

EFFECT OF MONENSIN SODIUM
ON THE REPRODUCTIVE ENDOCRINOLOGY
OF PREPUBERTAL BEEF HEIFERS

A Thesis
Submitted to the Faculty
of
Graduate Studies
The University of Manitoba
by
Murray James Pettitt

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science
Department of Animal Science
May 1991



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ISBN 0-315-76890-8

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MURRAY JAMES PETTITT

A thesis submitted to the Faculty of Graduate Studies of
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ABSTRACT

Pettitt, Murray James. M.Sc., The University of Manitoba, May, 1991. Effect of Monensin Sodium on The Reproductive Endocrinology of Prepubertal Beef Heifers.

Major Professor; Dr. W. M. Palmer.

The feeding of monensin sodium to prepubertal beef heifers at 200 mg/head/day has been demonstrated to reduce age at puberty in these animals. This is thought to occur due to the changes in volatile fatty acid (VFA) production in monensin-fed animals. While the increase in propionate production can account for the improvement in feed efficiency, it is unknown how these changes in VFA production influence the age of puberty. This experiment was carried out to monitor prepubertal hormone levels in beef heifers and to determine if monensin affected puberty through changes in these hormones.

Twelve Angus crossbred and twelve Simmental crossbred prepubertal heifers were used in this trial. One half of each breed was fed a control diet (C), the other one half the identical diet plus 200 mg/head/day monensin sodium (M). Feed intake was similar for both groups, however M heifers had improved weight gains over C ($p < 0.065$).

Monensin increased propionate production ($p < 0.001$) and total VFA production ($p < 0.03$) while depressing butyrate production ($p < 0.065$). Mean serum progesterone concentrations were similar between M and C animals until day 165 of the experiment when progesterone levels of C animals rose significantly higher than M animals ($p < 0.008$). Serum LH patterns were not significantly affected by the feeding of monensin.

The data suggests that the effect of monensin in reducing age at puberty is not mediated through a change in episodic LH release or serum progesterone concentrations. Further research will be required to determine which physiological mechanism(s) monensin acts upon in order to influence puberty.

ACKNOWLEDGMENTS

Sincere gratitude is extended to Dr. W.M. Palmer for his advice, guidance and patience throughout these studies. I am also grateful to all of the Faculty, staff and graduate students of the Department of Animal Science who assisted me during this program, without whose help this study would not have been possible. The assistance of Dr. G.H. Crow and Dr. M.M. Buhr in the design of the project is especially appreciated. Special thanks to Dr. M.A. Sheikheldin for his support, assistance and encouragement throughout the project. I would also like to thank Lorne Dawydiuk, Bob Lavallee, Dale Rosner and Gilbert Perron for taking excellent care of the animals at the Glenlea Research Station. Many thanks to Mary Cheang, M. Math., Department of Community Health Science for additional statistical consultation and sincere thanks to Mrs. Irene Tatsumi for excellent assistance in the preparation of this manuscript. I cannot thank my parents and family enough for the constant encouragement and belief in me as I completed these studies.

The donation of the monensin sodium (Rumensin®) premix by

Elanco, Eli Lilly and Co. is acknowledged as is the donation of the LH reference preparation by the USDA Reproduction Lab, Beltsville, MD. Financial support was provided by Natural Sciences and Engineering Research Council and the Department of Animal Science, University of Manitoba.

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in the herd.

This experiment was carried out to try to determine how monensin influences puberty by monitoring reproductive hormone levels in beef heifers as they approached and achieved puberty.

LITERATURE REVIEW

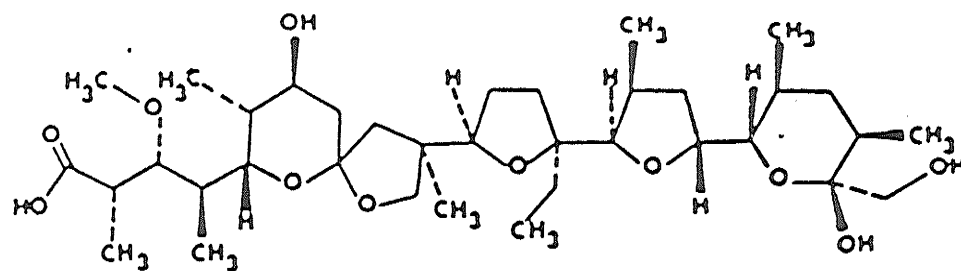
Monensin sodium, a carboxylic polyether ionophore antibiotic (Figure 1) produced by a strain of Streptomyces cinnamomensis (Herberg et al, 1978) was originally developed for use as an anticoccidial feed additive in poultry (Bergen and Bates, 1984). When added to the diet of growing and finishing cattle, studies indicated that monensin improves production efficiency and that this is mediated through modification of several physiological systems of the animal (Schelling, 1984).

Changes in these physiological systems are most likely a result of the basic mode of action of monensin, which is modification of ion transport across the membranes of rumen microbes (Bergen and Bates, 1984; Schelling 1984; Russell and Strobel, 1989).

2.0 Effects of Monensin on Volatile Fatty Acid Production

The most widely documented system affected by monensin is volatile fatty acid (VFA) production within the rumen (Bergen and Bates, 1984). Metabolic hydrogen is produced by fermentation of glucose and pyruvate by rumen micro-organisms. The majority of this metabolic hydrogen is utilized in the

FIGURE 1. Chemical Structure of Monensin Sodium



From Painter and Pressman, 1985.

synthesis of propionate and butyrate from pyruvate and in the reduction of carbon dioxide to methane (Chalupa, 1977). Increasing the amount of propionate produced in the rumen has been a goal of researchers for many years for several reasons. Propionic acid production from hexose in the rumen is more efficient than either butyric or acetic acid formation. Thus, an increase in propionate to acetate ratio represents an increase in the efficiency of removing useful energy from the feedstuff (Chalupa, 1977). Another reason is that animal tissues may utilize propionate more efficiently than acetate since propionate has a higher enthalpy than acetate and can be oxidized by the animal (Russell and Strobel, 1989). Finally, propionate is the only VFA which is gluconeogenic (Judson, 1973; Schelling, 1984) and having more substrate available for glucose synthesis may be advantageous to the animal by sparing amino acids from glucose synthesis and maintaining their availability for protein synthesis.

When monensin is fed to cattle in the range of 100-200 mg/head/day, molar percentages of propionic acid increase while those of acetic and butyric acids decrease (Perry et al, 1976; Richardson et al, 1976; Mowat et al, 1977; Oscar et al, 1987; Beacom et al, 1988). This effect of monensin is widely accepted as one of the major physiological results of its use. However, it is unlikely that it is responsible for all the improvements in animal performance associated with feeding

monensin. Raun et al (1976) demonstrated that increased ruminal efficiency due to monensin could not account for all of the improvement in feed efficiency observed in their experiments. Thus, it appears that monensin may exercise some of its benefits through other physiological systems.

2.1 Effects of Monensin on Nitrogen Utilization

2.1.1 Protein Synthesis

Nitrogen utilization in the rumen involves two major processes: protein synthesis and methane gas production. The rumen contains a dynamic pool of nitrogen whose sources include: 1) microbial degradation of dietary protein and hydrolysis of dietary non-protein nitrogen (NPN), 2) hydrolysis of urea recycled to the rumen, and 3) breakdown of microbial protoplasm. The destinations of this ruminal nitrogen include: 1) microbial incorporation, 2) absorption through the rumen wall, and 3) the omasum (Owens and Bergen, 1983). A certain proportion of dietary sources of preformed proteins can escape ruminal digestion and are termed bypass proteins. The amino acids from these proteins are available for absorption directly in the small intestine (Bergen and Bates, 1984) and are then available for protein synthesis. Increasing the amount of bypass protein available to the animal improves production efficiency due to the increase in direct absorption of dietary amino acids (Chalupa, 1977).

Poos et al (1979) fed monensin to cannulated steers at a level of 200 mg/head/day and found an increase in dietary protein reaching the abomasum and a reduction in bacterial nitrogen reaching the abomasum. Monensin supplied at 200 mg/head/day is equivalent to a concentration of 33 ppm of the ration as fed. Similar results were reported by Isichei and Bergen (1980). Faulkner et al (1985), found that feeding monensin at a level of 18.3 ppm decreased bacterial protein concentration (diaminopimelic acid - DAP) and increased the ratio of total nitrogen:DAP in rumen dry matter. The response was quadratic and the larger ratio suggests that less protein was broken down and/or less bacterial protein synthesized. An increase in the protein gain/protein intake ratio was accompanied by a higher average daily gain (ADG) and lower feed:gain for monensin fed animals compared to controls (McCarthy et al, 1979). This "protein-sparing" effect due to monensin is most significant in animals fed a low protein diet (Beede et al, 1980a; Hanson and Klopfenstein, 1979) compared to higher levels of dietary protein.

When propionate is administered in the abomasum of growing goats, the amount of L-threonine undergoing gluconeogenesis and oxidation is reduced as measured by isotope dilution techniques (Beede et al, 1980b). This suggests a greater availability of L-threonine for protein synthesis and agrees with the data which suggests that increased propionic acid

production may have an amino acid sparing effect as intravenous infusion of propionate will increase nitrogen retention in growing lambs (Eskeland et al, 1974). Presumably, this increase in nitrogen retention results in an increase in protein synthesis or cellular uptake of amino acids (Potter et al, 1968). Therefore, monensin has a direct effect on nitrogen retention in the ruminant by increasing the amount of dietary protein available for tissue synthesis. This is most likely because the increased availability of propionic acid for gluconeogenesis decreases the use of amino acids for this purpose (Schelling, 1984).

2.1.2 Methane Gas Production

The effects of monensin on methane gas production have also been documented. Acetate, hydrogen, carbon dioxide and formate (which is easily converted to carbon dioxide and hydrogen by many microbes) are the major precursors for the methanogenic microbes which convert the carbon dioxide and hydrogen to methane (Baldwin and Allison, 1983). The loss in feed energy due to methane production can be as high as 12%, as this gas is ultimately eructated (Russell and Strobel, 1989). Monensin added to in vitro ruminal fluid batch cultures can reduce methane formation by as much as 49% (Chalupa et al, 1980; Katz et al, 1986; Oscar et al, 1987; Sauer and Teather, 1987). In vivo studies in growing lambs have confirmed these results (Poos et al, 1979). When added

to in vitro cultures containing substrates specific for methane bacteria, monensin decreased methane production in substrates containing formate but not in substrates containing carbon dioxide and hydrogen (Van Nevel and Demeyer, 1977; Dellinger and Ferry, 1984). Both of these substrates (formate; carbon dioxide and hydrogen) are specific for methane bacteria (Baldwin and Allison, 1983). Since hydrogen production is less than expected in monensin-containing cultures with lowered methane production (Van Nevel and Demeyer, 1977) and one of the major ruminal methanogenic bacteria is not sensitive to monensin (Oscar et al, 1987), it has been concluded that the methane-depressing action of monensin is not due to a direct toxic effect on the methanogenic flora in the rumen, but due to the inhibition of the organisms which metabolize formate to carbon dioxide and hydrogen, the major precursors for methane synthesis (Van Nevel and Demeyer, 1977). This would allow for a diversion of metabolic hydrogen from methane production to propionate production (Chen and Wolin, 1979) which is consistent with the shifts in ruminal VFA profiles already discussed and can partially account for the improvement in energy management by the animal fed monensin.

2.2 Effects of Monensin on Feed Efficiency

Modified feed efficiency is another physiological factor affected by monensin which is most likely due to the changes

in VFA production and bypass protein levels. Raun et al (1976) found that as the level of monensin fed to feedlot steers increased, feed consumption progressively decreased and daily gains were equal to or superior than non-treated controls. When finishing steers were fed a ration consisting mostly of alfalfa silage, feed efficiency improved due to an increase in ADG with no significant change in feed intake per unit gain (Mowat et al, 1977). When the diet was changed to include a source of readily fermentable carbohydrate in addition to the alfalfa silage, feed efficiency was improved because feed intake was reduced with no effect on ADG (Mowat et al, 1977). Other studies (Perry et al, 1976; Raun et al, 1976; Oscar et al, 1987) confirm that when monensin is fed to feedlot animals receiving a high carbohydrate ration, feed efficiency is improved through reduced intake with no significant change in ADG. In those animals consuming primarily a forage ration, feed efficiency increases because of improved daily gains with no significant change in feed intake (Potter et al, 1976a; Turner et al, 1977; Faulkner et al, 1985; Beacom et al, 1988). These improvements in feed efficiencies are accompanied by similar carcass characteristics when finishing animals are fed monensin compared to controls (Perry et al, 1976; Potter et al, 1976b; Mowat et al, 1977). In a review of over 200 trials involving nearly 16,000 head of cattle fed a wide range of rations those

fed monensin gained, on average, 1.6% faster, consumed 6.4% less feed and required 7.5% less feed per 100 kg gain with no significant influence on carcass characteristics compared to controls (Goodrich et al, 1984). For these reasons, monensin is widely used in the feedlot industry today.

2.3 Effects of Monensin on Reproductive Stock

2.3.1 Influence on Feed Efficiency

Another major area of investigation has been the influence of monensin on reproductive stock. Including monensin in the pre-calving diet of mature beef cows allows for equal weight gains on less feed (Turner et al, 1980; Walker et al, 1980; Clanton et al, 1981) or improved gains at the same feed level (Turner et al, 1977; Grings and Males, 1988). Weight loss post-partum has been demonstrated to be similar between control and monensin-fed animals. However, the monensin animals consume less feed during this time (Lemenager et al, 1978a; Turner et al, 1980; Walker et al, 1980) resulting in an improvement in feed efficiency.

2.3.2 Influence on Reproductive Parameters

The effects of monensin on reproductive parameters when fed to mature cows during the pre- and post-calving period have been variable. Post-partum interval to estrus (or days post-partum to insemination) is not influenced by monensin in those animals whose body condition score did not change

throughout the trial (Turner et al, 1980; Walker et al, 1980; Clanton et al, 1981; Pendlum et al, 1981; Wagner et al, 1983; Grings and Males, 1988). When cow body condition is improved throughout the trial though, feeding monensin shortens the post-partum interval to estrus by 12 to 21 days (Turner et al, 1977; Hixon et al, 1982; Hardin and Randel, 1983). Animals in a heavier or "fleshy" body condition have a shorter post-partum interval than cows in thin condition (Sprott et al, 1988). Thus, when monensin feeding increases body weight and improves condition, post-partum interval to estrus will be reduced.

Monensin supplementation has no effect on first service conception rate (Walker et al, 1980), total % conception (Pendlum et al, 1981; Grings and Males, 1988), calving interval (Turner et al, 1980; Pendlum et al, 1981), calf birth weight (Walker et al, 1980; Pendlum et al, 1981) or calf weight gains (Turner et al, 1980; Grings and Males, 1988). Clanton et al (1981) demonstrated increased calf birth weight in monensin-fed animals, however, these differences were not significant at weaning. Hixon et al (1982) also reported heavier calf birth weights due to monensin accompanied by an increase in dystocia. Increased energy levels to the dams due to the monensin effect of improving feed efficiency may account for the increase in calf birth weights (Hixon et al, 1982).

2.4 Endocrinology of Puberty

2.4.1 Gonadostat Hypothesis

The classical "gonadostat" hypothesis suggests that the onset of puberty is due to a decrease in sensitivity of the hypothalamo-pituitary axis to the negative feedback effects of estradiol (Ramirez and McCann, 1963). As the animal matures, pituitary gonadotrophin secretion increases, as the sensitivity to steroid negative feedback is reduced, resulting in follicular growth and the first ovulation. In cattle, this hypothesis is based largely on the fact that gonadotrophin secretion escapes steroid inhibition at the pubertal age in ovariectomized heifers which have received estradiol implants (Day et al, 1984).

2.4.2 Effect of Nutrition on Puberty

Luteinizing hormone (LH) secretion and LH pulse frequency gradually increase in a linear manner as puberty approaches (Day et al, 1984). Prepuberal heifers receiving a low plane of nutrition (0.21 kg ADG) throughout the expected time of puberty demonstrate significantly reduced mean LH concentration, LH pulse frequency and LH pulse amplitude compared to controls (0.79 ADG) during the time immediately prior to puberty (Day et al, 1986). This was accompanied by minimal weight gains in the energy-stressed heifers, none of which reached puberty during the experiment. All but one of the control animals, which all had normal weight gains during

this period, reached puberty during the trial. Thus, restriction of dietary energy prevented the prepubertal rise in LH secretion and delayed the occurrence of puberty (Day et al, 1986). Other researchers have also demonstrated a delay of puberty in nutritionally stressed heifers (Wiltbank et al, 1969; Arije and Wiltbank, 1971; Ferrell, 1982).

Since energy level of the diet fed can influence LH secretion and thus puberty, and the feeding of monensin results in more feedstuff energy being available to the animal through increased propionate production (Chalupa, 1977), a possible mechanism by which monensin exerts its effects on puberty in the heifer may be through modification of prepubertal LH patterns. To date, no one has investigated this possibility.

2.5 Influence of Monensin on Puberty

The effects of monensin on puberty in the heifer have been the subject of many studies. Moseley et al (1977) determined that feeding monensin to prepuberal Brahman x Hereford crossbred heifers resulted in a significantly higher proportion of monensin-fed heifers reaching puberty during the trial than controls. This was accompanied by a 10.9% decrease in feed to gain ratio in the monensin heifers compared to controls. No significant difference was found in weight gains or conception rates between the two groups. In a trial by McCartor et al (1979) heifers fed 200 mg monensin/ head/day

reached puberty 29.5 days sooner and weighed 17.2 kg less than controls. Significant differences were not found between monensin and control groups for initial experimental weight, final experimental weight, ADG while on test, initial condition score and final condition score. When all the factors that are known to affect age at puberty are controlled by experimental design, monensin appears to decrease the age at puberty, presumably by shifting ruminal fermentation towards greater propionate production and therefore increasing available energy from the feedstuff (McCartor et al, 1979).

This conclusion is substantiated by the fact that a third group of heifers, who were fed a high concentrate diet without monensin, achieved puberty at a younger age and lighter weight than did controls. This high concentrate diet also shifted ruminal fermentation towards greater propionate production, as it did in the monensin-fed group (McCartor et al, 1979). The fertility of the heifers, measured by first service pregnancy rate, was not significantly affected by treatment.

A similar study found that heifers fed monensin were significantly younger at puberty and that this difference was not due to increased ADG or increased body weight. Neither weight at puberty or conception rates were affected by monensin (Moseley et al, 1982). Granger et al (1990) also demonstrated a decrease in age at puberty in monensin-fed heifers. Weight at puberty and calving rate were not affected

by monensin.

2.6 Alteration of Endocrine Systems in Animals Fed Monensin

The previous studies indicate that while monensin decreases the age at puberty, this is not due to increased ADG or body weight. It has been suggested that if age at puberty is lowered, then perhaps the maturation of the endocrine system responsible for puberty occurs sooner (Moseley et al, 1982) and that this may be due to the higher energy level supplied to the animal fed monensin. While it is still unclear as to what mechanism(s) trigger puberty it is known that many of the individual components of the reproductive endocrine system are functional prior to the onset of estrous cycles. Several of these have been explored after the feeding of monensin to prepuberal heifers.

Bushmich et al (1980) administered a 1.0 mg porcine follicle stimulating hormone (FSH-P) / 2500 IU human chorionic gonadotrophin (HCG) challenge to control and monensin-fed prepuberal heifers and observed an ovarian response in both groups. The response in the monensin group was much greater than that of controls. Heifers fed monensin had more corpora lutea (C.L.), greater total luteal weight, more follicles, greater ovarian weight and greater weight of follicular fluid and stroma than controls. Monensin-fed heifers had slightly larger C.L. than controls which contained similar progesterone concentrations as controls. This gave greater luteal

progesterone per C.L. and greater luteal progesterone per heifer in the monensin heifers than the controls.

Another study, in which multiple gonadotrophin releasing hormone (GnRH) injections were administered to prepuberal heifers, measured LH release from the pituitary. Two 100 μ g injections of GnRH, four hours apart, were given to monensin-fed and control heifers. Blood was collected at ten minute intervals for nine hours following the first GnRH injection. Amount of LH released after each GnRH injection, peak LH after the first GnRH challenge, peak LH after the second GnRH challenge, duration and area under the second GnRH-induced LH curve were significantly greater for monensin-fed heifers than controls. Area under the first GnRH-induced LH curve tended to be greater for monensin than control heifers, but this was not significant. Therefore dietary monensin supplied at 200 mg/head/day enhances the ability of the pituitary to release LH after a multiple GnRH challenge (Randel and Rhodes, 1980).

