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Kiyma, Zekeriya, Feed Efficiency and Carcass Traits of Ram Lambs Actively Immunized Against GnRH, M.S., Department of Animal Science, December, 1998.

It was hypothesized that ram lambs immunized against GnRH possess the desirable production and carcass traits of intact ram lambs. Intact male lambs received no treatment (R), were immunized with GnRH linked to keyhole limpet hemocyanin in either Freund's complete adjuvant (FCA) or ISA adjuvant (ISA), or were castrated (C). Anti-GnRH titers were higher in FCA immunized rams than ISA rams. Testicular weights, function and sexual behaviors were reduced by immunization. Days on feed were greater in C lambs than in R lambs, with FCA and ISA lambs intermediate. Average daily gains were greater in R than C, FCA and ISA lambs. Feed efficiencies for R lambs were better than in C, ISA and FCA lambs, but did not differ among C, FCA or ISA lambs ($p>.05$). Ribeye area, lean and bone maturity, overall quality, muscling score, flank streaking and color of fat did not differ among groups. Intact males, FCA, and ISA lambs had more ($p<.05$) desirable yield grades, less ($p<.05$) back fat, and less ($p<.05$) marbling than C lambs. Body wall thickness for R, ISA and FCA lambs was less ($p<.05$) than C lambs, and for FCA were greater ($P<.05$) than R lambs.

In summary, immunization against GnRH decreased testicular weight and reduced ($p<.05$) live animal performance and sexual behaviors to values comparable to those of castrated males. Partitioning of nutrients for growth and deposition of fat appears to differ among immunologically castrated and mechanically castrated lambs probably due to residual testicular activity.

FEED EFFICIENCY AND CARCASS TRAITS OF RAM LAMBS ACTIVELY IMMMUNIZED AGAINST GNRH

by

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A thesis submitted to the Department of Animal Science and The Graduate School of the University of Wyoming in partial fulfillment of the requirements for the Degree of Master of Science in Reproductive Biology

University of Wyoming

Laramie, Wyoming

December, 1998

ACKNOWLEDGMENTS

I would like to take this opportunity to offer my sincerest appreciation for all those who have helped me in this study. None of this would be possible without the guidance of Dr. Gary Moss who was always there to provide advice in times of need. In addition, I would like to recognize Ed Van Kirk whose expertise was indispensible. My thanks also go out to Dr. Melvin L. Riley, Dr. Bret W. Hess, Dr. Thomas E. Adams, Dr. William J. Murdoch, and finally Dr. Robert W. Atherton who provided invaluable assistance in conducting this research. In the course of accomplishing my Master's degree, I have made many friends. I would like to express my appreciation for their kindness and consideration shown during my course of studies. Most of all, I give thanks to Allah, the Most Merciful and Most Beneficent, the One who makes the impossible possible.

The last but not least, my wife Esma, the inspiration behind all my efforts and endeavors.

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I. INTRODUCTION

Male cattle, sheep and swine have long been castrated for meat production in the United States (Seideman et al., 1982). The main reasons for castration are to improve meat quality and reduce aggressive male behaviors. Keeping intact males isolated from females to eliminate unwanted pregnancies is a management problem. Castration, however, requires additional labor, increases stress to the animals and results in castrated males which utilize feed less efficiently and have lower rates of gain than intact males (Field et al., 1971: Seideman et al., 1982). Intact rams (Purchas, 1978) and bulls (Landon et al, 1978) produce higher yielding carcasses with less fat and more red meat, but poorer quality grades than castrated males. Undesirable meat colors, less desirable quality grades, lower palatability, market biases, and poor consumer acceptance at the retail level are the other important reasons for red-meat production from castrated farm animals. (Seideman et al., 1982).

In a variety of mammalian species, active immunization against gonadotropin releasing hormone (GnRH) suppresses secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). When gonadotropins are suppressed, steroid hormone production declines along with the primary and secondary actions of these steroid hormones. Immunization against GnRH results in atrophy of gonadal tissue, inhibition of gametogenesis, and decreased reproductive behavior (Fraser and Baker, 1978; Schanbacher et al., 1983; Falvo et al., 1986). Therefore, it was proposed that immunization against GnRH in male lambs may be a viable alternative to mechanical or surgical castration provided the desirable production and carcass traits of intact ram lambs are attained.

Immunization against GnRH would be expected to arrest secretion of LH and FSH from the pituitary gland and block gonadal functions (D'Occihio, 1993). Initial studies which examined effects of immunization against GnRH were conducted in laboratory animals and verified that reproductive functions were suppressed (Fraser and Gunn, 1973). Furthermore, as a result of blocked gonadal functions, desirable production and carcass quality could be achieved. In cattle and sheep, immunization against GnRH appeared to diminish or abolish the negative effects of feeding intact males (Adams et al., 1995;1996). The current study examined in more detail the effects of a GnRH-keyhole limpet hemocyanin (KLH) conjugated protein in two different adjuvants (Freund's complete adjuvant and ISA) on feedlot performance, carcass traits and reproductive development and behavior in ram lambs fed to a constant end point (weight).

II. REVIEW OF THE LITERATURE

Reproductive Endocrinology

Hpothalamic-Pituitary-Gonadal Axis.

The hypothalamic decapeptide, gonadotropin releasing hormone (GnRH), plays a primary role in mammalian reproduction. The existence of GnRH was predicted in the 1950s and the structure of this 10 amino acid peptide was reported in the 1970s. Similar to many other neuropeptides, GnRH is produced in the brain and may function as a neurotransmitter, neuromodulator or local hormone (Sherwood et al., 1993). In rats, GnRH cell bodies in the septal-preoptic-hypothalamic region send 50-70 % of their axons to the median eminence and the remainder to different parts of the brain (Silverman et al., 1987). Once released, GnRH enters the hypophyseal-portal vessels and is transported to the anterior pituitary gland where it binds to specific cell surface receptors primarily

located on the gonadotrophs (Marian and Conn, 1983). Similar receptors have also been reported to be present in gonadal tissue (Clayton, 1980), the human placenta (Washita et al., 1986) and in several tumors and neoplastic cell lines (Pahwa et al., 1989). When released at nerve terminals in the median eminence, GnRH traverses the hypothalamo-hypophyseal portal system to the anterior pituitary gland and binds to specific receptors on gonadotropic cells and causes release of the gonadotropins (Vickery et al., 1987). The GnRH-receptor (GnRH-R) complex is then internalized by endocytosis resulting in a decrease in the number of available receptors. Occupancy of GnRH-R causes a prompt elevation of cytoplasmic calcium concentrations and concomitant activation of protein kinase C. These changes are initiated by activation of phospholipase C and cleavage of IP3 (inositol triphosphate; Morgan et al., 1987). Gonadotropin release is biphasic with an initial peak that occurs in the first minute, and a second more sustained phase (Turgeon, 1986).

Following binding to gonadotrophic cells of anterior pituitary gland, GnRH regulates the synthesis and release of the gonadotropins, LH and FSH, which regulate testicular and ovarian steroid hormone and gamete production (Hamernik and Nett, 1988). Gonadal steroid hormones have various functions such as, maintenance of secondary sex characteristics and accessory sex tissues, metabolic effects, stimulation of brain centers associated with sexual and aggressive behavior, and feedback regulation of GnRH, LH and FSH.

Structure and Functions of GnRH

Synonyms for GnRH are LHRH, LHRH/FSHRH, gonadoliberin and luliberin. GnRH, which is cleaved from a pro-hormone during post-translational processing, is a

neurohormone containing the amino-acid sequence of pyro-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2. The first and last amino acids (N and C termini) of GnRH recognize binding regions on the receptor while the first three amino acids of GnRH are involved in release of LH and FSH from the pituitary (Bouchard et al., 1992; Sherwood et al., 1993).

As its name implies, the most widely recognized action of GnRH is on the anterior pituitary gland (Arimura et al., 1972; Schally et al., 1972). The importance of GnRH to reproductive function is illustrated by the fact that anestrus induced by transection of the infundibular stalk can be partially reversed by pulsatile delivery of exogenous GnRH (Awotwi et al., 1984). The cellular actions of GnRH are numerous, complex, and should be explained in terms of the time of their onset after cellular activation. After gonadotrophs are activated by GnRH, onset of LH release takes seconds to minutes, onset of GnRH receptor synthesis and LH biosynthesis takes hours to days, and an increase in gonadotroph size and secretory apparatus number takes days to weeks (Clayton, 1989). Also, GnRH increases the synthesis of FSH and the glycosylation of both gonadotropins. There is little evidence for short loop feedback regulation between GnRH and its own synthesis and (or) storage in the hypothalamus (Rabb et al., 1990). In the same study, LH secretion was more dependent on normal bioactive GnRH activity than was FSH. Plasma concentrations of LH and FSH in ponies immunized against GnRH decreased by 86 and 59%, respectively. Active immunization of stallions (Schanbacher and Pratt,1985) and mares to GnRH (Garza et al., 1986; 1988) reduced LH secretion by 90% or more whereas FSH was reduced only 60%. These data have been interpreted to suggest that two different cell types produce FSH, one being dependent on

GnRH input and the other being relatively independent. GnRH was shown to be equally effective in releasing both LH and FSH in vitro. However, different levels of LH and FSH released in response to GnRH appeared to be due to feedback actions of gonadal steroids. Gonadal regulatory hormones such as inhibin or activin also may have a role in the differential release of LH and FSH (Vickery et al., 1987). GnRH pulse frequency influences the release of LH and FSH. The release of FSH is favored at high pulse frequencies and LH secretion is optimized at lower GnRH pulse rates (Moenter et al., 1991). Gonadotroph desensitization was induced by continuous infusion of GnRH or super-agonist (Clayton, 1989). This reduction in activity was partially caused by occupancy of receptors by exogenous GnRH and receptor loss or down-regulation (Clayton, 1982). Hazum et al. (1988) also reported that GnRH regulates number of its own receptors (up and down regulation) in vivo and in vitro.

GnRH Pulse Generator

GnRH neurons spontaneously depolarize and release GnRH. There are relatively few GnRH neurons. Significant numbers of GnRH soma are located in the medial-basal hypothalamus in some species. The cell bodies of these neurons, which are located mainly in the septal-preoptic-hypothalamic region in rats and domestic animals, send 50-70 % of their axons to the median eminence. The remaining axons are widely distributed in the brain (Silverman et al., 1987). Pulsatile secretion of GnRH is regulated by a pulse generator located in the preoptic area of the hypothalamus. In rhesus monkeys electrical lesion of the hypothalamus abolished gonadotropin release and, infusion of GnRH once every hour, but not constant infusion, restored gonadotropin release (Belchets et al.,

1978). Lesion of the arcute nucleus also abolished the pulsatile gonadotropin secretion in rats and monkeys (Blake and Sawyer, 1974).

Effects of Testosterone on Muscle Growth

It was hypothesized that high circulatory concentrations of testosterone in male farm animals results in a larger mature size and more muscling, especially in the neck and forequarter, than in females (Jost and Magre, 1984). Lohse (1973) reported that growth rate of neck muscles in male cattle and sheep was higher relative to total muscle growth. Combined muscle weights from intact rams and wethers exposed to testosterone were larger than those from wethers. Field et al. (1989) and Arnold et al. (1997) reported that neck muscle growth (i.e. splenius muscle) was increased by treatment with testosterone in rams.

Immuno-Castration Studies

Studies, which examined the effects of active immunization against reproductive hormones have been conducted for more than two decades. Such immunization studies have suggested new approaches to control fertility and enhance production in domestic animals. Antibodies specific for a unique hormone in the circulation should bind that hormone and blocks its biologic actions (D'Occihio, 1993).

GnRH and steroid hormones need to be conjugated to an antigenic carrier protein (i.e. KLH) to get an effective response to the immunization (Ladd et al., 1990). It was suggested that immunization against GnRH-protein conjugates can produce sequence specific or confirmation specific anti-sera for GnRH. A modified GnRH analog-protein conjugate was immunologically recognized with greater affinity than the unmodified GnRH molecule conjugated to a carrier protein (Nett et al.,1973; Copeland et al., 1979).

Adams and Adams (1986) proposed that immunization of ewes against GnRH linked to KLH generated anti-sera that recognized both amino acid sequence and three-dimensional confirmation.

