Chapter 4

Active ACTH / GnRH Immunization of XB Ram Lambs

1. Introduction

The immuno-suppressive and anti-inf ammatory effects of high levels of glucocorticoids in mammals and other species is well recognised (Baxter and Rousseau,1979;Liddle,1981). Most of the elements of both cellular and humoral immunity are compromised by chronic stress, an effect that has been replicated by the administration of glucocorticoids or their analogues to animals. Excess glucocorticoid secretion arising from HPA dysfunction (Cushing's syndrome) can increase the patients susceptibility to infection (Liddle,1981).

A negative correlation has been shown to exist between cortisol and antibody titre to ovalbumin in calves (Cumins and Brunner,1991) and a similar relationship between dexamethasone and antibody titre to an antigen in sheep has been demonstrated (Minton et al.,1991). Similarly red deer, undergoing transport stress, had lowered antibody titres as well as fatal disease manifestations as a result of bacterial infection (Griffin et al.,1992). Dietary corticosterone has also been shown to reduce antibody titres in chickens (Takahashi et al.,1992).

Administration of ACTH daily to lambs can also compromise other immune mechanisms, by suppressing lymphocyte blastogenesis by 50% (Kuhlman et al.,1991).

In view of the stressors that grazing animals are subjected to, it is possible that the chronic suppression of plasma cortisol levels by active immunization against ACTH may lead to enhanced immuno-competence.

To study this concept and to further investigate the impact of chronically suppressing cortisol on body composition, we have investigated the effect of active ACTH immunization on the ability of animals to generate antibodies to another production related antigen, GnRH. This second antigen has been used commercially as a vaccine to immunocastrate animals (Hoskinson et al.,1990) and by itself has been shown to alter carcase composition of lambs.

2. Materials and Methods

2.1 Design

The lambs (Dorset x Border Leicester-Merino) were randomized into five groups based on a stratified liveweight basis. A factorial design for ram lambs was established when

the group of castrates was omitted fro n the analysis. One group was not treated with immunogen to act as a control group. This group was immunized against the protein carrier molecule only using the same adjuvants. Two groups of entire animals were immunized against ACTH:OA with o ie group receiving a second immunogen against GnRH after the booster vaccination tc ACTH. Another group was immunized against the GnRH immunogen only. A final grou ɔ underwent surgical castration in order to compare the efficacy of the anti-GnRH vaccine in decreasing carcase fatness relative to the control intact group.

Therefore the treatments were as follows:

1/ Control rams

2/ ACTH immune

3/ GnRH immune

4/ ACTH/GnRH immune

5/ Surgical castrates

2.2 Animals

Eighty five male second cross lambs with a mean birth date of 1/9/1991 were grazed with the ewes on phalaris/white clover pasture at the CSIRO, Chiswick property. The animals were weaned at 100 days of age.

2.3 Protocol

The eighty five ram lambs were randc mised into five experimental groups of 17 animals. ACTH immune groups were given a primary vaccination at a mean age of 44 days and a boost at day 73. A second boost vaccination to ACTH was given at 100 days of age. The primary GnRH vaccination was given at 79 days of age and a boost at day 130. Blood samples were collected from all animals from the jugular vein using venepuncture on the day of vaccination, prior to the time of injection and 7 days after vaccination. These were placed on ice immediately after collection and centifuged (1200g for 15min.) prior to the separation of plasma which was stored at -20°C pending analysis.

Animals were weighed monthly throughout the trial and at the same time an assessment of testicular size and weight was determined on entire animals as a bio-assay for gonadotrophin levels. A set of 10 egg shaped graduated wooden beads ranging in diameter from 18mm to 58mm and calibrated to testes weight was used to assess testis development in the live animal (Oldham et al.,1978). A strong correlation between testicular diameter and sexual maturity and activity has been demonstrated previously (Mattner,unpublished data).

At the end of the growth study on 23.4.1992 a sub-group of 6 animals from each of groups 2, 3, 4, 5 were transported to the tomography room at the UNE for CAT scanning to determine body composition.
Several days later on day 196 and 198 of the experiment, the groups were divided equally into two with half the animals fasted overnight and slaughtered the following day for carcase analysis.

2.4 Immunogens

ACTH 1-24 was conjugated to ovalbumin using carbodiimide chemistry (Goodfriend et al.,1964). Animals received 0.5mg of antigen per injection. GnRH 1-10 was conjugated to human serum albumin using the same conjugatory chemistry as above and used at a dose level of 1mg per animal. The immunogens were dissolved in saline and emulsified in an equal volume of adjuvant. The resultant 2ml dose was administered intramuscularly, 1ml into each hind leg.
Primary vaccinations were prepared using Freund's complete adjuvant, while Freund's incomplete adjuvant was utililized for booster vaccinations.

2.5 Assays

2.5.1 ACTH Antibody Titre

Blood samples from the immune lambs were analysed for antibody titre levels using the ELISA technique described in the previous chapter.

2.5.2 Plasma Cortisol

Cortisol levels in the plasma were determined using the RIA method described in the previous chapter.

2.5.3 Plasma Testosterone

Distilled water (180µl) was added to duplicate plasma samples (20µl) pipetted into glass tubes. A serial dilution (x8) of known standard, ranging from 0 to 16ng/ml testosterone was included for the standard curve as well as duplicate tubes to measure non-specific binding.
Hexane (1ml) was added to all of the tubes, and they were vortexed for 5min and then placed in dry ice to fractionate by freezing. The solvent layer was decanted into fresh tubes and the solvent evaporated at 40°C in the presence of nitrogen gas in an evaporator. Radiolabelled 3H testosterone (100µl) was added to all tubes then an equal volume of phosphate buffer (0.05M,ph 7.4) was added. Separate tubes (x2) received label and

phosphate buffer alone. Samples were incubated for 14h at 23°C, following which 500µl of charcoal solution (Ajax)(0.005m) was added and the tubes were vortexed and left to stand for 15min. at 23°C. Samples were centrifuged at 1000g for 15min and the supernatant decanted into scintillation vials. Scintillation fluid (High-safe,LKB Laboratories;4ml) was added and samples placed in ß scintillation counter.

2.5.4 GnRH Antibody Titre

Antibodies specific for GnRH were estimated by ELISA. Microtitre plates (96 well, round bottom, Dynatech,Virginia,USA) were washed in phosphate buffered saline (pH 8.0)(PBS) azide (1h at 23°C) then GnRH conjugated to gelatin (1:5;w:w)(100µl) was placed in the wells and sealed and incubated over night at 4°C. Following three washes with PBS, a blocking solution (200µl) (0.05% gelatin)(300 bloom;Sigma) was added to the wells for 1h at 23 C. Diluted plasma (1:2500) was added to the wells (100µl) which were covered and left to stand for 1.5 h at 23°C. A known standard was serially diluted 8 times and replicated in the last two columns of the microtitre plates. The wells were subsequently washed three times again and peroxidase conjugated rabbit immunoglobulins to sheep immunoglobulins (Dako)(100µl, diluted 1:1000 in PBS) was added to the wells and incubated for 1.5 h at 23°C. Following washes (3x PBS) and the addition of 100µl of substrate (O-Phenylenediamine)(12mg, Sigma) with hydrogen peroxide (10µl)(BDH) in citrate buffer (0.01M,pH4.9,100µl), plates were incubated for 7 min and the reaction stopped by the addition of sodium azide solution (0.4%). The optical density was read at 410 nm on the microplate reader (Dynatech).

2.6 CAT Scanning

Animals were stratified on liveweight and randomly selected across a weight range from the ACTH immune, GnRH immune, ACTH\GnRH immune and the castrate groups and taken to the tomography room at the University of New England, Armidale for scanning to estimate body composition as described in chapter 3. Scanning was done at the end of the growth period on days 191 and 192. All animals, including those not scanned, were fasted for 24h over this period so as not to introduce a bias into the experiment. Approximately 20 transverse scans per animal were taken at intervals of 40mm commencing at the distal end of the femur bone. Images were downloaded to a PC and using the CATMAN software (Thompson and Kinghorn,1993), the weights of muscle, bone, fat and non carcase protein calculated.

2.7 Carcase Analysis

At the completion of the growth study on days 196 and 198, animals were fasted overnight and slaughtered as described previously. Testes were removed from the entire animals immediately after the skins had been removed. The epididymus and associated blood vessels were removed and tissue dissected from the dorsal end of each testis. Both testes from each animal were weighed and recorded.

After the rumen and alimentary canal were removed from the animals, the omental fat was carefully removed from the associated gut and weighed. Likewise, kidney and channel fat was removed manually from around the kidneys and the pelvic arch and weighed. Carcases were weighed and placed in the chiller at 4°C.

The following morning carcases were reweighed to assess moisture loss and ultimate pH values recorded. pH was measured on the M.semimembranosus in the upper hind leg by inserting a probe from a pH meter into an incision made into the muscle.

After splitting the carcases in half longitudinally on the band saw, a GR measurement was taken over the 12th rib 110mm from the dorsal side of the vertebrae. The side was quartered at the 12-13th rib interface to photograph the M.longissimus and subcutaneous fat for measurement with a planimeter.

Opposing half carcase sides from the sub group of animals that underwent CAT scanning were placed in large plastic bags and frozen.

These were later cut into pieces on the band saw and ground into mince with a cutter-grinder (Jeffco Industries,Brisbane), thoroughly mixed and sub-sampled for chemical fat analysis incorporating the same method as per the previous experiment.

2.8 Chemical Fat Analysis

Duplicate mince samples from the half carcases were taken by coring (approximately 10g each) and freeze dried after weighing. Dry matter percentage was calculated. Ether extraction was performed as per the previous experiment (chapter 3) to determine percentage fat content and to estimate total fat present in the half carcase.

2.9 Statistical Analysis

A least square means analysis was conducted on the data in similar fashion to the previous chapter. A repeated measures analysis was only performed on the growth responses whereas muscle pH and CAT scan data were taken at a single point during the experiment.

3. Results

3.1 Growth

Lambs commenced the trial with a mean body weight of 11.1 ± 0.75kg at day 1 (Figure 4.1 and 4.2). Throughout the 204 day growth period, there was no difference in body weight or liveweight change between the ACTH immunized animals and the non-immunized control animals.

GnRH immunization significantly reduced liveweight ($P<0.05$) at day 162 and this decrease assumed greater significance ($P<0.01$) at day 189 and at the completion at day 204. Although there was a tendency for GnRH immune ram lambs to grow more slowly throughout the trial, the period between day 162 and day 189 was the only time period when this was significant ($P<0.01$). When adjusted using initial weight as a covariate there was a 2.2kg difference between these two groups at the completion of the experiment.

The contrast between GnRH immune rams and the surgically castrated males showed no difference in liveweight. However, for the third growth period (days 45 to 69) castrates grew 52g/day faster ($P<0.05$), while over the final period (days 190-204) the GnRH immune rams grew more quickly ($P<0.05$). Not only was this difference in growth rate reversed but it was of a similar magnitude.

It can be concluded that growth rates of immunologically castrated rams and surgically castrated rams were not significantly different over the duration of the experiment.

3.2 Assays

3.2.1 ACTH Antibody Titre

There was a 6-7 fold difference in the magnitude of the ACTH antibody titre response to the boost vaccination. As Figure 4.4 clearly demonstrates, the combined ACTH/GnRH immunization had a higher ACTH titre at day 40, but this declined to similar levels to that of the ACTH immune group by day 130. However, the group receiving the ACTH immunization alone had consistently lower ACTH titres throughout the experiment which declined over time.

3.2.2 Plasma Cortisol

Mean blood cortisol levels were found to be between 65-80ng/ml at the start of the experiment in all animals. These levels dropped over the three subsequent sampling periods in all animals, including the control animals (Figure 4.3). However, the ACTH and the ACTH/GnRH immunized groups had even lower cortisol levels than the controls

Table 4.1 Repeated measures analysis for growth rate (g/day) for control lambs and those immunized against ACTH with half of each group of lambs having a second immunogen (GnRH) injected into them at a later time period of the experiment.