These findings are supported by the results of Randel et al (1982) who measured the estradiol-17 β (E2) -induced LH surge in prepuberal heifers. After 14 days of a ration containing 0 mg or 200 mg monensin/head/day, heifers were injected with 5 mg estradiol-17 β . Blood samples were taken every two hours for 48 hours following the E2. Peak LH concentration was not affected by the feeding of monensin. However, monensin heifers had a longer duration of LH surge, greater area under

the LH curve and reached peak LH concentrations earlier than did controls. Thus the authors concluded that monensin alters the E2-induced LH response in prepuberal heifers (Randel et al, 1982).

In contrast, the feeding of monensin to mature cows prior to a GnRH challenge resulted in reduced LH release from the pituitary (Saturnino et al, 1985). Ovarian size and number of follicles were increased in the monensin group. Similarly, mean LH concentration, LH pulse frequency and LH pulse amplitude was not affected by monensin after administration of prostaglandin F-2 α (PGF-2 α) to mature cows (Peterson et al, 1984). These discrepancies may reflect the differences in maturational status of the hypothalamic-pituitary axis that exist between prepuberal heifers and mature cows.

2.7 Effect of Propionate on Reproductive Endocrinology

While the mechanism by which monensin exerts its effects on the prepuberal heifer is not understood, it can be demonstrated that monensin enhances both pituitary and ovarian response to exogenous hormones which may reflect an earlier maturation of the hypothalamic-hypophyseal axis in heifers fed monensin. This early maturation could be due to the shifts in VFA patterns. LH response was enhanced after multiple GnRH challenges in prepuberal heifers which were infused with propionate via an abomasal cannula (Rutter et al, 1983). Amount of LH released after the first of two GnRH injections

was significantly higher for propionate-infused heifers compared to controls. There was also a trend for area under the LH curve and peak LH concentration to be greater for the propionate-infused animals. Thus, this study supports the hypothesis that monensin, through its effect of increasing propionate production in the rumen, enhances pituitary response to exogenous gonadotrophins.

2.8 Onset of Puberty

It is not possible at this time to state how monensin enhances the onset of puberty in the heifer as the mechanism by which puberty is triggered in the natural state remains unknown. While the gonadostat theory states that GnRH escapes from estradiol inhibition at puberty, the cause of this decrease in pituitary sensitivity to ovarian influence is undetermined. The preovulatory LH surge is not responsible as prepubertal heifers given pulsatile infusions of GnRH demonstrate preovulatory-like LH surges, but this is not followed by puberty (Skaggs et al, 1986). Similarly, creating a preovulatory-like estradiol surge in prepubertal heifers does not result in puberty (Gonzalez-Padilla et al, 1975b). Puberty can be initiated in heifers given a combination of estradiol and progesterone, and this is followed by normal pregnancy rates (Gonzalez-Padilla et al, 1975c). However, since this treatment is effective only in animals near their expected age of puberty and not younger

animals, it has recently been suggested that the onset of puberty is due to the final maturation of some central mechanism which controls gonadotrophin secretion. This hypothesis states that this central mechanism is not responsive to ovarian steroids until just prior to the onset of puberty, presumably when the final maturation event occurs (Dodson et al, 1988). What this central mechanism and maturation event are remains undetermined at this time, and further investigation is required to determine if this is indeed the trigger for puberty.

MATERIALS AND METHODS

3.0 Management of Animals

Twelve Simmental crossbred and twelve Black Angus crossbred heifers born during March and April 1986 at the Glenlea Research Station, University of Manitoba were used in this study. Six animals of each breed were placed on a ration consisting of corn silage and barley concentrate containing 0 g monensin/tonne (control). The remaining six of each breed were placed on the same ration in which the concentrate contained monensin at a level to provide 11 g/tonne of ration (monensin). After 55 days of this introductory ration, the level supplied in the monensin ration was increased to the final concentration of 33 g/tonne. Water and salt were provided ad libitum. At the beginning of the trial, animals were fed daily nutrient requirements and energy content as recommended by the National Research Council recommendations (National Research Council, 1984) (Table 1). Energy content of the ration was increased on day 75 of the trial in order to increase the daily weight gains of the animals. The test rations (Table 2) were fed from November 22, 1986 to May 26, 1987.

TABLE 1. Nutrient Requirements of Experimental Animals*

	<u>Medium Frame Heifer Calves</u>	<u>Large Frame Heifer Calves</u>
Body weight (kg):	250	273
Average daily gain (kg):	0.455	0.682
^a Requirements:		
DM intake (kg):	5.82	6.73
Protein intake (kg):	0.52	0.64
Protein (%):	9.1	9.6
Me (Mcal/kg):	2.24	2.31
NE _m (Mcal/kg):	1.39	1.43
NE _g (Mcal/kg):	0.79	0.86
TDN (%):	62.0	64.0
Ca (%):	0.29	0.33
P (%):	0.21	0.19
Vitamin A (I.U./kg):	2200.0	2200.0
Vitamin D (I.U./kg):	275.0	275.0

*Nutrient Requirements of Beef Cattle, National Research Council, 1984.

^aCalculated requirements based on expected average body weight over duration of experiments

TABLE 2. Experimental rations (kg Dm offered/head/day)

DAYS OF TRIAL FED:	<u>1 - 66</u>	<u>67 - 74</u>	<u>75 - 150</u>	<u>151 - 161</u>	<u>162 - 175</u>	<u>176 - 186</u>
<u>Angus - Cross</u>						
Corn silage	4.5	5.0	4.5	-	-	-
Barley silage	-	-	-	4.5	4.5	-
Brome hay	-	-	-	-	-	4.5
Barley	1.06	1.16	1.84	1.84	1.06	1.06
Soybean meal	0.15	0.16	0.25	0.25	0.15	0.15
Co I - Salt	0.005	0.009	0.009	0.009	0.005	0.005
Wheat middlings	0.009	0.014	0.02	0.02	0.009	0.009
<u>Simmental - Cross</u>						
Corn silage	5.45	6.8	4.5	-	-	-
Barley silage	-	-	-	4.5	5.45	-
Brome hay	-	-	-	-	-	5.45
Barley	1.05	1.31	2.94	2.94	1.05	1.05
Soybean meal	0.14	0.18	0.40	0.40	0.14	0.14
Co I - Salt	0.006	0.009	0.02	0.02	0.006	0.006
Limestone	0.007	0.009	-	-	0.007	0.007
Wheat middlings	0.009	0.014	0.03	0.03	0.009	0.009

- Control and monensin rations identical except that wheat middlings of monensin ration contained sufficient monensin premix to supply 11 g activity/tonne from days 1 - 55 and 33 g activity/tonne from days 56 - 186.

Animals were grouped into pens of three containing the same breed and ration. The pens were balanced for weaning weight, age of dam and sire. Pen space was 10 m² and bunk space was 1.0 m per animal in a three-sided barn. Age and weight of heifers at the beginning and at the end of the feeding trial are shown in Table 3.

Feed consumption per pen was recorded weekly. Average body weight, taken from two consecutive weigh days each month, was used to monitor weight gains.

Estrus behavior was monitored visually from 0800-0820 and 1600-1620 hours daily. Ovarian examination by rectal palpation was carried out June 2, 1987. At the end of the experiment on May 26, 1987 all animals were placed in drylot with the other replacement heifers from the herd and were subsequently placed on pasture with a bull on June 15, 1987. Pregnancy checks were done September 30, 1987.

3.1 Volatile Fatty Acid Analysis

Rumen samples were obtained from each animal twice throughout the trial (days 145 and 186) for volatile fatty acid (VFA) determination. Sample collection was approximately 24 hrs after the last feeding and pH was determined immediately after obtaining the sample. One ml of 25% metaphosphoric acid (HPO₃) was added to 5 ml of each sample and frozen overnight at -20°C. After thawing, samples were centrifuged at 10,000 rpm for 10 min. The supernatant was

TABLE 3. Average Age and Weight of Heifers on Trial.

	<u>Beginning of Trial</u>		<u>End of Trial</u>		<u>Breed Average for Attainment of Puberty*</u>	
	Age (days)	Weight (kg)	Age	Weight	Age	Weight
Angus-Cross	232	221	418	335	410	309
Simmental-Cross	237	218	423	336	348	328

*Ferrell, 1982

decanted to a clean vial and stored for gas chromatography (G.C.) analysis of VFA's. Analysis was performed on a Varian 6000 G.C. linked to a Varian Vista 402 data processor. Chromatography was done by using a 2 m long (2.0 mm I.D.) column packed with G.P. 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb[®] W AW which was set at an operating temperature of 125°C. Carrier gas was helium and sample size was 1.0 μ l (Bulletin #749E, 1975, Supelco, Inc., Oakville, ON, Canada).

3.2 Radioimmunoassays

3.2.1 Progesterone

Blood samples, obtained by jugular venipuncture two times per week, were analyzed for serum progesterone to monitor ovarian activity. Serum was stored at -20°C until radioimmunoassay (RIA) was performed.

Progesterone was determined by the assay originally reported by Abraham et al (1971) as modified by Yuthasastrakosol et al (1974) and Sheikheldin et al (1988). Progesterone standard (4-pregnen-3,20-dione: Steraloid Inc., Wilton, NH, USA) was prepared in charcoal-stripped serum from an ovariectomized ewe. Standards and unknowns were extracted with petroleum ether.

Labelled progesterone ($[^3H]$ progesterone: New England Nuclear, Boston, MA, USA) was prepared in the assay buffer to give approximately 8000 c.p.m. per 100 μ l per tube.

Progesterone antibody (obtained from Dr. N. C. Rawlings, Department of Veterinary Physiological Sciences, W.C.V.M., University of Saskatchewan, Saskatoon, Saskatchewan, Canada) was used at a dilution of 1:10,000. Cross-reactivity with cholesterol, testosterone, hydrocortisone and estradiol 17-B was 0.47%, 0.04%, 0.01% and 0.01%, respectively.

Samples were assayed in volumes of 0.5 ml per tube in duplicate and counted for 4.0 minutes in a liquid scintillation counter (Rackbeta #1217: LKB, Wallac OY, Turku, Finland). The sensitivity of the assay at 95% binding was 26.0 pg/tube (n=8 assays). Repeated assays of heifer serum pools containing known amounts of progesterone resulted in an intra-assay coefficient of variation (C.V.) of 1.7%. The corresponding inter-assay C.V. was 5.4% (Robard et al, 1968). Unknown concentrations were calculated from a smoothed splined standard curve generated by the instrument RIA program (Wold, 1974). All samples from each animal were estimated in the same assay.

3.2.2 Luteinizing Hormone

Blood samples were obtained by jugular catheter for serum LH determination. Samples, collected every 20 min for 7 hrs beginning at 1200 hr, were obtained every three weeks beginning on day 75 of the trial at an average animal age of 309 days. A total of six sampling periods were carried out. Serum was stored at -20°C until RIA for LH was performed.

LH was determined by the assay originally described by Niswender et al (1968) as modified by Howland (1972). LH standard (USDA-bLH-I-1, USDA Reproduction Lab, Beltsville, MD, USA) was prepared in the assay buffer. Labelled USDA-bLH-I-1 (^{125}I -bLH) was prepared in assay buffer according to Greenwood et al (1963) to provide approximately 7500 c.p.m. per 0.1 ml.

Ovine/bovine LH antiserum (obtained from Dr. N. C. Rawlings, Department of Veterinary Physiological Sciences, W.C.V.M., University of Saskatchewan, Saskatoon, Saskatchewan, Canada) was used at a dilution of 1:36,000. Sheep anti-rabbit gamma-globulin (produced in our laboratory) was used at 5% as the second antibody.

Assays were performed on 0.2 ml serum aliquots and were counted for 1 min in a micro-computer controlled gamma counter (CompuGamma #1282: LKB, Wallac OY, Finland). Total binding was 23.0% and non-specific binding (NSB) was 3.0% (n=11 assays). Sensitivity of the assay at 95% binding was 0.04 ng/tube. Serum pools containing 0.78 and 12.50 ng/ml USDA-bLH-I-1 assayed repeatedly gave intra-assay C.V. of 5.3% and 10.2% respectively. The corresponding inter-assay C.V. were 8.1% and 22.8% respectively (Robard et al, 1968).

Concentration of the unknowns from the standard curve was calculated by a micro-computer curve fitted by spline functions (Wold, 1974). All samples from any one animal were analyzed in the same assay.

3.3 Statistics

Feed intake, ADG and VFA data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, Inc., 1985). Mean progesterone concentrations between treatments were analyzed by ANOVA.

LH data was initially analyzed by the Pulsar program (Merriam and Wachter, 1982) which identifies baselines, peaks, amplitudes, frequency of peaks, duration of peaks and interpeak interval in episodic hormone patterns. This program removes long-term trends, such as circadian rhythms, from the data series to generate a smoothed data series. Each point of the smoothed series is subtracted from the original raw data point and the residual series is used to determine peaks (Merriam and Wachter, 1982). Peaks are defined based on their amplitude from the previous nadir point (Santen and Bardin, 1973). Values generated by the Pulsar program for each LH data series were subsequently analyzed by GLM ANOVA (SAS, Inc., 1985). All analysis of variance means were compared by using the least-squared means after performing the Bonferroni adjustment.

RESULTS

4.1 Feed Intake and Weight Gains

Dry matter (DM) intake was similar for both breeds and both treatments throughout the trial (Table 4). Calculated net energy maintenance (NE_m) intake and net energy gain (NE_g) intake were not significantly different between breed or treatment (Table 5).

Daily weight gains over the entire experiment did not differ significantly ($p>0.05$) between the two breeds, however monensin-fed animals did gain weight at a faster rate than did controls during the entire trial (Table 6).

4.2 Volatile Fatty Acid Production

Volatile fatty acid production in the rumen for each treatment is shown in Table 7. The feeding of monensin increased the percentage of propionate produced, decreased the percentage of butyrate produced and enhanced total VFA production. Production of the remaining VFA's did not differ between treatments. Table 8 illustrates VFA production by each breed. Angus-cross animals had a lower percentage isobutyrate and a greater percentage isovalerate than did the

TABLE 4. Dry Matter Intake*

<u>Angus-Cross</u>	<u>Simmental-Cross</u>	<u>Significance</u>
16.6 ± 0.21	17.06 ± 0.33	NS
<u>Control</u>	<u>Monensin</u>	
16.6 ± 0.26	17.0 ± 0.30	NS

*kg/pen/day (mean ± sem) over entire trial.

TABLE 5. Calculated NE_m and NE_g Intake*

NE _m Intake		
<u>Angus-Cross</u>	<u>Simmental-Cross</u>	<u>Significance</u>
29.0 ± 0.39	30.1 ± 0.64	NS
<u>Control</u>	<u>Monensin</u>	
29.2 ± 0.48	29.9 ± 0.57	NS
NE _g Intake		
<u>Angus -Cross</u>	<u>Simmental-Cross</u>	<u>Significance</u>
18.8 ± 0.26	19.6 ± 0.43	NS
<u>CONTROL</u>	<u>MONENSIN</u>	
18.9 ± 0.32	19.4 ± 0.38	NS

*Mcal/pen/day (mean ± sem) over entire trial

TABLE 6. AVERAGE DAILY GAINS*

<u>Weigh Period</u>	<u>Angus-Cross</u>	<u>Simmental-Cross</u>		<u>Control</u>	<u>Monensin</u>	
Day 1-Day 28	0.48 ± 0.05	0.40 ± 0.05		0.46 ± 0.05	0.41 ± 0.06	
Day 29-Day 56	0.39 ± 0.05	0.17 ± 0.08		0.23 ± 0.08	0.33 ± 0.06	
Day 57-Day 84	0.40 ± 0.05	0.36 ± 0.05		0.37 ± 0.06	0.39 ± 0.04	
Day 85-Day 112	1.02 ± 0.03	1.19 ± 0.06		1.06 ± 0.04	1.15 ± 0.06	
Day 113-Day 140	0.91 ± 0.07	0.92 ± 0.09		0.90 ± 0.08	0.92 ± 0.08	
Day 141-Day 168	0.90 ± 0.05	1.04 ± 0.05		0.92 ± 0.06	1.01 ± 0.04	
Day 169-Day 186	0.04 ± 0.09	0.22 ± 0.07		0.09 ± 0.07	0.27 ± 0.07	
			<u>Signif.</u>			<u>Signif.</u>
Overall	0.60 ± 0.03	0.63 ± 0.04	NS	0.57 ± 0.03	0.65 ± 0.03	p<0.065

*Kg/head/day(mean ± sem)

TABLE 7. Rumen Volatile Fatty Acid Production, by Treatment (mean \pm sem.)
(n = 48)

	<u>Control</u>	<u>Monensin</u>	<u>Significance</u>
Total (mg/gm)	232.3 \pm 16.03	282.8 \pm 17.41	p <0.03
Acetate ^a	54.3 \pm 2.25	56.8 \pm 0.9	NS
Propionate ^a	15.6 \pm 0.60	17.6 \pm 0.50	p <0.001
Butyrate ^a	19.2 \pm 2.50	14.3 \pm 0.53	p <0.065
Isobutyrate ^a	4.3 \pm 0.55	4.7 \pm 0.75	NS
Isovalerate ^a	5.2 \pm 0.53	5.3 \pm 0.39	NS
Valerate ^a	1.5 \pm 0.13	1.4 \pm 0.08	NS

a = percentage of total

TABLE 8. Rumen Volatile Fatty Production, By Breed. (mean \pm sem)
(n = 48)

	<u>Angus- Cross</u>	<u>Simmental-Cross</u>	<u>Significance</u>
Total (mg/gm)	245.9 \pm 14.99	269.2 \pm 19.46	NS
Acetate ^a	56.2 \pm 2.14	54.8 \pm 1.23	NS
Propionate ^a	16.3 \pm 0.66	16.9 \pm 0.50	NS
Butyrate ^a	17.0 \pm 2.54	16.4 \pm 0.76	NS
Isobutyrate ^a	3.4 \pm 0.37	5.5 \pm 0.80	p <0.002
Isovalerate ^a	5.6 \pm 0.53	5.0 \pm 0.38	NS
Valerate ^a	1.5 \pm 0.10	1.4 \pm 0.12	NS

a = percentage of total

Simmental-crosses. There were no other breed effects on VFA production. Several two-way interactions by sampling period occurred and these are listed in Appendix I, Tables 3-9.

4.3 Estrus Behaviour and Calving Data

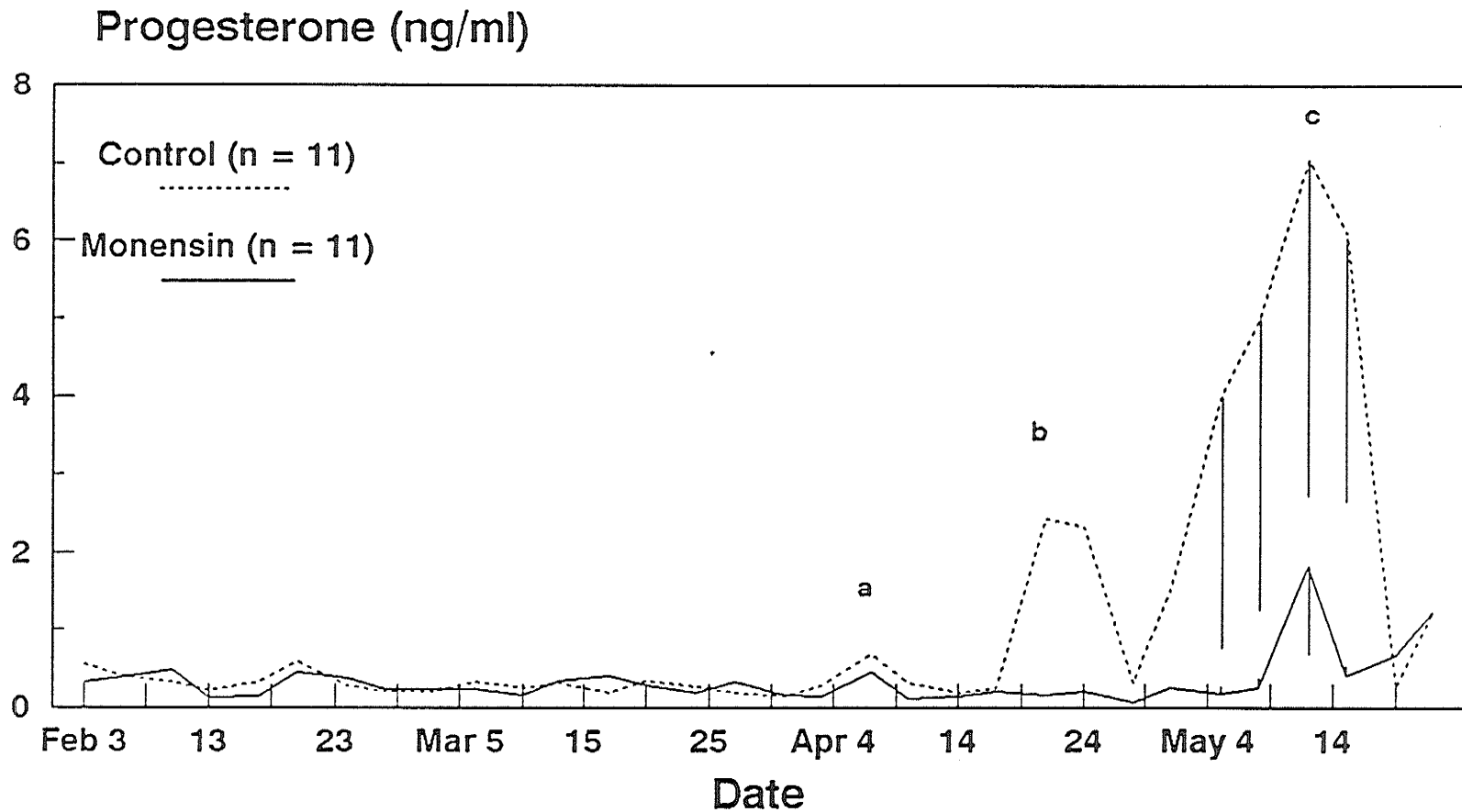
Behavioral estrus was not observed in any of the animals in this trial even though both breeds had reached the target age and weight required for the onset of puberty (Table 3) and mean serum progesterone concentrations had risen over 1.0 ng/ml (Figure 2). All animals except one Angus-cross-monensin, two Angus-cross-controls and two Simmental-cross-controls had active ovaries as indicated by palpation on June 2, 1987.

