half the maximum number of antibodies as assessed by the colorimetric reaction in the assay. Microtitre plates(96 well, round bottom, Dynatech: Dynatech Laboratories, Virginia) were coated with an ACTH-gelatin (1:2, w/w) conjugate in coating buffer (15mM Na2CO3, 35 mM NaHCO3, pH 9.6) at a concentration of 0.03 mg/ml (100 µl) and sealed for 18h at 4°C. Plates were washed 3 times with PBS (140 mM NaCl, 2.7 mM KCl, 10.1 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4). Wells were blocked with 0.3% gelatin (Sigma type A: Sigma Chemicals, St Louis) in phosphate buffer solution (PBS) containing 0.05% Tween (BDH Chemicals). Test plasma samples were serially diluted (100µl/well), including an internal standard for each of the plates and incubated for 1h at 23°C. Plates were washed with PBS-Tween (3 times) and a goat anti-rabbit antibody conjugated to alkaline phosphatase (100µl of 1/100; Sigma) added for 1h at 23°C. Wells were again washed with PBS-Tween (3 times) and the enzyme substrate, p-nitrophenyl phosphate added (100µl of 1mg/ml in 0.5 mM MgCl26H2O, 9.7% diethanolamine pH 9.8; Sigma). The reaction was allowed to develop for 30 min in the dark and was stopped with 3M NaOH (50µl/well) and the optical density of the wells recorded with a microplate reader (Dynatech Laboratories, Virginia). The between-assay coefficient of variation was 21%.

2.8 Carcase Analysis

At the completion of the growth study (day 189), one animal from each of the four treatment groups was slaughtered at random, so as to eliminate any possible bias at the point of slaughter. After the aorta and carotid were severed and the body suspended, adrenal glands, kidney and channel fat, omental fat and mesenteric fat were removed and weighed. Hot carcase weight as per AUSMEAT standards was recorded before chilling. Chilled carcase weight was recorded, after 16h at 4°C, and then a GR measurement taken at the 12th rib and 110mm from the midline of the vertebrae. A clean incision on the left hand side between the 12th and 13th rib exposed the eye muscle, which was then photographed for analysis of eye muscle area and subcutaneous fat thickness at position C (defined as the thickness of fat measured perpendicular to the deepest part of the M.longissimus) (Palsson,1939).

Carcases from animals previously undergoing CAT scan analysis were split down the backbone with a band saw into identical halves and the right hand side retained in plastic bags in the freezer for chemical fat analysis.

2.9 Muscle pH

At the completion of the slaughter process, and prior to carcases being placed in the chiller, three distinct skeletal muscles underwent pH measurements. An incision was made with a scalpel blade into the M.longissimus, M.semimembranosis and M.supraspinatus and the probe from a pH meter (Digital,T.P.S.,Brisbane) inserted and the reading taken when the readout stabilized. The following morning, after the carcases had been chilled overnight a second pH reading was taken to ascertain the ultimate pH values.

2.10 Chemical fat Analysis

The right side half carcase from animals that underwent CAT scanning were frozen at -18°C in large plastic bags and several weeks later were cut into small cubes while frozen and then minced through a cutter and grinder (Jeffco Industries, Brisbane) with a 15mm seive. The resultant minced tissue was homogenized in a dough mixer (Hobart Industries, Sydney) for 15 min. A sub-sample of approximately 2 kg was taken and refrozen. Duplicate core samples (approximately 10g) were subsequently taken and weighed before being placed in cellulose thimbles for freeze drying. Samples were reweighed at the completion of drying and the percentage of moisture calculated. The dried sample was placed in soxhlets for 6 h with the solvent petroleum ether (boiling point 40-60°C)(Field et al.1985). Meat samples were again dried and a final weight recorded for the estimation of percentage fat present.

2.11 Statistical Analysis

The growth rate of the lambs was analysed in a least squares model for repeated measures which included terms for ACTH immunization, stress, initial liveweight and all first order interactions. After dropping non-significant interactions (P>0.05) from the model, the simplest significant model was incorporated in the statistical analysis.

The CAT scan data allowed the statistical analysis of body composition to be conducted on a nested design over time to test animals within treatment variation on an individual animal basis. The model included terms for immunization treatments, scan over time and possible interactions. The least squares model used to assess rate of change of fat, muscle and bone relative to the sum of the components, and the rate of change of individual fat depots was log transformed as the variances were proportional to the means. The sum of

the three components mentioned was selected as the covariate being the best estimate of empty body weight. Results are presented in backtransformed geometric means, apart for standard errors.

Estimated feed intake was analysed incorporating the simplest least squares model for immunization and stress.

Carcase composition and chemical weights of carcase were analysed in a least squares model which included terms for treatment (immunisation versus non immunised), stress (stress and no stress), hot carcase weight and all first order interactions. Data were analysed after log transformation to minimize the relationship between the means and variance. Non-significant interactions ($P>0.05$) were sequentially dropped from the model until the simplest significant model was obtained. Results are presented in backtransformed geometric means, apart for standard errors.

3. Results

3.1 Liveweight

At the beginning of the experiment, the mean weight was 17.3 ± 0.51kg and increased to an overall liveweight of 44.0 ± 0.52kg at day 208 of the experiment. At the completion of the experiment ACTH immune lambs were significantly lighter ($P<0.05$). The imposition of stress exerted a greater significant impact on liveweight than the immunization procedure. At day 66 ($P<0.001$) and day 154 ($P<0.05$) stressed animals were lighter, but not so across all growth periods (Figure 3.1).

A repeated measures analysis for growth rates of the lambs over the period of the experiment showed a highly significant interaction between initial liveweight and time (Table 3.1). The interaction between initial weight and time was highly significant as was the interaction between time and the constant. The stress by initial weight interaction was less significant ($P<0.05$). At the third growth period, initial liveweight had a significant effect on individual growth rates, with the heavy lambs growing more slowly than the lightest lambs. This period coincided with weaning with a marked decrease in growth rates (Figure 3.2).

3.2 Feed Intake

The estimation of dry matter feed consumption of forage (digestability 74%) was determined from the concentration of chromic oxide in the faeces of the lambs sampled at day 90. ACTH immunization had no effect on dry matter intake (1.23 ± 0.04kg versus 1.24 ± 0.04kg). Feed intake was significantly reduced in stressed lambs ($P<0.001$)

Table 3.1 Repeated measures analysis for growth rate (g/day) for control lambs and those immunized against ACTH which were either stressed or not over the period of the experiment.