Independent Variable		Whole plot analysis		Mean regression coefficients	Repeated measures analysis	
		df	F ratio		df	F ratio
Constant				118.8		
					7,77	203.95***
GnRH		1,33	6.44			
					7,77	0.86
	Control			5.23		
	Immune			-5.23		
ACTH		1,33	2.03			
					7,77	1.19
	None			-2.94		
	Lots			2.94		
GnRH x ACTH		1,33	0.31			
					7,77	2.58*
Initial weight		1,33	12 41			
					7,77	10.86***

Table 4.1 (Cont.)
Predicted means for treatments on nean growth rate (g/day) over the six growth periods of the experiment.

	LWC1	LWC2	LWC3	LWC4	LWC5	LWC6
Non Immunized	212	212	213	179	24	143
GnRH Immune	155	215	202	179	3	139
ACTH Immune	172	218	207	178	9	174
GnRH/ACTH Immune	191	208	193	167	8	155
Std.Err.	12.9	8.3	13.5	9.8	14.7	12.7

Growth rates adjusted to a mean initial weight of 11.06kg.

Table 4.2 Liveweight and carcase traits for ram lambs immunized against ACTH, GnRH or a combination of immunogens, after adjustment for the appropriate covariate. Data was analysed using a log transformed model and predicted means calculated by taking the antilog.

	CONTROL	ACTH IMMUNE	GnRH IMMUNE	ACTH/GnRH IMMUNE	Std. error	'b' coefficient
Liveweight (kg)	39.8	40.7	37.6	38.5	(0.7)	24.5
Hot Carcase Wt.(kg)	15.8	15.7	15.3	15.3	(.016)	1.19
Carcase Ratio HCW:LWT	0.40	0.39	0.41	0.40	(0.007)	0.40
Kidney Fat (gm) #	272	242	302	269	(.028)	-3.16
Omental Fat (gm) #	454	424	545	508	(.025)	-0.14
Fat Depth # 12/13Rib (mm)	1.85	1.74	2.02	1.90	(.058)	-2.04
G.R. (mm)	5.19	5.22	5.24	6.27	(.031)	-1.64
Eye Muscle Area (sq.cm)	9.40	9.30	9.69	9.59	(.013)	0.32
Muscle pH	5.83	5.81	5.86	5.85	(.0035)	0.77

Group means adjusted to the same hot carcase weight.

Standard errors have been presented in the transformed mode.

Table 4.3 Liveweight and carcase traits for the contrast between lambs immunized against GnRH and castrates, after adjustment for the appropriate covariate. Data was analysed using a log transformed model and predicted means calculated by taking the antilog.

	Castrate	GnRH Immune	Std. error	'b' coefficient	Significance
Liveweight (kg)	41.6	38.7	.201 1.34	1.569	ns
Hot Caracse Wt. (kg)	15.5	15.8	.019 .029	.004	ns
Carcase Ratio HCW:LWT	.38	.41	.010 .007	.047	*
Kidney Fat (gm) #	218	289	.047 .031	.061	ns
Omental Fat (gm) #	333	561	.03	.134	**
Fat Depth # 12/13 Rib (mm)	1.36	2.41	.097 .065	.124	ns
G.R. (mm) #	4.13	6.32	.04	.092	*
Eye Muscle Area (sq.cm.) #	9.51	9.57	.02	.0014	ns
Muscle pH	5.86	5.77	.005	.765	ns

Group means adjusted to the same hot carcase weight.
Standard errors have been left transformed.

Table 4.4 Weight of body components for experimental male lambs either immunized against ACTH, GnRH or a combination of the two immunogens and castrates. Animals underwent CAT scanning one week prior to slaughter and the data log transformed and adjusted to the same total weight of body tissue.

Body Tissues (kg)

Treatment	Internal Fat	Subcutaneous Fat	Intermuscular Fat	Muscle	Non Carcase Fat	Bone
Castrates	1.416	1.311	1.173	9.389	5.683	1.534
ACTH Immune	1.276	1.070	0.973	9.247	6.566	1.514
GnRH Immune	1.408	1.205	1.089	9.251	6.148	1.505
ACTH/GnRH Immune	1.325	1.193	1.010	9.009	6.574	1.481
Std. Err.	.039	.037	.028	.009	.014	.015

Table 4.5 Weight and percentage chemical fat in the carcases of experimental male lambs estimated from solvent extraction and adjusted to the same carcase weight. Weight of tissue underwent log transformation and the figures presented are taken from the antilog. Standard errors are presented in the transformed mode.

Treatment	Weight of Fat (kg)	Std. Err.	% Carcase
Castrates	3.78	.017	23.6
ACTH Immune	3.40	.017	21.2
GnRH Immune	3.82	.017	23.8
ACTH/GnRH Immune	3.80	.017	23.7

Figure 4.1 Mean liveweight of lambs (Dorset Horn x Border Leicester-Merino;XB) immunized against ACTH and or GnRH or surgically castrated after adjustment to initial liveweight. Arrows denote time of vaccination.

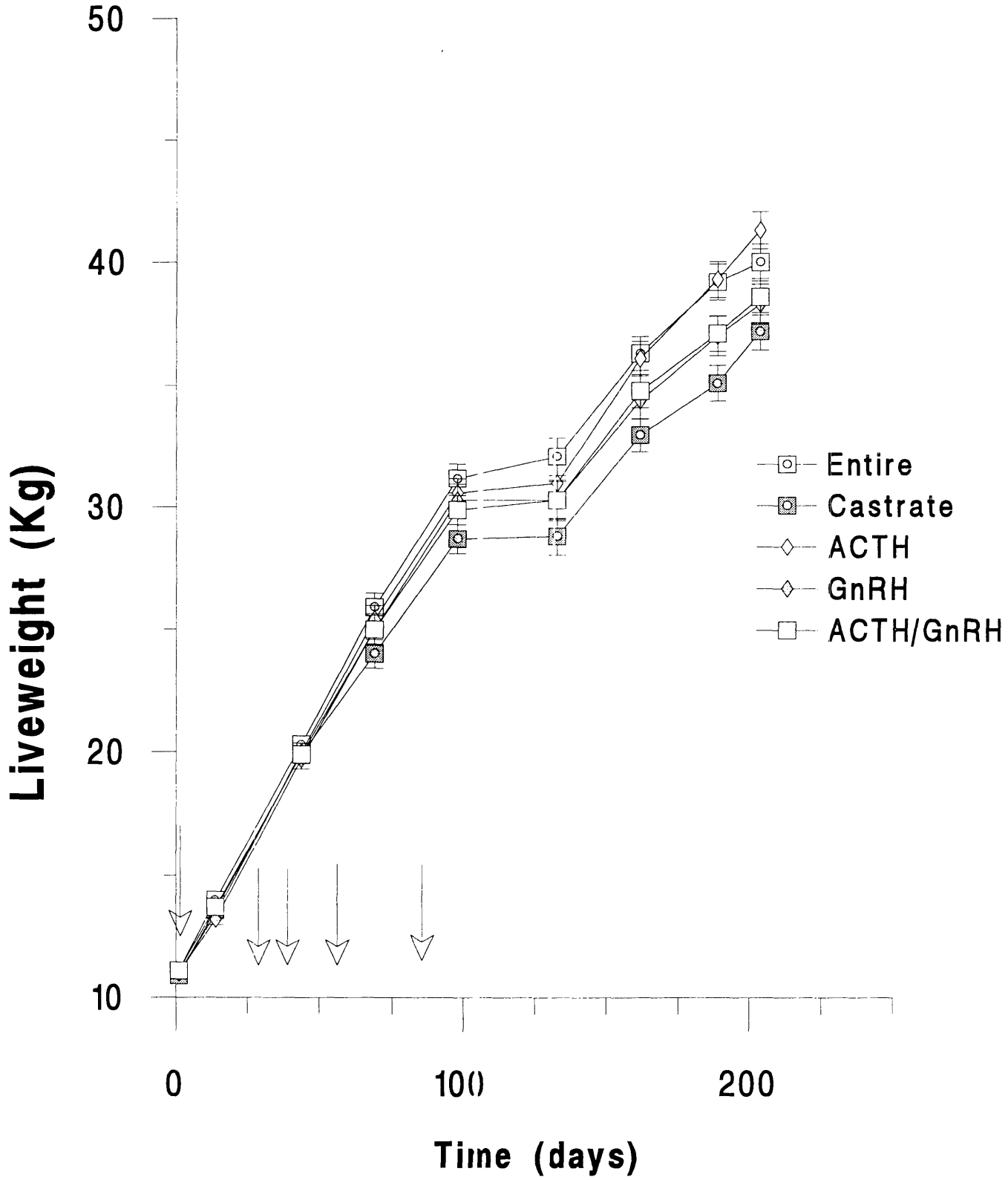


Figure 4.2 Growth rates (liveweight change) of XB male lambs expressed as group means for the five treatments. Arrows denote time of vaccination.

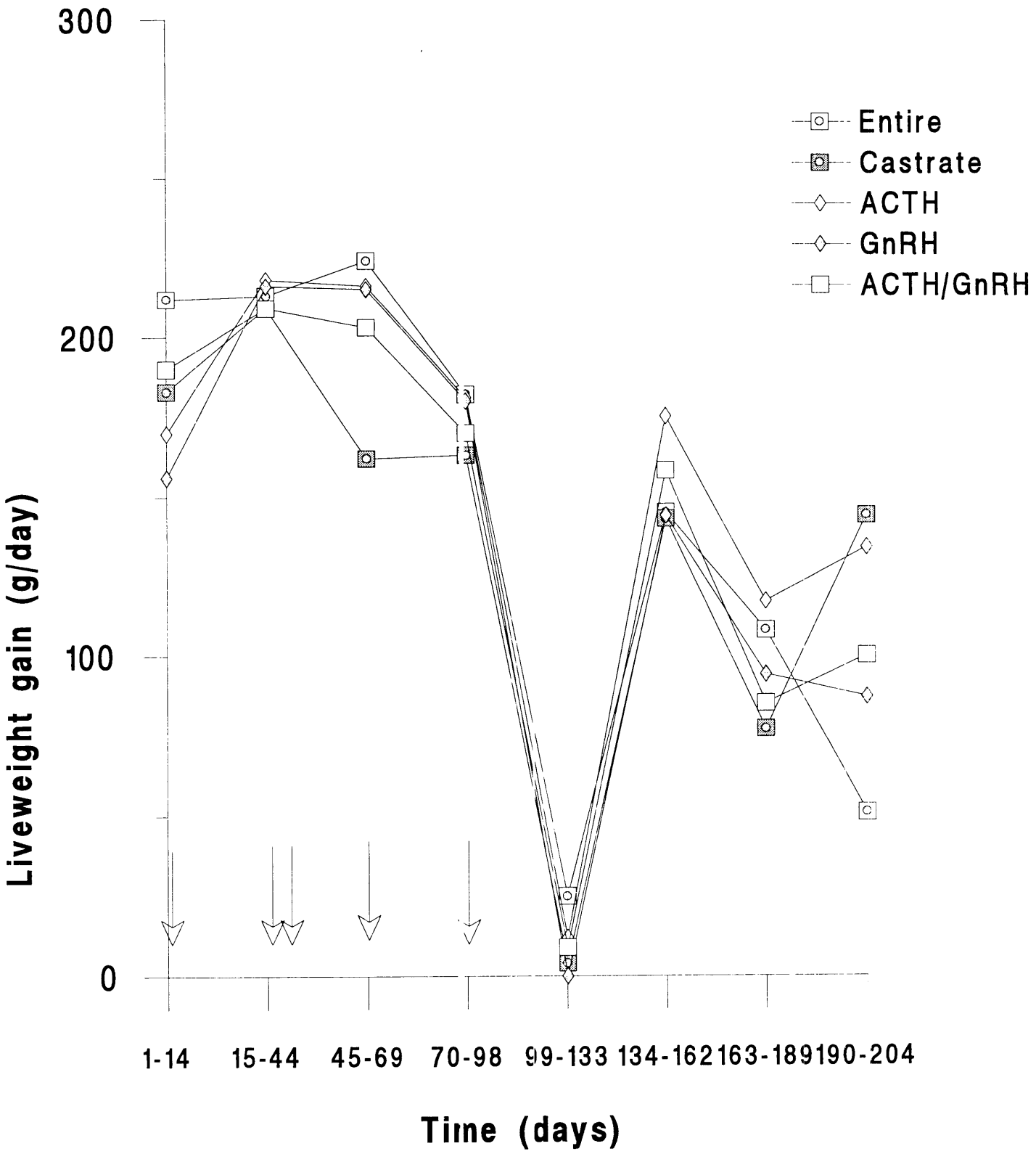


Figure 4.3 Plasma cortisol levels in male lambs either immunized against ACTH, ACTH/GnRH or non-immunized controls. Arrows denote time of vaccination.