Antibodies created by active immunization against a GnRH-KLH conjugate established a selective immunological barrier between the hypothalamus and anterior pituitary gland which blocked the biological actions of GnRH (Adams and Adams, 1986). This approach was used in laboratory animals (Fraser and Gunn, 1973; Fraser and Baker,1978) and livestock (Robertson et al., 1979; Jeffocate et al, 1982). Immunization against GnRH-KLH resulted in a significant decline in pituitary stores of LH and FSH, but did not affect prolactin stores in the pituitary gland of ewes (Adams and Adams, 1986). In order to study the physiological effects of the isolated anterior pituitary gland, physical methods of separating the pituitary gland from the hypothalamus have been used in rodents (Norman et al., 1982) and domestic animals (Awotwi et al., 1984). These methods (i.e. pituitary stalk disconnection or hypothalamic lesions), however, affect all interactions between the hypothalamus and pituitary gland, and therefore, could induce effects which differ from immunization (Clark et al., 1983).

Responses to Active Immunization Against GnRH at Different Ages

In ram lambs, a single administration of GnRH-KLH conjugate was sufficient to produce a high titers of GnRH antibodies at week 8. These titers were maximal at week 16 and resulted in reduced serum concentrations of testosterone, suppressed scrotal circumferences and testicular weights (Daley et al., 1995). Age seemed to influence the response to active immunization. In bulls, the percentage of animals with a robust immune response to immunization with GnRH were larger in bulls immunized at 4, 8 and

12 months of age than at 1.5 months of age (80, 100, 100, and 53%, respectively). However, bulls immunized at 7 months of age had higher final anti-GnRH titers than bulls immunized at 1.5, 4 and 12 months of age. In bull calves, immunocastration at 7 months of age or at weaning was suggested. Attenuated immune responses at a younger age (1.5 month) was indicative of an immature immune system (Adams et al., 1996). Immunization of bull calves at 4 months of age suppressed testicular growth and functions, but not at 12 months of age (Adams and Adams, 1992; Adams et al., 1993). Immunization twice at 6 and 10 months of age did not delay puberty in heifers. However, when heifers were immunized three times at 6, 10 and 16 months, puberty (sexual maturity) was delayed. In both groups, LH was suppressed after a second immunization at 10 months. Most immunization regimens for cattle require primary immunization and one or more secondary immunizations (Jeffcoate et al., 1982; Robertson et al., 1982; Johnson et al., 1988). However, a single immunization at 3 or 7 months of age resulted in anti-GnRH titers that remained at maximal for 10 to 12 months. Therefore, a single handling of cattle could be possible in an immuno-castration protocol performed before or at weaning (Adams et al., 1996).

Gonadotropin Levels, Gonadal Development, Steroidogenesis, Gametogenesis

The growth and development of testicular tissue is dependent on the episodic release of GnRH. Administration of anti-GnRH antibodies in serum blocked episodic LH secretion in rams (Lincoln and Fraser, 1979). Active immunization against GnRH decreased serum LH and FSH in male adult rats (Awoniyi et al., 1989;1992) and in ram lambs. In addition, testosterone concentrations were decreased to castrate levels (Schanbacher, 1982). A single immunization with a GnRH-KLH conjugate suppressed

testicular growth and development in young ram lambs (Daley et al., 1995). Bull calves immunized against GnRH had reduced testicular volume, serum testosterone and semen production. In these animals, the effects of immunization lasted about six months and, as antibody titer decreased, testicular volume, testosterone levels and libido recovered (Robertson et al., 1982). Immuno-neutralization of GnRH also reduced testicular growth and secretion of testosterone in rats (Shiota et al., 1981). Adams and Adams (1992) reported that immunization against GnRH-KLH reduced testicular development, however, serum LH and testosterone levels as well as feedlot performance were not affected compared to animals immunized against the carrier protein, KLH.

Active immunization of ten marmoset monkeys against GnRH-bovine serum albumen (BSA) resulted in antibody production. Although, all animals did not respond to the immunization to the same degree, testicular atrophy and decreased testosterone levels were observed in monkeys with high antibody titers (Hodges and Hearn, 1977). Male and female rabbits immunized against GnRH-BSA had slightly decreased testis size after 8 weeks and notable testicular atrophy after 16 weeks. Degeneration of seminiferous tubules and complete absence of spermatogenesis were observed by histological examination. Ovulation in rats was inhibited after treatment with antisera obtained from the immunized female rabbits (Fraser and Gunn, 1973).

Falvo et al., (1986) immunized boars against GnRH and LH. In these animals, testis and accessory sex gland weights and concentrations of LH and testosterone were reduced. Immunized gilts also had decreased serum concentrations of gonadotropins and gonadal steroids (Esbenshade and Britt, 1985). Rams immunized against testosterone had elevated serum concentrations of LH and testosterone due to lack of negative feedback on

the gonadotrophs (Schanbacher, 1982). Bulls immunized against testosterone exhibited increased concentrations of LH, testis size and sperm production (Walker et al., 1984).

Active immunization against GnRH caused cessation of estrous cycles in rats (Fraser, 1975), gilts (Essenshade and Britt, 1985), ewes (Clarkeet al., 1978), mares (Garza et al., 1986), and heifers (Johnson et al., 1988; Wetteman and Castree, 1994). After immunization, morphologically distinct follicles were not present on the ovaries of gilts (Traywick and Esbenshade, 1988). Infusion of analogs of GnRH into anestrus gilts and heifers immunized against GnRH stimulated release of LH and FSH (O'Connell et al., 1990). However, the treatment did not reinitiate estrous cycles. The later studies indicated that treatment with exogenous gonadotropins stimulate follicular growth, increase concentration of estradiol in plasma, and induce ovulation in anovulatory heifers. Treatment of rats with antiserum from rabbits immunized against GnRH-BSA inhibited ovulation and pre-ovulatory gonadotropin surges. Treatment with an inactive fragment of GnRH (3-10 octapeptide) caused the rats to ovulate. It was proposed that the antibodies were saturated by the exogenous GnRH fragments, and endogenous GnRH was able to reach and bind to the receptors on gonadotrophs.

Adams and Adams (1986) reported that immunization of ewes against GnRH decreased the content of LH and FSH in the pituitary gland and serum and resulted in cessation of estrous cycles. Estrus and fertility was suppressed in heifers immunized against GnRH-ovalbumin conjugate (Johnson et al., 1988; Wetteman and Castree 1994). Adams et al. (1990) also detected increased fat deposition in beef heifers that developed high antibody titers. Immunization of mares against GnRH resulted in cessation of estrous cycles and fewer follicles were detected (Garza et al., 1986).

Testes Functions

The relative contribution of FSH and testosterone to the control of spermatogenesis is not clear. In humans, FSH and testosterone seem to be required for normal spermatogenesis (Matsumoto et al., 1986). After gonadotropin deficiency, testosterone restored spermatogenesis induced by active immunization against GnRH in adult male rats (Caleb et al., 1992; McLachlan et al., 1994). In these studies, active immunization against GnRH resulted in nonmeasurable serum concentrations of FSH and LH without affecting other hormonal systems (Awoniyi et al., 1989:1992). Mclachlan et al. (1994) concluded that GnRH immunization procedures resulted in decreased serum concentrations of gonadotropins and severe regression of spermatogenesis in about 90% of adult male rats. At 12 weeks after active immunization against GnRH, histological examination of adult rat testicles showed a reduction of diameter of seminiferous tubule from 272 ± 6 μm to 129 ±12 μm and atrophic leydig cells. The same tissues contained no spermatogenetic cells beyond the round spermatid stage cells. In the same study, testosterone administration restored spermatogenesis. They also concluded that restoration of spermatogenesis might be due to increased serum testosterone concentrations and/or restoration of serum FSH concentration levels through stimulation of FSH secretion by GnRH-independent mechanisms.

Feedlot Performance in Immunocastrated and Physically Castrated Animals

Different studies indicated that immunization against GnRH may affect feedlot performance and carcass traits. Ram lambs immunized against GnRH conjugated to human serum albumen (HSA), lambs immunized against testosterone, and castrated lambs had slower growth rates and lower feed efficiency than intact ram lambs or lambs

immunized against human serum albumen (Schanbacher, 1982). Similar average daily gains for immunized and intact lambs were reported by Daley et al. (1995). Adams et al. (1993) concluded that sustained high rates of growth in GnRH immunized bulls could be caused by residual testosterone secretion. In this study serum testosterone concentrations in intact, immunized and castrated bulls were 3.4, 0.8 and 0.1 ng/ml, respectively. Adams and Adams, (1990) reported that active immunization against GnRH resulted in reduced weight gain of feedlot heifers to values comparable to spayed heifers. However, administration of anabolic steroid implants reversed the decreased feedlot performance in immunized animals. In another study, Adams and Adams (1992) reported that immunization against GnRH did not affect feedlot performance of intact bulls, but castration reduced average daily gain. The final live weights and gain during a feedlot trial using GnRH immunized bulls were intermediate between the control animals and steers (Adams et al., 1996). Immunization of prepubertal beef heifers against GnRH resulted in decreased average daily gains compared to control animals (Prendiville et al., 1995).

Intact rams and bulls utilize feed more efficiently than castrates (Field et al., 1971; Seideman et al., 1982). Deweese et al. (1969) and Jacobs (1970) reported that rams convert feed into live weight 12 to 15 % more efficiently than wethers and 13 % more than ewes (Orskov et al. 1974). Intact ram lambs and bulls had higher growth rates than castrates (Seideman et al., 1982; Andersen and Ingvartsen, 1984) and rams were heavier than wethers at a similar age and management conditions (Andersen et al., 1991). In Field's review (1971), unadjusted means of 13 cited references indicated that rams had an average daily gain of .23 kg and wethers had an average daily gain of .20 kg. At weaning,

ram lambs weighed 5% more than wethers. Yearling rams had 15 and 23% more weight when provided high and low planes of nutrition, respectively (Bradford and Spurlock, 1964).

Carcass Traits in Immunocastrated and Physically Castrated Animals

Immunization of lambs against GnRH and testosterone was reported to decrease back-fat thickness compared to castrates, but it was not statistically significant (Schanbacher, 1982). Daley et al., (1995) concluded that immunization against GnRH resulted in commercially acceptable lamb carcasses intermediate to castrated and intact lamb carcasses and suggested the immunization procedure as a non-invasive alternative to surgical castration in lamb production. In this study, back fat thicknesses of immunized (3.0 mm) lambs were intermediate between control (2.3 mm) and castrated (4.3 mm) lambs at 5 months of age. Dressing percentages of immunized lambs (52.4%) were higher than both intact (50.1%) and castrated (50.3%) lambs. Castration or immunization did not affect loin eye area. The masculinity of carcasses from bulls immunized against GnRH at seven months of age was comparable to the masculinity of steer carcasses and less than the masculinity of bulls (Adams et al., 1996). In this study, mechanical or immunological castration did not change dressing percentages, longissimus muscle areas, marbling scores, backfat thicknesses, internal abdominal fat and yield grades. However, steers had reduced longissimus muscle areas and increased internal fat compared to immunized and intact bulls. Some carcass traits of GnRH immunized bulls were intermediate between steers and intact bulls (Klastrup et al., 1984). Falvo et al., (1986) immunized boars against GnRH and LH. Boar taint was reduced in GnRH and LH immunized animals.

However, fat deposition was higher in the GnRH immunized group than castrates, intact boars, and boars immunized against LH.

In the United States, lamb carcasses are sold on a quality grade basis. Intact rams generally yield leaner carcasses and have higher yields of retail cuts than wethers (Wilson et al., 1970, 1972; Glimp, 1971; Schanbacher and Crouse, 1980). Ram lambs produced carcasses with lower fat percentages than wethers at all ages (Purchas, 1978). The rate of fat deposition in wethers was much higher at heavy weights (64 kg live weight) than in rams (Shelton and Carpenter, 1972). Unadjusted means of fat thicknesses from different studies were 5.2 mm and 6.9 mm for rams and castrates, respectively (Field et al., 1971). Andersen et al. (1991a) measured fat depth of rams and wethers at 5 months of age as 3 and 2.9 mm and at 12 months of age as 2 and 4.5 mm, respectively. Back fat thickness for rams and wethers were, respectively, similar at 5 (4.8 mm; 5.7mm) and 7 (7 mm; 8.4mm) months of age, but at 9 (5.5; 8.8mm) months of age rams were leaner than wethers. In the same study, body-wall thicknesses of rams were less than wethers at 9 (25.9 mm; 32.4mm) months of age, but not at 5 and 7 months (Andersen et al., 1991b). Backfat in intact and castrate rams was 1.3 and 4.3 mm at 9 months of age (Field et al., 1989). Crouse et al. (1981) also reported that carcasses from ram lambs were leaner than ewe or wether lamb carcasses. Wethers were likely to yield higher dressing percentages than castrates (Bradford and Spurlock, 1964; Carpenter et al., 1964; Field et al., 1967). Field et al. (1989) reported mean for dressing percentages for intact rams and castrates at 9 months of age as being 53.1 and 55.8 %, respectively. In Field's review (1971), unadjusted mean dressing percentages of rams and wethers from other studies were 49.6 % and 51.3 %, respectively.