Independent Variable	Whole plot analysis		Mean regression coefficients	Repeated measures analysis	
	df	F ratio		df	F ratio
Constant	-	-	140.66		
Time x constant				5,88	141.19***
Treatment	4,92	0.61			
Time x treat				5,88	1.85
Control			1.42		
Immune			-1.42		
Stress	4,92	0.80			
Time x stress				5,88	0.95
None			-10.06		
Lots			10.06		
Initial weight	4,92	2.82	-1.08		
Time x initial weight				5,88	5.70***
Stress x initial weight	4,92	5.40*			
Time x stress x initial weight				5,88	1.09
None			1.49		
Lots			-1.49		

Predicted means for stress treatments on mean growth rate (g/day) over the period of the experiment.

Stress Treatment	Initial weight (kg)		
	15.0	17.5	20.0
Unstressed	136.8	137.9	138.9
Stressed	112.1	105.7	99.2
aver.se	(3.3)	(2.6)	(3.6)

Table 3.2 Mean daily dry matter intake for XB lambs measured by the chromic oxide method .

Treatment	Intake (Kg)	Significance
Control No stress	1.37	
ACTH immune No stress	1.41	
Control Stress	1.09	***
ACTH immune Stress	1.08	***

Std. error 0.05
*** (P<0.001)

Table 3.3 Liveweight and carcase t raits for lambs immunized against ACTH and subjected to stress, after adjustmen for the appropriate covariate. Data were analysed using a log transformed m odel and predicted means calculated by taking the antilog.

	Control No stress	Immune No stres	Control Stressed	Immune Stressed	Std. error	'b' coefficient
Liveweight (kg)	47.0	45.7	42.3	41.0	(.766)	1.64
Hot Carcase Wt. (kg)	20.4	20.0	18.5	17.9	(.009)	1.28
Carcase Ratio HCW:LWT	.489	.486	.513	.510	(.004)	0.44
Kidney Fat (gm) #						
15.0kg Carcase Wt.	393	366	309	385	(.04)	0.22
26.0kg Carcase Wt.	1239	1153	974	1214	(.05)	
Omental Fat (gm) #						
15.0kg Carcase Wt.	614	561	528	618	(.03)	0.68
26.0kg Carcase Wt.	1713	1564	1473	1724	(.04)	
Mesenteric Fat (gm) #	432	439	415	421	(.01)	1.29
Fat Depth # 12/13 Rib (mm)						
15.0kg Carcase Wt.	2.3	2.0	1.7	2.4	(.06)	-1.91
26.0kg Carcase Wt.	7.1	6.0	5.2	7.3	(.07)	
G.R. (mm)						
15.0kg Carcase Wt.	7.5	7.1	5.6	7.1	(.04)	-1.69
26.0kg Carcase Wt.	25.9	24.6	19.5	24.5	(.04)	
Eye Muscle Area (sq.cm.) #	11.95	11.77	12.27	12.09	.009	0.40

Group means adjusted to the same hot carca se weigh.

Standard errors have been presented in the tra sformed mode due to their change in distribution from the normal curve after log transformation.

Table 3.4 Predicted weights of various tissues estimated from CAT scan analysis and adjusted to the same body weight. Lambs came from the stressed groups (n=10) and were either non-immunized or immunized against ACTH. Lambs were scanned one week prior to slaughter.

Treatment	Subcut. Fat	Intermus. Fat	Interr al Fat	Mammary Fat	Total Fat	Muscle	Bone
Non Immune	1.803	1.267	2.269	0.079	5.586	10.309	1.891
ACTH Immune	1.927	1.367	2.151	0.071	5.687	10.058	2.017
Log std. errors	(.03)	(.02)	(.02)	.(.06)	(.01)	(.01)	(.01)

Table 3.5 Predicted weights of tissues (kg) calculated by the CAT scan method and averaged over scan 1 and scan 2. Data was transformed and the results presented as the antilog.

	Subcutaneous Fat	Intermuscular Fat	Internal Fat	Mammary Fat	Muscle	Bone
Non Immune	1.947	1.377	2.062	0.109	11.143	2.119
ACTH Immune	1.987	1.420	2.053	0.108	10.977	2.234
Std.err.#	0.029	0.016	0.044	0.008	0.010	0.018

Standard errors have not been back transformed

Table 3.6 Analysis of variance for chemical fat content in the half carcase of the stress groups, either immunized against ACTH or not immunized.

FACTOR	LEVEL	REGN. COEFF	Std. Err.
Constant Term	1	-1.62028	0.3138
Treatment	1		
Non-immune	1	-0.02592	0.0113
ACTH immune	2	0.02592	0.0113
Log Hot Carcase	1	1.8377	0.2513

Table 3.7 Estimated carcase fat adj ısted to the same carcase weight for control and ACTH immunized lambs.

	Carcase Fat (kg)	Std. Error
Non-immune	4.475	(0.015)
ACTH immune	5.247	(0.015)

P <0.05

Table 3.8 Predicted means for muscle pH and adjusted for the muscle group for high and low stress treatment.

	No Stress	Stressed
Non-immune	5.99	6.17
IMMUNITY		
ACTH Immune	6 17	6.70

(0.045Std.Err.)

Table 3.9 Predicted mean PH values averaged over the three muscle groups at slaughter and 24hrs post slaughter.

		Hot	Cold***
STRESS	No Stress	6.47 (0.04SE)	5.69 (0.05SE)
	Stressed	6.46 (0.04SE)	5.88 (0.05SE)

***(P<0.001)

Table 3.10 Regression coefficients for the three muscle groups at slaughter and 24hrs post slaughter.

		Hot	Cold
MUSCLE	M.supraspinatus	0.218 (0.201 SE)	0.184 (-0.024 SE)
	M.longissimus	0.038 (-0.015 SE)	-0.068 (-0.075 SE)
	M.semimembranosus	-0.256 (-0.186 SE)	-0.115 (0.0995 SE)

Table 3.11 F ratios for the time interaction for hot and cold muscle pH measurements across treatments and their interactions.

Independent variable	Mean	Time interaction
Constant		2.279***
ACTH	3.72	
x time		2.23 (ns)
Stress	3.95*	
x time		5.16*
Muscle	25.00***	
x time		2.7 (ns)
ACTH x Stress	3.94*	
x time		0.05 (ns)

Statistics

* P<0.05
** P<0.01
*** P<0.001
ns = non significant

Figure 3.1 Mean liveweight of lambs actively immunized against ACTH (immune) and subjected to stress (s) after adjustment to initial weight. Arrows denote time of vaccination.