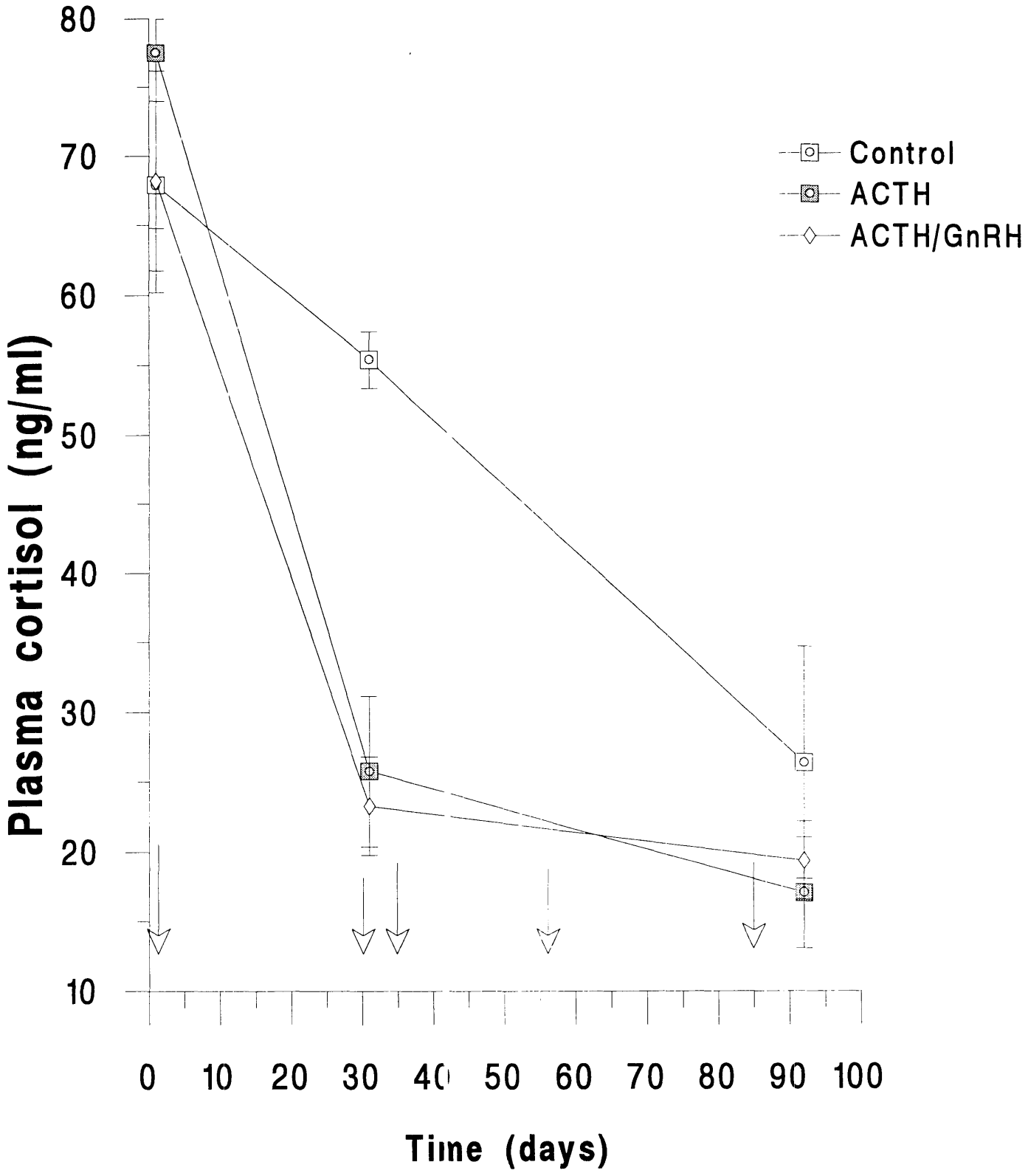


Figure 4.4 Plasma ACTH antibody titre for XB lambs actively immunized against the hormone or in combination (ACTH/GnRH). Time of vaccination is depicted by arrows.

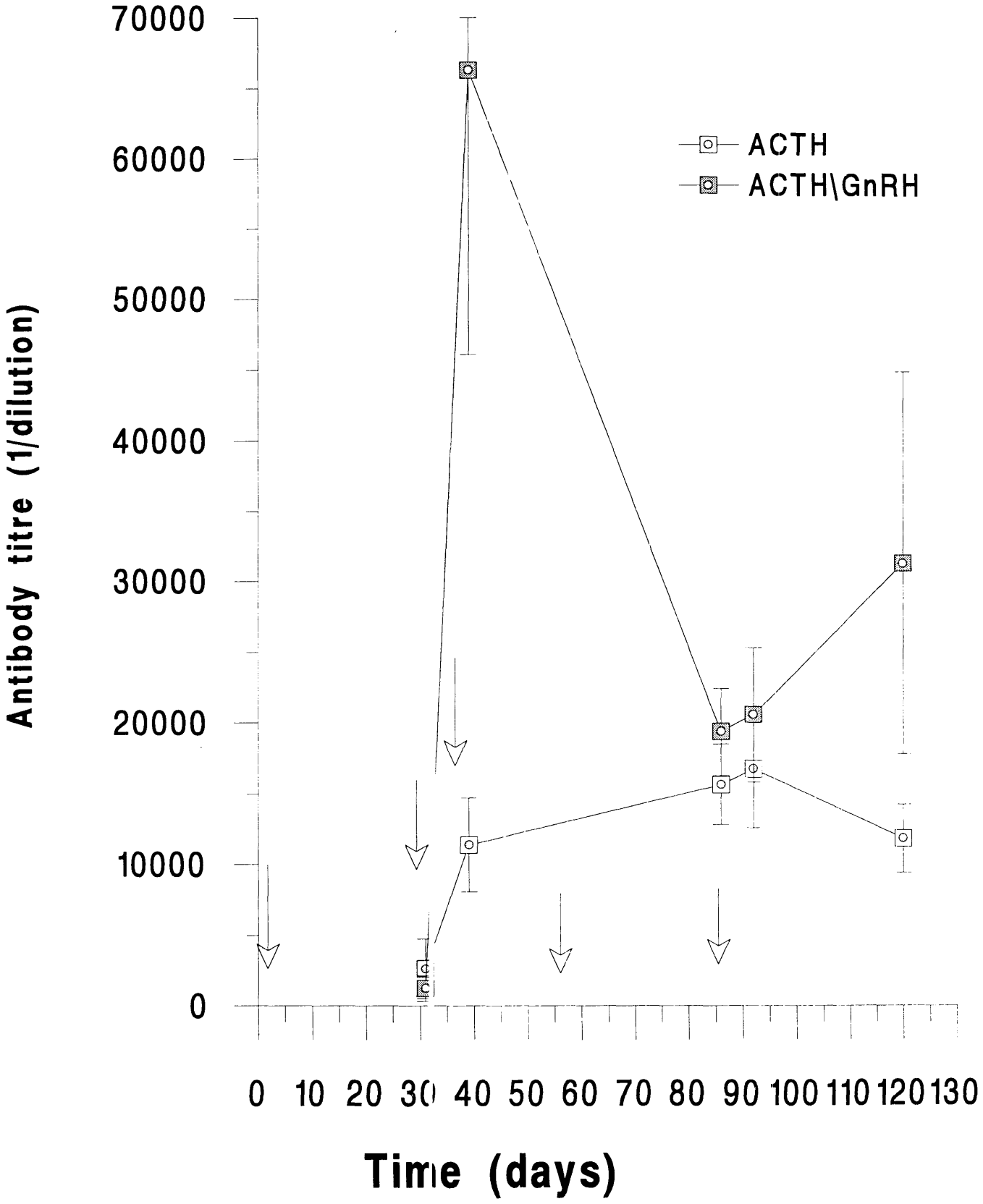


Figure 4.5 GnRH antibody titre for XB lambs immunized against GnRH or a combination of immunogens (ACTH/GnRH). Arrows denote time of vaccination.

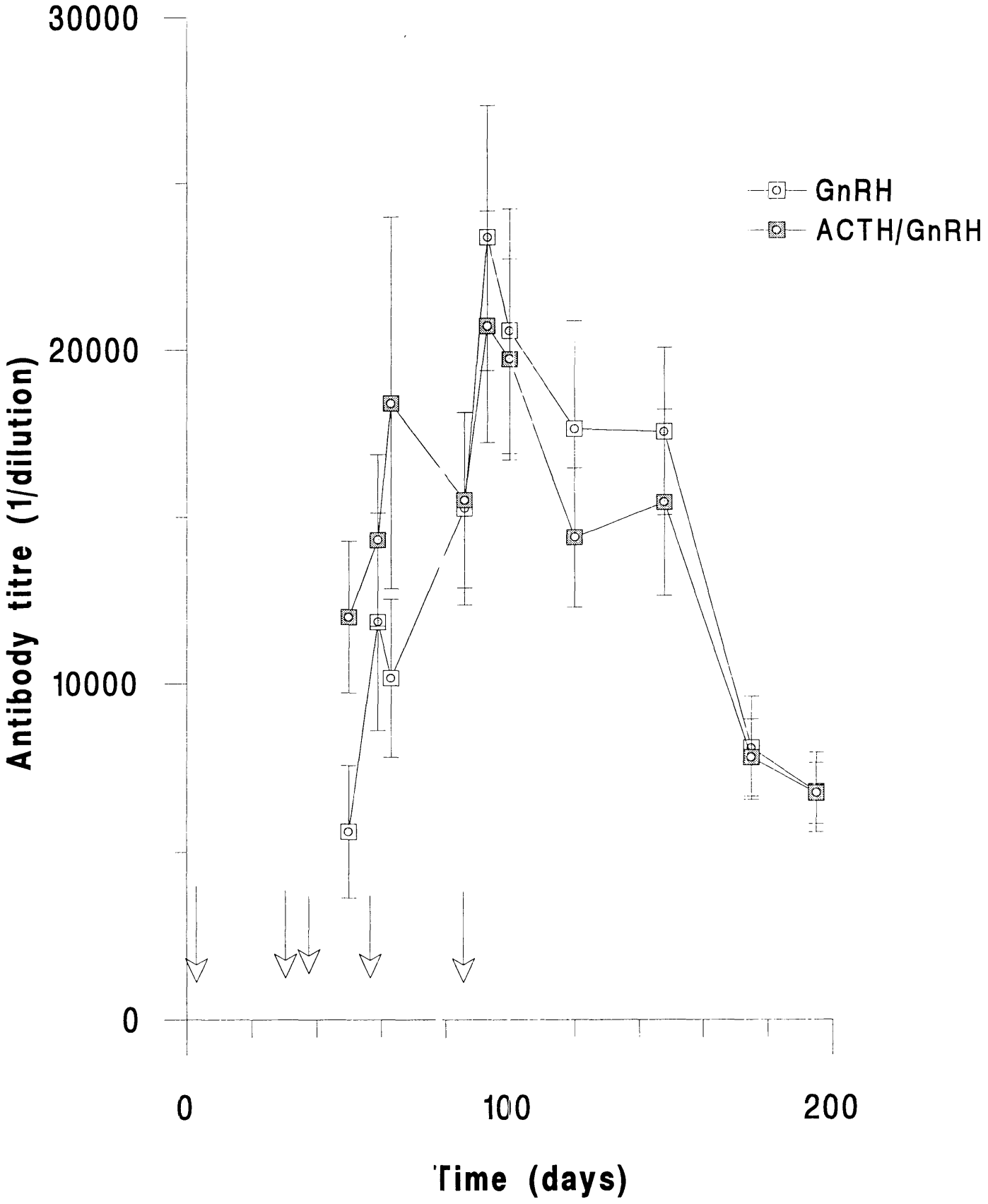


Figure 4.6 Plasma testosterone levels for treatment groups of entire male lambs. GnRH immune ram lambs had testosterone levels too low for the sensitivity of this graph.