As onset of behavioral estrus was not available to estimate onset of puberty, date of conception was estimated from calving dates assuming a mean 282 day gestation period. Average age at conception was 456 ± 17 days (mean \pm sd) for control animals and 448 ± 12 days for monensin fed animals (Table 9). All animals conceived except for four of the Simmental-cross-controls.

4.4 Progesterone

Mean progesterone values for control and monensin-fed animals throughout the trial are shown in Figure 2. Two animals, one Angus-cross-monensin and one Simmental-cross-control were omitted from the data due to problems with one

Figure 2. Mean Serum Progesterone Concentrations
During Trial, by Treatment *



* Standard error bars included only where means significantly different ($p < 0.02$) to reduce clutter.

a = mean animal age of 370 days.

b = mean animal age of 384 days.

c = mean animal age of 405 days.

TABLE 9. Average Age At Conception of Experimental Animals *

<u>Control</u>	<u>Monensin</u>
456 \pm 17	448 \pm 12

* Days (mean \pm sd)

assay run. Serum progesterone concentrations in the control animals rose significantly higher than the monensin-fed heifers after day 165 of the experiment. Two well defined peaks occurred in the control animals, one beginning on day 147 and the second, larger peak beginning on day 158. Similarly, in the monensin group, a smaller peak of several days duration began on day 168. A second peak appears to have started on day 174, but is incomplete due to termination of the experiment.

As behavioral estrus was not observed, progesterone concentrations were used to estimate luteal function. Luteal function was defined as beginning on the first of at least three consecutive twice weekly serum samples in which the progesterone concentration was > 1.0 ng/ml (Richards et al, 1989). Any three twice weekly serum samples covered a period of eight days. While the number of animals with luteal activity by the end of the trial was too small to allow for statistical analysis (7 of 24), there are some trends. Angus-cross heifers demonstrated luteal function at an earlier age than did the Simmental-crosses, with the monensin-fed Angus-cross animals being the earliest of all groups. The monensin-fed Simmental-cross heifers were the last group to demonstrate luteal function.

4.5 Luteinizing Hormone

Mean values for basal, peak amplitude, number of peaks and

duration of LH peak secretion are given in Table 10. One Angus-cross-monensin, one Angus-cross-control and two Simmental-cross-monensin animals were not serially sampled due to problems with restraining the animals. There was no overall breed or treatment effect ($p>0.05$) for basal LH secretion. When segregated by sampling period, Angus-cross heifers had higher basal LH levels at day 75 and day 180 and lower levels at day 138 than the Simmental-crosses (Figure 3). Control animals had higher basal LH concentrations than monensin-fed animals during four of the six sampling periods (Figure 4) but the overall effect was non-significant ($p>0.05$) (Table 10). There was no overall period effect for basal LH ($p>0.05$) (Figure 5).

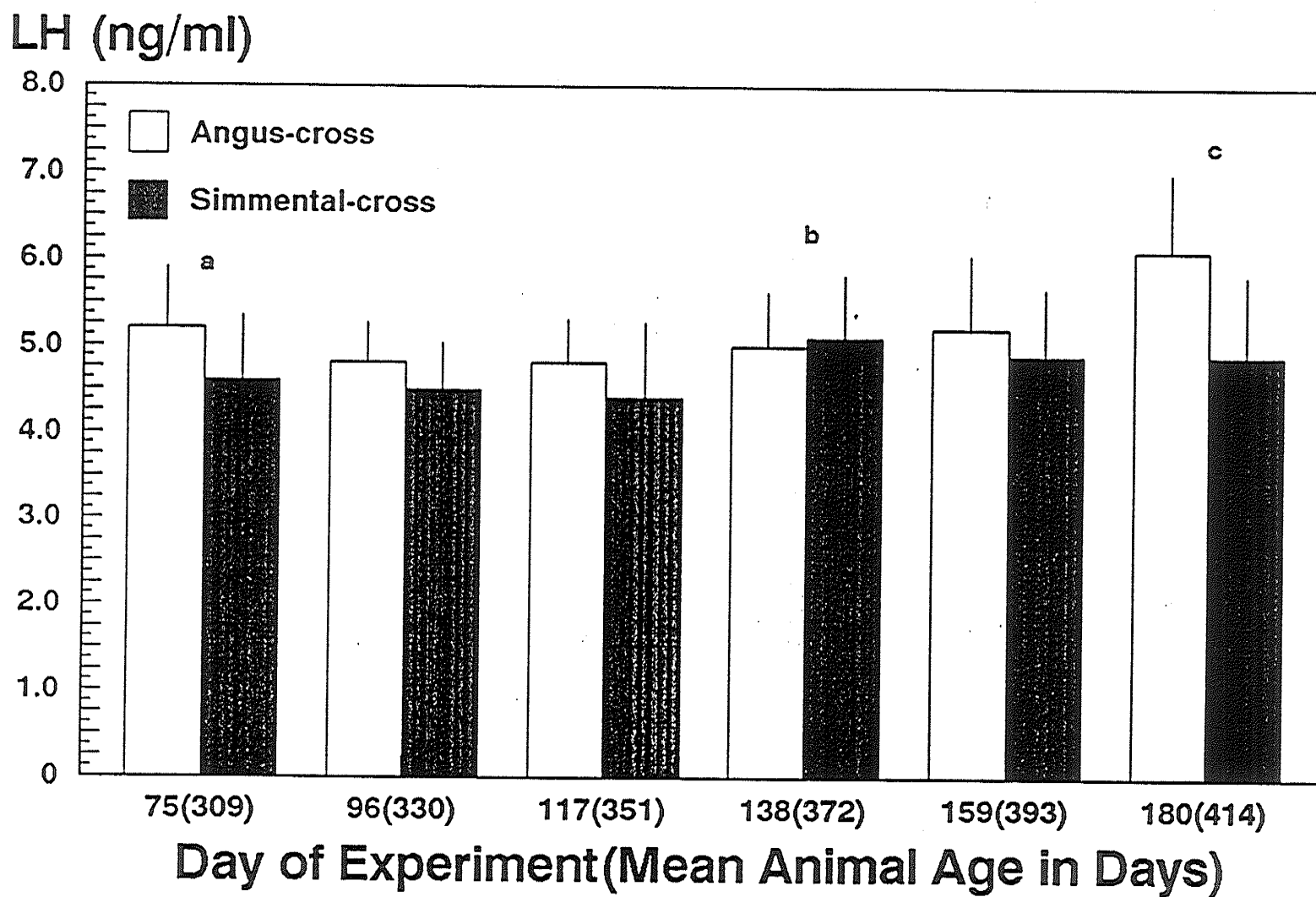
Overall amplitude of LH peak secretion was not significantly different between breeds or treatments ($p>0.05$) (Table 10). Simmental-cross and Angus-cross heifers show almost the same pattern of LH peak amplitude over time except at day 96 (Figure 6) where amplitude is much greater for the Simmental-crosses than the Angus-crosses ($p<0.03$). Control and monensin-fed animals do not demonstrate a significantly different ($p>0.05$) amplitude of LH secretion at any of the sampling periods (Figure 7). Amplitude of LH secretion was similar during most sampling periods but was greater at the end of the experiment than at the beginning (Figure 8).

Overall mean number of LH peaks during each sampling period

TABLE 10. Serum Luteinizing Hormone Profile Characteristics

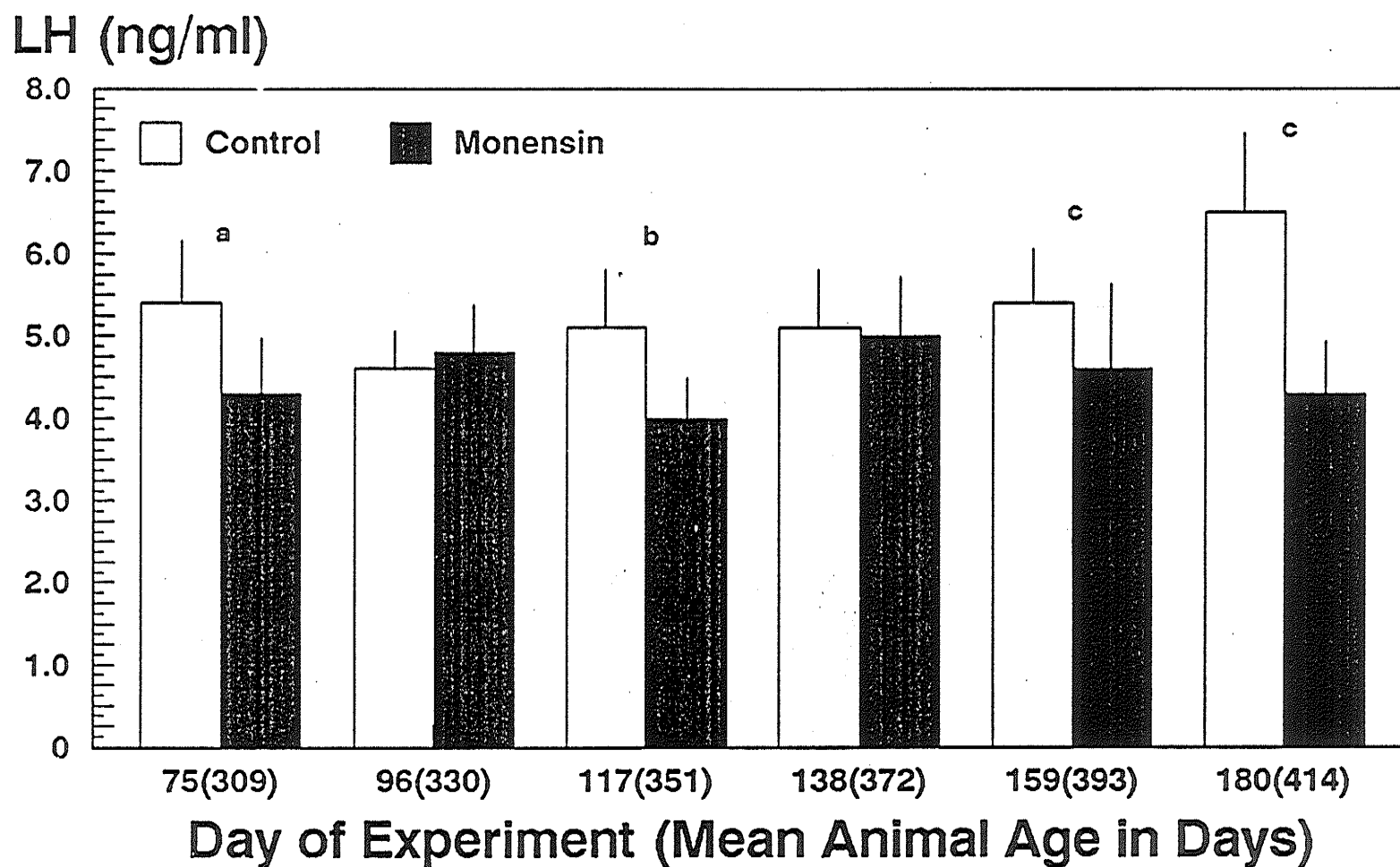
	<u>Angus -Cross</u> (n = 56)	<u>Simmental-Cross</u> (n = 54)	<u>Significance</u>
Basal LH concentration (ng/ml)	5.2 ± 0.27	4.8 ± 0.30	NS
Amplitude of LH peak (ng/ml)	18.2 ± 2.89	22.1 ± 2.92	NS
Number of peaks (per 7 hr. sampling period)	0.9 ± 0.12	1.4 ± 0.14	p<0.024
Duration of LH peak (min)	31.4 ± 4.94	41.9 ± 4.57	NS
	<u>Control</u> (n = 64)	<u>Monensin</u> (n = 51)	<u>Significance</u>
Basal LH concentration (ng/ml)	5.4 ± 0.29	4.5 ± 0.27	NS
Amplitude of LH peak (ng/ml)	18.4 ± 2.92	22.3 ± 2.86	NS
Number of peaks (per 7 hr. sampling period)	1.0 ± 0.12	1.3 ± 0.15	NS
Duration of LH peak (min)	32.7 ± 4.83	1.2 ± 4.68	NS

Figure 3. Basal LH Concentration According to Breed
(mean \pm sem)



Breed means significantly different: a $p < 0.05$; b $p < 0.02$ c $p < 0.0001$

Figure 4. Basal LH Concentration According to Treatment
(mean \pm sem)

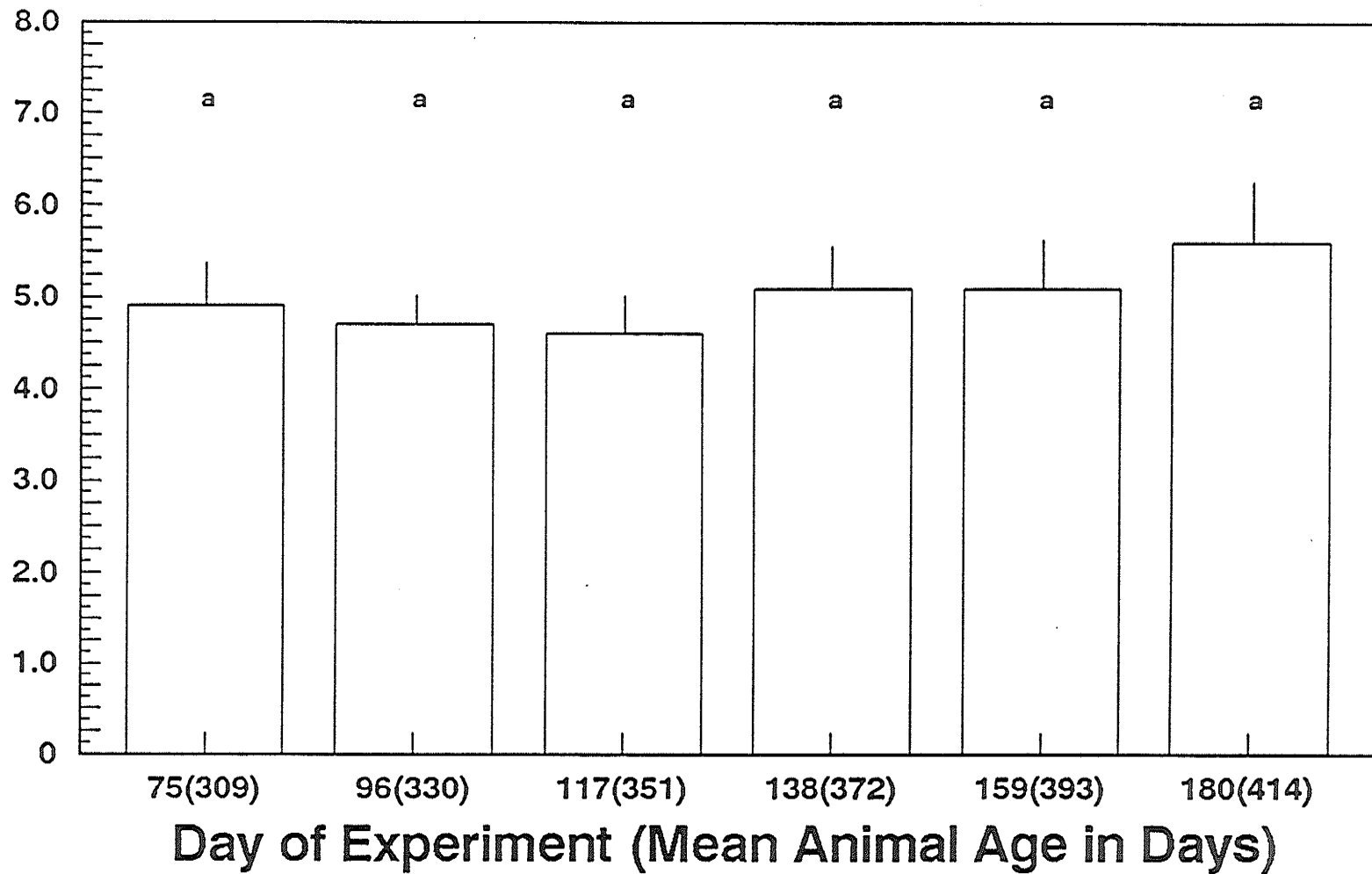


Treatment means significantly different: a $p < 0.0002$; b $p < 0.0004$; c $p < 0.0001$

Figure 5. Basal LH Concentration by Sampling Period

(mean \pm sem)

LH (ng/ml)



Means with different superscript differ significantly ($p < 0.05$)

Figure 6. LH Peak Amplitude According to Breed

(mean \pm sem)

LH (ng/ml)

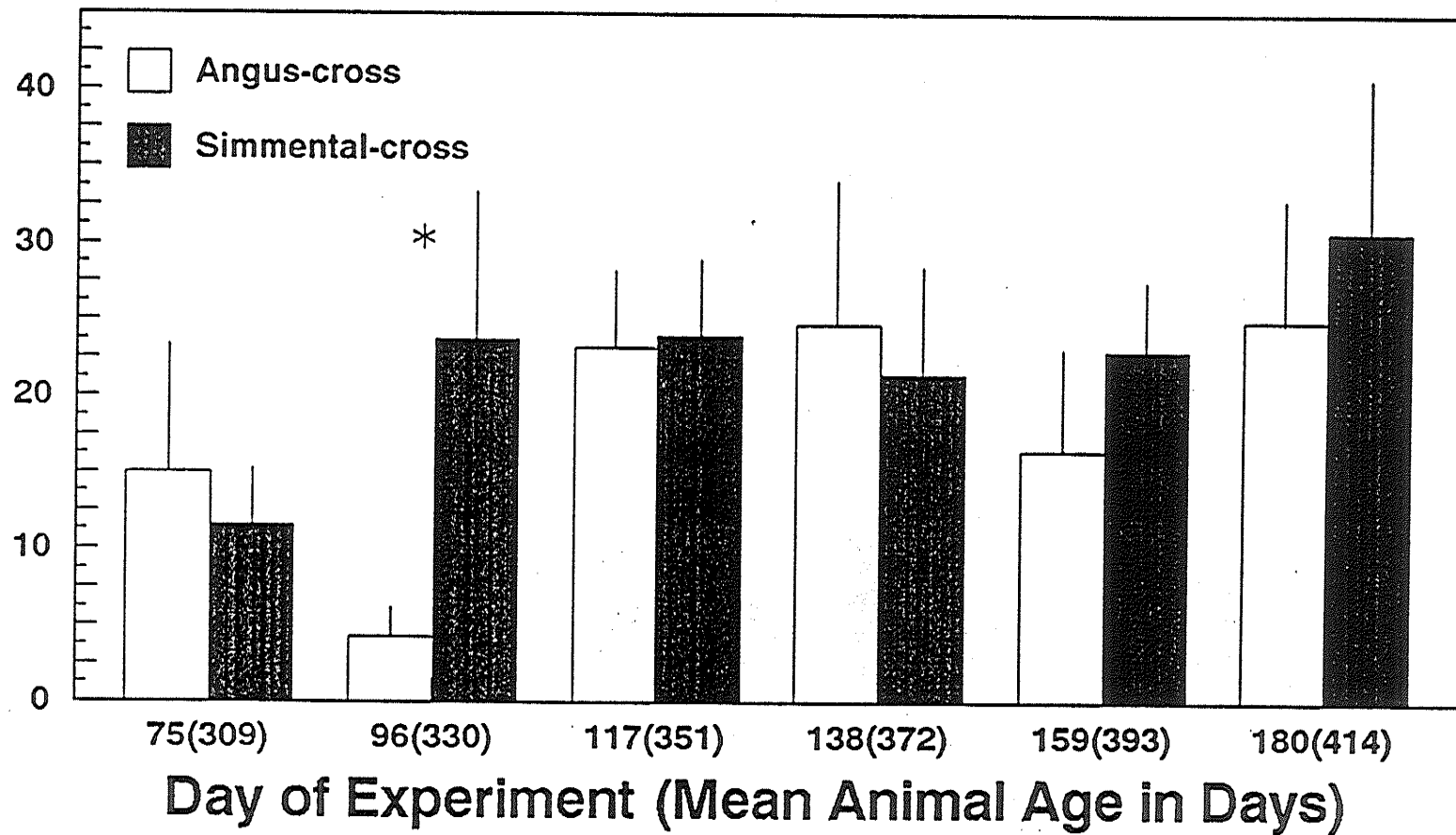


Figure 7. LH Peak Amplitude According to Treatment

(mean \pm sem)