Fat from castrates was firmer (2.0) and whiter (2.0) than in intact rams which had softer (3.2) and closer to yellow (2.6) fat at 9 months of age (1=white, 5=yellow; 1=extremely firm, 5=extremely soft). This was true at all ages evaluated (361, 459, 557, 652 days of age; Field et al., 1989). Other studies also reported softer and oily fat from ram carcasses compared to wether carcasses (Field 1971; Busboom et al., 1981; Anderson et al., 1991), a condition which makes these carcasses subject to price discounts (Seideman et al., 1982).

Carcass quality grades were better in castrates than intact rams at 271, 361, 459, 557 and 652 days of age. Carcass maturities were similar at 9 months of age (Field et al., 1989). Ram lambs have a higher proportion of neck and shoulder cuts than wethers at all ages (Field et al., 1989). This different distribution of carcass lean in rams and wethers was attributed to testicular androgen stimulation of muscle growth in the neck and shoulder regions of intact rams (Bradford and Spurlock, 1964; Jacops, 1970; Kemp et al., 1970). Hammond (1932) documented that muscles of rams contained the largest muscle fibers, while ewes had smallest and wethers intermediate-sized muscle fibers. Carcasses with noticeable neck and shoulder muscle hypertrophy are described as "bucky" and are assigned lower USDA quality grade.

Pelt removal from ram carcasses is more difficult than from wethers at both 5 and 12 months of age (Andersen et al., 1991a). This difference in force required for pelt removal among ram and wethers increased with age resulting in increased processing costs at slaughter plants for labor, equipment maintenance, and slower slaughter line speeds (Andersen et al., 1991b). The increasing testosterone concentrations that occured

with age (Field et al., 1989) increase collagen synthesis (Miller et al., 1989) which may cause difficulty with pelt removal.

Castration of bulls at birth results in carcasses with higher marbling and carcass quality grades, but lower yield of retail cuts than bulls castrated at weaning (Landon et al., 1978). Bull carcasses had slightly more maturity in bone and lean than steer carcasses (Crouse et al., 1985). In spite of the advantages mentioned above, intact males are not routinely fed to slaughter weights in U.S. because of their aggressive behavior, need to keep isolated from females, decreased quality grades and consumer acceptance.

Sexual Behaviors

GnRH may directly regulate sexual behavior at the brain level (Pfaff, 1973). Infusion of GnRH into midbrain enhanced sexual receptivity in rats and advanced sexual receptivity in birds reptiles and amphibians (Shivers et al., 1983). Less aggressive behavior was reported in immunized animals with suppressed gonadotropin secretion and development of the gonads (Fraser and Bacher, 1978; Falvo et al., 1986). Male bull calves that exhibited an immune response against GnRH had reduced libido. However, after six months the antibody titer decreased and testicular volume, testosterone level and libido recovered (Robertson et al., 1982). There was no cyclic reproductive activity in GnRH immunized heifers (Adams and Adams, 1990). Prendiville et al. (1995) reported that GnRH immunization before puberty delayed the onset of puberty and expression of estrous behavior.

One of the diverse functions of sex steroids is stimulation of brain functions associated with sexual and aggressive behavior (D'Occihio, 1993). Intact bulls and boars have more aggressive behaviors than castrates (Seideman et al., 1982) which can result in

serious injuries to other animals and more labor requirements for their maintenance. Bull carcasses are more subject to produce dark cutting meat which results from fighting during transport and prior to slaughter (Price and Tennesen, 1981). Heifers in estrus are very active and spend energy seeking and mounting other cattle. Heifers in heat can also cause reduced feedlot performance of other cattle in the same feedlot by chasing and disturbing them. Average daily gain and feed efficiency of ovariectomized beef heifers can be increased by reducing estrous activity and the occurrence of pregnancy. Ovariectomy, however, is not commonly practiced due to high labor costs, and decreased rates of gain and feed efficiencies (Horstman et al., 1982; Crouse et al., 1987).

III. Materials and Methods

Animals

Forty-eight spring born crossbreed ram lambs weighing 32.6 ± 1 kg were randomly assigned at weaning to: an intact control group (R; n=12), immunized with a GnRH-KLH conjugate in Freund's complete adjuvant (FCA; n=12), immunized with a GnRH-KLH conjugate in ISA (Seppic Inc.) adjuvant (ISA; n=12) or castrate (C; n=12) groups. Lambs were weaned, and randomly allotted to the 4 groups based on live weights. The next day, all the ram lambs were moved to the confinement building at the Livestock Center (University of Wyoming), weighed again, and placed in individual pens (1.15x2.30 m). The beginning experimental weight was calculated as the average of the two weights obtained at the initiation of the study. Testicular circumferences of all lambs were measured at the initiation of the study. Lambs were mechanically or immunologically castrated using elastorators or GnRH-KLH in FCA or ISA.

Diets were formulated and fed to achieve a weight gain of .30 kg/head/day. The lambs were fed once every day in the morning in individual feeders which allowed the amount of feed consumed by each lamb to be determined. Every morning refusals (unconsumed feed) were collected and weighed. The amount of feed was increased 0.1 kg every 3 days until the lambs refused about 10% of feed given the preceding day.

Diet #1 was a start-up ration fed for the first two weeks of the experiment. Ingredients of the first diet were: alfalfa hay (40.1%), cracked corn (30.9%), alfalfa dehydrated pellets (26.8%), sodium tripolyphosphate (.8%), trace minerals in NaCl (.5%), vitamin premix (.04%) and lasalocid (16.9 g).

Diet #2 was fed for 4 weeks after diet #1. This diet had a higher energy content than diet #1 and consisted of: cracked corn (57.6%), alfalfa hay (26.3%), alfalfa dehydrated pellets (13.5%), soybean meal (1.6%), sodium bicarbonate (.50%), trace minerals in NaCl (.50%), vitamin premix (.04%) and lasalocid (43.1 g).

Diet #3, the finishing diet, was initiated on the seventh week of the experiment and fed until the lambs reached their target weight of 59 ± 2.3kg. Ingredients of this diet were: cracked corn (83.0%), alfalfa hay (11.5%), soybean meal (3.1%), sodium bicarbonate (1.0%), limestone (.8%), trace minerals in NaCl (.5%), vitamin premix (.04%) and lasalocid (60.1 g.)

Vaccines and Anti-GnRH Titer Assay

Vaccines were provided by Dr.Thomas E. Adams (Department of Animal Sciences, University of California at Davis). These vaccines consisted of GnRH linked to keyhole limpet hemocyanin (1mg) in 2 ml of either Freund's complete adjuvant (FCA) or ISA adjuvant (Seppic, Inc; Paris, France). ISA is composed of mineral oil and an emulsifying agent, but its exact composition is proprietary. The vaccines (2ml/injection) were shipped overnight on ice and delivered s.c. equally in four different sites in the upper neck, just behind the ears.

The method of conjugation of GnRH to KLH was described by Adams and Adams (1986). Anti-GnRH titers were measured from blood serum samples (collected as described below) and anti-GnRH titers were determined as described by Adams et al. (1997). The anti-GnRH titer is expressed as the percentage of total [^{125}I] GnRH bound to antibody in a 1:1000 dilution of serum.

Measurements (Weight, Testicular circumferences)

Animals were weighed every 2 weeks to monitor weight changes. After week 10 of the experiment, animals were weighed weekly to identify the date for the behavior studies. Behavior studies were conducted at 52 kg and as close as possible to the slaughter target weight of 59 kg. Feed efficiencies were calculated using the last weight obtained in the confinement building. Dressing percentages were calculated using live and carcass weights obtained in the meat laboratory at the University of Wyoming. Scrotal circumferences were measured using a scrotal tape on the first day of the experiment and at four weeks interval throughout the experiment.

Sample Collections (Blood, Hypothalamus, Pre-Optic Area (POA), Stalk of Median Eminence (SME), Pituitary and Testes)

Blood samples (10 ml) were collected by jugular venipunctures at the time of placement of animals in their individual pens, and at two weeks intervals throughout the experiment. An additional blood sample was collected immediately prior to slaughter. All blood samples were allowed to clot overnight at 4 °C after which serum was harvested following centrifugation for 20 min at 1500 g. Serum samples were divided into two equal aliquots and stored frozen at –20 °C prior to analysis for testosterone, LH and anti-GnRH titer. One aliquot of serum samples was sent to Dr. Adams at the University of California at Davis for determination of anti-GnRH titer concentration (Adams et al., 1997). The other aliquot was used to determine concentrations of testosterone and LH.

At slaughter, lambs were stunned by electric shock and exanguinated. Skullcaps were removed within 10 min. after killing. Anterior pituitary glands and hypothalamic areas were collected as described by Moss et al. (1980). First, each stalk-median

eminence (SME) was separated from the anterior pituitary gland and hypothalamus, placed in a cryovial, labeled and immediately frozen in liquid nitrogen. The other two areas of the hypothalamus, the preoptic area (POA) and medial basal hypothalamus (HYP), were separated from each other. The HYP and POA were bisected midsagitally and each piece was wrapped in aluminium foil, labeled and frozen in liquid nitrogen. Each pituitary gland was removed from the sphenoid bone, trimmed of connective tissue and pars nervosa, and weighed. Each anterior pituitary gland was bisected midsagitally and a single slice was obtained from one half of the pituitary using a Stadie-Riggs tissue slicer for future morphological examination. The remaining halves of anterior pituitary tissue were separately wrapped and frozen in liquid nitrogen for hormone analysis.

Testicles were removed immediately after slaughter of the intact lambs, trimmed of the muscles (tunica dartos) and weighted. Three pieces (3x2x3 mm) were obtained from one of the testicles and placed into fixative (Histochoice, Amresco Solon, OH + 20% ethanol) for morphological examination. Two additional pieces (3x3x3 mm) were frozen in cryovial for hormone analysis.

LH and Testosterone Assays

Concentrations of LH and testosterone in serum were determined in duplicate for all samples by radioimmunoassay. Sensitivity of the assays were 0.15 ng/ml and 0.025 ng/ml for LH and testosterone, respectively. Coefficients of variations (C. V.) for LH and testosterone radioimmunoassays (RIA) were 10.8 %and 13.5 %. The RIA for determination of LH in serum was described by Alexander et al. (1994) and the RIA for serum testosterone was described by Murdoch and Dunn (1982).

Carcass Evaluation

Lambs were scheduled for slaughter when they reached a target weight of 59 ± 2.3 kg. Hot carcass weights were obtained on the day of slaughter and carcass traits were evaluated 24 hours later according to USDA methodology for lamb grading (USDA, 1992) by Dr. Melvin L. Riley (Department of Animal Science, University of Wyoming). Carcass characteristics evaluated were; dressing percentage, back fat thickness, yield grade, body wall thickness, rib eye area (REA), marbling, maturity of lean and bone, flank streaking, muscling score, overall quality score, kidney fat weight, fat color and firmness. Testis and pituitary weights were also measured.

Yield grades were calculated as: yield grade = {back fat thickness (inch) X 10} + .4. Fat firmness and color were based on a scale of 1-5 where 1=soft and 5=firm or 1=yellow 5=white for firmness and color, respectively. Grades for marbling score, flank streaking, maturity of bone and lean, muscling score and quality score were converted into numbers for statistical analysis. The scores and their equivalent numeric values for different traits are as follows: marbling score and flank streaking scores; practically devoid=0, traces=100, slight=200, small=300, modest=400, moderate=500: maturity in bone and lean scores; A=100 and B=200: muscling and overall quality sores; low choice=10, average choice=11, high choice=12, low prime=13, average prime=14, high prime=15.

Rib eye areas (REA) were measured by placing a plastic grid over the rib-eye muscle between 12^{th} and 13^{th} ribs. The squares on the plastic grid were counted and total square inches were converted into square centimeters. Back fat and bodywall thicknesses

were measured in the middle of the longissimus dorsi muscle cut between 12^{th} and 13^{th} ribs.