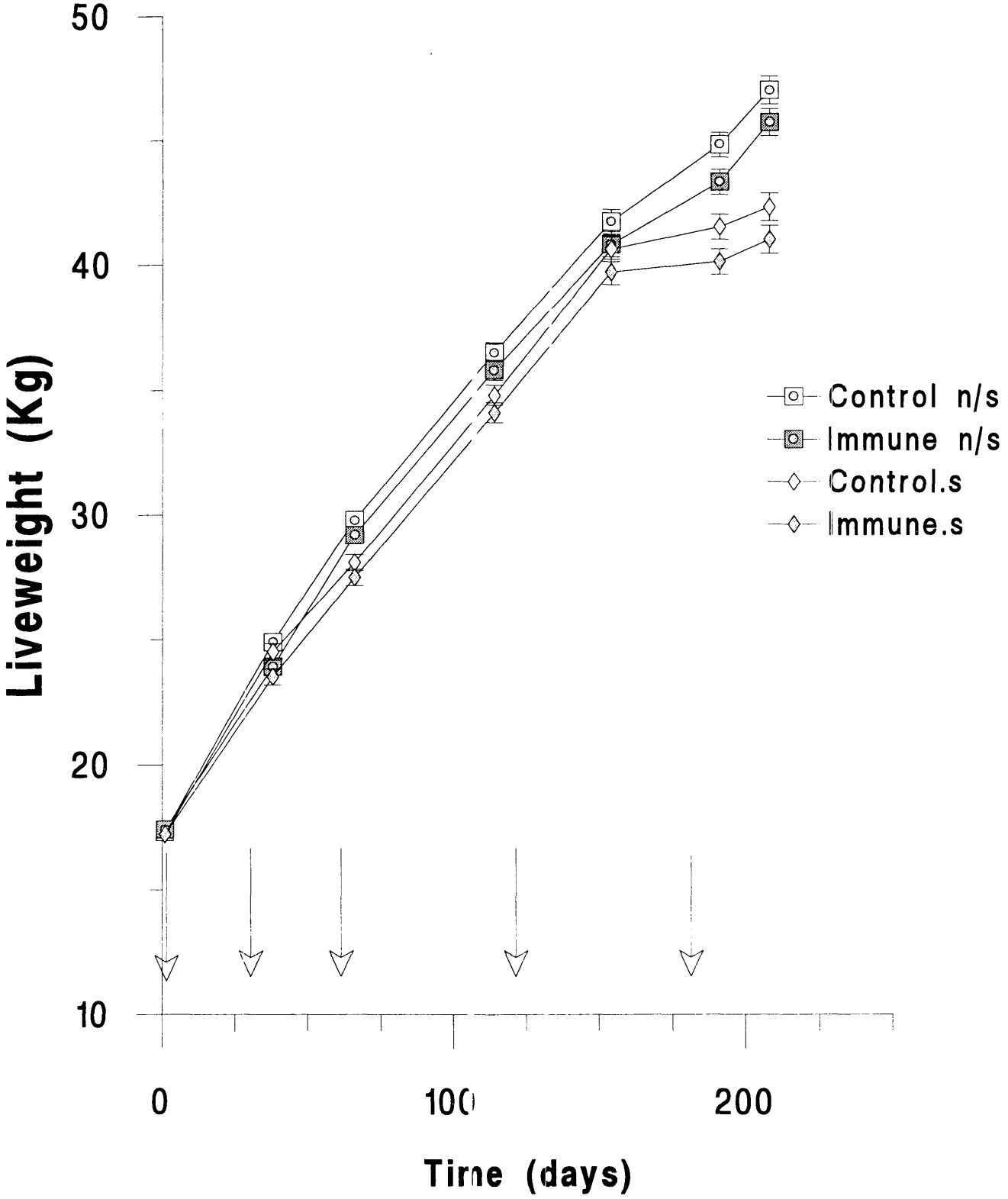


Figure 3.2 The influence of active immunization against ACTH (Immune) and the imposition of chronic psychosocial stress (s) on changes in liveweight gain in XB lambs. Arrows denote the time of immunization.

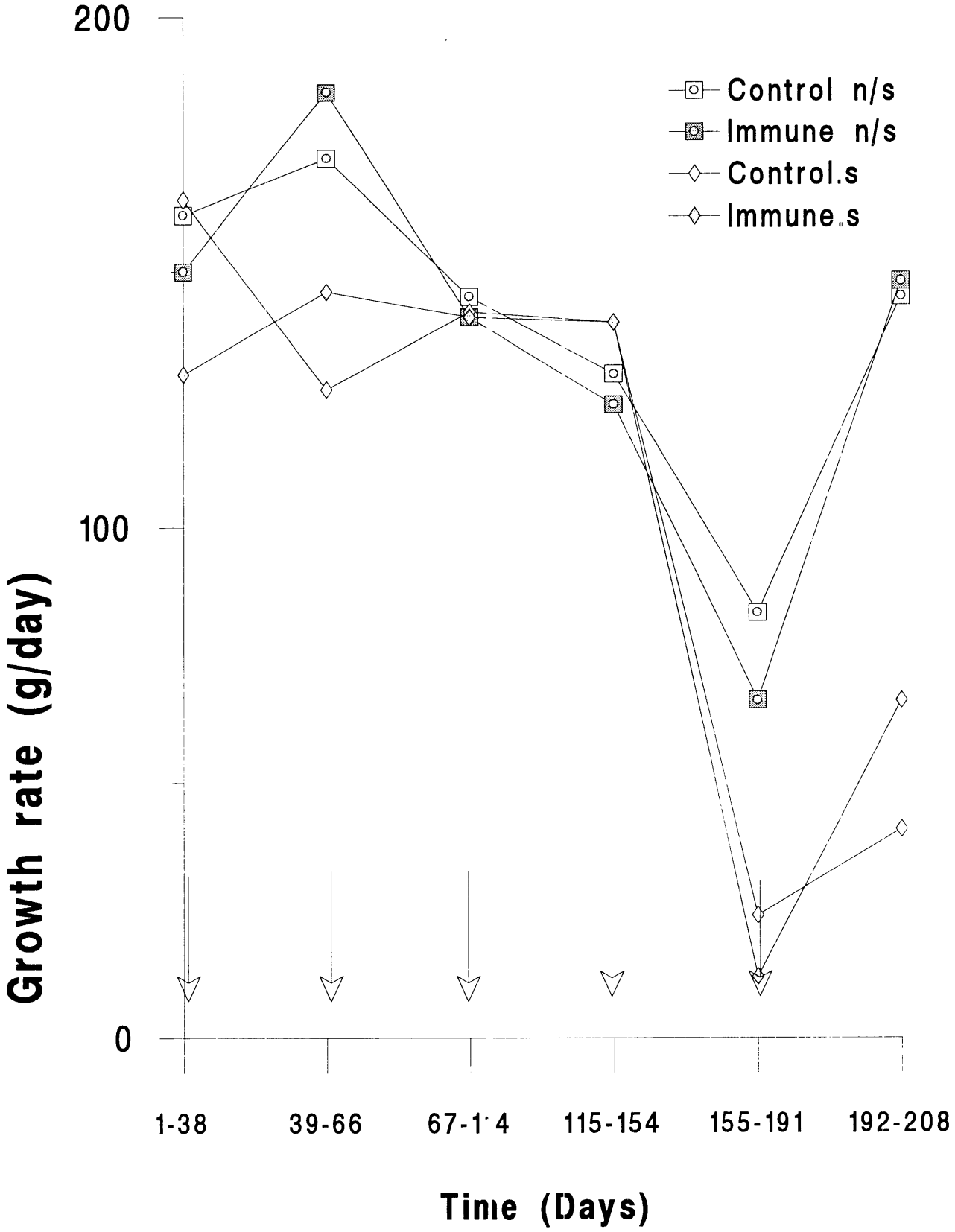


Figure 3.3 Mean ACTH antibody titre for immunized groups which were either stressed or not over the period of the experiment. Arrows denote times of vaccination.