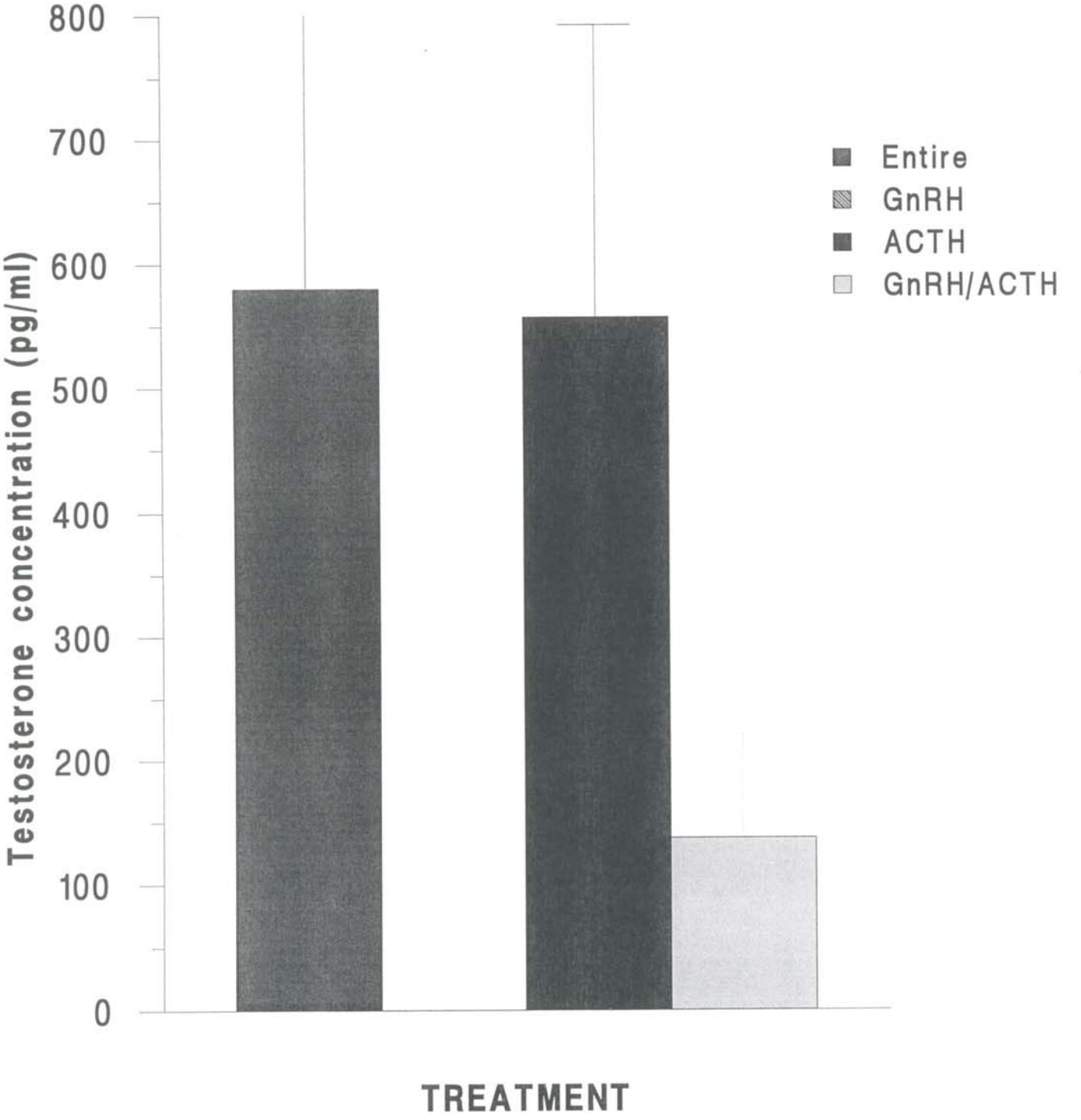
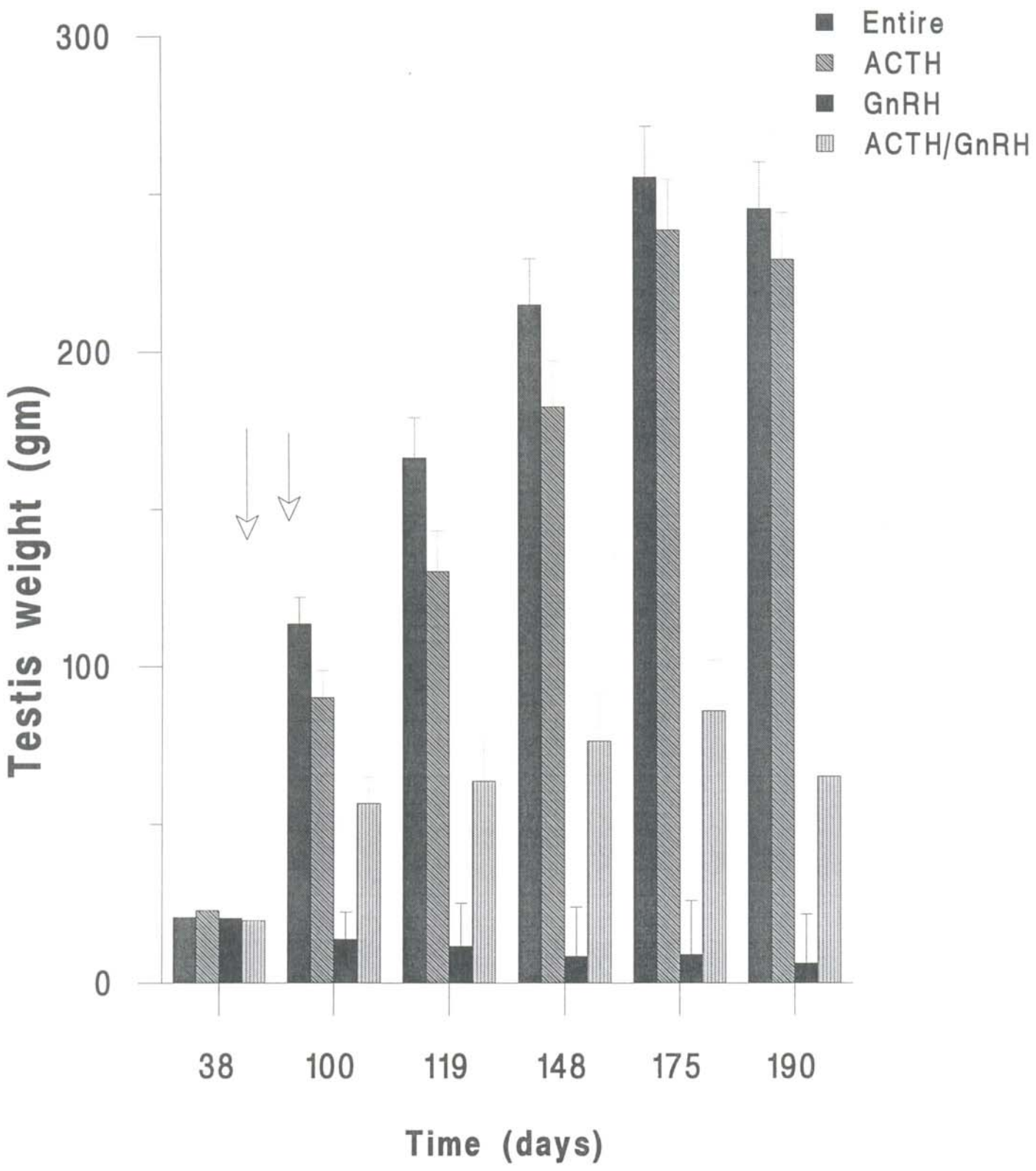


Figure 4.7 Estimated weights of combined testes adjusted to initial testis weight for entire male lambs. Arrows denote GnRH vaccination.



at the second sampling and these leve s remained lower until day 92 at which time the levels were similar in all three groups

Remarkably, the two ACTH immuniz ed groups had very similar cortisol profiles despite having vastly different antibody titres in the early stages of the experiment. Insufficient cortisol assays were performed, howe ver, to characterize thoroughly the glucocorticoid status of both control and ACTH imm une animals.

3.2.3 Plasma Testosterone

GnRH immunization significantly rec uced testosterone levels to an overall mean of 0.1pg/ml ($P<0.05$) with the levels in r nany samples from the GnRH immune group being close to the detectable limit of the ass ay (Figure 4.6). ACTH immunization had no effect on androgen levels and the combined treatment compromised the effectiveness of the GnRH immunization rather than pote ntiating it. This resulted in higher circulating testosterone levels in this group wher compared to the group immunized against GnRH alone.

3.2.4 GnRH Antibody Titre

Lambs pre-immunized against ACTH had higher GnRH antibody titres until day 63 (Figure 4.5). Three weeks later their titres were no different and both groups reached a peak at day 93. From this point in tim e, these titres declined, with the combined ACTH/GnRH group and the GnRH i nmunized group following a similar profile to the completion of the experiment at day 200.

3.3 Testis Development

The combined weight of testes for the control rams was estimated to be 20.6 ± 3.45g at the commencement of the trial and increased to a maximum size of 255.1 ± 16.09g at day 175. Thereafter the weights did not c nange (Figure 4.7).

From day 50 there was a highly significant difference ($P<0.001$) between the size of testes between the treatments. When adjusted to initial testis size, the testes from the GnRH immunized rams regressed ov er time rather than developing with age and liveweight. The ACTH/GnRH immu ne rams had larger testes than the GnRH immunized animals, while ACTH immunization per se suppressed testis development only slightly when compared to the control entire rams.

The final testis measurement recorde d at the completion of the growth period was later plotted against the weight of testes m easured at slaughter, resulting in a significant regression relationship.

Y= -4.746 + 0.781X (correlation coefficient r = 0.983)
Although there was a tendency to under-estimate the actual size of the smaller testes, the above regression demonstrates a strong relationship between estimated and actual testis weight, accounting for 96.6% of the variation as the weight increased.

3.4 CAT Scan Analysis

When the weight of the various fat depots, subcutaneous, intermuscular, internal fat (including omental, mesenteric, kidney and channel fat) and total fat, was calculated on the live animal there was no significant difference between the treatments when these variables were adjusted to equal weight of total body components (excluding rumen and reticulum contents). The ACTH immune rams tended to have the lowest weight of fat in all depots measured in comparison to the other treatments (Table 4.4).
The weight of carcase muscle and bone did not vary significantly between treatments. Viscera, including kidneys, liver, spleen, lungs, heart and parts of the alimentary canal minus the contents did differ significantly ($P<0.05$). The castrates and the GnRH immune rams had significantly less weight of non-carcase viscera.
The castrates and the GnRH immune rams were depositing more fat in the intermuscular depot compared with the other treatments when calculated from the regression coefficients for these parameters.

3.5 Carcase Analysis

Although there was a tendency for GnRH immunization to depress liveweight at slaughter, it did not reach significance at the 5% level. Neither ACTH nor GnRH immunization influenced the hot and cold carcase weights post slaughter, nor did they influence the weights of each of the internal fat depots. There was a significant interaction between ACTH and GnRH immunization on the weight of the omental fat depot ($P<0.05$) suggesting that these depots varied in weight between the treatments depending on carcase weight.
GnRH immunization significantly increased the GR measurement ($P<0.01$) when compared to the entire groups that were not immunized against this immunogen. This response was not as apparent ($P<0.05$) when animals were co-immunized against ACTH and GnRH.
The treatments had no effect on eye muscle area for the semimembranosus group when measured on the cold carcase (Table 4.2). Direct carcase measurements, including the eye muscle area of the M.longissimus and subcutaneous fat thickness were not affected by the

treatments. This result is also not consistent with the significant differences in GR measurements between the entire and GnRH immune groups.
When the contrast was performed for the immuno-castrated rams and the surgically castrated wethers, there was a significant decrease in omental fat for the wethers ($P<0.01$), and a similar trend existed for carcase GR ($P<0.05$). Parameters not affected by treatment in the contrast included both hot and cold carcase weights, kidney fat and muscle pH.
Muscle mass, which is reflected in eye muscle area, was unaltered and although castrates tended to have less subcutaneous fat at the 'C' position, this did not reach significance.