LH (ng/ml)

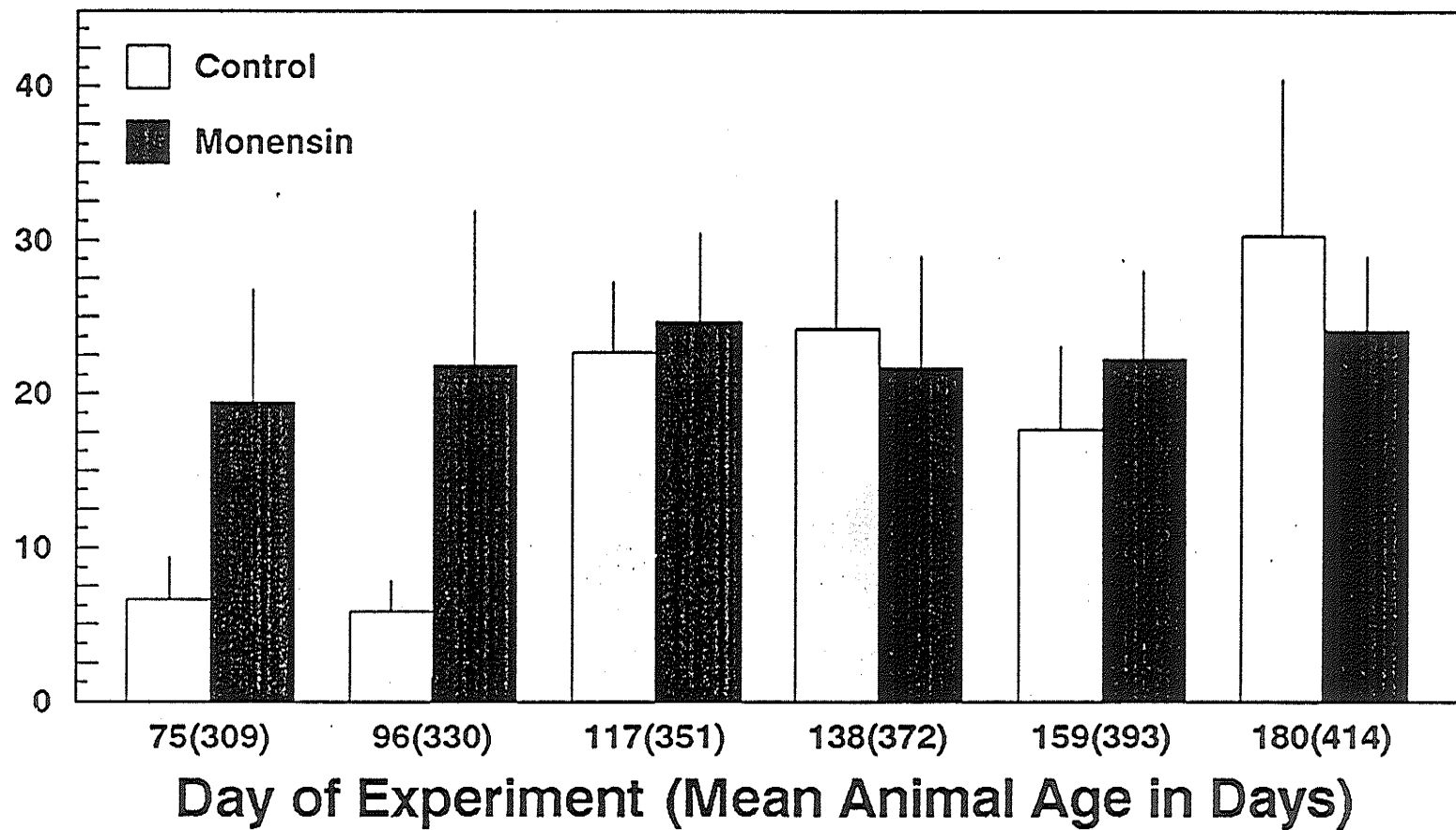
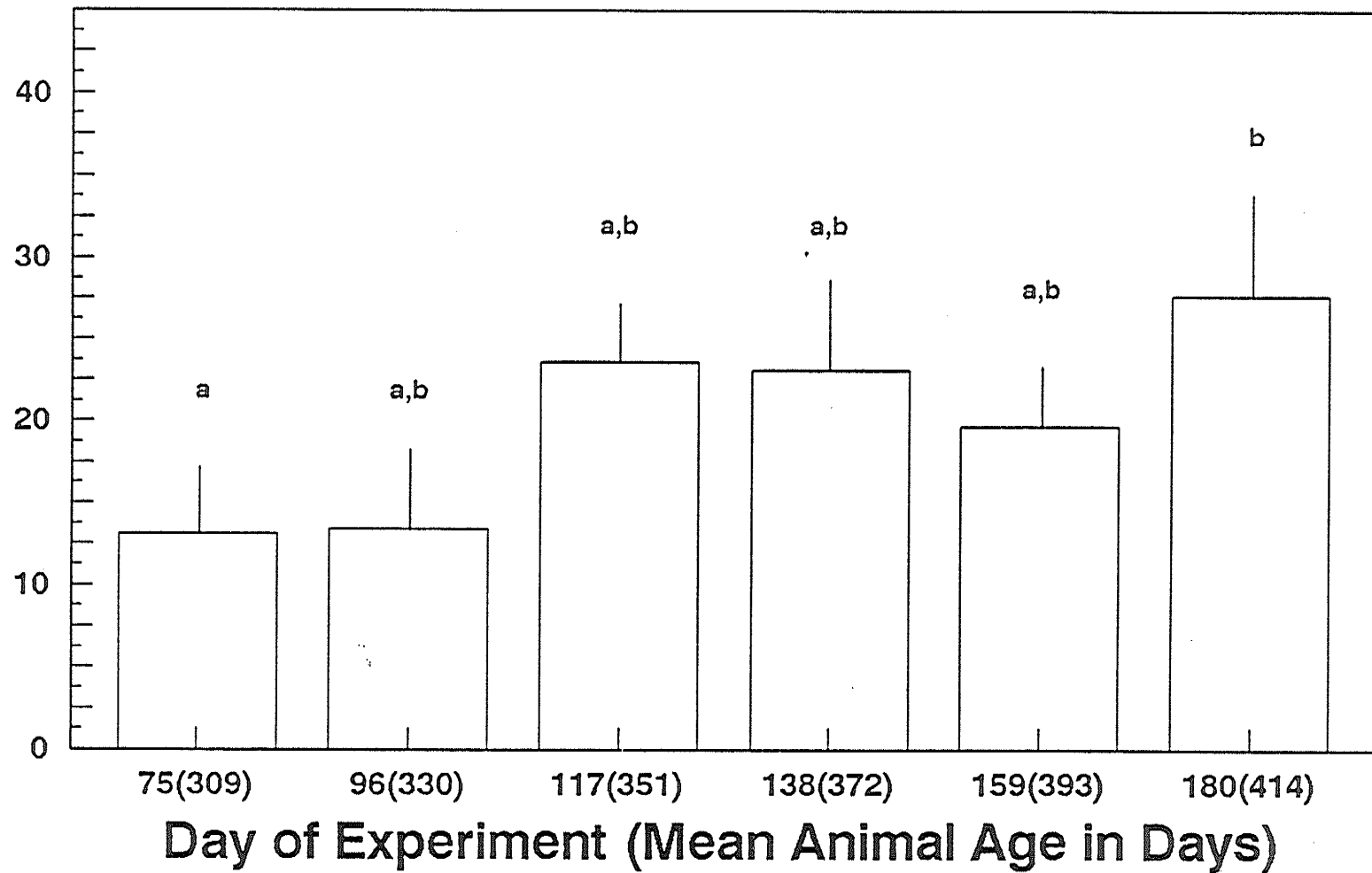


Figure 8. LH Peak Amplitude by Sampling Period

(mean \pm sem)

LH (ng/ml)



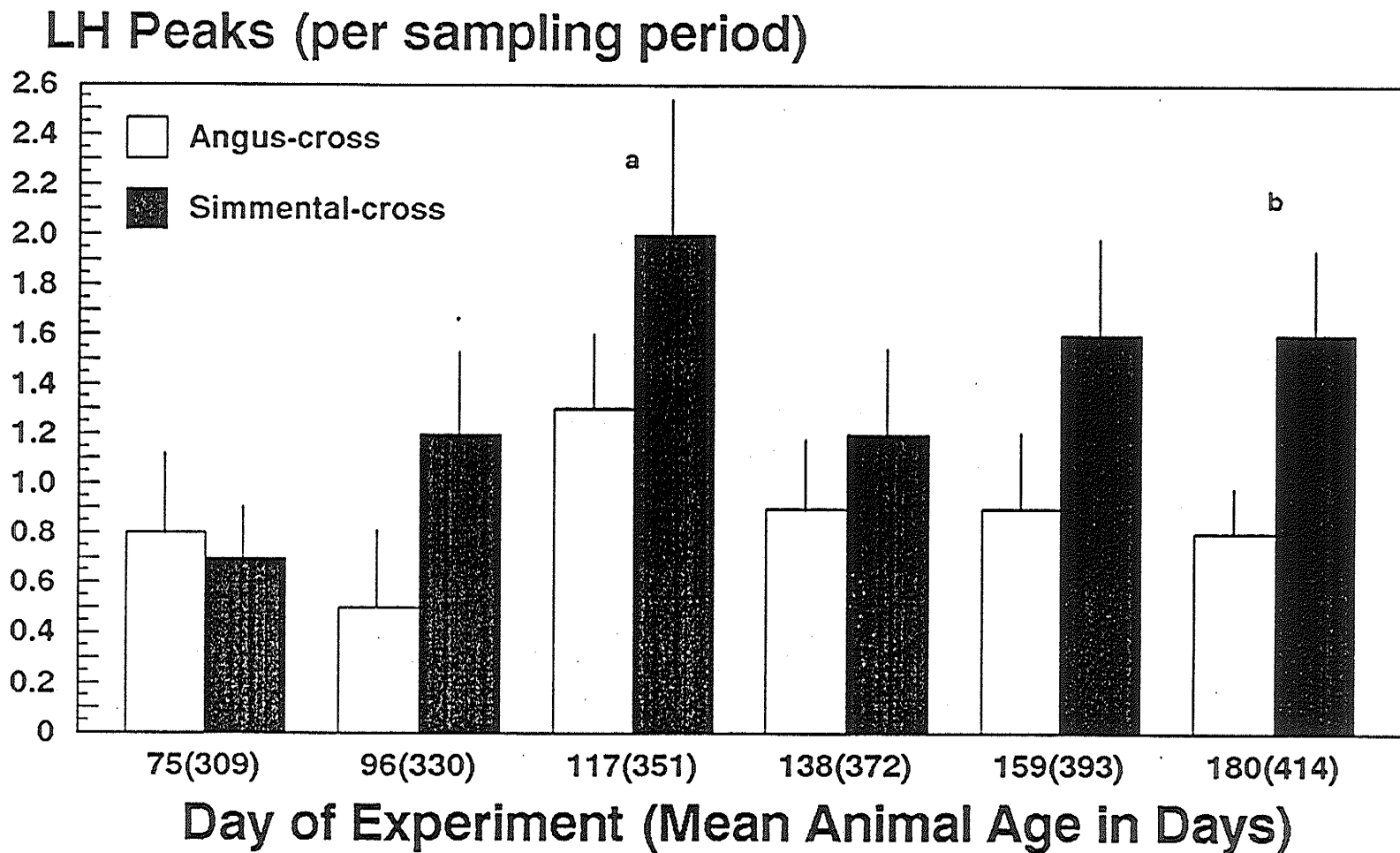
Means with different superscripts differ significantly ($p < 0.05$)

was significantly different between breeds but not by treatment (Table 10). Simmental-cross animals had a significantly greater number of LH peaks than the Angus-crosses at day 117 ($p < 0.03$) and day 180 ($p < 0.05$) (Figure 9) while there was a trend for greater number of peaks for the Simmental-crosses at day 96 and day 159. Control and monensin animals followed much the same rise in number of LH peaks over time as there were no differences between control and monensin-fed animals at any of the sampling periods (Figure 10). There was a significant ($p < 0.05$) period effect, with number of LH peaks being the lowest during the first two sampling periods (Figure 11).

Duration of the LH peaks showed no overall significant ($p > 0.05$) breed or treatment effect (Table 10). Angus-cross and Simmental-cross animals were not different from each other for duration of peak except at day 96 (Figure 12). Control and monensin animals did not differ significantly ($p > 0.05$) for LH peak duration at any of the sampling periods (Figure 13). A significant period effect for duration of LH peak was observed, as duration of the peak was significantly different ($p < 0.05$) between days 75 and 138, and days 75 and 180 (Figure 14).

Figure 9. Number of LH Peaks According to Breed

(mean \pm sem)



Breed means significantly different: a $p < 0.03$; b $p < 0.05$

Figure 10. Number of LH Peaks According to Treatment
(mean \pm sem)

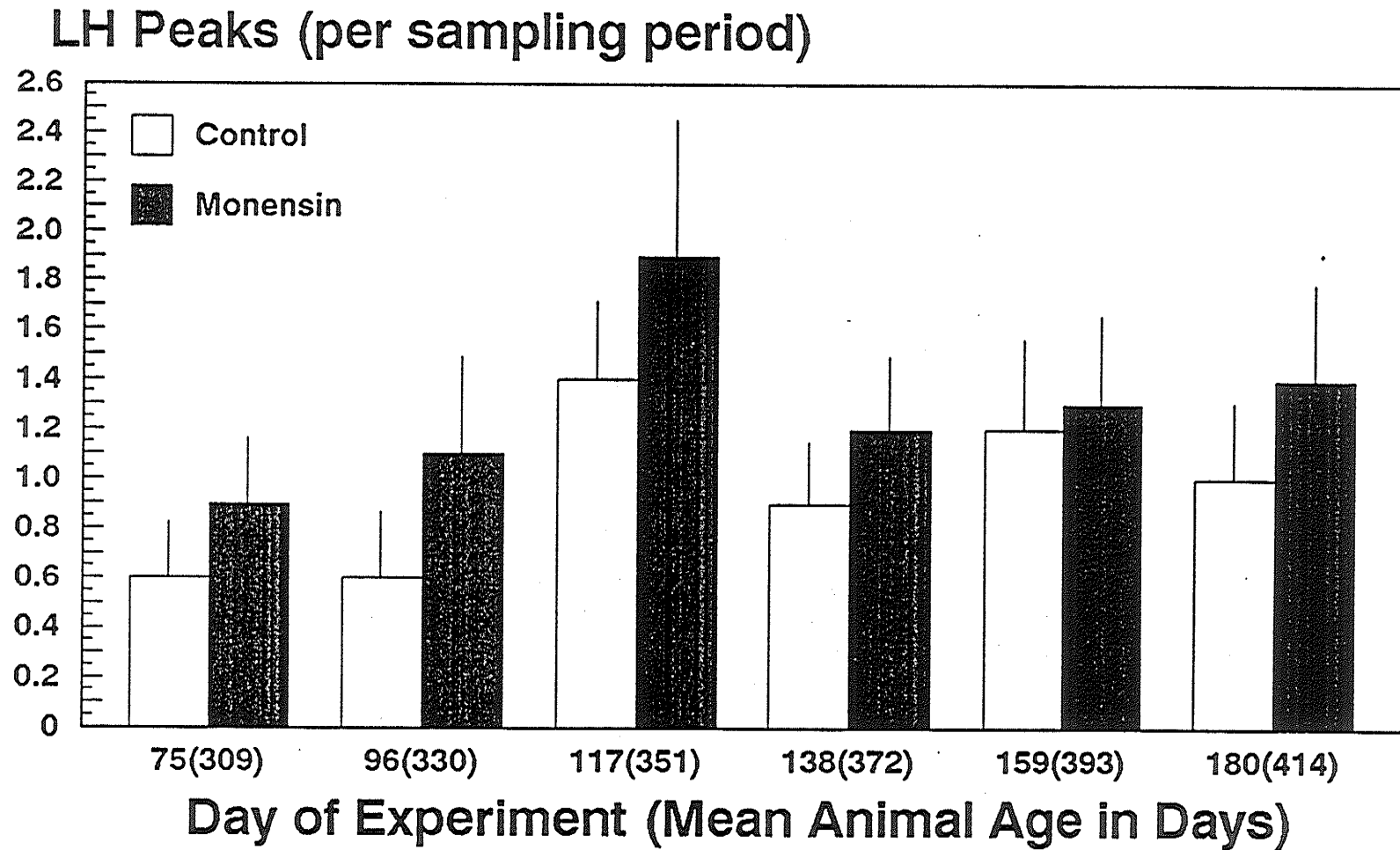
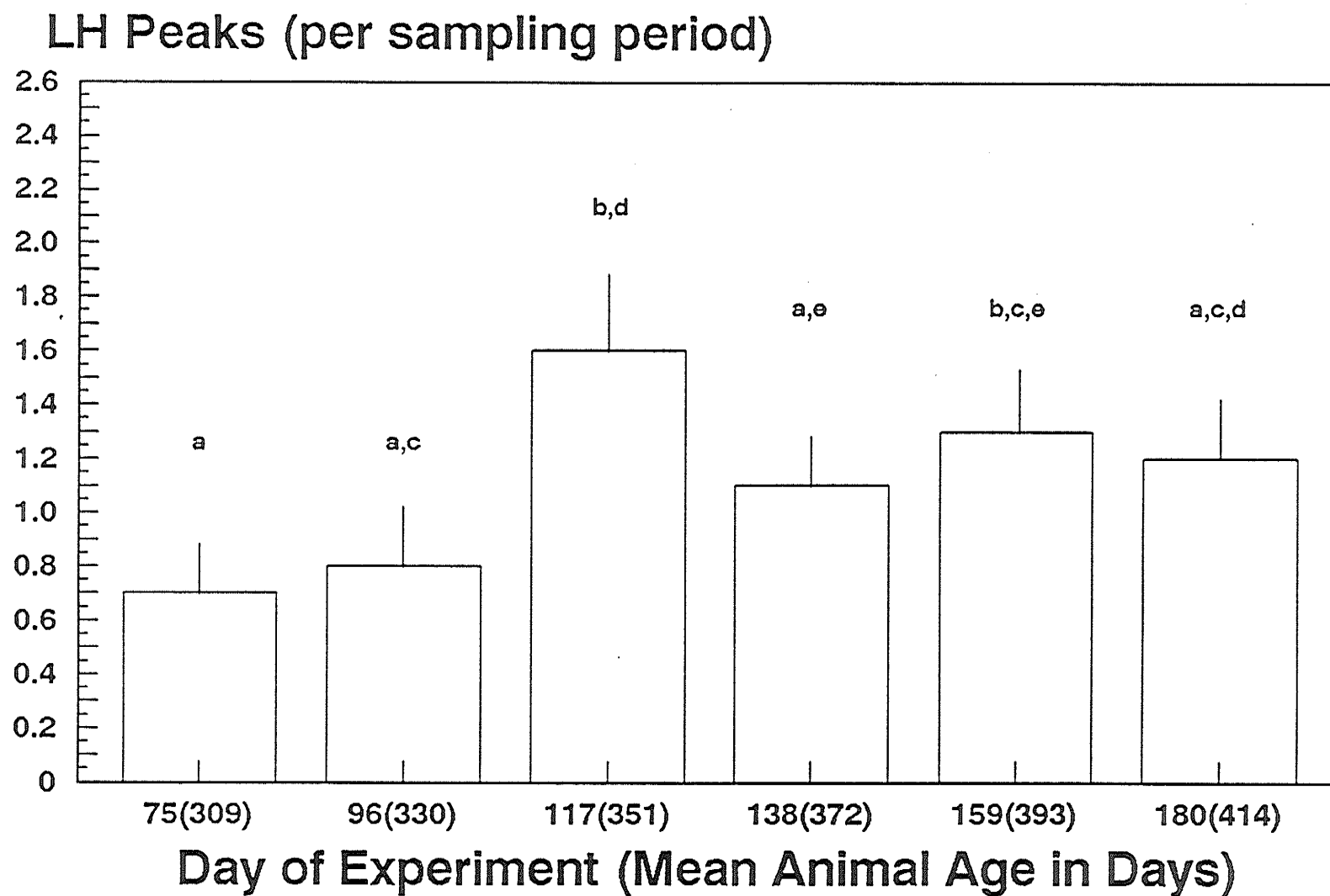