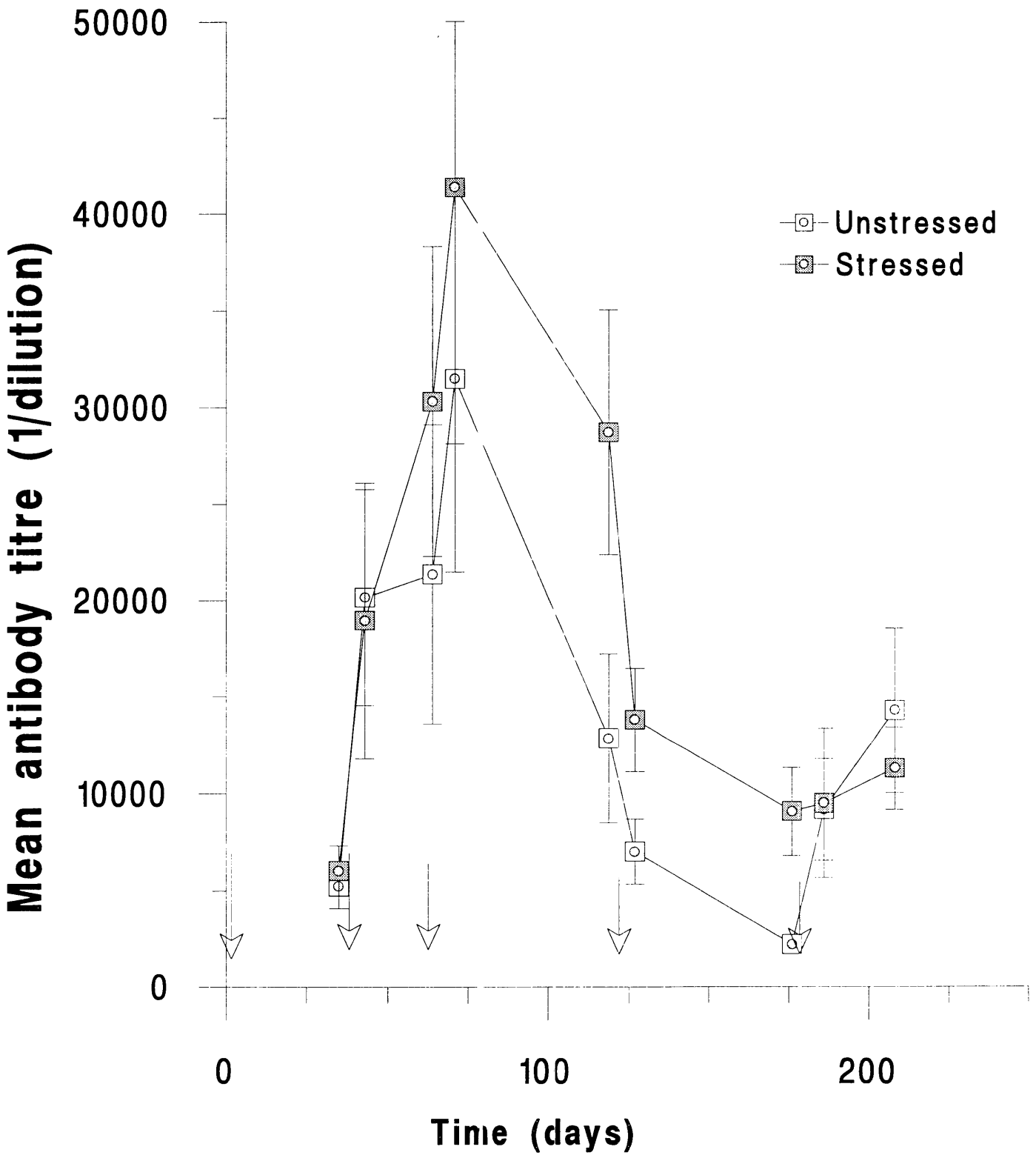


Figure 3.4 Plasma cortisol levels for XB lambs actively immunized against ACTH which were either stressed or not over the period of the experiment (n=stress,n/s=unstressed). Arrows denote the time of vaccination.