3.6 Chemical Fat Analysis

Chemical fat determinations were made on samples from the ACTH immune, GnRH immune, ACTH/GnRH immune and the surgically castrated lambs. The results were analysed in a one way layout for the least square means and the predicted means adjusted to the same carcase weight and the percentage fat in the carcase presented (Table 4.5). The ACTH immune rams contained 3 percentage units more water in the carcase than the castrates and GnRH immune rams. This figure did not reach significance. As expected this same group contained on average the least amount of chemical fat in the carcase with a lower weight of fat (400g) than the other groups. Once again, statistical significance was not quite attained.

4. Conclusions

The significant lower liveweight in the GnRH immune male lambs as compared to the control males and ACTH immune lambs could be attributed to depressed plasma androgen levels post immunization, especially after the booster injection. The anabolic effects of testosterone per se have been well documented in the male. Surgical castration or highly effective active immunization against GnRH could have cumulative effects through the growing phase of the animal and result in these lower body weights. The lowering of plasma cortisol by active immunization against ACTH was not expected to have an impact on growth in non-stressed animals as was observed. Not having been subjected to chronic stress, the male lambs would be expected to exhibit cortisol levels within the normal physiological range rather than the high levels associated with muscle degradation and increased fatness.
The depressed growth rates for the castrates from days 45-69 may well have resulted in the failure to synthesise testosterone as they approached puberty. By this time the GnRH antibody titres had not been established in the GnRH immune groups and the exposure to

testosterone may have provided the impetus to maintain growth rates of around 200g/d in these intact groups of animals. From day 98 the growth of the lambs was severely depressed across all experimental groups as the pastures hayed off and the quantity and quality of feed on offer was limiting. This also corresponded with the weaning of the lambs which lead to lower growth rates as the animals adjusted to the change in diet. Subsequently there was an improvement in growth rate which coincided with summer rains and the growth of higher quality pasture. However, the growth rates were not of the same magnitude as before weaning and declined over the last couple of months to approximately 100g/d.

It would appear that both ACTH immunization or a combination with the GnRH immunogen were effective in depressing basal cortisol levels in the short term at least up until day 90. Yet over the same time period the levels of cortisol were lower in the control group as well and these were only slightly higher than in the immune groups at this time. This most likely is a response to increased familiarity of the animals to yarding and handling as the experiment progressed. Fell et al.(1991) observed similar findings with decreased plasma cortisol levels over time with experimental sheep. The adaptation of animals to routine procedures can sometimes be observed by changes in behaviour (less flighty) when handled which has been associated with lowered cortisol levels with conditioned handling by Fell and Degabriel (pers.comm.).

The variance between the two immune groups in ACTH antibody titres after the first boost can only be attributed to biological variation. The ACTH/GnRH immune group had not been injected with the second immunogen at that stage but this group contained five animals with very high titres compared to the rest of the group as is evident by the large standard error. These same high responding animals had titres closer to the group mean at the next blood sampling.

Differences between the two groups were not evident at day 90 but the titres digressed at day 100. The impact of the GnRH immunogen could possibly account for this observation towards the end of the experiment.

The experiment failed to demonstrate greatly enhanced antibody titres to the GnRH immunogen following the lowering of basal cortisol through ACTH immunization. Although there was a slight increase in GnRH antibody titre in the short term until day 63, any superiority in this group compared to the other diminished resulting in lower titres in the longer term. However there is a significant body evidence to show that chronic hyper-activity of the HPA axis is immunosuppressive. For example Griffin et al. (1992) showed that deer subjected to transport stress resulted in activation of the HPA axis resulting in a depressed immune response to bacterial infection. Likewise Kuhlman

et al. (1991) depressed the immunoresponsiveness of lambs with ACTH treatment, resulting in decreased T lymphocytes levels circulating in the blood. Similarly, Minton et al. (1991) depressed antibody product on to ovalbumin in dexamethasone treated lambs. However, the use of synthetic analogues such as dexamethasone must be viewed with caution, as they have a 15-fold greater biological activity and slower clearence rates from the body than cortisol.

Considering that the basal levels of adrenal steroid hormone were declining in all groups at the time of the primary injection of the second immunogen and thereafter plateaued, it is likely that they were not sufficiently high to limit the function of either the cellular or humoral elements of the immune system.

GnRH immunization was very effective in significantly decreasing plasma testosterone levels in these male lambs almost to undetectable levels. This indicates that the level of luteinizing hormone reaching the testes and stimulating the Leydig cells was very low. The low levels are consistent with the very small testes observed in this group (Figure 4.7). The vaccination was very effective in suppressing pubertal testicular development in these animals through to the end of the experiment. At day 190 their testes had actually regressed to be smaller than at the early stages of the experiment at day 38. Although the GnRH antibody titres for the combined immunogen treatments were similar to those observed in animals receiving the single immunogen, their testes sizes did not reflect this observation. Testes in this group developed over time, attaining an intermediate weight between the GnRH immune group and the control entire males. This would seem to be an anomoly as the testosterone levels were closer to those in the entires than to the immunologically castrates and yet their testis weights were intermediate. It would appear that the strong relationship between testosterone, GnRH antibody titre and testes weight established in previous studies (Hoskinson et al.,1990) did not hold in this group of animals. The combined immunogens may have modified LH receptors in the testis resulting in their up-regulation. Alternatively the ACTH immunization may have changed the nature of the GnRH antibody response, by for example altering the ratio of anti-GnRH isotypes or promoting anti-idiotypic clones. There is little doubt that there are many interactions between the reproductive and stress endocrine axes and it is possible that these effects may be mediated by the peptides related to ACTH, such as ß-endorphin and α MSH, the circulating levels of which are increased markedly by the ACTH immunization procedure (Behrendt and Wynn,1992).

Moberg (1976) has shown in dairy cattle that stress induced high levels of ACTH and glucocorticoids can impair reproduction. Further research in cattle (Li and Wagner,1981;Matteri and Moberg,1982) suggested that elevated ACTH and possibly

cortisol can depress the pituitary gonadotroph response to GnRH and thereby limit luteinizing hormone secretion. Similar results have been observed in the ram (Fuquay and Moberg,1983). There is some conjecture as to whether stress induced elevated HPA hormones act at the hypothalamus as well as at the pituitary level (Moberg,1976). In this present study, the decrease in free ACTH hormone induced by immunization may have increased the responsiveness of the pituitary to GnRH resulting in more LH being released despite the second immunization against GnRH. This phenomenon would account for the elevated testosterone levels in the group as compared to the GnRH immune group. This being the case, the increased LH levels would stimulate the Leydig cells to synthesise and secrete testosterone resulting in the increased testes size that was observed.

Although both immunogens exerted highly significant effects on testis weight, there was a significant ACTH x GnRH interaction. Thus, although the GnRH antibody titres did not appear to vary between groups, it is possible that the titres in the ACTH/GnRH group were either lower or less effective at suppressing gonadotroph function in the pituitary. In these studies we have not assessed LH and follicle stimulating hormone (FSH) status because of the extensive nature of the experiment. Intensive blood sampling over a 12-24h period to determine the plasma profiles of these two gonadotrophins was not possible in this production oriented experiment.

Although there was no significant difference in body composition when measured by CAT scan analysis, there was a tendency for the castrate and GnRH immune lambs to have more fat stored in the carcase depots, namely the subcutaneous and intermuscular depots. A similar trend was apparent for non-carcase fat depots for these two groups. Having been adjusted to the same weight, the removal or depression of testosterone has resulted in both these groups depositing fat at an earlier stage than the other two groups. This observation is in agreement with Butterfield (1988), who indicated that castrates are earlier maturing than entire males. If the original model had been retained for CAT scan analysis and the number of replicates in each treatment increased, then significant differences may have become evident. As could be expected, both castrate groups had less non-carcase protein and bone than the other treatment groups, however the trend in skeletal muscle content of carcases is not so clear.

The observations observed in the two androgen restricted groups for carcase fat was supported by chemical fat levels. These treatments had the highest weight of fat in the carcase. Intramuscular fat is accounted for in this analysis and not in the CAT scans, although the trends are similar. Intramuscular fat is the latest maturing depot and in lambs only accounts for a small proportion of total fat stored in the body and thus differences

observed have little impact on total fa per se. Neither immunization against ACTH or GnRH had significant effects on most of the carcase traits measured except for GR and omental fat. GnRH immune male lambs had significantly higher GR values, suggesting that they had more fat stored in the carcase which is supported by CAT scans and solvent extraction. The increase in GR measurement could be the combined result of more intermuscular and subcutaneous fat rather than an effect on a single fat depot.
There is also a suggestion that these GnRH immune animals had more fat in the omental depot around the rumen, omasum and abomasum reaching significance at the 5% level. The removal of androgen may well have reduced ß-adrenergic receptors on the adipocytes from these fat depots resulting in a reduction in lipolytic activity which has been demonstrated in other species (Bjorntorp et al.,1990). This trend is not so evident for the kidney fat depot.
Ultimate muscle pH was not significantly altered by immunization, although there was a tendency for ACTH immune ram lambs to have lower pH values. Lowering cortisol could have spared muscle glycogen utilisation prior to slaughter by suppressing adrenergic receptor levels in muscle cells but this trend was not supported by the measurements taken in the combined immunized group. It would be expected that lower cortisol levels would lead to lower blood glucose as a result of a decrease in peripheral tissue resistance to glucose uptake. This would make available more energy substrate for muscle requirements and therefore lead to a decrease in glycogen utilisation.
The contrast between castrate and GnRH immune lambs produced some interesting results. GnRH immune lambs had a significantly greater carcase yield, more omental fat and a superior GR. It is difficult to explain why these animals had significantly more fat in these depots particularly as the omental and subcutaneous depots are not functionally related. This trend, although not significant also occurred in the other non-carcase fat depot measured. The marked reduction in testosterone levels should result in similar fat levels between these two groups. Previous experience with immunological castration has shown that as anti-GnRH antibody titres decrease, the immune lambs assume an intermediate level of fatness between entire and castrate lambs (unpublished data). This can be explained by intermediate levels of androgen being present in these animals as a consequence of more free GnRH reaching the pituitary gland to stimulate gonodotrophin synthesis and secretion.
In conclusion, normal physiological levels of glucocorticoids do not appear to inhibit the primary humoral immune response. Reports of the suppression of the immune response by elevated glucocorticoids are often the result of the use of supraphysiological levels of these hormones, synthetic analogues or hypersecretion through acute stress. However

there is little doubt that a chronic stress of high intensity can lead to similar responses. These situations are not common occurrences in normal production systems and where present are minimized by the livestock producer.

However, there would appear to be close functional interactions between the activity of the hypothalamic-pituitary-adrenal axis and the gonadal regulatory hormones, although the nature of these relationships is yet to be determined.

Chapter 5

Active Immunization of Feedlot Lambs against fragments of ACTH (1-24 and 22-39)

1. Introduction

The intensive husbandry of ruminants has become a feature of modern day animal production systems as producers strive to maximize the growth rates of animals to meet specific commercial market requirements. Grazing animals selected for superior growth performance, specific meat quality and carcase characteristics may not be able to cope with the stressors imposed on them when they are placed in a novel environment such as a feedlot, due to an elevation in stress hormones when they are removed from the extensive grazing system such as the Australian pastoral zone. In this production system the repertoire of stressors that animals are subjected to is different to those experienced by grazing animals and therefore it is not surprising that a portion of these animals are unable to display the growth rates recorded in extensive grazing systems.

The incidence of dark cutting meat which occurs in sheep and cattle is related to high muscle ultimate pH after slaughter and is due to a mobilisation of muscle glycogen prior to slaughter resulting in less substrate being available for lactic acid production (Lister,1988). An elevation in plasma adrenaline levels, excessive fasting or starvation and prolonged exercise are all factors related to to depletion of muscle glycogen either independently or in combination (Devine and Chrystall,1988). The blockade of the muscle receptor for adrenaline has only a minor effect on the incidence of dark cutting meat (Tarrant,1989) and therefore other stress related factors must also be responsible for glycogen mobilisation.