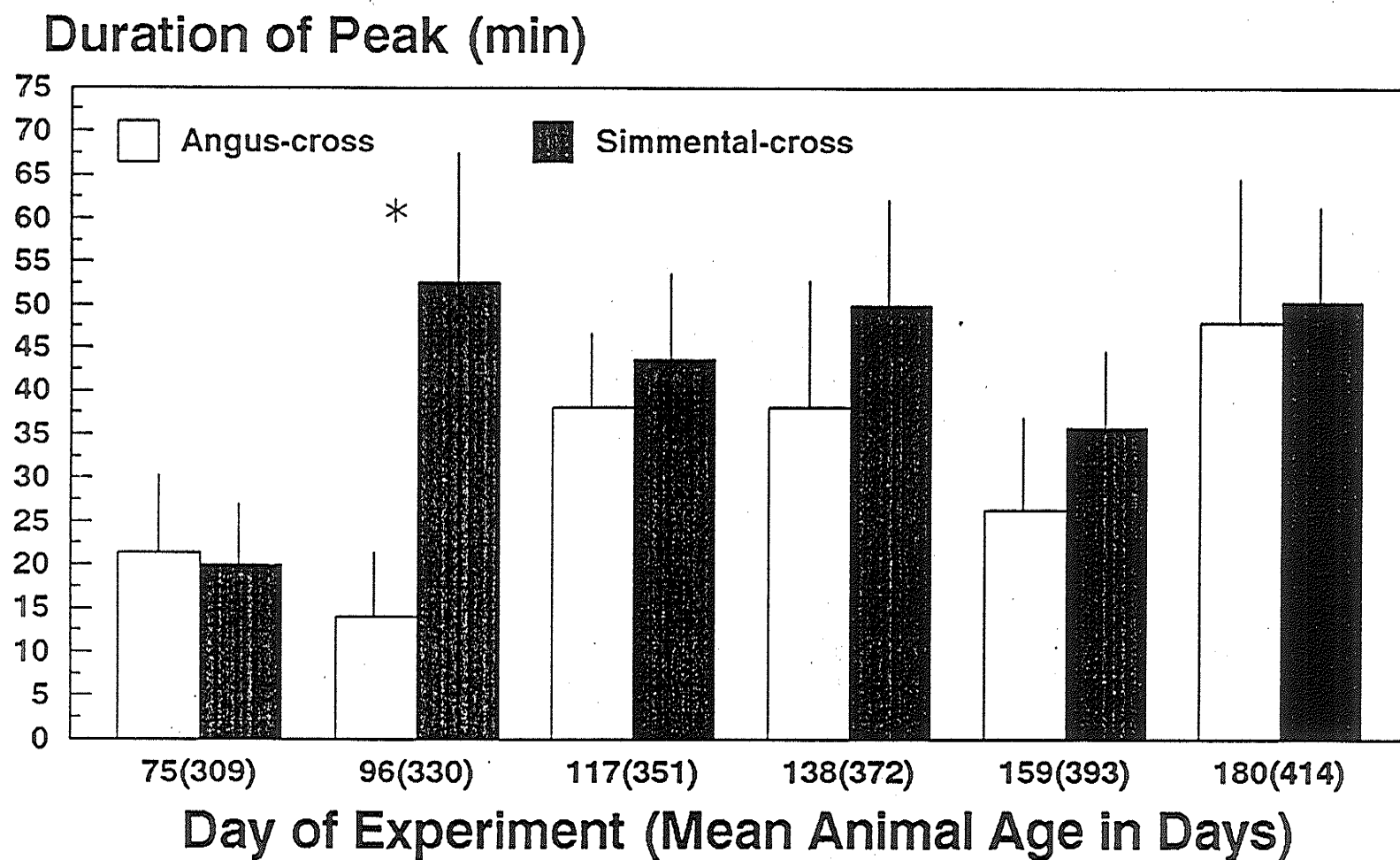
Figure 11. Number of LH Peaks by Sampling Period

(mean \pm sem)



Means with different superscripts differ significantly ($p < 0.05$)

Figure 12. Duration of LH Peak According to Breed
(mean \pm sem)



* Breed means significantly different ($p < 0.02$)

Figure 13. Duration of LH Peak According to Treatment

(mean \pm sem)

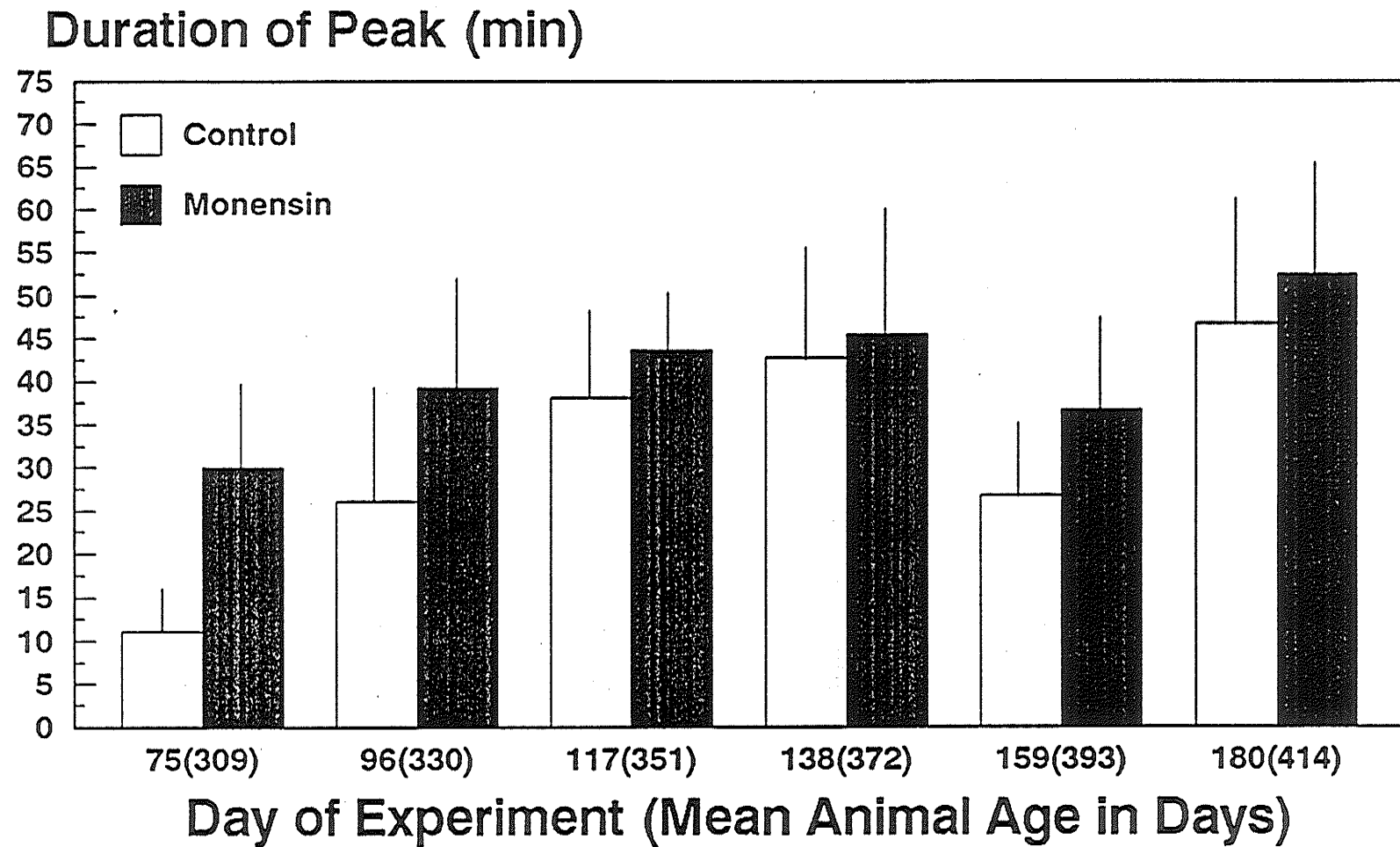
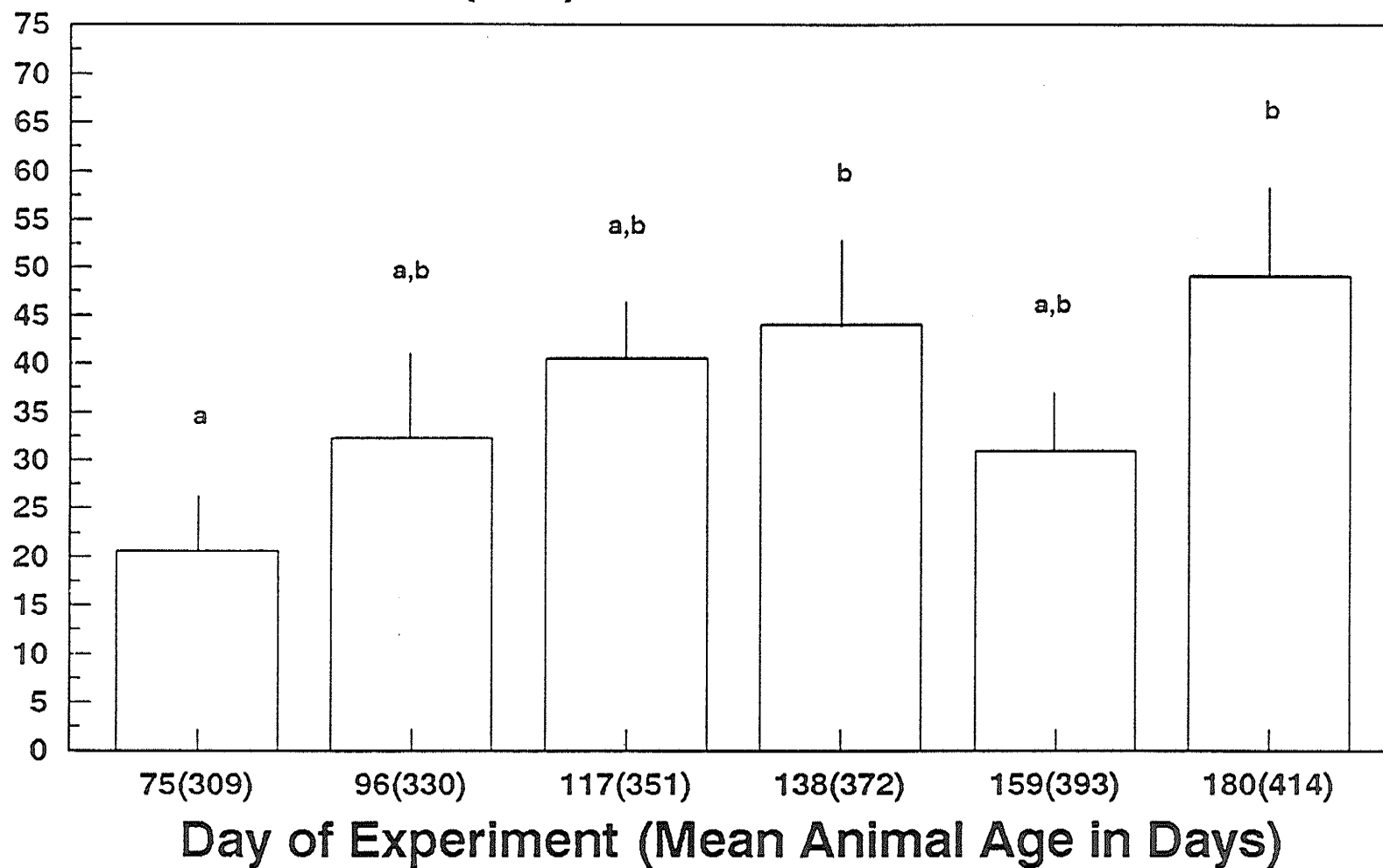


Figure 14. Duration of LH Peak by Sampling Period

(mean \pm sem)

Duration of Peak (min)



Means with different superscripts differ significantly ($p < 0.05$)

DISCUSSION

This study represents the first trial in which hormonal patterns were monitored in monensin-fed heifers during the prepubertal period in an attempt to explain the physiological reason why monensin reduces age and weight at puberty in these animals.

5.0 Feed/Gain Efficiency

Monensin in the ration of both the Angus-cross and Simmental-cross prepubertal heifers improved feed efficiency. This was due to an improvement in daily gains, with no effect on feed intake. This is in agreement with the studies in which monensin supplied to feedlot animals consuming a high forage ration demonstrate improved feed efficiencies due to increased daily gains with no significant change in feed intake (Potter et al, 1976a; Turner et al, 1977; Faulkner et al, 1985; Beacom et al, 1988).

The lack of a significant difference in ADG between Angus-cross and Simmental-cross heifers may have been the result of the feeding management of the animals. Energy intake and composition of each breed's ration (Table 1) was based on NRC

guidelines (National Research Council, 1984) in order to allow for gains such that each breed would reach its inherent pubertal weight at the target age in the spring (Table 3). Average age and weight at puberty for Angus heifers is 410 days and 309 kg and that for Simmental heifers is 348 days and 328 kg respectively (Ferrell, 1982). However, the rations obtained from these calculations resulted in an actual ADG of the heifers that was less than expected for the first two weigh periods of the trial (Table 6). Intake was then increased for both breeds on day 67 of the trial (Table 2) in order to correct this but neither breed would consume more than 4.5 kg DM/head/day of the corn silage. Thus energy intake and ADG remained below requirements during weigh period three. On day 75, silage offered was decreased to the maximum the animals would consume and the amount of barley was increased to make up the energy shortfall. This ration was fed until day 162 when intake was reduced in order to keep daily gains at the desired rate of 0.455 kg for the Angus-crosses and 0.682 kg for the Simmental-crosses. All remaining ration changes were based on the availability of the feed source.

The very low ADG for the Simmental-crosses during the first 74 days of the experiment resulted in a lower overall ADG during the entire trial for this group than was required while the ADG for the Angus-cross heifers was greater than required

due to the ration manipulations (Table 1, Table 6). This eliminated the expected breed difference in ADG.

5.1 Volatile Fatty Acid Production

Monensin supplementation resulted in an increase in propionate production from 15.6% to 17.6% and a decrease in butyrate production from 19.2% to 14.3% within the rumen (Table 7) which is consistent with earlier findings (Perry et al, 1976; Richardson et al, 1976; Mowat et al, 1977; Beacom et al, 1988). While there was no overall decrease in acetate production as reported previously, monensin increased total VFA production. This is in contrast to earlier reports as the feeding of monensin at 33 ppm generally has no effect on total VFA production (Richardson et al, 1976; Mowat et al, 1977; Turner et al, 1977). The increase in total VFA production of the monensin group in this experiment demonstrates an improvement in the utilization of energy from the feedstuff (Chalupa, 1977) since feed intake was similar between treatment groups but gains were superior for monensin-fed animals. Breed differences did exist for isobutyrate and isovalerate production (Table 8), but the effect was minimal since these two combined only account for approximately 10% of total VFA production and no significant breed by treatment interactions were found for any of the VFA's or total VFA's produced. Several two-way interactions by sampling period did occur (Appendix I, Tables 3-9). However, these interactions

are relatively unimportant because even though production of some of the VFA's were different between the two sampling periods, the proportion of each VFA to total VFA production was similar within sampling periods.

Overall, the feed intake, ADG and VFA data indicate that the monensin was being supplied to the animals in sufficient quantities such that the documented effects of monensin did occur.

5.2 Estrus Behavior

Behavioral estrus was not observed in any animals from either treatment group, even though both breeds were at an age and weight at which the onset of puberty should have occurred by the end of the experiment (Table 3). Level of nutrition and prepubertal gains are known to influence age at puberty. Prepubertal heifers maintained on an energy restricted diet are older and lighter at puberty than controls (Wiltbank et al, 1969; Arije and Wiltbank, 1971; Ferrell, 1982). Puberty is delayed in heifers undergoing compensatory gains after being removed from a low energy diet to a normal diet, compared to animals receiving a normal energy diet during the entire trial (Short and Bellows, 1971). Thus, the low rate of gain during the first 74 days of the present experiment may have caused a delay in puberty past the end of the trial even though gains after day 74 were adequate. This is substantiated by the observation that by five weeks after the

end of the feeding trial, by which time the heifers were on pasture, 80% of the heifers were actively cycling as determined by ovarian palpation.

Another factor which may have contributed to the lack of observed behavioral estrus is the environment in which the animals were kept. At the end of the experiment, the heifers were removed from the small pens of three animals each and placed together with the rest of the replacement heifers in the Glenlea herd. Within days, several of the heifers that were in the experiment began to show behavioral estrus. However, records were not kept at this time. Lack of behavioral estrus in animals from this herd which were penned in small groups has been documented previously (White, 1977).

5.3 Progesterone

5.3.1 Prepubertal Patterns

Gonzalez-Padilla et al (1975a) were among the first to describe serum progesterone concentrations in prepubertal heifers as they approached and achieved puberty. Levels were below 1.0 ng/ml in all animals until approximately 20 days prior to the onset of the first estrous cycle. From day -20 to day 0 (estrus), two elevations in progesterone occurred. The first between days -18 to -11 and the second, larger peak between days -9 to 0. The amplitude of these peaks were in the order of 1.0 to 2.5 ng/ml (Gonzalez-Padilla et al,

1975a). Dodson et al (1988) reported that progesterone levels in the prepubertal heifer are undetectable until just before the first estrus, when a small elevation of progesterone of approximately six days duration precedes first estrus. Serum progesterone levels in the present study follow the same pattern, with levels being below 1.0 ng/ml until the time period of expected puberty, when the mean concentration of progesterone then rose above 1.0 ng/ml.

5.3.2 Luteal Function

As day of first behavioral estrus was not available to allow for determination of the day of puberty, the progesterone data was used to give an estimate of luteal function of the heifers. An increase in circulating progesterone concentration > 1.0 ng/ml is indicative of luteal function if this rise persists for several days (Day et al, 1984; Skaggs et al, 1986; Richards et al, 1989; Dyer et al, 1990; Kurz et al, 1990). This rise may or may not be followed by estrous cycles of normal duration. In this experiment, luteal function was considered to have begun on the first of three successive biweekly samples in which progesterone concentration was ≥ 1.0 ng/ml (Stabenfeldt et al, 1969; Wettemann et al, 1972; Richards et al, 1989). According to this definition, only four control and three monensin animals demonstrated luteal function by the end of the experiment. Of the remaining 15 animals, progesterone profiles ranged from $<$

1.0 ng/ml for the duration of the entire trial, to isolated peaks of progesterone > 1.0 ng/ml with interpeak levels < 1.0 ng/ml. Thus the number of animals achieving luteal function was too few for statistical analysis and the impact of monensin on the onset of luteal function cannot be assessed. The delay in the onset of luteal function would be due to the same reasons as the delay in puberty, nutritional stress during the first 74 days of the trial (Short and Bellows, 1971).