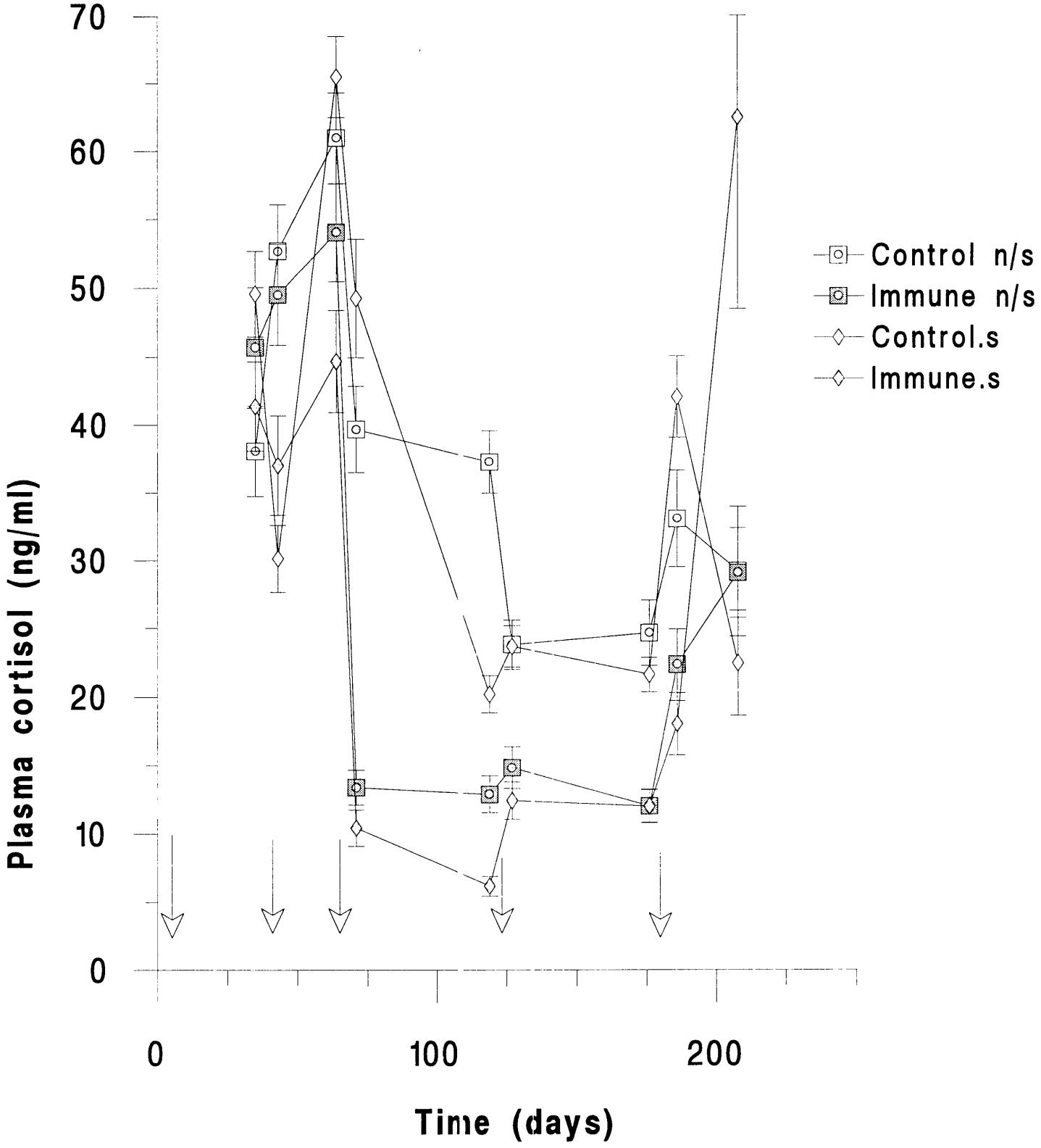
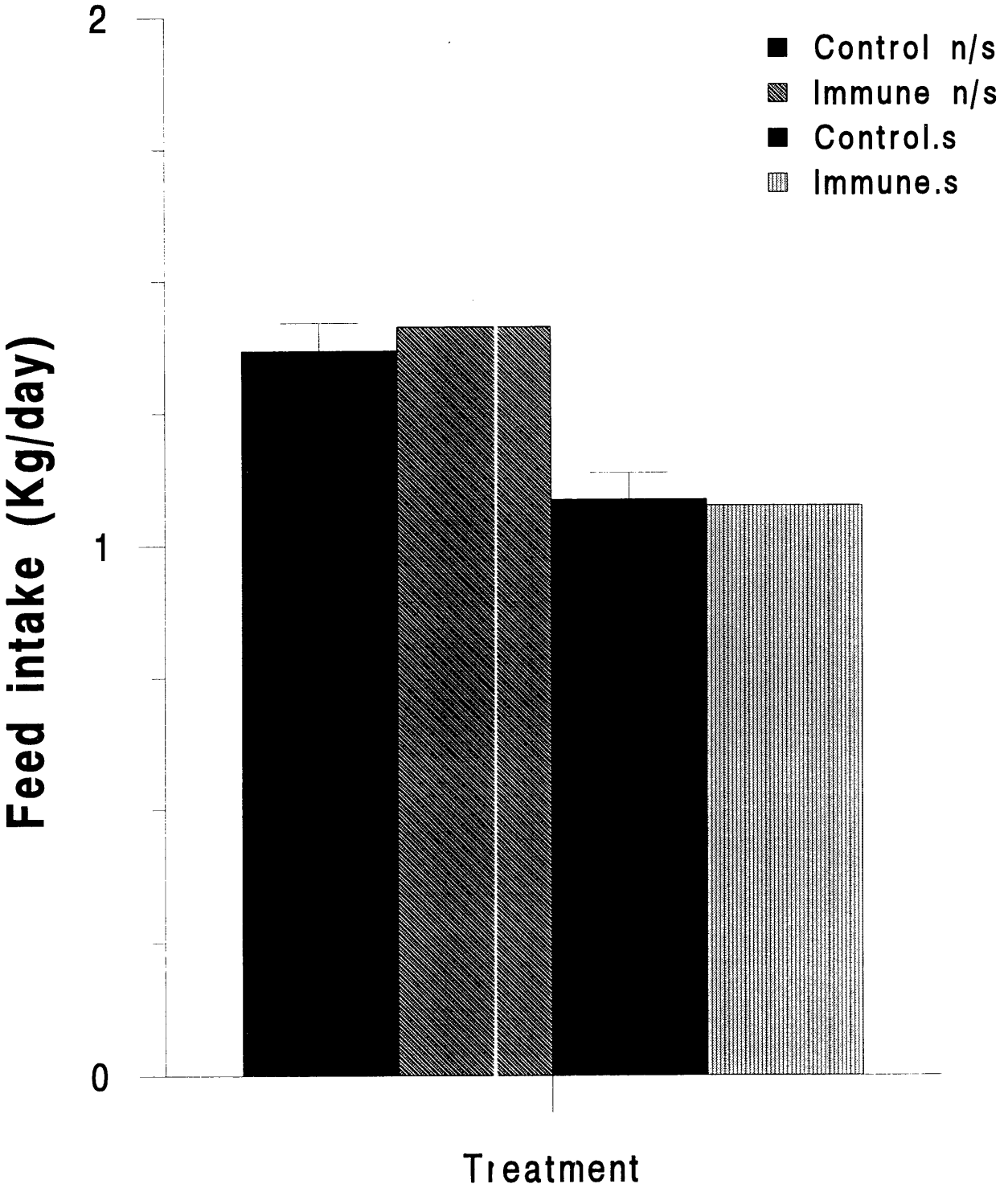


Figure 3.5 Dry matter intake for XB lambs, estimated using the chromic oxide method