It is not known what impact the interaction of the autonomic nervous system with the HPA axis has on this phenomenon and body composition in such a production system. There are obvious differences between lot fed animals and grazing livestock in terms of behaviour, energy expenditure and the social interactions resulting from an increase in crowding of animals and from the nature in which feed is provided in intensive feeding systems.

In view of of this novel range of stressors to which animals are exposed, it is possible that the suppression of circulating glucocorticoid levels may result in more consistent growth rates and superior carcase composition (muscle:fat) and improved meat quality.

The failure to maintain a high antibod / titre to ACTH for the duration of the first experiment (Chapter 3), in which anim als were maintained at pasture may have been due to the lower nutritional status of the ar imals, particularly towards the end of the experiment, when their diets were sup plemented with a concentrate ration. A decrease in immune function can result from nutritional deprivation (Van Houtert et al.,1995).
In this experiment, a number of optior s were explored with different ACTH antigens in order to maintain high antibody titres for the duration of the post-weaning growth phase of the animals. Firstly, the nutritional status of the animals was maintained at a high level to ensure that this was not limiting for immune function. Secondly, differences in the ACTH epitope density of the antigen were compared and the efficiency of the different carrier proteins for the immunogenic conjugate were evaluated.
Thirdly, the ACTH antigens that have been tested to date have used the amino terminal of the peptide (ACTH 1-24) as the basis for a conjugate. In this study we have assessed the role of the carboxyl terminal 17 amino acids (ACTH 22-39) as an antigen to modulate ACTH function. The impetus for this approach was provided by the studies of Morton (1989), who showed that this fragment was lipogenic in primates and therefore it is proposed that the immuno-neutralisation of this portion of the ACTH molecule may actually decrease lipogenic activity induced by other endocrine factors.

2. Materials and Methods

2.1 Design

Ninety six lambs were randomly allocated to one of six treatments in a non-orthogonal design, with 16 replicates per treatment. The treatment groups for the experiment were:

1. vehicle treated control
2. ACTH 1-24(HCl):HSA (1:)
3. ACTH 1-24(oAc):HSA (1:)
4. ACTH 1-24(HCl):OA (1:2)
5. ACTH 22-39:HSA (1:2)
6. ACTH 22-39:HSA (1:2) + ACTH 1-24 (HCl):HSA (1:1)

2.2 Animals

Second cross ewe lambs (Dorset x Border Leicester-Merino) with a mean birth date 10/8/1992 were stratified on body weight and allocated to the six treatments at seven weeks of age. They grazed pasture at the CSIRO Chiswick field station until weaning and were supplemented with sheep nuts twice weekly (Fielders,16% crude protein) with their mothers to condition the animals to their final feedlot ration.

2.3 Protocol

At a mean age of eight weeks, lambs received their primary vaccination followed by a booster vaccination four weeks later. Weaning took place at 13 weeks of age. Fifty six days post primary immunization, experimental animals received a second booster vaccination and were moved into the feedlot. The feedlot consisted of four large sheep pens with feed and water on offer ad l bitum. Animals were randomly allocated to 4 pens, each containing 24 animals. The pens were shielded from each other using a solid hessian barrier so as to restrict visual contact for animals between pens. Individual lambs were exchanged between the pens at irregular intervals varying from 3-8 days to disrupt their social hierarchy. Additionally, the length of the feeding troughs was shortened to allow 20 animals to feed simultaneously so that a psychological stress of the inability to feed was imposed on less dominant animals. The aim of this was to establish conditions for the expression of the social hierarchy within the flock with the submissive animals unable to feed until dominant animals had completed their time of feeding at the trough.
At the same time sufficient feed was always on offer for the submissive lambs to receive adequate feed to maintain their potential growth.
Animals were blood sampled on the day of vaccination, 1 week post injection and at monthly intervals to the day of slaughter at day 112. Blood samples were chilled on ice until centrifugation (1200g for 15min) and the plasma separated and frozen until assayed for antibody titre and cortisol levels.
Body weights were recorded approximately every month for liveweight gain determinations.

2.4 Statistical Analysis

A least square means analysis was performed on the data in the one way layout and the appropriate covariate used to compare lambs at a constant weight. Interactions in the model were tested and dropped if they did not reach significance.
Liveweight change was analysed in a repeated measures analysis with the model including terms for treatment, liveweight at day 1, and the interaction. Treatment contrasts were performed comparing the growth rates between the regular ACTH immunogen (ACTH:OA) with control animals and with other ACTH immunogens (groups 2,3,5,6).

2.5 Immunogens

The hapten ratio to the protein carrier molecule was either 2:1 or 1:1(w:w) as well as a change of carrier molecules. Haptens were conjugated to either human serum albumin or

ovalbumin using the carbodiimide chemistry (Goodfriend et al.,1964). The ACTH peptide was de-acetylated prior to conjugation and injected into groups 2 and 4 to determine the influence of this procedure on the effectiveness of the conjugate. Primary immunogens were prepared using Freund's complete adjuvant and booster immunogens with Freund's incomplete adjuvant. The antigen (0.5mg per injection) was dissolved in saline (1ml) and emulsified with same volume of Freund's adjuvant. Vaccines were administered intramuscularly (1ml in each hind leg).

2.6 Assays

2.6.1 ACTH Antibody Titre

The ELISA technique was adopted for this assay to assess the antibody titres for the various ACTH fragments used in the experiment. The titres are specific to either ACTH1-24 or the terminal end of the molecule ACTH22-39. Refer to details in chapter 3.

2.6.2 Plasma Cortisol

These were determined using RIA, as previously discussed.

2.7 Carcase Analysis

Animals were slaughtered at random within a batch of the six treatments to avoid any bias. The procedures were the same as in previous experiments. Measurements for hot carcase weight, kidney fat and omental fat were taken on the hot carcase. The following morning carcases were removed from the chiller (4°C) and the ultimate pH recorded in M.longissimus, M.supraspinatus and M.semimembranosus with a pH meter. Subcutaneous fat depth at the 12th rib, eye muscle area and GR measurements were all measured on the cold carcase.

3. Results

3.1 Growth Rates

Lambs commenced the experiment at a mean liveweight of 18.8 ± 1.1kg with growth rates of 150 g/d at pasture prior to weaning. The transfer of animals to the feedlot and weaning depressed growth rates markedly for the next two weekly periods. Within 14 days of moving into the feedlot the lambs were fully accustomed to the ration and changed environmental conditions resulting in growth figures in excess of 300g/d for 17 days which subsequently fell to a consistent 200g/d for the remainder of the study. On days 75,93,134 and 153 there was a significant interaction ($P<0.05$) between initial liveweight and treatments.

Table 5.1 Repeated measures analysis for growth rate (g/day) for control lambs and those immunized against various ACTH fragments. Contrasts were performed on the control animals and the ACTH immunogen used in experiment 1 and subsequent contrasts performed with this immunogen and the various ACTH fragments.

Independent Variable	Whole plot analysis		Least Squares differences	Repeated measures analysis	
	df	F ratio		df	F ratio
Constant	-	-	196.0	8,81	71.04***
Treatment Contrasts					
Control v ACTH:OA	1,38	1.61	8	8,81	0.82
ACTH:OA v ACTH:HSA (hcl)	1,38	0.11	-2	8,81	1.16
ACTH:OA v ACTH:HSA (oAc)	1,38	0.57	5	8,81	0.95
ACTH:OA v ACTH terminal fragment	1,38	2.62	-11	8,81	1.50
ACTH:OA v Combination of fragments	1,38	3.07	-11	8,81	0.50
Initial Wt.#	1,88	5.84*	-1.7	8,81	2.16

* $P<0.05$

Animals adjusted to an initial weight of 18.8kg.

Figure 5.1 (Cont.)
Predicted means for the six treatme ıts on mean growth rate (g/day) over the period of the experiment.

Treatment	Grow th rate g/ lay
Control	1ᵕ 5.3
ACTH:HSA (HCl)	1ᵋ 5.4
ACTH:HSA (oAc)	1ᵕ 8.0
ACTH:OA	1ᵕ 2.2
ACTH Terminal fragment (22-39)	1ᶜ 3.9
Combination of ACTH fragments	1ᶜ 5.0
Average Std. Err.	(7.3)

Initial weight (18.8kg)

Table 5.2 Liveweight and carcase traits for lambs immunized against various ACTH immunogens and β-cell trophin after adjustment for the appropriate covariate. Data was analysed using a log transformed model and predicted means calculated by taking the antilog.

	TREATMENT							
	1 Control	2	3	4	5	6	Std. error	Reg. Coeff.
Liveweight (kg)	38.9	40.8	39.9	39.4	42.0	41.9	(0.9)	25.5
Hot caracse Weight.(kg)	18.5	19.3	18.7	18.5	19.0	18.6	(0.02)	1.27
Carcase Ratio HCW:LWT	.477	.471	.467	.477	.467	.459	(.005)	-0.664
Eye Muscle # Area (sq.cm)	12.7	12.6	12.9	12.5	12.4	12.2	(.01)	0.25
Kidney Fat (gm) #								
12.0kg Carcase Wt.	276	289	90	85	305	190	(.03)	.03
28.0kg Carcase Wt.	889	808	1500	1424	691	1044	(.03)	.03
Omental Fat (gm) #	802	742	687	665	792	781	(.03)	.69
Fat Depth #								
12/13 rib(mm)	4.7	4.6	3.8	4.1	4.6	4.3	(.03)	-0.188
G.R. (mm)	15.3	14.2	11.9	13.7	14.9	14.0	(.03)	-0.68

Group means adjusted to the same hot carcase weight.

Standar errors have been presented in the transformed mode.

Treatments numbered as per the protocol.

Table 5.3 Predicted means for muscle pH for the three sets of muscles measured. Values represent ultimate pH obtained from carcases 24h post slaughter. Data was transformed and is presented as the antilog for each treatment group.

	MUSCLE		
Treatment	M.semimembranosus	M.longissimus	M.supraspinatus
Gp.1	5.94	5.97	6.06
Gp.2	5.95	5.93	6.00
Gp.3	5.91	5.93	6.05
Gp.4	5.93	5.91	6.01
Gp.5	5.91	5.89	6.02
Gp.6	5.93	5.92	6.00
Std. Err. #	(.001)	(.001)	(.001)

Standard errors are presented in log transformation.

LEGEND
Gp.1 =Control
Gp.2 =ACTH 1-24(HCl):HSA
Gp.3 =ACTH 1-24(oAc):HSA
Gp.4 =ACTH 1-24(HCl):OA
Gp.5 =ACTH 22-39:HSA
Gp.6 =ACTH 1-24:HSA + ACTH 22-39:HSA

Figure 5.1 Mean liveweight of XB lambs adjusted for initial weight. Treatments are numbered (1=Control; 2=ACTH 1-24HCl:HSA; 3=ACTH 1-24oAc:HSA; 4=ACTH 1-24HCl:OA; 5=ACTH 22-39:HSA; 6=ACTH 1-24 & ACTH 22-39). Arrows denote time of vaccination.