Behavior Study

A target weight of 52 ± 3 kg was determined for the first replicate of behavior monitoring and the second replicate was conducted as close as possible to the slaughter target weight of 59 ± 2.3kg. Both behavior trials were recorded by a cam corder for 30 minutes. The number of reproductive behaviors (i.e. mounts, attempted mounts, investigatory sniffs, nose to nose sniffs, udder sniffs, ejaculation, foreleg-kicks, flehmen, ram and ewe butting) exhibited during the 30 min period were counted and recorded for statistical analysis.

The behavior trials were conducted by placing the test lambs with two estrous ewes in a 2.3 x 4.6 m. pen. The view of the behavior trials by the remaining rams was blocked by a solid fence. Estrus was induced in three ovariectomized mature ewes by treatment with progesterone and estradiol. CIDRs, an intra-vaginal device containing progesterone, were inserted in the ewes for 12-14 days. Three days before the behavior study, the CIDRs were removed and 100 μg estradiol in oil was administered daily. Estrus was confirmed in each progesterone / estradiol treated ewe by one or two mature rams. Two ovariectomized ewes that exhibited estrous behavior were utilized for the behavior studies.

Statistical Analysis

Data were analyzed by general linear model (GLM) procedures as a split plot in time design to account for repeated measures using the SAS computer program. For anti-GnRH titers, LH, testosterone and behavior data, the main effects included in the model

were treatment, time and interaction of treatment by time. After significant treatment or time effects were detected, differences among means were tested by Fisher's protected lsd. All the data in tables and text are reported as mean ± standard error of mean unless otherwise specified.

IV. RESULTS

Anti-GnRH Titer

The anti-GnRH titers achieved in the FCA and ISA groups are illustrated in **Figure 1**. Titers are presented as the percentage of radioactive GnRH bound by antibodies in a 1:1000 dilution of serum samples taken every two weeks. Antibodies to GnRH were not detected in the two serum samples (first and last samples) analyzed from R and C lambs. Overall, FCA immunized rams produced higher ($p < .05$) GnRH titers at an earlier time during the experiment than ISA immunized rams. From day zero through week 6 there were no differences ($p > .05$) in the amount of antibodies produced by the FCA and ISA immunized rams. Antibody production steadily increased from week 8 through end of the experiment for both groups. Titers, however, differed among FCA and ISA groups, respectively, at week 8 (12.9 ± 2.8 % and 2.6 ± 0.5 %; $p < .005$), 10 (20.1 ± 3.7% and 8.2 ± 2.5 %; $p < .0005$), 12 (23.6 ± 3.7 % and 13.9 ± 4.9 %; $p < .005$), 14 (27.2 ± 4.7 % and 17.8 ± 6.4 %; $p < .005$), and 16 (30.7 ± 4.9 % and 21.6 ± 7.3 %; $p < .005$). Serum samples collected at slaughter showed a large difference in anti-GnRH titers between FCA and ISA immunized rams, 36.3 ± 5.4 % and 19.3 ± 5.4 %, respectively ($p < .05$; **Figure 2**). Within the 11 rams in the FCA group, 1 rams failed to produce high levels of anti-GnRH titers (i.e. 3 %), while 6 of 12 in the ISA group failed to produce high levels of antibodies (i.e. <11.4 %).

LH Concentrations

Concentrations of LH at the initiation of the study were similar ($p > .05$) for C, FCA, ISA and R groups (.58 ± .20, 1.30 ± .73, .33 ± .07 and .26 ± .05 ng/ml,

Figure 1. The percentage of radioactive GnRH bound by antibodies in a 1:1000 dilution of serum samples taken every two weeks.

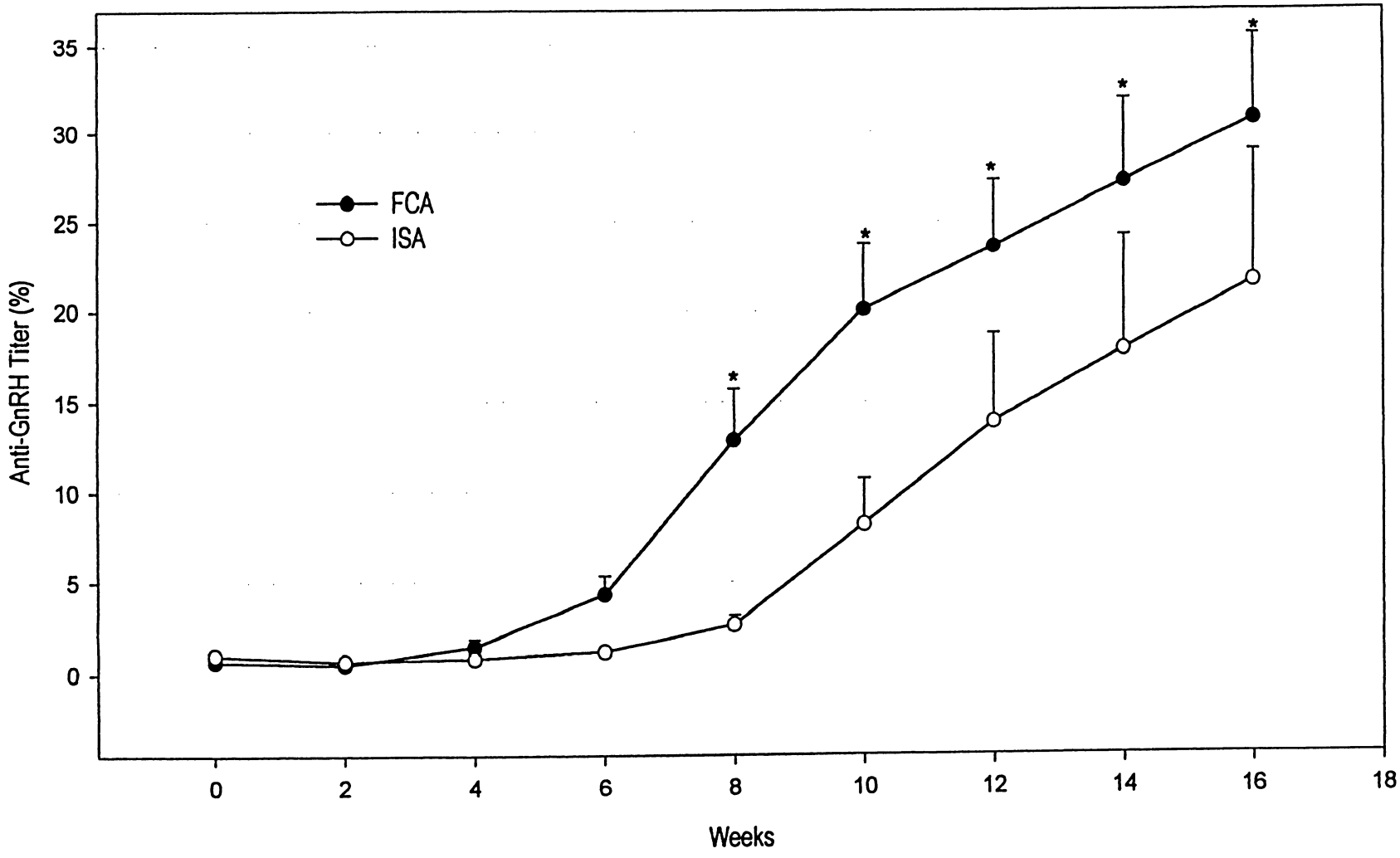
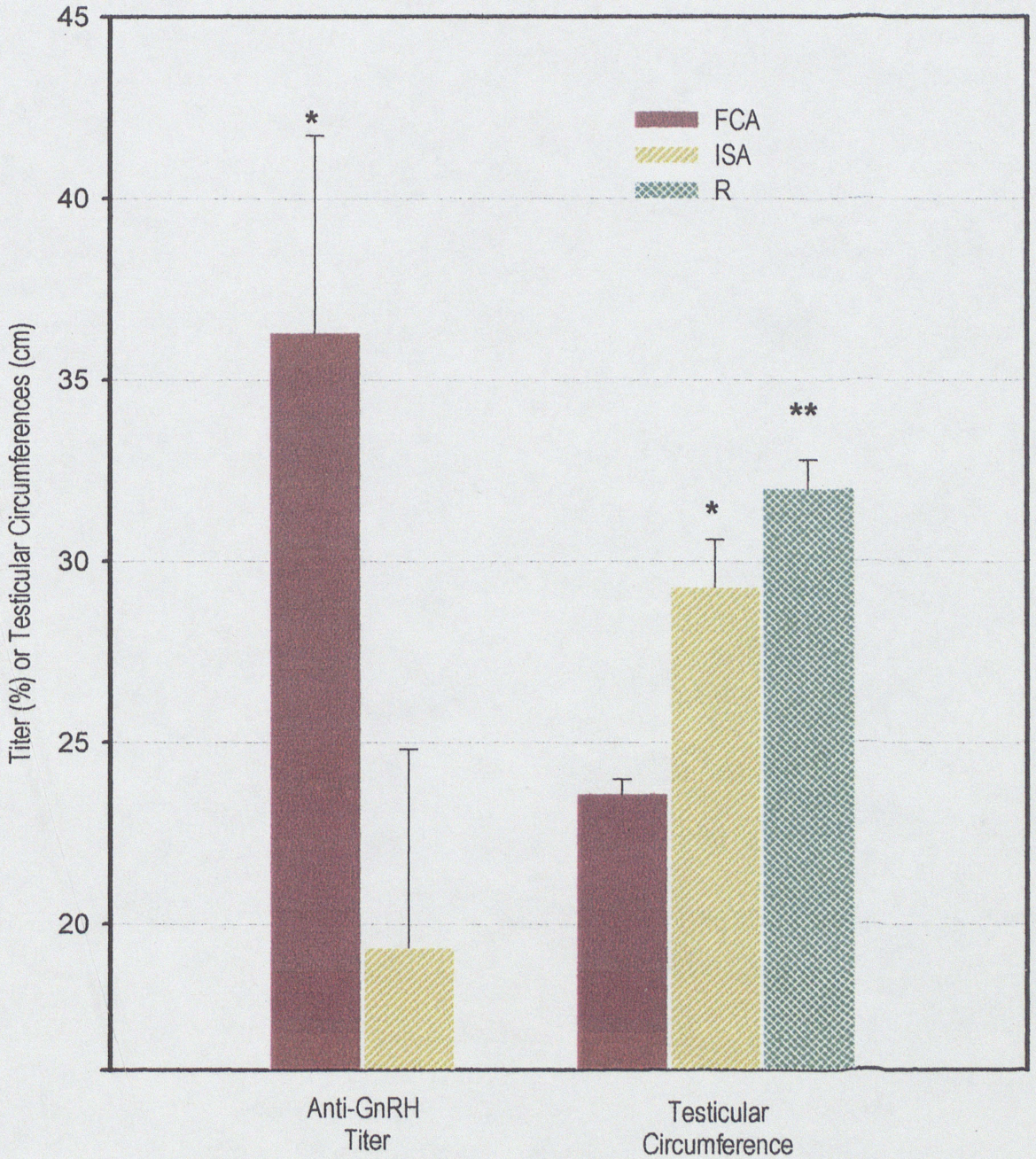


Figure 2. Anti-GnRH titers from the final serum samples of ram lambs immunized against GnRH-KLH conjugate in FCA or ISA adjuvants and final testicular circumferences of the immunized (FCA and ISA) and intact (R) ram lambs.



respectively). Concentrations of LH increased in castrated rams at week 2 (2.83 ± .2 ng/ml; $p < .0005$), 4 (4.80 ± .52 ng/ml; $p < .0005$) and remained elevated and higher than in the other groups through the end of the experiment. However, concentrations of LH in FCA, ISA and R groups did not differ with time or among groups ($p > .05$; **Figure 3**). Serum samples collected immediately prior to slaughter contained concentrations of LH similar to those collected earlier during the experiment. That is, C lambs (4.71 ± .72 ng/ml) had greater ($p < .0005$) serum LH concentrations than FCA (.22± .06 ng/ml), ISA (.29 ± .07 ng/ml) and R (.79 ± .29 ng/ml) lambs, but concentrations were similar ($p > .05$) among ISA FCA and R lambs **(Figure 4).**

Testosterone Concentrations

Overall, concentrations of testosterone in serum were similar in R and ISA groups ($p > .05$), lower in FCA groups than ISA ($p < .01$) and R ($p < .005$) groups, and lower in the C group than FCA ($p < .05$) ISA ($p < .005$) and R ($p < .005$) groups **(Figure 5).**

Testosterone concentrations were similar ($p > .05$) among groups at time zero **(Figure 5).** At week 2 of the experiment, testosterone concentrations decreased in the castrated group and remained low through the end of the experiment. In the FCA group, testosterone concentrations increased from week 2 to week 6, then decreased ($p < .005$) from week 6 through week 10 ($p < .0005$), and remained low thereafter. Testosterone concentrations in the ISA group increased ($p < .0005$) from week 2 to week 6, decreased between weeks 6 and 10 ($p < .05$), increased between weeks 10 and 12 ($p < 005$) and again decreased between weeks 12 through 16. Concentrations of testosterone in intact rams

Figure 3. Concentrations of luteinizing hormone (LH) in serum from castrated (C), immunized against GnRH-KLH conjugate in FCA or ISA adjuvants, and intact (R) ram lambs.