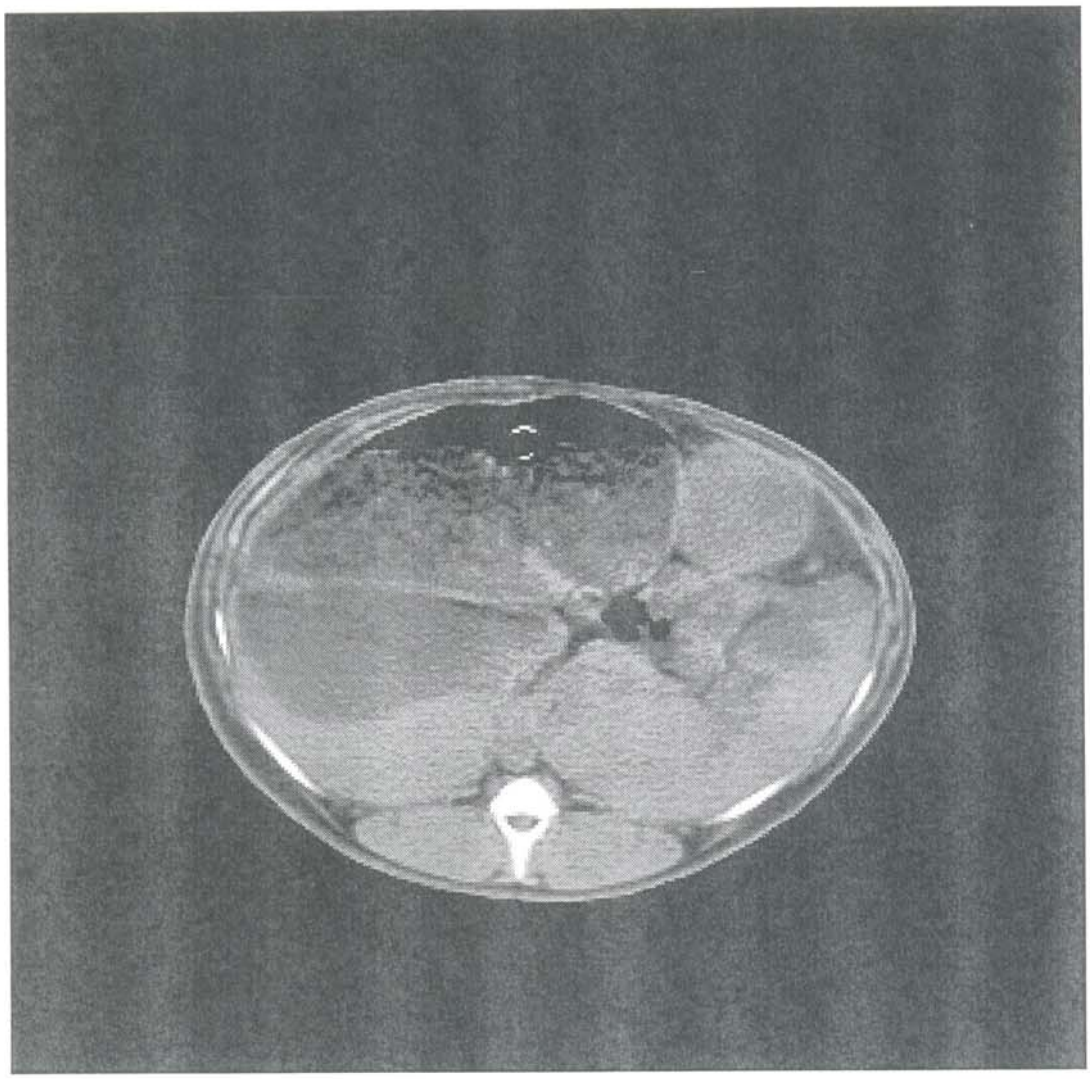


Figure 3.6 Transverse CAT scan image of the abdominal region of a XB lamb with the chromic oxide capsule visible at the interface between the liquid and gas contents in the rumen. The eye muscle and subcutaneous fat are clearly visible at the bottom of the image with the rumen situated at the middle to upper left hand side of the image.

compared to non-stressed animals. On average the stressed group consumed 0.31kg less feed (Figure 3.5).

3.3 CAT Scans

A repeated measure analysis was performed on the two scannings performed on the same set of animals separated by a period of 84 days. Unfortunately the lambs did not grow as well as could be expected over that period. They were just entering the fattening phase after the first scan and had not progressed much further into their final stage of development by the second scan. On average these animals only gained 4.3kg over the 80 day period.

There was no significant difference in the quantity of fat in the various depots, or muscle or bone between the vaccine treatments over time.

The ACTH immune sheep tended to have more carcase fat (0.233kg) than controls at the final scan just prior to slaughter and this was evident in the intermuscular fat depot. The same group had less internal fat than the controls and less muscle. The difference in bone weight was 126g in favour of the immune group but none of these differences reached significance.

An attempt was made to discriminate and calculate the partitioning of fat in non-carcase fat depots from the first set of images Due to poor visual demarcation between these depots, results were very subjective and did not give reliable estimates and hence the non-carcase fat was pooled as a single entity.

The CAT scanning technique did not demonstrate significant energy partitioning between any of the fat depots or differences in the proportion of skeletal muscle or bone between treatments.

3.4 ACTH Antibody Titre

Plasma antibody titre increased significantly after the first booster vaccination, while the control groups had no detectable binding. The antibody titre peaked after the second boost at day 66 and declined sharply hereafter (Figure 3.3). Following the third booster injection there was a marked decrease in the antibody titre in the subsequent week and further booster injections were not capable of reversing this decline. The vaccination protocol failed to maintain high antibody titre levels for the duration of the experiment and after the fourth boost there was in fact no response in titre at all, with final titres lower than the first boost response.

3.5 Plasma Cortisol

Cortisol levels were negatively correlated with ACTH antibody titres. Cortisol levels in response to the maximal titres at around day 70, were at their lowest for the two immune groups with absolute values 10-15ng/ml compared to non immunized lambs with plasma cortisol values 4-5 times higher (Figure 3.4). Toward the completion of the experiment cortisol levels were actually stimulated in the stress immune group to be 100% greater than the circulating concentrations in the non-immune groups. The plasma cortisol levels in this group were higher than those measured at the initial sampling. This may well have been in response to the stressors imposed prior to slaughter and blood sampling, although levels in the non-immunized stressed group only marginally increased in response to these factors.

3.6 Carcase Analysis Measured After Slaughter

Neither hot carcase weight measured after slaughter or cold carcase weight (measured after storage at 4°C for 24hr) were affected by treatments (Table 3.3).

3.6.1 Fat Depots

Both ACTH immunization and the imposition of stress had no effect on preferential partitioning of lipid substrate into kidney, omental and mesenteric fat. There was a significant interaction between immunization and stress for two of the fat depots, with kidney fat ($P<0.05$) and omental fat ($P<0.01$) being reduced in the stressed non-immunized group of animals.

Subcutaneous fat thickness at the 'C' position was measured in order to assess possible fat partitioning. When adjusted for the hot carcase weight covariate, subcutaneous fat thickness was not significantly different.

3.6.2 GR Measurement

While ACTH immunization had no effect on the GR measurement, stressed animals had a significantly lower GR ($P<0.01$). Although this would indicate lower carcase fatness, it was not evident in non-carcase fat depots.