5.3.3. Effect of Monensin on Serum Progesterone

Monensin did affect serum progesterone as mean progesterone levels did not rise as dramatically after day 161 as they did in the control group. There is a lack of information in the literature concerning serum progesterone levels in monensin-fed prepubertal heifers. The feeding of monensin to post-pubertal, cycling feedlot heifers does not alter progesterone concentrations compared to controls (Horton et al, 1981). Superovulated heifers, when fed monensin, have a greater number of corpora lutea and a higher luteal progesterone content than controls 11 to 15 days after a (FSH-P) challenge or a combined FSH-P - HCG challenge. This is true in prepubertal heifers (Bushmich et al, 1980) and in cycling, pubertal heifers (Harrison et al, 1982). Thus, while monensin allows for an enhanced ovarian response to exogenous pituitary gonadotrophins, results of the present study indicate that

progesterone production was lower in monensin-fed heifers during the prepubertal period. Since serum progesterone concentrations are indicative of C.L. function (Stabenfeldt, 1969), this would indicate that the development of C.L. tissue was delayed in the monensin group. First behavioral estrus usually occurs 11 to 18 days after the first of two consecutive increases in serum progesterone above 1.0 ng/ml (Gonzalez-Padilla et al, 1975a), but it is difficult to determine if the delay observed in this study would affect onset of first estrus since the difference in first progesterone peak between the two groups was 21 days and behavioral estrus was not observed prior to the end of the study.

5.4 Luteinizing Hormone

LH data in this experiment agrees with previous reports that LH secretion and LH pulse frequency increase as puberty approaches in beef heifers (Day et al, 1984; Day et al, 1986; Dyer et al, 1990; Kurz et al, 1990). Amplitude of LH secretion and duration of LH peak were both greater at the end of the experiment than at the beginning. Number of peaks per sampling period also increased during the later stages of the experiment. Although estradiol concentrations were not monitored in this trial, this increase in LH secretion is presumably due to decreased estradiol inhibition on LH secretion (Day et al, 1984) which is in agreement with the

"gonadostat" hypothesis presented by Ramirez and McCann (1963).

5.4.1 Effect of Nutritional Stress on Serum LH Levels

Energy restriction can delay puberty in heifers as well as reduce LH pulse frequency and concentration during the prepubertal period (Day et al, 1986). LH pulse frequency and concentration are also reduced in mature beef cows who are nutritionally stressed to the point of becoming anestrus (Richards et al, 1989).

Kurz and co-workers (1990) demonstrated that suppression of the prepubertal rise in LH due to a low energy diet may involve two components. First, suppression may be due to prolonged steroidal/ovarian inhibition of LH secretion since both intact, and ovariectomized then subsequently estradiol-implanted heifers, fed a low plane of nutrition do not demonstrate a decrease in estradiol inhibition of LH. LH secretion remained basal and non-pulsatile throughout the trial. Intact and ovariectomized/estradiol-implanted animals fed an adequate level of energy did experience a decrease in estradiol inhibition since LH secretion increased throughout the experiment. Secondly, ovary independent suppression of LH secretion, presumably by direct action on the hypothalamo-pituitary axis may occur. Ovariectomized heifers fed a low plane of nutrition demonstrated a reduced increase in LH pulse frequency compared to those fed control diets. This was

accompanied by an increase in GnRH-induced LH peak amplitude and frequency compared to animals fed sufficient energy diets (controls). The author's conclusion was that undernutrition in ovariectomized prepubertal heifers resulted primarily in a decrease in the rate of GnRH release from the hypothalamus rather than an alteration in GnRH synthesis, pituitary response to GnRH or pituitary stores of LH (Kurz et al, 1990).

Inclusion of monensin in the diet may have a beneficial effect in this regard. As removal of useful feedstuff energy is enhanced in animals fed monensin (Chalupa, 1977), these animals are in a superior energy balance compared to controls on the same ration. This could serve to eliminate any nutritional stress inadvertently imposed on the animals by the ration. Since undernutrition has an adverse effect on LH secretion by decreasing the rate of GnRH release from the hypothalamus and thus delaying puberty, monensin may help prevent this in animals by eliminating or reducing nutritional stress.

During the first 74 days of the present experiment, both breeds of heifers were nutritionally stressed; the Simmental-crosses to a much larger extent than the Angus-cross. This deficiency in energy level supplied could have caused the delay of puberty in both breeds (Short and Bellows, 1971), but the difference in plane of nutrition was probably not great enough to affect LH release patterns (Day et al, 1986). No

overall breed effect was present for basal LH, LH peak amplitude or duration of LH peak. Number of peaks was significantly greater in the Simmental-cross heifers although these animals were energy stressed to a larger degree than the Angus-crosses. This can be interpreted as a lack of an influence of undernutrition on LH secretion as the amount of feed energy taken in by each animal was different between breeds, not treatments. This was most evident during the first sampling period done on day 75 of the experiment. The Simmental-cross heifers were still nutritionally stressed to a greater degree than the Angus-crosses as the ration correction had not been made at this time, but no significant differences were present in LH release characteristics except for basal levels. Even though basal LH concentration was lower in nutritionally-stressed Simmental-cross group at this time, it was not due to undernutrition as underfeeding reduces basal LH concentrations to below 1.0 ng/ml (Day et al, 1986).

Thus, any change in LH secretion between control and monensin-fed heifers should be due to the ionophore itself, and not because of differences in energy supplied to the animals.

5.4.2 Effect of Monensin on Serum LH Levels

LH secretion characteristics followed the same general trends in both the monensin and control groups, indicating that monensin did not radically alter the physiology of LH

secretion. Amplitude of LH peak, number of LH peaks per sampling period and duration of peaks were not significantly affected by feeding monensin during any of the six sampling periods. However, for these three variables, there was a general non-significant trend for the values to be greater in the monensin group than controls. This trend may have affected basal LH concentrations at several of the sampling periods in the monensin group. The pulsar program used to calculate the release characteristics of episodic LH secretion removes values considered as peaks from the calculation of basal concentration. Thus the trend towards a greater number of peaks in monensin-fed animals resulted in the removal of a greater number of high concentration data points from the basal concentration calculations and may account for the lower basal levels in the monensin group. Although this trend may have affected basal LH concentrations at several of the time periods, the overall effect of monensin on basal LH was non-significant (Table 10).

Moseley et al (1982) put forth the hypothesis that monensin decreases the age of puberty in beef heifers by causing an earlier maturation of the endocrine system(s) responsible for puberty. This is based largely on the observations that feeding monensin or infusing propionate enhances pituitary release of LH after multiple GnRH injections (Randel and Rhodes, 1980; Rutter et al, 1983) or an estradiol-17 β

injection (Randel et al, 1982) in prepubertal heifers. Data from the present experiment is in direct contrast with these findings as monensin did not significantly affect serum LH concentrations or release patterns. However, in those experiments that measured pituitary response after GnRH or E2 injection in monensin-fed or propionate-infused animals, pharmacological doses of hormones were administered. These challenges were approximately 10,000 times greater in concentration than that normally found in the peripheral circulation (Randel and Rhodes, 1980; Randel et al, 1982; Rutter et al, 1983). Thus it is difficult to expect the same pituitary response under two greatly different hormone concentrations.

Therefore, while monensin does increase pituitary LH release after administration of pharmacological doses of exogenous hormones, it does not appear to carry out its effects on puberty through modification of endogenous serum LH levels. As the mechanism by which puberty is triggered remains unknown, it is possible that monensin does affect this trigger to enhance the onset of puberty. Further research is required to identify the event responsible for the onset of puberty and to determine if monensin does affect it in some way.

SUMMARY

Feeding monensin sodium at 33g/tonne to prepubertal heifers improved daily gains but had no effect on feed intake compared to controls.

Total VFA production and propionate production was increased in heifers fed monensin while that of butyrate was decreased.

Behavioral estrus was not observed in the experimental heifers, and this was partially due to a shortfall in dietary energy consumed during the first 74 days of the trial.

The rise in mean serum progesterone concentration after day 161 of the trial was greater in control animals than monensin-fed animals.

Monensin did not significantly alter serum LH concentrations or release characteristics compared to controls.

It is concluded that the effect of monensin in decreasing age at puberty is not mediated through a change in episodic LH release or serum progesterone concentrations.

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TABLE 1: Analysis of Variance Table for Dry Matter Intake

Source	df	MS	F	p Value
Breed	1	6.19	0.30	0.6135
TRT	1	6.51	0.31	0.6047
Breed*TRT	1	69.02	3.34	0.1417
Error	4	20.67		
Period	22	42.16	44.05	0.0001
Breed*Period	22	3.25	3.39	0.0001
TRT*Period	22	0.91	0.95	0.5345
Breed*TRT*Period	22	1.52	1.59	0.0679
Error	88	0.96		
Total	183			

TABLE 2: Analysis of Variance Table for Average Daily Gain

Source	df	MS	F	p Value
Breed	1	0.004	0.35	0.5580
TRT	1	0.037	3.81	0.0652
Breed*TRT	1	0.017	1.74	0.2027
Error	20	0.010		
Total	23			

TABLE 3: Analysis of Variance Table for Total Volatile Fatty Acid Production

Source	df	MS	F	p Value
TRT	1	30545.02	5.77	0.0262
Breed	1	6542.14	1.23	0.2797
TRT*Breed	1	1296.08	0.24	0.6263
Error	20	5298.06		
Period	1	21188.56	2.70	0.1163
TRT*Period	1	15233.09	1.94	0.1792
Breed*Period	1	981.97	0.12	0.7275
TRT*Breed*Period	1	695.04	0.09	0.7693
Error	20	7860.64		
Total	47			

TABLE 4: Analysis of Variance Table for Acetate Production

Source	df	MS	F	p Value
TRT	1	76.16	1.41	0.2492
Breed	1	23.07	0.43	0.5211
TRT*Breed	1	146.04	2.70	0.1159
Error	20	54.07		
Period	1	504.39	8.01	0.0104
TRT*Period	1	265.12	4.21	0.0536
Breed*Period	1	13.39	0.21	0.6498
TRT*Breed*Period	1	13.19	0.21	0.6522
Error	20	63.00		
Total	47			

TABLE 5: Analysis of Variance Table for Propionate Production

Source	df	MS	F	p Value
TRT	1	48.31	14.53	0.0011
Breed	1	3.13	0.94	0.3438
TRT*Breed	1	3.99	1.20	0.2864
Error	20	3.33		
Period	1	162.01	38.04	0.0001
TRT*Period	1	0.07	0.02	0.9023
Breed*Period	1	8.32	1.95	0.1776
TRT*Breed*Period	1	1.45	0.34	0.5659
Error	20	4.26		
Total	47			

TABLE 6: Analysis of Variance Table for Butyrate Production

Source	df	MS	F	p Value
TRT	1	286.17	3.83	0.0645
Breed	1	3.83	0.05	0.8232
TRT*Breed	1	125.73	1.68	0.2093
Error	20	74.72		
Period	1	72.42	0.88	0.3582
TRT*Period	1	173.44	2.12	0.1610
Breed*Period	1	72.86	0.89	0.3567
TRT*Breed*Period	1	29.94	0.37	0.5521
Error	20	81.87		
Total	47			

TABLE 7: Analysis of Variance Table for Isobutyrate Production

Source	df	MS	F	p Value
TRT	1	1.91	0.47	0.4990
Breed	1	54.91	13.61	0.0015
TRT*Breed	1	8.21	2.03	0.1691
Error	20	4.04		
Period	1	214.29	57.26	0.0001
TRT*Period	1	0.57	0.15	0.7006
Breed*Period	1	44.08	11.78	0.0026
TRT*Breed*Period	1	2.60	0.70	0.4142
Error	20	3.74		
Total	47			

TABLE 8: Analysis of Variance Table for Isovalerate Production

Source	df	MS	F	p Value
TRT	1	0.13	0.09	0.7664
Breed	1	5.29	3.71	0.0685
TRT*Breed	1	0.87	0.61	0.4443
Error	20	1.43		
Period	1	132.32	69.07	0.0001
TRT*Period	1	10.68	5.57	0.0285
Breed*Period	1	19.65	10.25	0.0045
TRT*Breed*Period	1	2.60	1.36	0.2580
Error	20	1.92		
Total	47			

TABLE 9: Analysis of Variance Table for Valerate Production

Source	df	MS	F	p Value
TRT	1	0.25	0.65	0.4298
Breed	1	0.01	0.04	0.8501
TRT*Breed	1	0.86	2.21	0.1531
Error	20	0.39		
Period	1	0.29	1.53	0.2307
TRT*Period	1	0.12	0.63	0.4373
Breed*Period	1	0.05	0.24	0.6262
TRT*Breed*Period	1	0.41	2.16	0.1569
Error	20	0.19		
Total	47			

TABLE 10: Analysis of Variance Table for Mean Serum Progesterone Concentration

Source	df	MS	F	p Value
TRT	1	92.40	7.10	0.0079
Date	31	21.69	1.67	0.0142
TRT*Date	31	14.99	1.15	0.2642
Error	575	13.01		
Total	638			

TABLE 11: Analysis of Variance Table for Basal LH Concentrations

Source	df	MS	F	p Value
Breed	1	743.61	0.36	0.5561
TRT	1	3752.76	1.82	0.1956
Breed*TRT	1	637.64	0.31	0.5854
Error	16	2056.95		
Period	5	37.49	0.50	0.7746
Breed*Period	5	271.93	3.63	0.0056
TRT*Period	5	386.50	5.16	0.0004
Breed*TRT*Period	5	131.36	1.75	0.1336
Error	70	74.85		
Total	109			

TABLE 12: Analysis of Variance Table for Amplitude of LH Peak

Source	df	MS	F	p Value
Breed	1	892.82	1.38	0.2577
TRT	1	845.33	1.30	0.2702
Breed*TRT	1	215.70	0.33	0.5720
Error	16	648.11		
Period	5	569.28	1.34	0.2591
Breed*Period	5	368.97	0.87	0.5083
TRT*Period	5	447.04	1.05	0.3958
Breed*TRT*Period	5	442.08	1.04	0.4024
Error	70	425.94		
Total	109			

TABLE 13: Analysis of Variance Table for Number of LH Peaks per Sampling Period

Source	df	MS	F	p Value
Breed	1	9.66	6.21	0.0241
TRT	1	5.29	3.40	0.0837
Breed*TRT	1	0.48	0.31	0.5877
Error	16	1.56		
Period	5	2.50	3.44	0.0077
Breed*Period	5	.51	0.71	0.6214
TRT*Period	5	0.18	0.25	0.9373
Breed*TRT*Period	5	1.73	2.39	0.0466
Error	70	0.73		
Total	109			

TABLE 14: Analysis of Variance Table for Duration of LH Peak

Source	df	MS	F	p Value
Breed	1	4043.84	2.43	0.1384
TRT	1	2926.70	1.76	0.2032
Breed*TRT	1	1003.87	0.60	0.4485
Error	16	1662.66		
Period	5	1703.48	1.42	0.2287
Breed*Period	5	1001.82	0.83	0.5303
TRT*Period	5	269.56	0.22	0.9508
Breed*TRT*Period	5	894.02	0.74	0.5933
Error	75	1201.80		
Total	114			

APPENDIX II
ORIGINAL DATA

Table 1. Feed Intake

(DMS = kg dry matter intake silage per weighback period; DMG = kg dry matter intake grain per weighback period; DAYS = days within each weighback period).

OBS	PEN	BREED	TRT	PERIOD	DMS	DMG	DAYS
1	1	A	M	1	94.98	26.31	9
2	2	S	C	1	91.80	20.61	9
3	3	A	M	1	90.12	24.99	9
4	4	S	M	1	96.18	21.66	9
5	5	A	C	1	96.72	26.75	9
6	6	S	M	1	93.66	21.66	9
7	7	A	C	1	96.24	26.31	9
8	8	S	C	1	78.60	18.49	9
9	1	A	M	2	157.02	43.82	15
10	2	S	C	2	160.05	36.80	15
11	3	A	M	2	157.35	45.03	15
12	4	S	M	2	171.99	39.25	15
13	5	A	C	2	166.05	46.68	15
14	6	S	M	2	172.20	39.60	15
15	7	A	C	2	176.67	50.28	15
16	8	S	C	2	140.34	32.24	15
17	1	A	M	3	137.76	38.63	12
18	2	S	C	3	134.91	31.33	12
19	3	A	M	3	133.86	38.19	12
20	4	S	M	3	137.82	32.73	12
21	5	A	C	3	134.61	38.19	12
22	6	S	M	3	153.30	35.53	12
23	7	A	C	3	150.84	43.33	12
24	8	S	C	3	116.85	28.53	12
25	1	A	M	4	90.66	26.05	8
26	2	S	C	4	83.37	20.77	8
27	3	A	M	4	86.88	26.03	8
28	4	S	M	4	85.20	20.42	8
29	5	A	C	4	92.70	27.21	8
30	6	S	M	4	83.61	20.34	8
31	7	A	C	4	94.41	27.98	8
32	8	S	C	4	74.25	19.72	8
33	1	A	M	5	80.40	22.53	8
34	2	S	C	5	87.60	20.42	8
35	3	A	M	5	90.45	25.61	8
36	4	S	M	5	89.55	20.77	8
37	5	A	C	5	96.30	26.77	8
38	6	S	M	5	96.90	21.82	8
39	7	A	C	5	95.25	26.77	8
40	8	S	C	5	86.40	20.42	8
41	1	A	M	6	148.08	41.45	13
42	2	S	C	6	163.50	38.17	13
43	3	A	M	6	149.49	42.68	13
44	4	S	M	6	154.50	36.07	13
45	5	A	C	6	154.95	45.04	13
46	6	S	M	6	183.93	42.11	13
47	7	A	C	6	157.53	44.65	13
48	8	S	C	6	136.41	31.52	13
49	1	A	M	7	87.72	25.97	7
50	2	S	C	7	86.40	20.93	7
51	3	A	M	7	85.14	24.73	7
52	4	S	M	7	87.99	21.47	7
53	5	A	C	7	94.65	29.79	7
54	6	S	M	7	100.35	23.48	7
55	7	A	C	7	90.00	27.22	7

Table 1, cont.

OBS	PEN	BREED	TRT	PERIOD	DMS	DMG	DAYS
56	8	S	C	7	84.90	20.58	7
57	1	A	M	8	64.35	27.61	7
58	2	S	C	8	57.72	37.58	7
59	3	A	M	8	61.20	26.91	7
60	4	S	M	8	58.02	37.58	7
61	5	A	C	8	68.61	29.10	7
62	6	S	M	8	64.95	40.57	7
63	7	A	C	8	62.10	28.32	7
64	8	S	C	8	56.55	37.58	7
65	1	A	M	9	73.89	36.24	7
66	2	S	C	9	63.30	53.67	7
67	3	A	M	9	72.60	36.34	7
68	4	S	M	9	63.75	53.67	7
69	5	A	C	9	74.70	37.66	7
70	6	S	M	9	80.10	64.86	7
71	7	A	C	9	78.60	40.30	7
72	8	S	C	9	60.45	52.54	7
73	1	A	M	10	86.16	41.27	8
74	2	S	C	10	69.36	54.66	8
75	3	A	M	10	87.12	41.88	8
76	4	S	M	10	75.18	59.22	8
77	5	A	C	10	92.01	43.20	8
78	6	S	M	10	90.93	69.54	8
79	7	A	C	10	91.86	43.81	8
80	8	S	C	10	66.66	51.21	8
81	1	A	M	11	69.57	33.09	7
82	2	S	C	11	53.64	44.53	7
83	3	A	M	11	78.48	36.95	7
84	4	S	M	11	59.28	45.67	7
85	5	A	C	11	82.26	39.08	7
86	6	S	M	11	80.40	61.71	7
87	7	A	C	11	86.46	40.91	7
88	8	S	C	11	59.55	45.68	7
89	1	A	M	12	83.88	39.59	7
90	2	S	C	12	77.88	60.47	7
91	3	A	M	12	92.97	43.83	7
92	4	S	M	12	71.49	58.16	7
93	5	A	C	12	84.96	39.59	7
94	6	S	M	12	90.90	70.22	7
95	7	A	C	12	92.22	43.83	7
96	8	S	C	12	69.87	53.67	7
97	1	A	M	13	116.79	55.54	9
98	2	S	C	13	104.31	80.72	9
99	3	A	M	13	100.92	47.95	9
100	4	S	M	13	99.24	77.27	9
101	5	A	C	13	107.82	50.77	9
102	6	S	M	13	117.57	89.23	9
103	7	A	C	13	110.46	52.19	9
104	8	S	C	13	92.70	72.60	9
105	1	A	M	14	79.50	36.95	7
106	2	S	C	14	72.00	53.69	7
107	3	A	M	14	73.50	34.31	7
108	4	S	M	14	70.50	52.54	7
109	5	A	C	14	94.50	43.78	7
110	6	S	M	14	87.00	64.78	7

Table 1, cont.