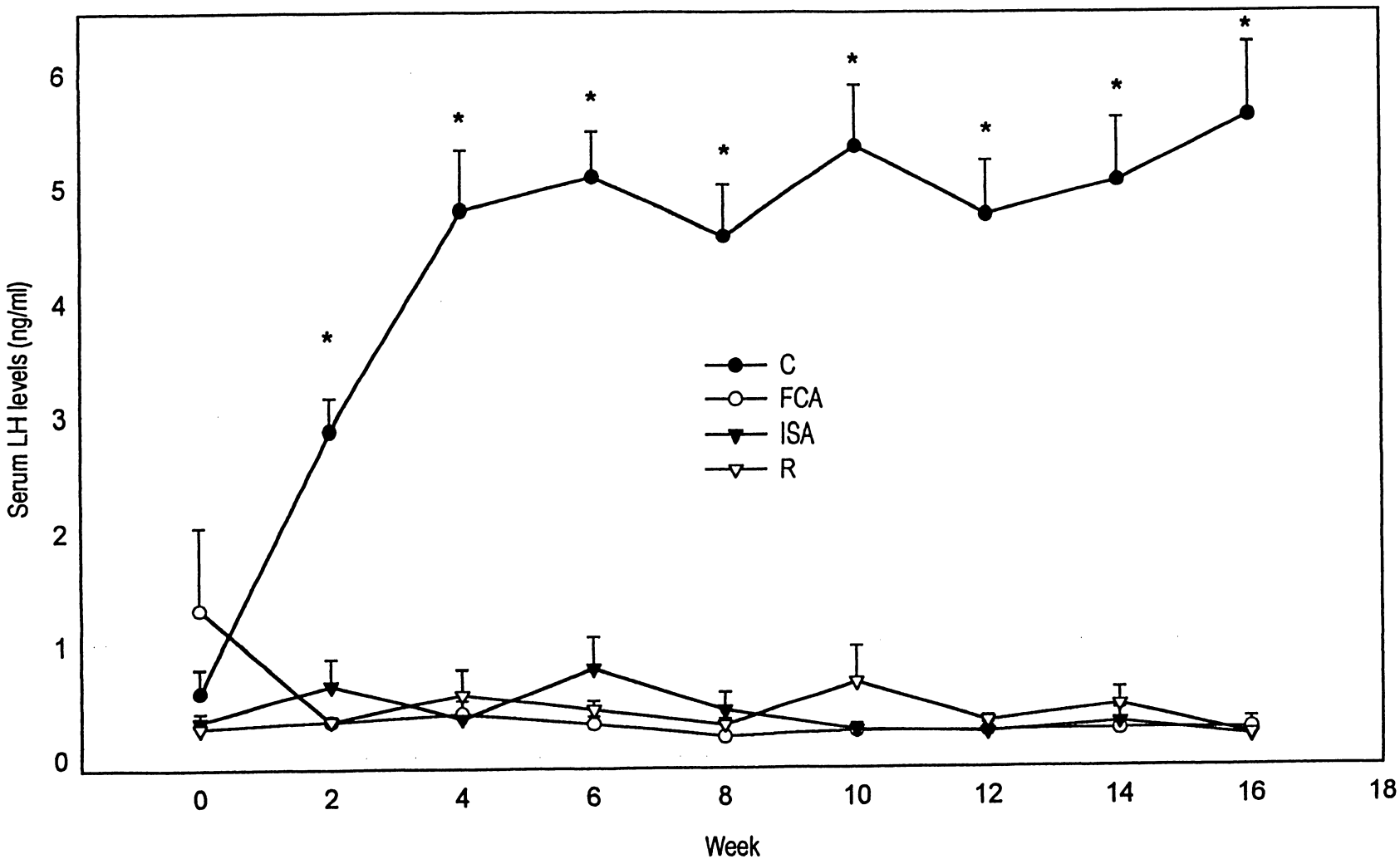


Figure 4. Luteinizing hormone (LH) and testosterone concentrations in the final serum samples collected from castrated (C), immunized against GnRH-KLH conjugate in FCA and ISA adjuvants, and intact (R) ram lambs.

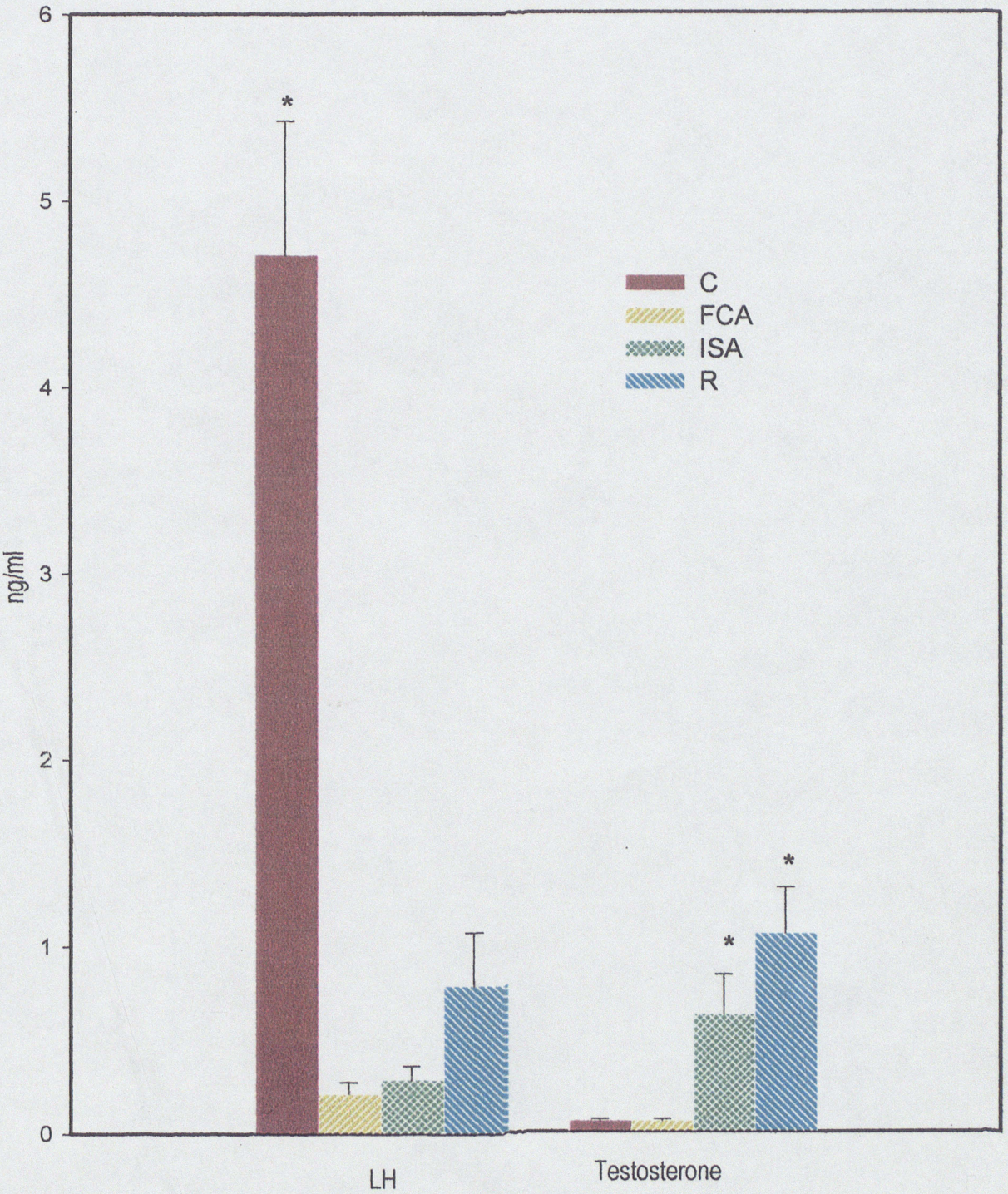
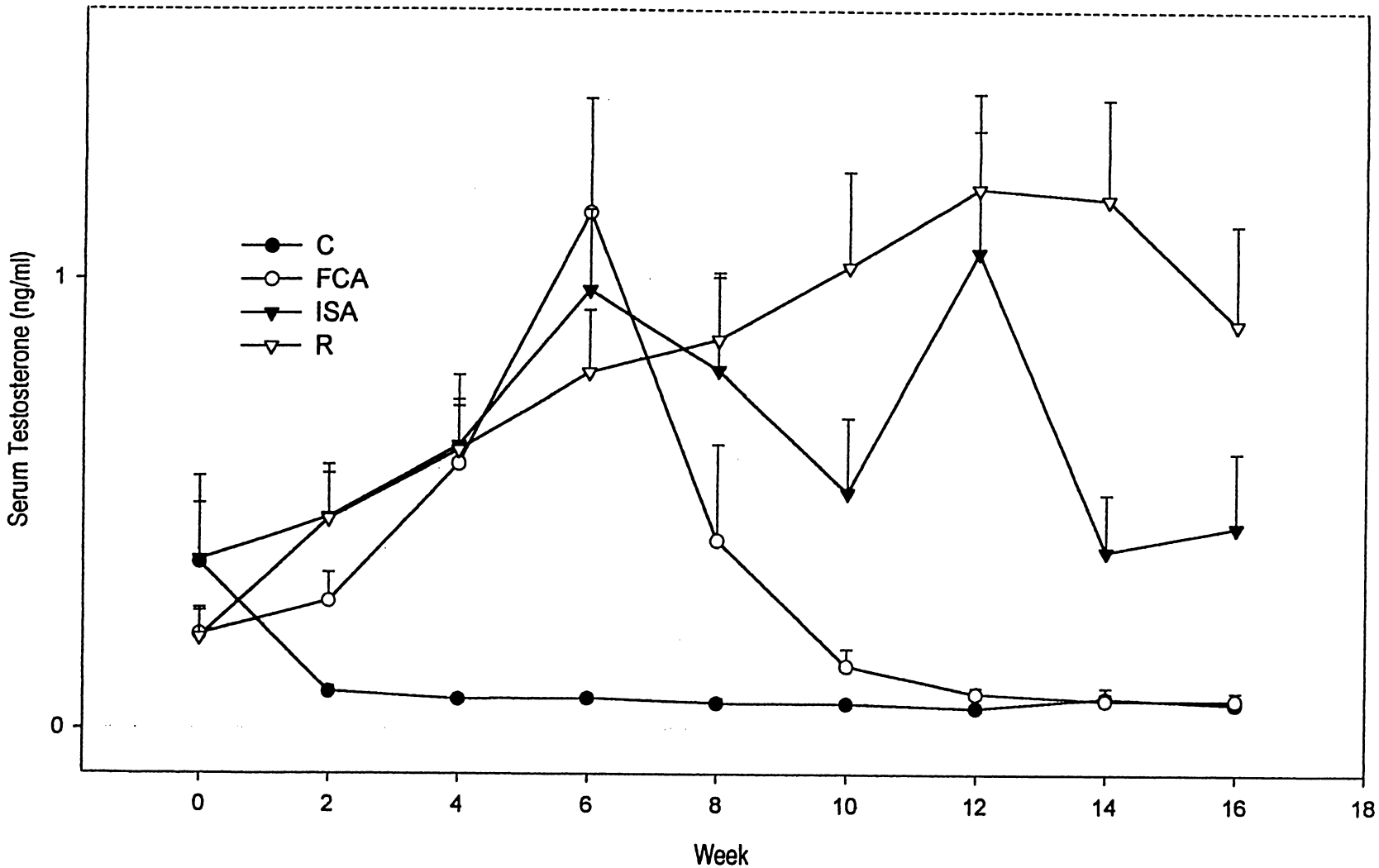


Figure 5. Concentrations of testosterone in serum collected from castrated (C), immunized against GnRH-KLH conjugate in FCA or ISA adjuvants, and intact (R) ram lambs.



steadily increased ($p < .005$) from day 0, through week 12. The decrease after week 12 was not significant ($p > .05$).