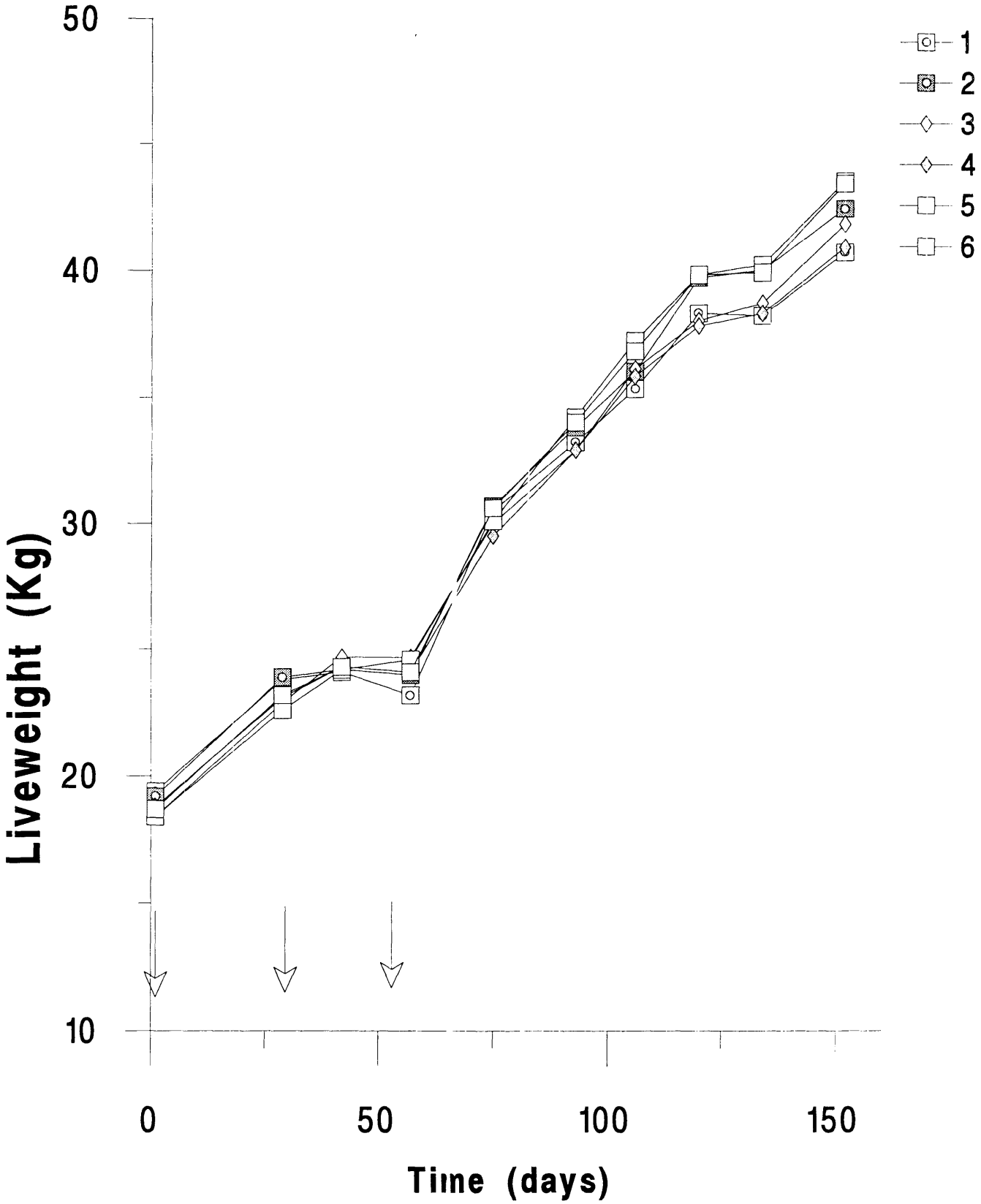


Figure 5.2 Growth rates of lambs immunized against various fragments of ACTH. Treatments are numbered as per the legend on figure 5.1

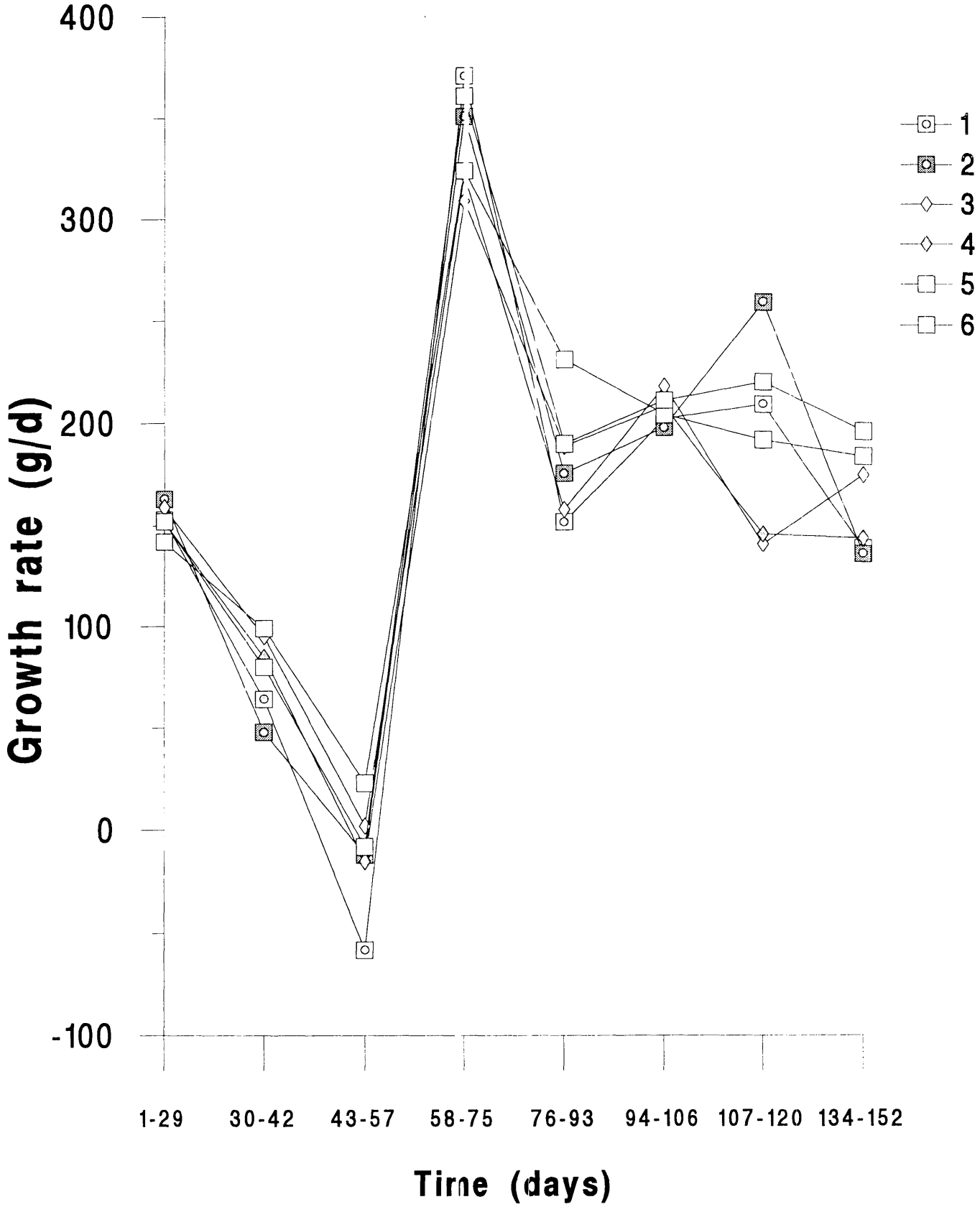


Figure 5.3 ACTH antibody titre for ewe lambs immunized against various ACTH related immunogens. Treatments are numbered as per legend in figure 5.1

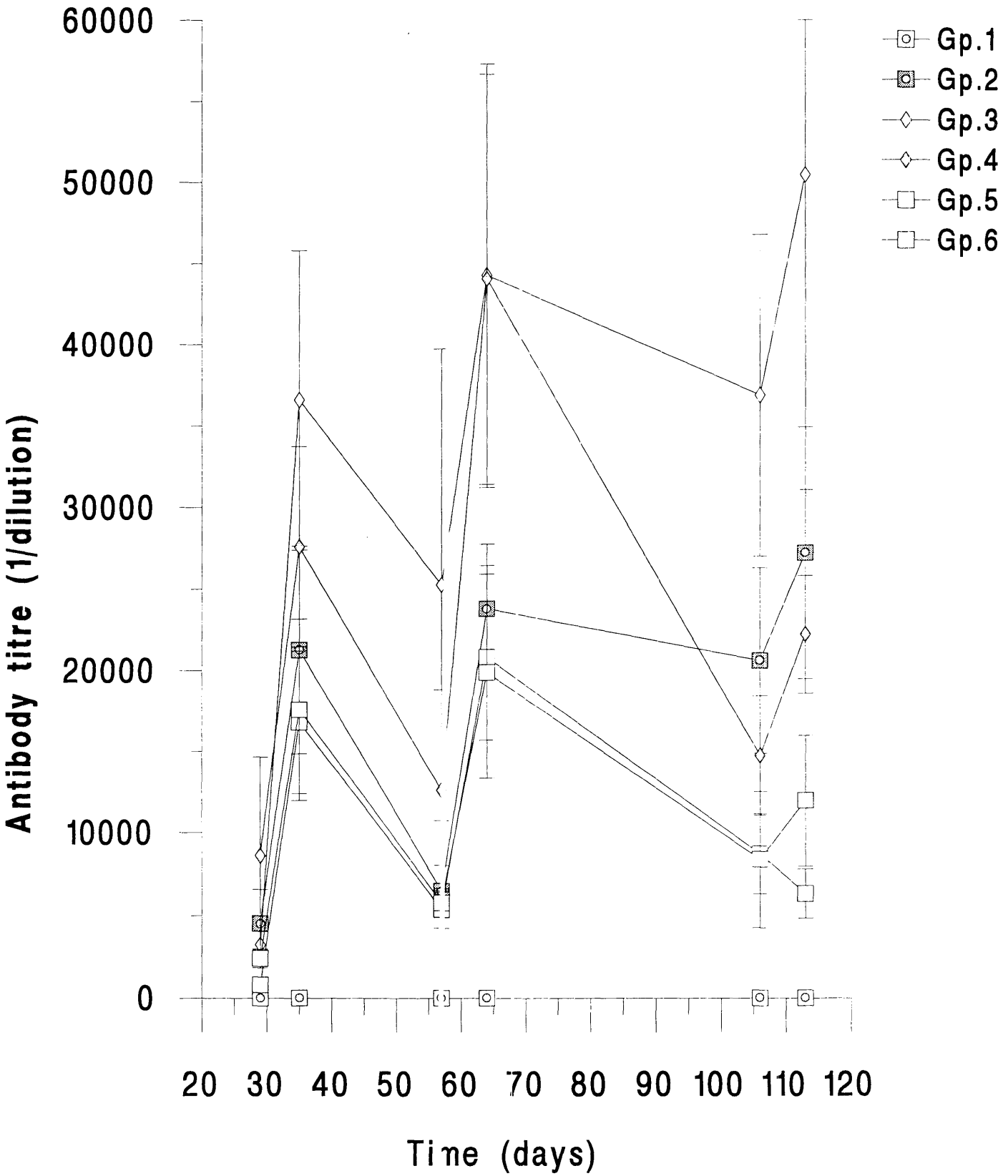
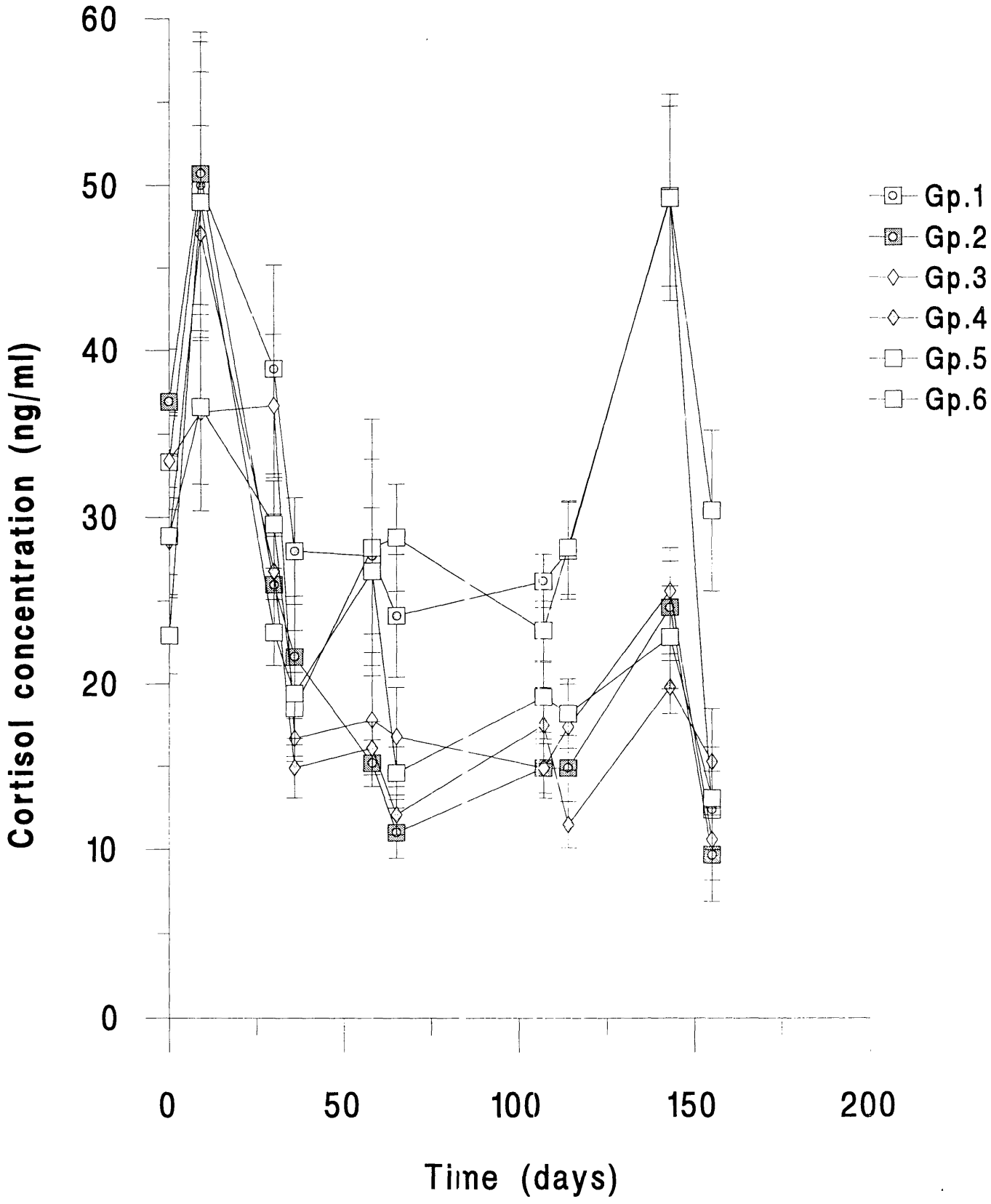