OBS	PEN	BREED	TRT	PERIOD	DMS	DMG	DAYS
111	7	A	C	14	90.0	41.62	7
112	8	S	C	14	67.5	50.24	7
113	1	A	M	15	85.5	39.49	7
114	2	S	C	15	66.0	49.11	7
115	3	A	M	15	85.5	39.49	7
116	4	S	M	15	76.5	57.11	7
117	5	A	C	15	96.0	44.54	7
118	6	S	M	15	87.0	64.96	7
119	7	A	C	15	96.0	44.54	7
120	8	S	C	15	72.0	53.67	7
121	1	A	M	16	94.5	43.75	7
122	2	S	C	16	73.5	54.81	7
123	3	A	M	16	94.5	43.75	7
124	4	S	M	16	76.5	57.12	7
125	5	A	C	16	100.5	46.91	7
126	6	S	M	16	94.5	70.21	7
127	7	A	C	16	72.0	33.80	7
128	8	S	C	16	72.0	53.67	7
129	1	A	M	17	79.5	36.85	7
130	2	S	C	17	69.0	51.38	7
131	3	A	M	17	78.0	36.15	7
132	4	S	M	17	73.5	54.81	7
133	5	A	C	17	93.0	43.04	7
134	6	S	M	17	93.0	69.16	7
135	7	A	C	17	85.5	39.49	7
136	8	S	C	17	72.0	53.67	7
137	1	A	M	18	87.0	40.20	7
138	2	S	C	18	76.5	57.11	7
139	3	A	M	18	87.0	40.20	7
140	4	S	M	18	73.5	54.81	7
141	5	A	C	18	90.0	41.62	7
142	6	S	M	18	90.0	67.06	7
143	7	A	C	18	87.0	40.20	7
144	8	S	C	18	76.5	57.11	7
145	1	A	M	19	94.0	43.75	7
146	2	S	C	19	87.0	64.96	7
147	3	A	M	19	94.0	43.75	7
148	4	S	M	19	87.0	64.96	7
149	5	A	C	19	94.0	43.75	7
150	6	S	M	19	94.5	70.21	7
151	7	A	C	19	94.0	43.75	7
152	8	S	C	19	87.0	64.96	7
153	1	A	M	20	82.5	30.77	7
154	2	S	C	20	82.5	41.01	7
155	3	A	M	20	82.5	30.77	7
156	4	S	M	20	70.5	34.33	7
157	5	A	C	20	93.0	35.54	7
158	6	S	M	20	93.0	48.74	7
159	7	A	C	20	88.5	34.06	7
160	8	S	C	20	84.0	43.84	7
161	1	A	M	21	91.5	24.73	7
162	2	S	C	21	91.5	20.23	7
163	3	A	M	21	91.5	24.73	7
164	4	S	M	21	79.5	17.43	7
165	5	A	C	21	91.5	24.73	7

Table 1, cont.

OBS	PEN	BREED	TRT	PERIOD	DMS	DMG	DAYS
166	6	S	M	21	91.5	20.23	7
167	7	A	C	21	90.0	24.34	7
168	8	S	C	21	79.5	17.43	7
169	1	A	M	22	90.9	24.34	7
170	2	S	C	22	106.2	20.93	7
171	3	A	M	22	95.4	25.50	7
172	4	S	M	22	98.7	19.18	7
173	5	A	C	22	95.4	25.50	7
174	6	S	M	22	106.2	20.93	7
175	7	A	C	22	90.9	24.34	7
176	8	S	C	22	101.7	19.88	7
177	1	A	M	23	86.4	21.86	6
178	2	S	C	23	102.6	17.94	6
179	3	A	M	23	86.4	21.86	6
180	4	S	M	23	102.6	17.94	6
181	5	A	C	23	86.4	21.86	6
182	6	S	M	23	102.6	17.94	6
183	7	A	C	23	86.4	21.86	6
184	8	S	C	23	102.6	17.94	6

Table 2. Average Daily Gains
 (where WTnn = weight in kg; first number in WTnn
 represents weigh period number and second number
 represents first or second consecutive weigh day)

OBS	ANIMAL	PEN	BREED	TRT	WT01	WT02	WT11	WT12	WT21	WT22	WT31	WT32
1	27	1	A	M	220	214	224	220	240	236	242	245
2	36	1	A	M	230	230	237	233	249	246	262	263
3	66	1	A	M	235	230	240	236	242	236	240	242
4	3	5	A	C	214	210	215	214	234	230	240	243
5	19	5	A	C	218	214	222	221	233	232	246	248
6	29	5	A	C	215	214	220	223	235	233	238	242
7	26	3	A	M	202	198	205	206	220	222	232	232
8	43	3	A	M	210	206	217	220	232	226	240	246
9	46	3	A	M	202	198	201	202	221	218	234	233
10	45	7	A	C	228	226	231	228	243	243	254	252
11	83	7	A	C	216	218	220	218	240	238	252	258
12	87	7	A	C	222	218	228	228	240	240	252	255
13	2	8	S	C	202	204	206	206	214	212	204	206
14	5	8	S	C	216	220	217	217	224	218	215	218
15	49	8	S	C	200	198	201	204	218	214	220	223
16	12	4	S	M	214	210	213	218	238	234	250	254
17	18	4	S	M	224	222	226	226	234	234	242	240
18	69	4	S	M	206	202	209	212	223	216	222	224
19	22	6	S	M	234	232	242	242	253	248	262	264
20	33	6	S	M	232	230	236	246	247	244	254	256
21	78	6	S	M	230	228	236	236	251	248	250	250
22	6	2	S	C	220	214	220	218	231	232	224	230
23	40	2	S	C	204	202	200	199	214	212	222	223
24	51	2	S	C	198	192	195	193	213	212	220	222

Table 2, cont.

OBS	ANIMAL	PEN	BREED	TRT	WT41	WT42	WT51	WT52	WT61	WT62	WT71	WT72	WT81	WT82
1	27	1	A	M	252	249	273	278	300	298	325	325	325	317
2	36	1	A	M	274	276	298	304	325	324	355	350	359	355
3	66	1	A	M	256	259	292	293	314	318	346	344	349	350
4	3	5	A	C	250	256	280	284	318	322	338	338	330	330
5	19	5	A	C	263	268	295	300	332	333	352	348	356	353
6	29	5	A	C	248	253	274	276	297	302	319	322	309	312
7	26	3	A	M	242	244	268	274	283	285	313	314	313	312
8	43	3	A	M	248	256	280	282	304	317	336	339	342	342
9	46	3	A	M	244	248	274	276	294	298	326	324	335	335
10	45	7	A	C	252	252	278	279	298	298	325	324	322	312
11	83	7	A	C	272	271	303	302	332	335	354	356	354	350
12	87	7	A	C	264	264	289	293	308	318	340	342	340	335
13	2	8	S	C	208	216	246	247	256	260	298	285	294	292
14	5	8	S	C	220	220	241	246	268	274	292	302	298	295
15	49	8	S	C	230	236	256	263	282	288	312	309	313	310
16	12	4	S	M	258	260	300	302	332	340	360	360	369	369
17	18	4	S	M	254	254	283	288	310	314	346	355	357	360
18	69	4	S	M	228	228	254	258	274	278	302	304	312	312
19	22	6	S	M	276	282	322	320	348	358	380	382	383	377
20	33	6	S	M	268	270	302	304	326	322	346	345	357	355
21	78	6	S	M	256	257	294	295	332	339	368	369	381	378
22	6	2	S	C	232	238	270	272	286	288	324	309	318	317
23	40	2	S	C	230	234	262	264	290	298	320	323	327	327
24	51	2	S	C	240	240	270	276	290	298	326	332	329	325

Table 3. Volatile Fatty Acids
(all values mg/g).

OBS	ID	TRT	BRED	Period	ACETIC	PROPION	ISOBUT	BUTYRIC	ISOVAL	VALERIC	TOTAL
1	2	C	S	1	180.373	53.5283	6.0594	55.0214	8.6564	5.0990	308.738
2	2	C	S	2	63.240	19.0569	12.1514	43.0687	14.7580	5.2181	157.493
3	3	C	A	1	110.347	33.1198	3.7439	27.6559	3.6467	2.2909	180.804
4	3	C	A	2	95.022	25.1736	8.9123	33.1664	17.9273	3.9711	184.173
5	5	C	S	1	158.561	51.6687	4.5636	50.5333	6.2054	4.3735	275.906
6	5	C	S	2	115.246	30.6384	14.7829	40.7617	16.6447	4.0601	222.134
7	6	C	S	1	147.675	39.5603	5.7032	36.9150	6.8532	2.8151	239.522
8	6	C	S	2	112.756	34.0789	18.5253	32.0198	11.9133	2.3422	211.635
9	12	M	S	1	85.754	26.5542	5.6261	35.2669	11.5030	2.1911	166.895
10	12	M	S	2	153.663	42.6048	21.5088	38.9188	20.7590	3.8261	281.281
11	18	M	S	1	180.825	60.9449	7.9402	48.3139	12.1508	3.5559	313.731
12	18	M	S	2	301.013	87.3832	24.4832	59.4358	18.9914	5.0919	496.398
13	19	C	A	1	108.947	30.9677	5.1829	29.3339	6.2544	2.2628	182.949
14	19	C	A	2	120.145	29.5744	10.1165	35.4606	17.5118	4.7689	217.577
15	22	M	S	1	174.741	60.1458	8.2249	46.7409	12.3009	3.3812	305.535
16	22	M	S	2	61.287	17.8273	22.8627	24.8631	10.2010	2.0031	139.044
17	26	M	A	1	157.745	51.1509	5.5341	41.0344	11.2708	3.4930	270.228
18	26	M	A	2	205.544	58.7762	13.0473	39.2639	21.7264	4.7646	343.122
19	27	M	A	1	148.329	53.7844	6.1854	36.9629	8.8779	5.1251	259.265
20	27	M	A	2	137.615	31.6327	10.4701	28.0275	21.0724	5.2572	234.075
21	29	C	A	1	239.953	70.9677	7.0661	61.4983	8.5818	6.2495	394.316
22	29	C	A	2	152.979	39.5883	12.5105	38.9850	19.7077	4.3278	268.098
23	33	M	S	1	208.964	77.4882	9.4975	52.4295	17.0780	5.1592	370.616
24	33	M	S	2	118.472	36.5583	20.3285	33.2574	11.6707	2.3494	222.636
25	36	M	A	1	166.879	55.1245	5.7474	28.6696	9.8481	4.3730	270.642
26	36	M	A	2	119.991	28.9999	9.2467	30.1424	19.5948	4.7732	212.748
27	40	C	S	1	197.537	59.6509	6.8388	50.5029	9.8399	3.7857	328.155
28	40	C	S	2	146.637	44.7966	18.8387	33.0181	12.7842	2.4158	258.490
29	43	M	A	1	218.702	71.4431	7.1318	50.7152	12.0600	3.4689	363.521
30	43	M	A	2	167.719	50.1274	12.7143	43.0887	20.0066	3.2991	296.955
31	45	C	A	1	180.742	53.7906	5.6933	42.8407	8.7065	3.4127	295.186
32	45	C	A	2	5.657	3.7709	0.0000	46.9852	6.5413	0.0000	62.955
33	46	M	A	1	167.236	57.0802	6.7032	35.3301	10.9277	3.6228	280.900
34	46	M	A	2	170.303	48.5631	11.5888	29.8585	18.6164	3.4428	282.373
35	49	C	S	1	221.991	63.2858	9.1506	55.5247	13.1540	6.7443	369.850
36	49	C	S	2	55.909	14.6279	11.5971	30.1892	9.3232	3.4476	125.094
37	51	C	S	1	123.676	32.5971	5.0381	30.0672	7.6396	2.5615	201.579
38	51	C	S	2	168.872	53.9542	17.8661	36.2253	11.8549	2.8482	291.621
39	66	M	A	1	179.957	52.0631	5.3740	35.0572	6.7884	3.0753	282.315
40	66	M	A	2	127.324	32.2465	9.1436	29.7123	16.8967	3.9869	219.310
41	69	M	S	1	107.311	41.6977	5.5600	37.1045	11.9069	3.3667	206.947
42	69	M	S	2	285.170	76.6155	24.7845	49.4276	17.3564	3.5213	456.875
43	78	M	S	1	194.141	65.0250	8.4202	55.9702	12.9328	4.4568	340.946
44	78	M	S	2	77.808	28.5777	23.2788	29.7472	8.7722	1.8434	170.027
45	83	C	A	1	168.368	48.2874	7.3939	46.3235	12.7393	5.1148	288.227
46	83	C	A	2	93.298	24.8386	14.2164	26.9808	11.8076	2.4785	173.620
47	87	C	A	1	109.391	30.8705	5.0865	33.7355	7.5146	1.8332	188.431
48	87	C	A	2	78.671	20.2520	10.2616	27.1242	10.5975	2.0746	148.981

Table 4. Serum Progesterone
 (Date = SAS date where 9895 = Feb. 3, 1987;
 P4 = ng/ml progesterone; 0.0 = undetectable
 levels)

OBS	ID	BREED	TRT	DATE	P4
1	49	S	C	9895	0.58447
2	49	S	C	9897	0.44886
3	49	S	C	9902	0.25428
4	49	S	C	9905	0.19045
5	49	S	C	9909	0.36156
6	49	S	C	9912	0.29159
7	49	S	C	9919	0.47568
8	49	S	C	9923	0.48160
9	49	S	C	9926	0.39329
10	49	S	C	9930	0.49784
11	49	S	C	9933	0.23608
12	49	S	C	9940	0.30306
13	49	S	C	9944	0.28022
14	49	S	C	9947	0.29687
15	49	S	C	9951	0.31344
16	49	S	C	9954	0.29125
17	49	S	C	9961	0.45313
18	49	S	C	9965	0.27035
19	49	S	C	9968	0.34233
20	49	S	C	9972	0.41119
21	49	S	C	9975	0.16995
22	49	S	C	9982	0.16685
23	49	S	C	9986	0.18917
24	49	S	C	9989	0.13964
25	49	S	C	9993	0.87774
26	49	S	C	9995	0.96140
27	49	S	C	10003	0.77672
28	43	A	M	9897	2.28274
29	43	A	M	9902	1.04673
30	43	A	M	9905	0.61293
31	43	A	M	9909	0.23546
32	43	A	M	9912	0.76117
33	43	A	M	9919	1.02951
34	43	A	M	9923	0.86564
35	43	A	M	9926	0.95815
36	43	A	M	9930	0.67901
37	43	A	M	9933	0.48233
38	43	A	M	9937	0.42954
39	43	A	M	9940	0.41757
40	43	A	M	9944	0.71728
41	43	A	M	9947	0.51115
42	43	A	M	9951	0.37623
43	43	A	M	9954	0.53288
44	43	A	M	9958	0.28390
45	43	A	M	9961	0.66920
46	43	A	M	9965	0.71619
47	43	A	M	9968	0.42736
48	43	A	M	9972	0.60931
49	43	A	M	9975	0.28236
50	43	A	M	9979	0.17250
51	43	A	M	9982	0.27557
52	43	A	M	9986	0.27081
53	43	A	M	9989	0.42910
54	43	A	M	9993	0.28904
55	43	A	M	9995	0.32954
56	43	A	M	10000	0.613865
57	43	A	M	10003	0.258886

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
1	19	A	C	9895	0.0000
2	19	A	C	9897	0.2581
3	19	A	C	9905	0.0000
4	19	A	C	9909	0.0000
5	19	A	C	9912	0.0954
6	19	A	C	9919	0.0000
7	19	A	C	9923	0.0000
8	19	A	C	9926	0.0000
9	19	A	C	9930	0.0000
10	19	A	C	9933	0.0000
11	19	A	C	9940	0.0000
12	19	A	C	9944	0.0000
13	19	A	C	9947	0.0000
14	19	A	C	9951	0.0000
15	19	A	C	9954	0.0000
16	19	A	C	9961	0.0000
17	19	A	C	9965	0.0000
18	19	A	C	9968	0.0000
19	19	A	C	9972	0.0000
20	19	A	C	9975	0.6389
21	19	A	C	9982	0.0798
22	19	A	C	9986	5.8649
23	19	A	C	9989	7.9382
24	19	A	C	9993	11.7720
25	19	A	C	9995	11.5862
26	19	A	C	10003	0.9942
27	2	S	C	9895	0.5417
28	2	S	C	9897	0.3176
29	2	S	C	9902	0.4436
30	2	S	C	9905	0.5121
31	2	S	C	9909	0.4461
32	2	S	C	9912	0.7843
33	2	S	C	9919	0.4892
34	2	S	C	9923	0.7208
35	2	S	C	9926	0.3807
36	2	S	C	9930	0.3654
37	2	S	C	9933	0.7031
38	2	S	C	9940	0.5181
39	2	S	C	9944	0.4730
40	2	S	C	9947	0.1814
41	2	S	C	9951	0.0946
42	2	S	C	9954	0.3106
43	2	S	C	9961	0.2917
44	2	S	C	9965	0.5335
45	2	S	C	9968	0.2525
46	2	S	C	9972	0.5044
47	2	S	C	9975	0.2647
48	2	S	C	9982	0.0000
49	2	S	C	9986	0.1690
50	2	S	C	9989	0.1074
51	2	S	C	9993	0.2121
52	2	S	C	9995	0.3627
53	2	S	C	10003	0.3760

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
164	69	S	M	9895	0.0000
165	69	S	M	9897	0.0000
166	69	S	M	9902	0.000000
167	69	S	M	9905	0.117084
168	69	S	M	9909	0.000000
169	69	S	M	9912	0.000000
170	69	S	M	9916	0.000000
171	69	S	M	9919	0.000000
172	69	S	M	9923	0.000000
173	69	S	M	9926	0.000000
174	69	S	M	9930	0.000000
175	69	S	M	9933	0.000000
176	69	S	M	9937	0.000000
177	69	S	M	9940	0.000000
178	69	S	M	9944	0.000000
179	69	S	M	9947	0.000000
180	69	S	M	9951	0.000000
181	69	S	M	9954	0.000000
182	69	S	M	9958	0.239700
183	69	S	M	9961	0.000000
184	69	S	M	9965	0.000000
185	69	S	M	9968	0.000000
186	69	S	M	9972	0.000000
187	69	S	M	9975	0.000000
188	69	S	M	9979	0.000000
189	69	S	M	9982	0.000000
190	69	S	M	9986	0.000000
191	69	S	M	9989	0.000000
192	69	S	M	9993	0.000000
193	69	S	M	9995	0.000000
194	69	S	M	10000	0.000000
195	69	S	M	10003	0.000000
196	51	S	C	9897	0.145796
197	51	S	C	9902	0.097263
198	51	S	C	9905	0.000000
199	51	S	C	9909	0.000000
200	51	S	C	9912	0.000000
201	51	S	C	9916	0.000000
202	51	S	C	9919	0.000000
203	51	S	C	9923	0.000000
204	51	S	C	9926	0.000000
205	51	S	C	9930	0.000000
206	51	S	C	9933	0.000000
207	51	S	C	9937	0.000000
208	51	S	C	9940	0.000000
209	51	S	C	9944	0.000000
210	51	S	C	9947	0.000000
211	51	S	C	9951	0.000000
212	51	S	C	9954	0.000000
213	51	S	C	9958	0.000000
214	51	S	C	9961	0.000000
215	51	S	C	9965	0.000000
216	51	S	C	9968	0.000000
217	51	S	C	9972	0.000000
218	51	S	C	9975	0.000000
219	51	S	C	9979	0.000000
220	51	S	C	9982	0.000000
221	51	S	C	9986	0.000000
222	51	S	C	9989	0.28399
223	51	S	C	9993	0.000000
224	51	S	C	9995	0.38774
225	51	S	C	10000	0.12682
226	51	S	C	10003	0.000000