At week 2 of the experiment, testosterone concentrations in the C group were less ($p < .05$) than in ISA and R groups, but similar ($p > .05$) with FCA group. The FCA group was intermediate ($p > .05$) between the C, ISA and R groups. By week 4 ($p < .01$) and 6 ($p < .0005$) the C group had lower testosterone concentrations than the other three groups. At week 8, concentrations of testosterone were lower in the C group than ISA and R groups ($p < .0005$) and similar with the FCA group ($p > .05$). At week 10, testosterone concentrations were similar in C and FCA ($p > .05$) and both groups were lower than ISA ($p < .05$) and R ($p < .0005$) groups. By week 12, testosterone concentrations were similar ($p > .05$) in C and FCA groups and ISA and R groups ($p > .05$). The C and FCA groups were less ($p < .0005$) than ISA and R groups. At weeks 14, testosterone concentrations were similar among ($p < .05$) C, FCA and ISA groups with concentrations in R greater than the other groups ($p < .005$). At week 16, testosterone concentrations were similar in C and FCA groups ($p > .05$) and ISA and R groups ($p > .05$), and lower in C and FCA groups than the ISA ($p < .05$) and R groups ($p < .0005$).

Testosterone concentrations in the last serum samples from the C ($.059 \pm .009$ ng/ml) group were similar ($p > .05$) to FCA animals ($.058 \pm .010$ ng/ml). The ISA ($.627 \pm 214$ ng/ml) group were similar ($p > .05$) to the R ($1.059 \pm .248$ ng/ml) group and both the C and FCA groups were less ($p < .05$) than ISA and R animals **(Figure 4).**

Testicular Circumferences

Testicular circumferences increased in all R, ISA and FCA animals from day 0 to week 4 ($p < .0005$). In the R group, testicular circumferences continued to increase at 8 ($p < .0005$), 12 ($p < .01$), and 16 weeks ($p < .05$). Testicular circumferences also slightly increased in the ISA group, and slightly decreased in FCA group after week 4, but not significantly ($p > .05$) **(Figure 6).**

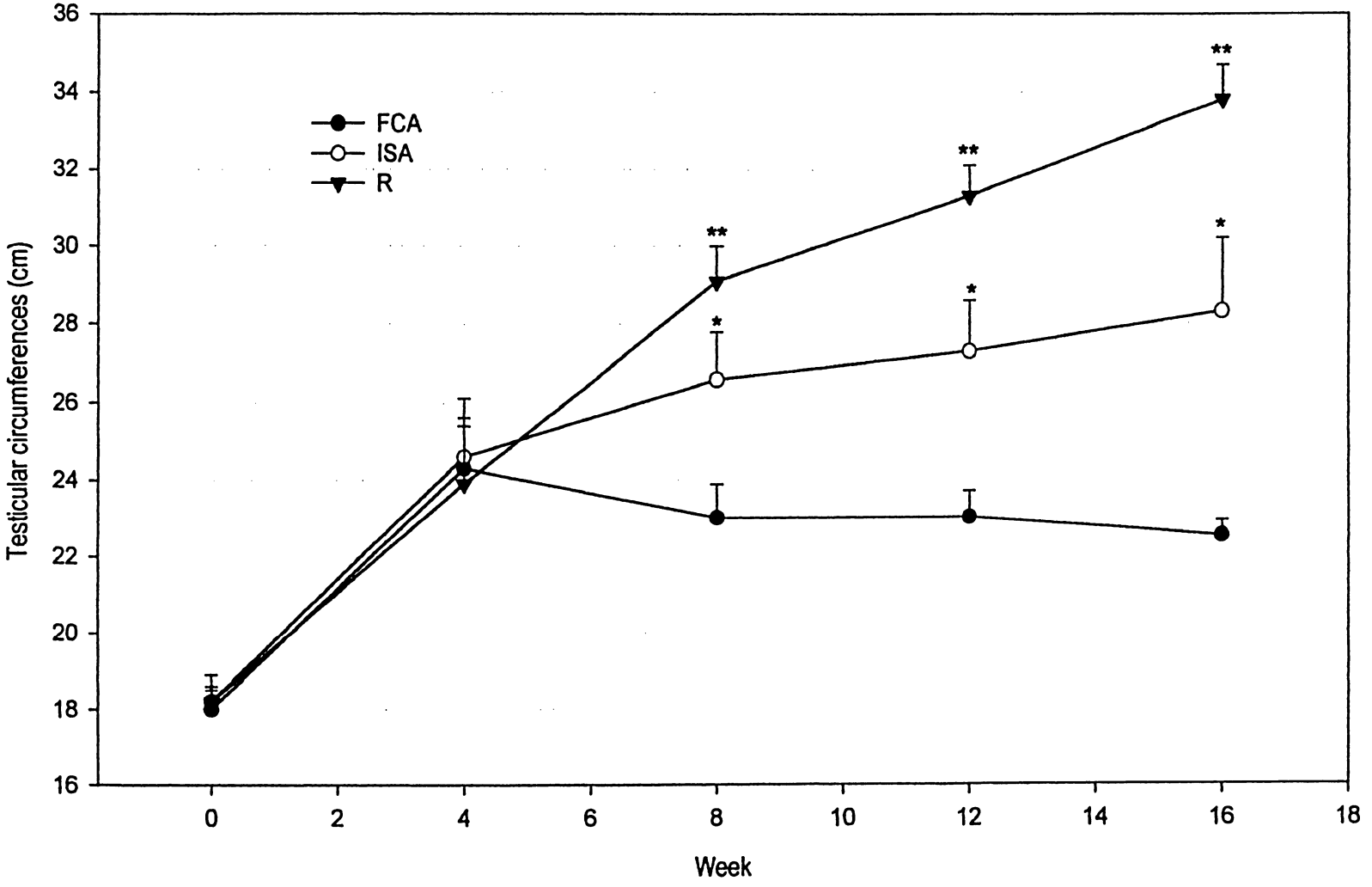
Overall testicular circumferences were higher in R ($p < .001$) and ISA ($p < .05$) than FCA lambs. At week 8, 12 and 16, R lambs had larger testicles than ISA ($p < .05$; $p < .0005$; $p < .005$) and FCA lambs ($p < .0005$; $p < .0005$; $p < .0005$), respectively. At weeks 8, 12 and 16, ISA lambs also had larger testicles than FCA lambs ($p < .01$), ($p < .0005$) ($p < .005$), respectively.

Testicular circumferences closest to slaughter age were similar ($p > .05$) in the ISA and R groups and smaller in FCA than both ISA and R animals ($p < .005$; **Figure 2**).

Testicular Weight

At slaughter, the R group had average testicular weights (402.5 ± 23 g) that were heavier than in the ISA (254.4 ± 4 g; $p < .005$) and FCA groups (65.2 ± 8 g; $p < .0005$). Testicular weights of ISA animals were also heavier ($p < .0005$) than in the FCA group. Anti-GnRH titers at slaughter were negatively correlated with testicular weights ($r = -0.70$; $p < 0.0005$, $n = 23$). Testicular weights were positively correlated with testosterone concentrations in serum at slaughter in the FCA, ISA and R groups ($r = 0.67$, $p < 0.001$, $n = 36$).

Figure 6. Testicular circumference of intact (R), and in lambs immunized against GnRH-KLH conjugate (1mg) in 2 ml FCA or ISA adjuvants.



Microscopic Examination of Testes

Diameters of seminiferus tubules from FCA lambs were smaller than in R lambs **(Figure 7)**. Tubular atrophy was observed in 82, 25 and 0 % of testicles from FCA, ISA and R lambs, respectively. Spermatozoa were detected in 0, 50 and 69 % of the testicles from FCA, ISA and R groups, respectively. Animals with high anti-GnRH titers exhibited decreased testicular weights, increased testicular atrophy and the absence of spermatozoa **(Table 1)**.

Pituitary Weights

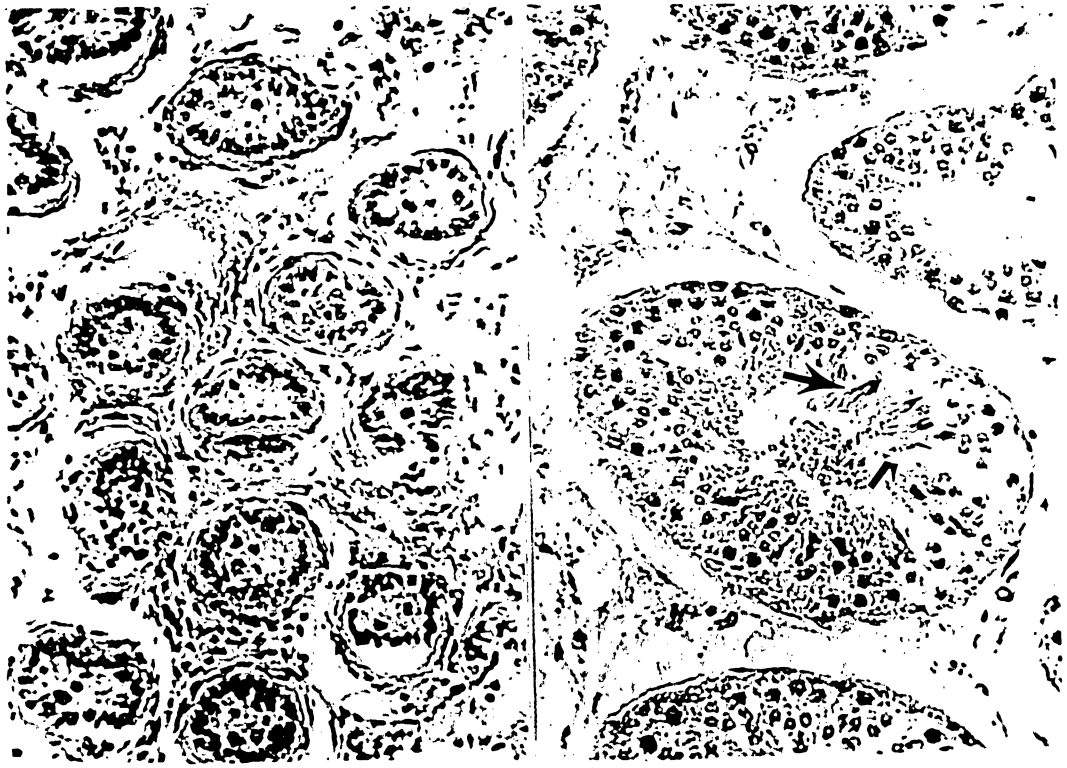
Anterior pituitary glands were heavier in R (643 ± 26 mg) than both ISA (561 ± 21 mg; $p<0.05$) and FCA lambs (521 ± 25 mg; $p<0.005$), and similar ($p>0.05$) with C lambs (599 ± 21 mg). Pituitary weights in FCA lambs were less ($p< .05$) than in C lambs. FCA and ISA lambs did not differ ($p > .05$).

Feedlot Performances

Overall weight gain did not differ ($p> .05$) among the groups because lambs were slaughtered at a pre-determined slaughter weight. Days on feed, total feed consumption, feed efficiency (total feed consumption: total gain) and average daily gains (ADG), however, were different among groups **(Table 2).**

Days on feed were greater ($p< .05$) in C than R lambs, similar ($p> .05$) in FCA and ISA lambs, and FCA and ISA lambs were intermediate to C and R lambs. Total feed consumed was higher ($p< .01$) in the C and FCA groups than the R group and intermediate in the ISA group. Feed efficiency (kg feed/kg gain) was better in the R group than C ($p< .01$), FCA ($p< .001$) and ISA ($p< .05$) groups, but similar in C, FCA

Figure 7. Testicular morphology of an FCA-immunized (Panel A) and non-immunized ram (Panel B) at 200x magnification. Note the tubular atrophy in panel A. It was not clear whether the staining cells in seminiferus tubules in panel A are spermatocyte cells or sertoli cells. In panel B arrows indicates spermatids.



Panel A Panel B

Table 1. Testis weights, anti GnRH-titers, and presence of testicular atrophy and spermatozoa in FCA, ISA and R groups.