3.6.3 Muscle Area

Estimates of eye muscle area using a planimeter were recorded for the M.longissimus and analysed. Although the stress groups had greater muscle areas, they were not significantly different from the non-stressed treatments. The ACTH immune groups were slightly

smaller in the cross-sectional area of the M.longissimus than in non-vaccinated groups but this difference was not significant

3.6.4 Carcase Ratio

Lambs from the stressed groups had higher carcase ratios, expressed as the ratio of hot carcase weight to liveweight (0.51± 0 004 versus 0.49 ± 0.004) than their control counterparts (P<0.001).

3.6.5 Muscle pH

The mean muscle pH (measured on the hot and cold carcase) was significantly different between the muscle groups (P<0.001) with M.supraspinatus displaying the highest pH followed by M.longissimus and M.semimembranosus respectively for both hot and cold muscle pH (Figure 3.8). Averaged over time, the ranking for pH between muscles did not change.

Although stress had a less significant effect on increasing muscle pH (P<0.05), there was a stress x time interaction and a stress x ACTH immunization interaction for this parameter (Table 3.11).

The drop in muscle pH from the hot carcase measurement to the ultimate pH measurement taken 24h post slaughter ranged from 0.69 for the M.semimembranosus to 0.89 for the M.longissimus. The interaction between ACTH immunization and stress may account for these differences in rate of pH decline from hot to cold carcase measurements.

3.6.6 Chemical Fat

Estimates of chemical fat in the half carcase were calculated on the sub-groups from the stressed animals. ACTH immune lambs contained 11.3% more fat when adjusted to the same carcase weight, which was statistically significant (P< 0.05). This group had the elevated cortisol levels at the time of slaughter, despite having been immunized against ACTH. The percentage fat present in the half carcase represents subcutaneous, intermuscular and intramuscular fat as well as fat in the long bones.

Results from the CAT scans suggest that the increased fat in the carcase was predominantly in the intermuscular depot. The slaughter data indicate that there was also an increase of 1.1mm in subcutaneous fat thickness for the immune lambs.

The data consistently points to increased fat in the stressed ACTH immune group when measured by a number variables.

4. Discussion

The experiment failed to achieve its p imary goal, that is to maintain high antibody titres against ACTH and therefore significa itly reduce basal cortisol in lambs over the post-pubertal growth phase up to 200 days of age. It was the latter part of this phase that a chronic psychosocial stressor was imp osed to activate the HPA axis in order to assess the efficiency of the ACTH immunizatior regime to modulate cortisol secretion.

In one group in fact the reverse was a:hieved, which confounded the results and may have been responsible for the ACTH immunization by stress interaction observed in some variables. A possible explanation for the dramatic down-regulation of the ACTH antibody titre profile could be the stimulation of anti-idiotypic antibodies which mimic the specific hormone, binding to the target recepto r on the adrenal cortex and therefore activating the signal transduction pathway to releas: cAMP and subsequently cortisol. Repeated booster injections may well increase this poss bility. A longer primary to boost interval with less frequent injections may have maintai ed elevated titres without inducing an anti-idiotypic response.

It would appear that ACTH immuniz: tion depressed the final liveweight. Stress definitely had an impact on the growth of the lambs, however it cannot be ascertained if this was due to the psychosocial stress due to c isruption of the social hierarchy of the animals or to the increased stocking pressure pla:ed upon them when grazing 1.4ha plots. The experimental design cannot separate t ese two variables within the stress regime. These results are at variance with the increas:d growth rates achieved in female rats passively immunized against ACTH (Sillence e: al.,1992), although the rats in this study were fed a high quality diet ad libitum.

A possible explanation for the signifi: ant negative relationship between initial liveweight and subsequent growth rates could be due to the greater impact of the withdrawal of maternal milk at weaning from the lai ger single and faster growing lambs. Twin lambs would be disadvantaged less at weani ig as a result of a lower milk intake before weaning as a greater proportion of nutrients w:uld have been obtained from the forage component of their diets.

Stressed lambs ate significantly less pasture which may be due to either pasture availability or quality. Given that ani nals we:e rotated frequently between plots to prevent establishing a social hierarch', it is likely that feed intake was reduced by psychosocial stress that animals were exposed to. Faecal samples were taken at day 100 when antibody titres were in decline although plasma cortisol levels were at their lowest at this period of the experiment. The poor negative correlation between ACTH antibody titre and plasma cortisol levels at this time of the experiment makes it difficult to

ascertain the true stress hormone status of the lambs at the time of measurement of pasture intake.

Although it is not possible to establish the causes of the stress induced decrease in feed intake, the fact that ACTH immunization had no effect on intake is inconsistent with the results of Spurlock and Clegg (1962) and Adams and Sanders (1992). In both of those studies, adrenal steroid supplementation resulted in increased feed intake, while conversely Sillence et al.(1992) increased feed intake in rats when cortisol levels were lowered by passively immunizing animals against ACTH. This disparity may be due to a species difference in the animals responsiveness in feed intake to a variation in plasma cortisol profiles and the subsequent effects on growth. These studies failed to demonstrate significant differences in feed conversion ratios.

Although there was a significant difference in chemical fat in the carcase of control and ACTH immune lambs, this result was not evident from the CAT scans due probably to the failure of the latter technique to differentiate between intramuscular fat content and to possible inconsistencies in image analysis by the author. There was a difference in carcase fat between the two experimental groups of 7.6% as measured by scanning which could be attributed to the increased cortisol levels in the immune group in the 30 days prior to slaughter. Clegg and Spurlock (1960) achieved similar increases in carcase fat content of lambs by injecting with cortisone acetate. When a value of 3% for intramuscular fat is added (non published data for lambs obtained from previous studies), then a value for the combined estimate of fat from from both of these depots of 10.6% does not differ greatly from the difference in chemical fat content of 11.3% between ACTH immune and control animals. These results support that there is good agreement in the results of both techniques and validates the accuracy of the CAT scanning. Methodology to assess carcase fat status, as discussed in Chapter 2, ether extraction of fat from meat samples tends to over-estimate the total of fat present due to the inclusion of a small proportion of structural lipids from damaged cell membranes.

Active immunization of lambs against ACTH only produced elevated titres for two booster injections and the failure to maintain the titre profile after the third and fourth injections is difficult to explain as steroid immunogens are capable of at least maintaining if not increasing antibody titres with repeated injections. The most plausible explanation is the formation of anti-idiotypic antibodies after the third or fourth injection. As the animals were repeatedly injected intramuscularly into each hind leg, it is remotely possible that some of the latter injections could have been placed directly into granulomas formed in the muscle from prior injections thus reducing the antigenicity of the immunogen.