Figure 5.4 Plasma cortisol levels from lambs immunized against various ACTH related immunogens. Treatments are numbered as per the legend on figure 5.1



The repeated measures analysis on growth rates of the lambs (liveweight change g/d) showed a significant effect of initial liveweight on subsequent growth during the experiment ($P<0.05$). Growth rates were not affected by any of the treatments. The differences in growth rates between the groups, although not significant, may have been cumulative in their effects on the final liveweight of the various treatment groups of lambs. Groups 5 and 6 were the heaviest of all the lambs with mean liveweights of 42.0 ± 0.9 and 41.9 ± 0.9kg respectively. These final weights (Table 5.2) include the animals fleece weight post-shearing in calculation of growth rates for the entire experiment.

3.2 Plasma Cortisol

The cortisol levels reflect the efficacy of the anti-ACTH titres, with all groups immunized against ACTH 1-24 having lower cortisol. Circulating cortisol levels were higher in both the non-immunized controls and the ACTH 22-39 immune group, with the difference being more pronounced in the second half of the study. The combined ACTH 1-24 and 22-39 immunogen group had lower cortisol levels than the single ACTH 22-39 immunogen (Figure 5.4), indicating that antibodies directed to the ACTH molecule had a greater efficiency than the specific immunoglobulin to the 24 amino acid sequence peptide and was more effective at neutralizing ACTH bioactivity.

3.3 ACTH Antibody Titre

All of the antigens produced anti-ACTH antibody titres in the animals treated. The level of the immune response to the immunogens was successfully maintained for the duration of the experiment (Figure 5.3). There was very little difference in the titres amongst the ACTH1-24 immunogen vaccinated lambs throughout the experiment, with no decline in group 4 following the second boost injection as observed in the earlier experiment (see section 3). ACTH antibody titres for the animals immunized against the terminal sequence of the ACTH molecule were lower than the other immune groups from day 65.

3.4 Carcase Analysis

The various immunization treatments had no effect on hot carcase weight, which is reflected in the very similar carcase ratios among the groups.

There were no treatment effects on the internal fat depots with the exception of a significant effect ($P<0.05$) on kidney fat with a positive interaction between treatment and hot carcase weight. It would appear that this fat depot developed at a rate different to that of the increase in carcase weight.

Subcutaneous fat thickness was not affected by the treatments but the other measure of carcase fatness (GR measurement) was influenced significantly ($P<0.05$) by treatment when corrected for carcase weight. Similarly the treatments were significantly influenced for this variable when corrected for fatness. Overall, for groups 3 and 4 (ACTH1-24(oAc): HSA (1:1) and ACTH1-24(HCl):OA (1:2)) there was a decrease in fatness per se, including subcutaneous fat thickness and GR measurement and the internal depots of omental and kidney and channel adipose tissue weights.

There was no difference in skeletal muscle mass between all groups when assessed by the measurement of eye muscle area, although there were slight differences between treatment groups (Table 5.2).

Ultimate pH of three muscle groups did not vary between treatments but did differ significantly between muscle groups ($P<0.001$). pH was highest in M.supraspinatus with the other two muscles markedly lower but not different from each other.

4. Conclusions

Despite the success of this experiment in maintaining reasonably high antibody titres for the duration of the experiment and subsequently lowering blood cortisol, statistically significant increases in growth rates were not achieved. Unlike the report of Sillence et al. (1992) in rats, ACTH immunization failed to alter the physiological responsiveness of target tissues in response to the suppression of circulating glucocorticoid levels. Wehrenberg et al. (1983) and Wehrenberg et al.(1990) demonstrated the potential suppression of growth hormone with glucocorticoids through their interaction with growth hormone releasing hormone and somatostatin in rodents. However these reports have to be interpreted with caution, since the potent analogue, dexamethasone was used in the study and hence may not provide physiological responses. The endocrine milieu controlling growth is extremely complex and it would seem that the pertubation of only one hormone within the system may be compensated for by other hormones. We do not know how the ACTH antibody complex may interact at the level of the receptor, however if active immunoneutralizat on is effective then it is likely that both receptor affinity and number may increase as the cells attempt to bypass the immunological blockade. Similarly, signal transduction pathways for ACTH may be sensitized and it is possible that the activity of hormones with related function may be up-regulated. Although immunological interaction has proved successful in manipulating the reproductive system in both sheep and cattle (Wilson et al.,1992;Hoskinson et al.,1990), the manipulation of the HPA axes appears not to provide a repeatable increase in the efficiency of animal productivity. In the longer term this technique does not seem to offer

the opportunity to manipulate growth immunologically. Lowering plasma cortisol by immunizing experimental lambs can be achieved as shown in these studies although biological responses assessed in this experiment such as fat deposition and distribution, enhanced growth rates and pH were not altered by the immunization procedure. Most of the research work in this area has been conducted in monogastric species with little information being available from scientific literature on ruminant studies. The capability of sheep and cattle to adjust and adapt to changes in environmental conditions and other stimuli may result in less extreme shifts in the responsiveness of the HPA axis to altered conditions when compared with other species. This may be due to the fact that the rumen "buffers" the animal against marked swings in plasma glucose levels. Therefore it is possible that that the HPA axis is less sensitive to such stressors, however I suspect that the major reason for a lack of a response was that animals were too well fed. Animals did not perceive the need to re-partition nutrients to the essential tissues. It is likely that glucocorticoids were not promoting peripheral insulin resistance and therefore the immunization was ineffective.

Cortisol levels in groups 3 and 4 were lowered by immunization between days 50 and 160 and this was associated with a decrease in body fat per se including both carcase and non-carcase fat components (15% lower for subcutaneous and GR). However these differences were not significantly different from other groups. Group 2 also exhibited low cortisol levels, but there was not the same trend in lower body fat in this treatment. Despite being able to lower blood cortisol experimentally from day 50 to day 160, there was no effect on body fat per se or in the distribution of adipose tissue around the body as is observed commonly in Cushings syndrome patients. Other studies (Clegg and Spurlock,1960;Carroll et al.,1963;Spurlock and Clegg,1962) observed significant increases in carcase fatness in both sheep and cattle treated with cortisone acetate and support the synergy between glucocorticoids and insulin observed by Vernon and Taylor (1988). The findings from the present studies do not support the hypothesis that the reverse may be achieved by lowering glucocorticoids. The administration of exogenous cortisone would obviously have a negative feedback effect on the hypothalamus and pituitary and suppress not only CRH and ACTH secretion but other POMC derived peptides as well. These hormones may have an impact in promoting adiposity.

Once again, immunization was not capable of modifying the ultimate muscle pH. It would appear that there is very little interaction of ACTH or glucocorticoids on adrenal catecholamines which are known to deplete muscle glycogen. May be the stress regime imposed on these animals was not sufficient to increase ultimate pH in the first place considering their high nutritional status. High cortisol levels per se in animals prior to

slaughter may have little influence on the expression of dark cutting meat in carcases. However, they may have a permissive role by modulating ß adrenergic receptor variables and by promoting glycogenolysis through the preferential partitioning of glucose to the brain, heart and other essential tissues. This experiment supports the findings of the first experiment with differences between muscle groups for pH values, which are in accordance with those findings of Devine and Chrystall (1989). There was no difference between the M.semimembranosus and the M.longissimus which are primarily muscles of support and posture, whereas the ultimate pH in the M.supraspinatus was significantly higher than in the former two muscles This latter muscle in the shoulder is responsible for the locomotion of the animal and would be expected to have a greater ratio of fast-twitch to slow-twitch muscle fibres. The greatest loss of glycogen is suffered by the complement of fast twitch oxidative and glycolytic fibres in muscle. Exercise may have lowered muscle glycogen in this muscle as more fast twitch fibres were recruited for movement, or glycogen reserves are generally more depleted in this muscle group.
It would appear that the influence of the HPA axis is more responsive to the grazing animal where there are fluctuations in nutrient quality and quantity, in order to maintain blood glucose levels for essential organs. In this study the lambs were fed a high quality ration ad libitum and despite the imposition of chronic psychosocial stress in order to activate the HPA axis, the animals had sufficient energy substrate present in the blood stream to maintain normal function without partitioning nutrients to essential tissues. In contrast when animals are grazing, they are generally maintained at a lower nutritional status and therefore when stress is applied, the HPA axis is sensitized resulting in the mobilisation of muscle tissue and glycogen.
The chronic stress imposed on these animals by changed environmental conditions, moderate crowding and changing hierarchical status may have been negated by elevated ß-endorphin levels in immunized animals (Wynn et al.,1995). The increase in this pituitary peptide is the result of decreased feedback of circulating adrenocortical steroids to the hypothalamus leading to an increase in the expression of the precursor molecule for ACTH (proopiomelanocortin). The reason for the difference in responsiveness of monogastrics and ruminants may be related to the difference in the nature of glucose metabolism (Vander et al.,1980;Van Houtert.1991). Ruminants do not absorb glucose from the gastro-intestinal tract and as mentioned earlier, they would appear not to suffer from marked variations in circulating concentrations of this essential energy source to the brain, heart and other neural tissue as compared to non-ruminants. Therefore the glucocorticoid sensitive glucose regulators are not as sensitive as those in monogastrics in which variations in dietary glucose intake can cause marked shifts in plasma glucose

levels. Thus it is likely that monogastr cs are more likely to respond to the ACTH immunization procedure.

The partitioning of fat within the body has been shown to be under endocrine control as the changes in the endocrine milieu re:ulting from altered physiological state such as pregnancy and lactation occur and are also governed by substrate availability. In addition, variations between early and late maturing breeds of animals, differences between male and female animals and the variance b:tween dairy and meat animals in musculature and fat partitioning are genetically control ed. The aspects responsible for the difference in genetic expression appear to be buffer :d against minor changes in any one factor and this is not surprising given the complex hc meostatic mechanisms that have developed to ensure the survival of the species.

For this technique to be effective in manipulating fat partitioning, it may require the immunization against several hormon:s rather than targetting just one or two regulatory hormones. Although this may sound plausible in theory, the results achieved with a second immunogen in chapter 4 are not encouraging as the two immunogens did not give an additive effect. On the other hand the studies of Wilson et al. (1992) to multiple steroid immunogens do provide hope for the future in this field of immunobiology.

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