Groups	Testis Weights (g)	Anti-GnRH Titers (%)	Tubular Atrophy	Spermatozoa
FCA	34.5	38.2	+	-
FCA	43.2	52.3	+	-
FCA	102	3.0	+	-
FCA	74.4	25.6	-	-
FCA	60.1	18.9	+	-
FCA	76.1	28.6	-	-
FCA	38.5	31.1	+	-
FCA	74.2	63.8	+	-
FCA	35.6	55.7	+	-
FCA	69.1	50.7	+	-
FCA	109.6	31.6	+	-
Means	**65.2 ± 7.8**	**36.3 ± 5.4**	**82%**	**0%**
ISA	339.8	0.8	-	+
ISA	28.1	33.8	+	-
ISA	270.2	6.7	-	+
ISA	53.9	32.2	+	-
ISA	344.2	1.4	-	-
ISA	373.9	19.8	-	-
ISA	199.7	17.0	-	+
ISA	488.3	4.9	-	+
ISA	136.9	58.3	+	-
ISA	42.9	44	+	-
ISA	338.5	11.3	-	+
ISA	437	2.3	-	+
Means	**254.4 ± 45.9**	**19.4 ± 5.5**	**33%**	**50%**
R	389.2		-	+
R	455.9		-	-
R	377.4		-	+
R	467.6		-	+
R	517.6		-	+
R	399.6		-	-
R	453.2		-	-
R	288.4		-	+
R	392.3		-	+
R	496.2		-	+
R	219.6		-	-
R	405.4		-	+
R	367.4		-	+
Means	**402.3 ± 22.7**		**0%**	**69%**

Table 2. Effects of castration (C), immunization against GnRH with GnRH-KLH conjugate in ISA and FCA adjuvants and intact (R) ram lambs on feedlot performances (Mean ± S.E.M.).

Traits	C	FCA	ISA	R
Start Weight (kg)	33.2±1	32.2±1	32.5±1	32.5±1.1
Slaughter Weight (kg)	58.4±0.6	56.9±1	57.1±0.6	58.6±0.5
Feedlot Gain (kg)	25.1±0.7	24.6±1.2	24.7±1	26.1±1.2
Total Days	130.8±8.1[a]	126.8±7.3[ab]	121.6±8.3[ab]	107.5±5.7[b]
Total Feed (kg)	174.7±5.2[a]	175.8±6.2[a]	165.4±6.9[ab]	152.5±6.6[b]
ADG (g)	199±12[b]	200±14[b]	210±12[b]	246±9[a]
Feed Efficiency[d]	6.97±0.17[a]	7.32±0.47[a]	6.76±0.28[a]	5.85±0.13[b]

[a,b] Within a row, values do not share a common superscript differ ($p<.05$).

[d] Feed Efficiency: Total Feed Consumed / Weight Gain.

and ISA groups (p> .05). Average daily gains (ADG) were greater in the R group than C, FCA (p< .01) and ISA (p< .05) groups, and similar among the C, FCA and ISA groups (p>.05).

Carcass Characteristics (Dressing %, Quality Grade, Muscling Score, Flank Streaking, Maturity in Bone & Lean, REA, Marbling Score, Back-Fat Thickness, Yield Grade, Body-Wall Thickness, Kidney Fat, Fat Firmness, Fat Color.)

Castration or immunization against GnRH did not change rib eye areas (REA), overall quality grades, muscle scores, flank streaking, maturity in bone and lean and fat colors among groups (p> .05; **Table 3**).

Dressing percentages, back fat thickness, yield grades, body wall thicknesses, kidney fat, marbling and fat firmness differed (p< .05) among groups **(Table 4)**. Dressing percentages were higher (p< .05) in C and FCA groups than the R group, similar (p> .05) among C, FCA and ISA groups and ISA and R groups. Back fat thicknesses and yield grades were higher (p< .0005) in the C group than FCA, ISA and R groups. These traits were similar among FCA, ISA and R groups (p> .05).

Body wall thicknesses were greater in the C group than FCA (p< .05), ISA (p< .0005), R (p< .0005) groups; greater in the FCA than R group (p< .05); and similar among the ISA and FCA groups and the ISA with R groups (p> .05). Amount of kidney fat was greater in the C group than in the ISA (p< .05) and R (p< .01) groups. The FCA lambs had amounts of kidney fat similar to, and intermediate between the ISA, R and C groups (p> .05). Marbling scores were higher in the C group than the other three groups (p< .0005) which were similar (p> .05). Fat was firmer in the C group than ISA (p< .05)

Table 3. Carcass traits which were similar in castrated (C), immunized with GnRH-KLH conjugate in FCA or ISA adjuvants and intact (R) control rams (Mean ± S.E.M.).

Traits	C	FCA	ISA	R
Lean Maturity	170±3	175± 3	175±4	169±3
Bone Maturity	176±3	171± 3	165±3	169±3
Rib Eye Area (REA)	15.5±0.4	15.4±0.7	15.2±0.4	15.7±0.5
Quality Score	11.2±0.2	11±0.1	10.8±0.1	10.9±0.1
Muscle Score	11.5±0.2	11.4±0.2	11.3±0.1	11.5±0.1
Flank Streaking	284±12	253±13	243±15	241±11
Fat Color*	3.1±0.3	3 ± 0.3	2.6±0.1	2.5±0.1

*Grade 1 is for yellow, 5 is for white color fat.

Table 4. Effects of castration, immunization against GnRH with GnRH-KLH conjugate in FCA or ISA adjuvants and intact (R) ram lambs on carcass characteristics testicle and pituitary weights (Mean ± S.E.M.).

Traits	C	FCA	ISA	R
Dressing %	50.8±0.65 [a]	50.79±0.56 [a]	49.23±0.73 [ab]	48.78±0.45 [b]
Back Fat Thickness (mm)	5.9±0.6 [a]	3.6±0.3	3.4±0.3	3.4±0.4
Yield Grade	2.71±0.24 [a]	1.8±0.11	1.73±0.12	1.73±0.14
Body Wall Thickness (mm)	24.1±1 [a]	21.4±0.8 [b]	18.8±0.9 [bc]	18.7±0.9 [c]
Kidney Fat (g)	1092±56 [a]	930±129 [ab]	815±60 [b]	761±70 [b]
Marbling	415±17 [a]	279±12	290±22	298± 20
Fat Firmness*	3.6±0.3 [a]	3±0.2 [ab]	2.8±0.2 [b]	2.7±0.2 [b]
Testicle Weights (g)		65.2±7.8 [a]	254.4±45.9 [b]	402.5±22.7 [c]
Pituitary Weights (mg)	599±21 [ac]	527±26 [c]	561±22 [bc]	643±26 [a]

* Grade 1 is for softest, 5 is for hardest fat.

[a,b,c] Within a row, values do not share a common superscript differ ($p<.05$).

and R ($p < .005$) animals and similar to the FCA group. Fat firmness was similar among the FCA, ISA and R groups ($p > .05$).

Sexual Behaviors

Behaviors exhibited during the first and second behavior trials were similar with no interaction between the behavior studies and groups ($p > .05$). Thus, only the statistical results of each behavioral trait are presented as an average of the first and second behavioral studies rather than as separate studies. Numbers of attempted mounts, mounts, ejaculations, investigatory sniffing, foreleg kicks, ram butting, ewe butting and nose to nose sniffing differed ($p < .05$) among groups, but number of flehmen behaviors and udder sniffing were similar among groups ($p > .05$; **Table 5**).

Numbers of attempted mounts were less in the FCA group than ISA ($p < .001$) and R groups ($p < .05$), less in the C group than the ISA group ($p < .005$), and similar among the R, ISA and C groups ($p > .05$). Numbers of mounts were less in the C than ISA ($p < .005$) and R ($p < .0005$) groups, less in the FCA than ISA ($p < .005$) and R ($p < .0005$) groups, similar among the C and FCA groups, and among the ISA and R groups ($p > .05$). Numbers of ejaculations were similar ($p > .05$) in C and FCA groups which were less than ISA ($p < .005$) and R ($p < .0005$) animals, and less in the ISA than R group ($p < .05$). Numbers of investigatory sniffs were less in the C than ISA ($p < .05$) and R ($p < .0005$) groups, less in FCA than ISA ($p < .05$) and R ($p < .0005$) groups, less in the ISA group than R ($p < .05$) group, and similar among the C and FCA groups ($p > .05$). Numbers of foreleg kicks were less in the FCA than C, ISA and R groups ($p < .05$), and similar among the C, ISA and R groups ($p < .05$). Ram butting was greater ($p < .01$) in the R group than C and

Table 5. Effects of castration, active immunization against GnRH with GnRH-KLH conjugate in FCA or ISA adjuvants and intact (R) ram lambs on sexual behaviors (Mean ± S.E.M).

Traits	C	FCA	ISA	R
Attempted Mounts	1 ± 0.5^{bc}	0.5 ± 0.4^{c}	4.1 ± 1.4^{a}	2.8 ± 0.5^{ab}
Mounts	0.7 ± 0.4^{b}	0.9 ± 0.5^{b}	5.1 ± 1.8^{a}	6.2 ± 1.3^{a}
Ejaculations	0^{c}	0.3 ± 0.3^{c}	2.6 ± 1^{b}	4.3 ± 0.9^{a}
Investigatory Sniffing	3.9 ± 1^{c}	5 ± 1^{c}	9.1 ± 1.7^{b}	13.1 ± 1.7^{a}
Foreleg Kicks	1 ± 0.6^{a}	0^{b}	1.3 ± 0.5^{a}	1.8 ± 0.8^{a}
Butting	0^{b}	0.7 ± 0.6^{b}	3 ± 2.7^{ab}	8.7 ± 4.7^{a}
Ewe Butting	0.4 ± 0.3^{b}	1.5 ± 0.6^{a}	0.1 ± 0.1^{b}	0.2 ± 0.1^{b}
Nose to Nose Sniffing	1.5 ± 0.3^{b}	1.7 ± 0.3^{ab}	1.5 ± 0.3^{b}	2.8 ± 0.7^{a}
Udder Sniffing	0.6±0.2	0.9±0.4	1.4±0.6	1.7±0.7
Flehmen Behavior	0	0	0.1±0.1	0.2±0.1

[a,b,c] Within a row, values do not share a common superscript differ ($p<.05$).

FCA groups, similar in the ISA with the other groups, and similar in C and FCA groups ($p>.05$). Numbers of ewe butting episodes were larger in the FCA group than C ($p<.05$), ISA ($p<.005$), and R ($p<.005$) groups, and similar in C, ISA and R groups ($p>.05$). Numbers of nóse to nose sniffing were greater ($p<.05$) in the R group than C and ISA groups, but similar with FCA group ($p>.05$), and similar among the C, FCA and ISA groups ($p>.05$). Amount of udder sniffing and flehmen behaviors did not differ among groups ($p>.05$).

V. DISCUSSION

The current study demonstrates that active immunization against GnRH and castration affects feedlot performance, carcass characteristics and reproductive behaviors in ram lambs. This study also examined effectiveness of two different adjuvants on stimulation of the immune system.

Similar to previous studies in sheep and cattle (Adams and Adams,1986; Johnson et al.,1988), FCA was effective in stimulating antibody production when used with the GnRH-KLH conjugate. GnRH and steroid hormones are not immunogenic molecules and have to be conjugated to an antigenic carrier protein to stimulate the immune system (Ladd et al., 1990). In the current study, a single injection of GnRH-KLH conjugate in FCA at weaning produced higher antibody titers than when ISA was used as the adjuvant. In FCA lambs, the anti-GnRH titers were sufficient to suppress testicular growth, serum concentrations of LH and testosterone. Although the variation in titer percentages was larger among lambs in the ISA group, titer production continuously increased in every lamb in both groups. However, 10 of 11 lambs in the FCA group and only 3 of 12 lambs in ISA group produced titers high enough to consistently decrease testicular weights and functions. Therefore, ISA does not appear to consistently evoke an immune response as robust as the FCA adjuvant. The high correlation ($r = -0.86$) between anti-GnRH titers and testicular circumferences illustrates that antibody production is necessary to retard testicular development in immunized animals.

Daley et al. (1995) immunized lambs at an earlier time (i.e. 30 days of age) with 2 ml of GnRH-KLH conjugate in FCA containing 1.65 mg GnRH-KLH conjugate. All immunized lambs produced anti-GnRH titers, which averaged 33.8 % at week 8 and 52.4

% at week 16 or the time of slaughter. In the current study, rams were immunized at 90 days of age and received a total of 1mg GnRH-KLH conjugate in 2 ml of vaccine. Average anti-GnRH titers from FCA lambs were 12.9 % at week 8 and 30.7 % at week 16. In both studies, anti-GnRH titers were expressed as the percentage of total [^{125}I] GnRH bound to antibody in a 1:1000 dilution of serum. In this study, anti-GnRH titers were less than those reported by Daley et al. (1995) possibly because of the lessor amount of GnRH-KLH in the vaccine, or alternatively, due to differences in specific activity of the iodinated hormone used for titer determination.