Alternatively the suppression of the titre may have been due to the complex interaction of stress, age of animal, nutritional status and the relatively low immunogenicity of the ACTH peptide conjugate with the cellular and humoral elements of the immune system. Given the stresses that the lambs were exposed to in the grazing systems, it is conceivable that initially the ACTH immunization procedures suppressed plasma cortisol levels to the point whereby their essential role in supporting normal immunological function was compromised (Kuhlman et al.,1991). Thus after subsequent booster injections, cellular components of the immune system were reduced even more, resulting in further suppression of immunoresponsiveness.

Despite this phenomenon the two immune groups had lowered plasma cortisol levels as compared to the controls until day 186. The rapid rise in steroid hormone occurred in the last three weeks of the experiment.

The results are not able to indicate whether elevated antibody titres are able to bind ACTH hormone sufficiently to suppress a stress induced acute rise in cortisol brought about by increased pituitary ACTH. However, blood sampling by venipuncture plus the manual restraint of animals is an acute stress in itself as the blood samples were not collected under local anaesthesia and the animals were not used to confinement. In general levels of cortisol were suppressed under chronic psychological stress when mean ACTH titres were greater than 1:20,000. As Figure 3.3 demonstrates, the wide range in individual antibody titres within immune groups has lead to standard errors of the means approaching 50% of the mean value, which makes the interpretation of results difficult. The titre values also reflect the tremendous biological variation in immuno-responsiveness between animals given the same dose of immunogen although the variation may also reflect limitations in accuracy in the ELISA assay (20%) measuring the individual responses.

The negative correlation between plasma cortisol and ACTH antibody titre is in agreement with other workers (Jones et al.,1990). Although the latter report indicated lower antibody titres in steers, it is recognised that cattle also have a lower response to steroid immunogens (Cox,unpublished). At the peak of the antibody titre response, plasma cortisol levels in the immune groups were 25% of their initial levels and were approximately 30% of the levels in their non-immune counterparts however, unfortunately the effectiveness of the ACTH immunization procedure could not be maintained until the completion of the experiment.

The significant differences in carcase ratio between the stressed and non-stressed groups can be accounted for by the extended fasting of the former groups which lead to reduced gut fill prior to slaughter and resulting in a higher ratio. Immunization of lambs had no

impact on this variable suggesting tha: there was no differential influence on fat partitioning between non-carcase and carcase fat components.

Yet the various fat depots associated with the viscera acted differently. For both omental and kidney fat there was a significant interaction between stress and immunity, indicating that the rate of development of these tissues was increasing at different rates and hence the predicted means are presented for light and heavy carcase weight. A possible explanation for this observation could be the extreme variance in cortisol levels between the two immune groups at the comple ion of the experiment. The data do not support the findings of Bjorntorp et al.(1990) in h.mans. Their study suggests a role for glucocorticoids in lipoprotein lipase a:tivity in internal adipose depots, accounting for the preferential increase in lipid accretion in this region. A comparison between humans and ruminants may not be valid since the sources of lipogenic substrate are quite different between the species, with ruminants reliant on acetate and humans on glucose for a source of carbon for fatty acid synthesis.

Estimates of carcase fat, using either the subcutaneous fat thickness or the GR measurement were influenced by an immune status by stress interaction. This would suggest that like the omental and kidney fat depots, they were increasing at differing rates between treatment groups and differed at the high and low carcase weights (Table 3.2).

The failure of the immunization treatment to demonstrate differences in skeletal muscle mass as indicated by the measurement of the area of M.longissimus contradicts the accepted view that elevated cortisol is capable of degrading muscle (Bassett,1963) but is in agreement with Jones et al.(1990) who demonstrated that the rate of skeletal muscle breakdown was not affected by ACTH immunization. In fact the stress immune group which displayed the elevated cortisol levels had an EMA of 12.1cm² which is only marginally smaller than the largest EMA area for the stressed non-immune group (group 3).

The significant decreases in muscle pH measured soon after slaughter and 24h post slaughter in the chilled carcase is consistent with other workers (Purchas,1989). The sudden loss of oxygen soon after the slaughter procedure switches energy utilization in skeletal muscles from oxidative to anaerobic process resulting in a marked increase in lactic acid formation which lowers muscle pH over time until the energy substrate is expended. Low muscle pH decreases the chance of bacterial proliferation in meat stored at 4°C as they replicate most effectively at physiological pH's. On the other hand, elevated muscle pH has been associated with darker meat colour score (McIntyre,1989) and greater moisture loss at storage and cooking (Hertzman,1989).

The significant differences between the three muscle groups measured have been observed by other researchers (Tarrant,1989). There is a definite relationship between the activity of the muscle group prior to slaughter and the corresponding ultimate pH of the muscle with the muscles used for protracted work containing predominantly red fibres and therefore having a higher pH than those used mainly for postural support. It is well known that working muscle groups utilize glycogen during the exercise period while resting muscles have no net glycogen depletion, with glycolysis equaling glycogenesis. The relative changes in ultimate muscle pH values between different muscle groups following exercise and adrenaline treatment (Tarrant,1989) suggest that different mechanisms of glycogen breakdown are operating when animals are exposed to exercise and stress. Medium increases in ultimate muscle pH have been associated with increased meat toughness (Purchas,1989) as determined by the Warner-Bratzler shear force. Table 3.8 shows that the stress-induced rise in muscle pH was not altered by ACTH immunization but in fact increased, possibly due to the elevation in cortisol levels. Reports from De Wied and Croiset (1991) have linked high CRH levels and associated increased glucocorticoid levels with increased adrenal catecholamine secretion which depletes muscle glycogen. Other studies (Wynn et al.,1995) have demonstrated lower oxygen consumption in ACTH immune lambs being exercised on a treadmill. The decrease in the amount of oxygen consumed under strenuous exercise may be the result of greater glycogen utilization and therefore would support these findings of higher muscle pH in the ACTH immune group that were stressed. Not only was the impact of stress and immunization on ultimate pH values additive but there was a suggestion of synergy in their actions.

The significant difference in chemical fat in the carcase between immunized and non-immunized animals is well supported by other researchers (see earlier section 4) who investigated the influence of elevated glucocorticoids on carcase fatness. It is unfortunate that chemical fat in the carcase was not measured in the non-stressed groups, however the increase in plasma cortisol observed in the immune groups could not be foreseen in the light of previous experimental results. At the time of planning the experimental design, it was reasonably expected that circulating cortisol levels would have been suppressed in both immune groups. It is however, possible that the imposition of chronic stress may have activated the pituitary corticotrophs sufficiently for ACTH to be present in concentrations that exceeded the binding capacity of the ACTH specific immunoglobulin. The results demonstrate that there is a need to either improve the efficiency of the ACTH-protein conjugate to maintain chronic high levels of immunity or to modulate the

nutritional and or stress status of the f ock to improve the functionality of the immune system.