Castration resulted in elevated serum concentrations of LH probably due to the lack of negative feedback effects of testosterone on the hypothalamus and anterior pituitary gland. Compared to castrates, concentrations of LH in intact rams should be suppressed. Schanbacher and Ford (1977) and Keel et al. (1987) found that castration reduced the amount of LH in the pituitary gland, but serum concentrations were increased. Synthesis of LH subunit peptides (Wise et al., 1985) and secretion of LH (D'Occhio et al., 1993) are dependent on secretion of GnRH in sheep. Although statistically not significant, active immunization against GnRH-KLH conjugate appeared to decrease serum LH concentrations to levels less than that observed in intact rams. Such an effect seems reasonable because GnRH molecules bound by the antibodies in the hypophyseal vessels should not be biologically active. The correlation coefficient ($r = 46$; $p<.05$) between GnRH and LH also indicates that animals which responded well to immunization had lower concentrations of LH. Concentration of LH, however, were not completely suppressed in the immunized lambs. These residual concentrations of LH may have been due to a combination of incomplete suppression of GnRH-activity and reduced

negative feedback of actions by testosterone. Alternatively, incomplete suppression of LH secretion may have occurred as a result of increased sensitivity of the anterior pituitary gland to GnRH. Low doses of GnRH may up regulate numbers of receptors for GnRH (Clayton and Catt, 1981). The reason LH concentrations varied within groups is most likely due to the pulsatile nature of LH release. Since LH is released as pulses at intervals of one pulse per 1 to 2 hours, it is not possible to determine whether the single samples collected every 2 weeks occurred during, or after, an LH secretory pulse.

Decreased testicular development, testosterone and sperm production in the present study (Table 1) are similar to previous studies (Robertson et al., 1982; Adams et al.,1993; Daley et al., 1995). Anti-GnRH titers were negatively correlated with testis weights ($r = -70$) and testosterone concentrations ($r = -54$). This provides support for the hypothesis that testicular development and functions were suppressed as a result of the antibodies produced against GnRH. Tubular atrophy and the absence of spermatozoa were observed only in rams with anti-GnRH titers whereas there was no testicular atrophy in intact rams.

Daley et al., (1995) reported smaller final scrotal circumferences, testicular weights and lower testosterone concentrations in lambs immunized at 5 months of age than in intact rams. Immunization decreased serum concentration of testosterone, but did not completely inhibit testosterone production. In the present study, testicular circumferences and weights and testosterone concentrations were decreased in rams with high antibody titers compared to intact rams. In ISA lambs with lower anti-GnRH titers, testicular development and functions were greater than in FCA lambs. Reasons for the increased serum concentrations of testosterone at week 12 in the ISA lambs are unclear.

However, variability was very high among testis weights in the ISA lambs. Only 20 % of ISA lambs had testicles less than 100 g and the others had testicles as large as the non-immunized lambs. At week 8, 12 and 16, testicular circumferences were smaller in ISA than R lambs. However, at the last measurement prior to slaughter, testicular circumferences among the ISA and R lambs were similar. This could have been a result of the decrease in titer levels in ISA lambs which declined from 21 % at week 16 to 19 % close to slaughter. In contrast, titer levels in FCA lambs increased during the same period from 30% to 36%.

In contrast to Daley's study, which indicated that endogenous testosterone production was not completely inhibited by immunization, concentrations of serum testosterone were undetectable in both C and FCA lambs near the end of the study. This difference may be due to the differences among ages at immunization in the previous study (30 days) and in the current study (90 days).

Robertson et al. (1982) found no live sperm in the semen obtained from the tail of the epididymis within 30 minutes of slaughter from bulls immunized against GnRH. In the current study, FCA lambs did not have any detectable spermatozoa in their seminiferous tubules. However, no spermatozoa were observed in 50 % of the ISA rams and 31 % of the intact rams. The reason some of the intact and ISA rams which did not have spermatozoa in their testicles might be due to the effects of a longer photoperiod in confinement building. Although not a strong light source, one lamp in the entrance was always on during the trial. Alternatively, because the average ages of R and ISA lambs were about 6.5 and 7 months, they may not have all reached sexual maturity. Some animals in R and ISA groups were slaughtered at a very young age. For example lambs

#5 (R), #18 (ISA) were slaughtered at 122 d. of age, #24 (R), #22 (ISA) were slaughtered at 144 d. of age, and #20 (R) was slaughtered at 155 d. of age. The average age at slaughter for all lambs was 211 d.

In agreement with previous studies (Field et al., 1971; Seideman et al., 1982), this study confirmed that intact rams convert feed more efficiently into meat and gain faster than castrate lambs. As Schanbacher (1982) concluded, feedlot performance of ram lambs immunized against GnRH-KLH conjugate was comparable to castrate male lambs and lower than intact rams. Intact ram lambs consumed 1.120 kg less feed for 1 kg increase in live weight and gained 0.048 kg/day or 24.2% more than castrate lambs. In contrast, Daley et al. (1995) reported similar average daily gains for immunized and intact lambs. The FCA and ISA groups in this study had ADG and feed efficiencies similar to the C group, but lower than intact rams. Daley et al. (1995) used age (5 months) as the end point, while in this study a slaughter weight of 59 ± 2.3 kg. was the end point when the lambs averaged of 7 month of age.

Feed efficiencies and weight gains may have been improved if the animals had been slaughtered at a lower live weight because lambs gain slower as they approach slaughter weight. Feed efficiency and feedlot gain of ISA lambs were better than FCA and C lambs probably due to the higher testosterone concentrations in ISA lambs. Testosterone stimulates protein deposition and bone growth (Oltjen, 1982; Daley et al., 1995). In support of this concept, serum testosterone concentrations were correlated with feed efficiency ($r = -0.38$; $p<.01$) and average daily gain ($r = 0.46$; $p<.005$). Adams et al. (1993) also concluded that, sustained high rates of growth in GnRH immunized bulls could be caused by a residual level of secretion of testosterone.

Lean lamb carcass production is one of the issues challenging the American Sheep Industry because consumers prefer meat with less fat (Snowder et al., 1994a). Back fat thickness over the center of ribeye between the 12th and 13th ribs is used for U.S. Yield Grading Standards (USDA, 1992) for lamb carcasses (Snowder et al., 1994b). Genetics, sex, and environment affect fat deposition, and therefore, these factors affect carcass yields. Similar to previous results (Wilson et al., 1970; 72; Field et al, 1971; Anderson et al., 1991a), back fat thicknesses were greater in C lambs than intact lambs. Back fat deposition in immunized rams was similar with intact rams and less than in C lambs. Bodywall thicknesses varied among groups similar to backfat thicknesses. Daley et al., (1995) however, reported that back fat thicknesses of immunized lambs was intermediate between intact and castrated lambs at 5 months of age. Greater back fat thicknesses in C lambs in this study was probably due to the older age and weight of the lambs at slaughter. Amount of kidney fat was intermediate in immunized lambs between C and R lambs suggesting differential deposition of fat occurred among groups.

The leaner condition of intact animals has been attributed to high levels of testosterone which stimulate protein deposition and bone growth. Castrated animals reach physiological maturity at lighter weights and start to deposit fat earlier than intact animals (Oltjen, 1982). Carcasses from intact rams produce more boneless, closely trimmed, retail cuts than castrated rams (Taylor, 1994). In this study, yield grades for intact rams and rams immunized against GnRH-KLH conjugate were better than for castrated lambs. As in previous studies (Adams et al, 1996), fat was firmer in castrates than intact lambs. Inter-muscular fat deposition (marbling) was similar to back fat deposition among groups and correlated with backfat thickness ($r = 0.44$) and bodywall thickness ($r = 0.56$).

In this study, dressing percentages were higher in C and FCA lambs than R lambs. This higher dressing percentage was most likely due to fatness of the castrate lambs. However, removal of the testicles from intact rams may also affect dressing percentages. For example, in this study the testicles were 1.4 % of the carcass weights of the R group. Daley et al. (1995) reported dressing percentages of immunized lambs were higher than intact (50.1 %) and castrated (50.3 %) lambs, respectively. Slaughtering at a younger age (i.e. 5 months) and lighter live weight might account for these similar dressing percentages for castrate and intact lambs. Daley et al., (1995) concluded that immunization against GnRH resulted in commercially acceptable lamb carcasses intermediate to castrated and intact lamb carcasses and suggested the immunization procedure as a non-invasive alternative to surgical castration in lamb production.

Certain muscles in the body respond differently to testosterone (Field et al., 1989). Arnold et al. (1997) implanted wethers with testosterone to achieve serum testosterone concentrations twice that of normal for intact rams. In that study, combined muscle weight and growth of the splenius muscle in the neck were larger in wethers exposed to testosterone and in intact rams than non-implanted wethers. They concluded that increased splenius muscle growth associated with sexual maturity of rams is caused by testosterone. Development of some muscles in the neck and forequarter in intact males results in a situation called buckyness, an unfavorable condition that decreases quality grades of carcasses. In the current study, there was no difference among groups in quality grades, maturity of carcasses, ribeye area and fat color. Immunization against GnRH or castration did not change quality and muscle score, maturity of bone and lean.

In the present study, ram lambs immunized against GnRH-KLH or physically castrated exhibited less sexual behaviors toward estrous ewes than intact ram lambs. These results confirm previous results in castrated or immunized animals. Immunization against GnRH resulted in decreased concentrations of LH, FSH, and testosterone in serum, decreased sexual behavior and azoospermia in male rats. Sexual behaviors were maintained in these rats after administration of low-dose testosterone (Caleb et al., 1993). In addition to steroids that are required for the expression of the courtship and copulatory behavior (Crews, 1984), GnRH may have a role in regulation of sexual behaviors as a neurotransmitter (Rissman, 1996). Decreased sexual behaviors in actively immunized animals might be a result of decreased GnRH, LH and/or testosterone concentrations which act on the brain to stimulate sexual behaviors. However, in the present study it was not possible to determine whether the behavior changes were due to a lack of testosterone or GnRH. Anti-GnRH titers were negatively, and concentrations of testosterone were positively correlated with attempted mounts, mounts, ejaculation, and investigatory sniffing. Low concentrations of testosterone or decreased GnRH bioactivity in lambs with high antibody titers, or both, may have caused the decrease in sexual behaviors. Unexpectedly, castrated lambs exhibited numbers of attempted mounts and foreleg kicks similar to intact rams and some of them mounted the estrous ewes. The reason for these sexual behaviors might be high levels of GnRH or LH. GnRH release is probably increased in castrates and may have a role in sexual behaviors as discussed earlier.

Robertson et al. (1982) studied behaviors of immunized bulls and steers immunized against GnRH. Ten immunized bulls were subdivided into poor responders (n=5) with low antibody titers and good responders (n=5) with higher antibody titers.

Good responders had low serum concentrations of testosterone, involuted testis, reduced libido and semen production and docile behavior. Behaviors were examined in week 29 after immunization by exposing each animal to a cow in estrus. Number of mounts and ejaculations were less in the bulls with high antibody titers. Ease of handling of these animals was similar to steers. Similarly, in the current study number of mounts, ejaculations and investigatory sniffing were less in castrate and immunized lambs than intact lambs.

VI. IMPLICATIONS

Similar to castration, immunization against GnRH also reduced weight gain, feed efficiency and sexual behaviors. However, the immunization procedure resulted in leaner carcasses similar to those of intact rams. Partitioning of nutrients for growth and fat deposition appeared to differ among immunologically castrated and mechanically castrated lambs probably due to residual testicular activity. Therefore, immunization against GnRH has the potential to be a non-invasive alternative to surgical or mechanical castration if high antibody titers to GnRH are consistently induced. In the current project, Freund's complete adjuvant more effectively induced antibodies then the ISA adjuvant.

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VIII. APPENDIX

Nutrient composition of diets.

	Diet 1	Diet 2	Diet 3
Dry matter (%)	89.5	89.0	88.6
	----------% of dry matter----------		
Crude Protein	14.7	13.4	11.9
TDN	65.1	73.9	81.4
ME (Mcal/kg)	2.30	2.65	2.95