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
551	40	S	C	9895	0.672263
552	40	S	C	9897	0.000000
553	40	S	C	9902	0.000000
554	40	S	C	9905	0.119243
555	40	S	C	9909	0.000000
556	40	S	C	9912	0.602850
557	40	S	C	9916	0.082399
558	40	S	C	9919	0.000000
559	40	S	C	9923	0.000000
560	40	S	C	9926	0.000000
561	40	S	C	9930	0.118921
562	40	S	C	9933	0.000000
563	40	S	C	9937	0.000000
564	40	S	C	9940	0.000000
565	40	S	C	9944	0.000000
566	40	S	C	9947	0.000000
567	40	S	C	9951	0.000000
568	40	S	C	9954	0.000000
569	40	S	C	9958	0.000000
570	40	S	C	9961	0.000000
571	40	S	C	9965	0.000000
572	40	S	C	9968	0.000000
573	40	S	C	9972	0.000000
574	40	S	C	9975	0.000000
575	40	S	C	9979	0.000000
576	40	S	C	9982	0.000000
577	40	S	C	9986	0.000000
578	40	S	C	9989	0.000000
579	40	S	C	9993	0.000000
580	40	S	C	9995	0.000000
581	40	S	C	10000	0.101983
582	40	S	C	10003	0.000000
408	29	A	C	9895	0.4436
409	29	A	C	9897	0.1733
410	29	A	C	9902	0.1141
411	29	A	C	9905	0.0000
412	29	A	C	9909	0.0000
413	29	A	C	9912	0.0000
414	29	A	C	9919	0.0000
415	29	A	C	9923	0.2131
416	29	A	C	9926	0.3303
417	29	A	C	9930	0.0924
418	29	A	C	9933	0.0000
419	29	A	C	9940	0.0000
420	29	A	C	9944	0.0000
421	29	A	C	9947	0.0844
422	29	A	C	9951	0.0000
423	29	A	C	9954	0.0000
424	29	A	C	9961	0.2086
425	29	A	C	9965	0.1388
426	29	A	C	9968	0.0000
427	29	A	C	9972	0.0000
428	29	A	C	9975	0.0000
429	29	A	C	9982	0.0000
430	29	A	C	9986	0.0000
431	29	A	C	9989	0.6161
432	29	A	C	9993	0.0000
433	29	A	C	9995	0.0000
434	29	A	C	10003	0.0000

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
351	66	A	M	9895	0.00000
352	66	A	M	9897	0.07618
353	66	A	M	9905	0.00000
354	66	A	M	9909	0.00000
355	66	A	M	9912	0.07664
356	66	A	M	9916	0.26744
357	66	A	M	9919	0.00000
358	66	A	M	9923	0.00000
359	66	A	M	9926	0.19016
360	66	A	M	9930	0.00000
361	66	A	M	9933	0.00000
362	66	A	M	9937	0.00000
363	66	A	M	9940	0.00000
364	66	A	M	9944	0.00000
365	66	A	M	9947	0.00000
366	66	A	M	9951	0.00000
367	66	A	M	9954	0.00000
368	66	A	M	9958	0.00000
369	66	A	M	9961	0.00000
370	66	A	M	9965	0.00000
371	66	A	M	9968	0.00000
372	66	A	M	9972	0.00000
373	66	A	M	9975	0.00000
374	66	A	M	9979	0.00000
375	66	A	M	9982	0.00000
376	66	A	M	9986	0.15792
377	66	A	M	9989	0.83783
378	66	A	M	9993	0.94631
379	66	A	M	9995	1.00737
380	66	A	M	10000	1.20743
381	66	A	M	10003	0.00000
382	87	A	C	9895	0.55638
383	87	A	C	9897	1.14427
384	87	A	C	9905	0.21707
385	87	A	C	9909	1.63075
386	87	A	C	9912	1.5545
387	87	A	C	9919	0.4555
388	87	A	C	9923	0.3806
389	87	A	C	9926	0.2890
390	87	A	C	9930	0.3521
391	87	A	C	9933	1.3054
392	87	A	C	9940	0.3728
393	87	A	C	9944	0.4560
394	87	A	C	9947	0.0759
395	87	A	C	9951	0.8622
396	87	A	C	9954	0.1237
397	87	A	C	9961	0.7713
398	87	A	C	9965	0.1230
399	87	A	C	9968	0.7340
400	87	A	C	9972	7.9251
401	87	A	C	9975	1.2923
402	87	A	C	9982	13.6878
403	87	A	C	9986	36.2568
404	87	A	C	9989	42.2236
405	87	A	C	9993	47.2343
406	87	A	C	9995	35.6303
407	87	A	C	10003	10.5641

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
292	3	A	C	9895	0.99517
293	3	A	C	9897	1.82407
294	3	A	C	9902	0.80953
295	3	A	C	9905	0.17303
296	3	A	C	9909	0.62424
297	3	A	C	9912	2.28468
298	3	A	C	9919	0.49674
299	3	A	C	9923	0.37534
300	3	A	C	9926	0.98478
301	3	A	C	9930	0.73959
302	3	A	C	9933	1.01096
303	3	A	C	9940	0.89559
304	3	A	C	9944	0.27232
305	3	A	C	9947	0.08943
306	3	A	C	9951	0.30204
307	3	A	C	9954	0.27980
308	3	A	C	9961	0.50029
309	3	A	C	9965	0.29677
310	3	A	C	9968	0.79353
311	3	A	C	9972	0.71280
312	3	A	C	9975	0.37661
313	3	A	C	9982	0.00000
314	3	A	C	9986	0.47746
315	3	A	C	9989	0.00000
316	3	A	C	9993	0.27603
317	3	A	C	9995	0.19718
318	3	A	C	10003	0.00000
319	6	S	C	9895	1.66294
320	6	S	C	9897	0.15122
321	6	S	C	9902	1.21945
322	6	S	C	9905	1.23189
323	6	S	C	9909	0.23656
324	6	S	C	9912	0.22915
325	6	S	C	9916	0.75553
326	6	S	C	9919	0.28496
327	6	S	C	9923	0.08472
328	6	S	C	9926	1.24278
329	6	S	C	9930	0.47746
330	6	S	C	9933	0.18603
331	6	S	C	9937	0.56416
332	6	S	C	9940	0.70215
333	6	S	C	9944	1.14060
334	6	S	C	9947	1.21081
335	6	S	C	9951	0.00000
336	6	S	C	9954	2.10822
337	6	S	C	9958	2.03461
338	6	S	C	9961	0.95395
339	6	S	C	9965	0.00000
340	6	S	C	9968	0.54115
341	6	S	C	9972	0.35149
342	6	S	C	9975	0.22485
343	6	S	C	9979	0.92267
344	6	S	C	9982	1.41120
345	6	S	C	9986	0.39276
346	6	S	C	9989	0.00000
347	6	S	C	9993	1.23043
348	6	S	C	9995	0.00000
349	6	S	C	10000	0.51524
350	6	S	C	10003	0.38097

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
227	26	A	M	9895	0.00000
228	26	A	M	9897	0.00000
229	26	A	M	9902	0.00000
230	26	A	M	9905	0.00000
231	26	A	M	9909	0.00000
232	26	A	M	9912	0.00000
233	26	A	M	9916	0.50454
234	26	A	M	9919	0.00000
235	26	A	M	9923	0.00000
236	26	A	M	9926	0.00000
237	26	A	M	9930	0.00000
238	26	A	M	9933	0.00000
239	26	A	M	9937	1.22089
240	26	A	M	9940	0.00000
241	26	A	M	9944	0.00000
242	26	A	M	9895	0.00000
243	26	A	M	9947	0.00000
244	26	A	M	9951	0.00000
245	26	A	M	9954	0.00000
246	26	A	M	9958	0.00000
247	26	A	M	9961	0.00000
248	26	A	M	9965	0.00000
249	26	A	M	9968	0.00000
250	26	A	M	9972	0.00000
251	26	A	M	9975	0.00000
252	26	A	M	9979	0.12360
253	26	A	M	9982	0.27473
254	26	A	M	9986	0.99584
255	26	A	M	9989	0.00000
256	26	A	M	9993	0.00000
257	26	A	M	9995	0.35220
258	26	A	M	10000	0.08426
259	26	A	M	10003	0.27103
260	36	A	M	9895	1.82577
261	36	A	M	9897	0.99922
262	36	A	M	9902	1.48344
263	36	A	M	9905	0.00000
264	36	A	M	9909	0.00000
265	36	A	M	9912	1.89952
266	36	A	M	9916	1.45509
267	36	A	M	9919	0.88340
268	36	A	M	9923	1.39163
269	36	A	M	9926	0.71208
270	36	A	M	9930	0.90427
271	36	A	M	9933	0.64180
272	36	A	M	9937	1.59932
273	36	A	M	9940	1.51182
274	36	A	M	9944	0.00000
275	36	A	M	9947	2.30272
276	36	A	M	9951	0.93414
277	36	A	M	9954	0.09053
278	36	A	M	9958	3.00019
279	36	A	M	9961	0.09912
280	36	A	M	9965	0.24686
281	36	A	M	9968	1.24932
282	36	A	M	9972	0.76881
283	36	A	M	9975	1.09892
284	36	A	M	9979	0.00000
285	36	A	M	9982	0.56263
286	36	A	M	9986	0.09993
287	36	A	M	9989	0.00000
288	36	A	M	9993	0.00000
289	36	A	M	9995	0.56073
290	36	A	M	10000	3.04302
291	36	A	M	10003	0.00000

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
435	18	S	M	9895	0.0000
436	18	S	M	9897	0.0000
437	18	S	M	9902	0.0000
438	18	S	M	9905	0.0000
439	18	S	M	9909	0.0000
440	18	S	M	9912	0.0000
441	18	S	M	9916	0.000000
442	18	S	M	9919	0.149190
443	18	S	M	9923	0.000000
444	18	S	M	9926	0.000000
445	18	S	M	9930	0.000000
446	18	S	M	9933	0.000000
447	18	S	M	9937	0.000000
448	18	S	M	9940	0.000000
449	18	S	M	9944	0.000000
450	18	S	M	9947	0.000000
451	18	S	M	9951	0.000000
452	18	S	M	9954	0.000000
453	18	S	M	9958	0.000000
454	18	S	M	9961	0.000000
455	18	S	M	9965	0.000000
456	18	S	M	9968	0.000000
457	18	S	M	9972	0.000000
458	18	S	M	9975	0.000000
459	18	S	M	9979	0.218989
460	18	S	M	9982	0.000000
461	18	S	M	9986	0.000000
462	18	S	M	9989	0.000000
463	18	S	M	9993	0.000000
464	18	S	M	9995	0.000000
465	18	S	M	10000	0.000000
466	18	S	M	10003	0.000000
467	45	A	C	9895	0.000000
468	45	A	C	9897	0.000000
469	45	A	C	9902	0.000000
470	45	A	C	9905	0.000000
471	45	A	C	9909	0.000000
472	45	A	C	9912	0.000000
473	45	A	C	9919	0.000000
474	45	A	C	9923	0.000000
475	45	A	C	9926	0.000000
476	45	A	C	9930	0.000000
477	45	A	C	9933	0.000000
478	45	A	C	9940	0.900151
479	45	A	C	9944	0.192040
480	45	A	C	9947	0.000000
481	45	A	C	9951	0.000000
482	45	A	C	9954	0.000000
483	45	A	C	9961	0.000000
484	45	A	C	9965	0.000000
485	45	A	C	9968	0.000000
486	45	A	C	9972	0.000000
487	45	A	C	9975	0.000000
488	45	A	C	9982	0.000000
489	45	A	C	9986	0.000000
490	45	A	C	9989	0.000000
491	45	A	C	9993	0.000000
492	45	A	C	9995	0.000000
493	45	A	C	10003	0.373188

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
112	83	A	C	9895	0.1227
113	83	A	C	9897	0.0000
114	83	A	C	9902	0.0000
115	83	A	C	9905	0.0000
116	83	A	C	9909	0.1900
117	83	A	C	9919	0.0000
118	83	A	C	9923	0.0000
119	83	A	C	9926	0.0000
120	83	A	C	9930	0.0000
121	83	A	C	9933	0.0000
122	83	A	C	9940	0.0000
123	83	A	C	9944	0.0000
124	83	A	C	9947	0.0000
125	83	A	C	9951	0.0000
126	83	A	C	9954	0.0000
127	83	A	C	9961	0.1536
128	83	A	C	9965	0.6410
129	83	A	C	9968	0.0879
130	83	A	C	9972	16.8262
131	83	A	C	9975	22.5248
132	83	A	C	9982	1.1162
133	83	A	C	9986	0.4803
134	83	A	C	9989	3.7324
135	83	A	C	9993	16.0539
136	83	A	C	9995	18.0583
137	83	A	C	10003	0.0000
138	78	S	M	9895	0.2798
139	78	S	M	9897	2.4000
140	78	S	M	9902	0.2983
141	78	S	M	9905	0.0856
142	78	S	M	9909	0.0000
143	78	S	M	9912	0.1522
144	78	S	M	9919	0.0000
145	78	S	M	9923	0.0000
146	78	S	M	9926	0.0000
147	78	S	M	9930	0.0000
148	78	S	M	9933	0.1427
149	78	S	M	9940	0.2747
150	78	S	M	9944	0.1095
151	78	S	M	9947	0.0000
152	78	S	M	9951	0.0000
153	78	S	M	9954	0.0000
154	78	S	M	9961	0.0000
155	78	S	M	9965	0.0000
156	78	S	M	9968	0.0000
157	78	S	M	9972	0.0000
158	78	S	M	9975	0.0000
159	78	S	M	9982	0.3494
160	78	S	M	9986	0.0000
161	78	S	M	9993	0.0000
162	78	S	M	9995	0.0000
163	78	S	M	10003	0.0000

Table 4, cont.

OBS	ID	BREED	TRT	DATE	P4
54	22	S	M	9895	0.6912
55	22	S	M	9897	0.3488
56	22	S	M	9902	1.95744
57	22	S	M	9905	0.48478
58	22	S	M	9909	0.27311
59	22	S	M	9912	0.25858
60	22	S	M	9919	0.34152
61	22	S	M	9923	0.18818
62	22	S	M	9926	0.22923
63	22	S	M	9930	0.00000
64	22	S	M	9933	1.53139
65	22	S	M	9940	0.00000
66	22	S	M	9944	0.57690
67	22	S	M	9947	0.79501
68	22	S	M	9951	0.21810
69	22	S	M	9954	0.79326
70	22	S	M	9961	0.37458
71	22	S	M	9965	0.21604
72	22	S	M	9968	0.00000
73	22	S	M	9972	0.25304
74	22	S	M	9975	0.80394
75	22	S	M	9982	0.19172
76	22	S	M	9986	0.26007
77	22	S	M	9989	0.26966
78	22	S	M	9993	8.21281
79	22	S	M	9995	1.00601
80	22	S	M	10003	3.21656
81	46	A	M	9895	0.00000
82	46	A	M	9897	0.00000
83	46	A	M	9902	0.00000
84	46	A	M	9905	0.00000
85	46	A	M	9909	0.68582
86	46	A	M	9916	0.00000
87	46	A	M	9919	0.00000
88	46	A	M	9923	0.00000
89	46	A	M	9926	0.00000
90	46	A	M	9930	0.00000
91	46	A	M	9933	0.79179
92	46	A	M	9937	0.00000
93	46	A	M	9940	0.00000
94	46	A	M	9944	0.10254
95	46	A	M	9947	0.00000
96	46	A	M	9951	0.00000
97	46	A	M	9954	0.00000
98	46	A	M	9958	0.00000
99	46	A	M	9961	0.00000
100	46	A	M	9965	0.08080
101	46	A	M	9968	0.00000
102	46	A	M	9972	0.00000
103	46	A	M	9975	0.00000
104	46	A	M	9979	0.00000
105	46	A	M	9982	0.74587
106	46	A	M	9986	0.00000
107	46	A	M	9989	0.52723
108	46	A	M	9993	0.00000
109	46	A	M	9995	0.53714
110	46	A	M	10000	0.32649
111	46	A	M	10003	0.2992

Table 4, cont.

OBS	ID	BREED	TRY	DATE	P4
494	12	S	M	9897	0.241982
495	12	S	M	9902	0.000000
496	12	S	M	9905	0.0000
497	12	S	M	9909	0.2567
498	12	S	M	9912	1.2412
499	12	S	M	9919	0.0862
500	12	S	M	9923	0.1341
501	12	S	M	9926	0.4622
502	12	S	M	9930	0.0000
503	12	S	M	9933	0.0000
504	12	S	M	9937	0.0000
505	12	S	M	9940	0.4718
506	12	S	M	9944	0.0000
507	12	S	M	9947	0.0000
508	12	S	M	9951	0.0000
509	12	S	M	9954	0.0000
510	12	S	M	9958	0.0000
511	12	S	M	9961	0.0000
512	12	S	M	9965	0.0000
513	12	S	M	9968	0.3236
514	12	S	M	9972	0.0000
515	12	S	M	9975	0.0000
516	12	S	M	9979	0.0000
517	12	S	M	9982	0.0000
518	12	S	M	9986	0.0000
519	12	S	M	9989	0.0000
520	12	S	M	9993	0.0000
521	12	S	M	9995	0.2124
522	12	S	M	10000	0.0801
523	12	S	M	10003	0.0000
524	33	S	M	9895	0.3814
525	33	S	M	9897	0.0000
526	33	S	M	9902	0.0000
527	33	S	M	9905	0.0000
528	33	S	M	9909	0.0000
529	33	S	M	9912	0.1181
530	33	S	M	9919	0.0000
531	33	S	M	9923	0.0000
532	33	S	M	9926	0.0000
533	33	S	M	9930	0.1691
534	33	S	M	9933	0.0916
535	33	S	M	9940	0.2928
536	33	S	M	9944	0.5621
537	33	S	M	9947	0.0000
538	33	S	M	9951	0.0849
539	33	S	M	9954	0.0911
540	33	S	M	9961	0.0000
541	33	S	M	9965	0.1792
542	33	S	M	9968	0.2320
543	33	S	M	9972	0.0000
544	33	S	M	9975	0.0000
545	33	S	M	9982	0.2197
546	33	S	M	9986	0.0000
547	33	S	M	9989	0.4077
548	33	S	M	9993	10.3837
549	33	S	M	9995	0.4595
550	33	S	M	10003	9.4441

Table 5. Serum Luteinizing Hormone
 (Time = minutes; LHP1 to LHP6 = ng/ml LH at
 sampling periods one to six; value of -5
 represents missing data point)

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5	LHP6
ID = 18 TRT = M BRD = S	1	20	4.40	1.65	38.60	3.65	13.00	2.64
	2	40	2.50	1.95	18.35	2.90	11.40	2.28
	3	60	2.55	14.40	17.05	2.25	5.60	2.94
	4	80	32.65	19.55	14.15	63.85	13.45	1.80
	5	100	9.65	3.10	6.10	9.95	2.65	2.16
	6	120	7.80	2.25	2.55	5.35	6.05	21.12
	7	140	3.40	2.65	2.85	5.30	5.50	16.74
	8	160	8.30	4.30	3.35	6.00	1.95	-6.00
	9	180	2.45	12.80	4.05	5.65	1.35	-6.00
	10	200	9.60	2.55	3.70	3.00	1.80	-6.00
	11	220	2.25	4.45	4.60	5.20	10.05	-6.00
	12	240	2.45	2.20	11.80	2.05	6.60	-6.00
	13	260	5.45	2.80	5.45	3.90	6.50	-6.00
	14	280	1.90	1.75	2.70	4.45	2.10	-6.00
	15	300	1.05	19.85	2.95	8.50	3.70	-6.00
	16	320	1.60	10.00	3.05	12.30	0.00	-6.00
	17	340	1.85	6.00	6.20	15.05	4.85	-6.00
	18	360	-5.00	5.35	1.75	6.80	1.90	-6.00
	19	380	-5.00	6.10	5.25	8.05	1.85	-6.00
	20	400	-5.00	3.15	25.10	7.05	1.85	-6.00
	21	420	-5.00	3.55	26.05	5.65	32.35	-6.00
	22	440	-5.00	3.55	6.70	3.55	5.45	-6.00
ID = 69 TRT = M BRD = S	23	20	2.40	4.85	2.80	6.40	1.75	43.38
	24	40	7.05	2.85	11.25	1.95	14.10	2.64
	25	60	1.90	3.75	2.55	9.05	8.65	1.62
	26	80	2.45	3.50	15.20	5.00	3.15	1.56
	27	100	3.50	4.75	2.95	6.35	3.95	1.86
	28	120	4.05	3.95	18.30	5.95	1.90	4.32
	29	140	8.00	5.55	2.40	2.75	2.85	6.18
	30	160	2.95	45.30	56.70	8.40	3.75	3.12
	31	180	4.00	20.35	18.00	4.30	2.90	3.12
	32	200	4.40	9.95	11.15	2.90	3.50	2.58
	33	220	2.80	10.10	6.25	1.70	2.75	14.52
	34	240	7.35	12.45	7.65	1.40	3.80	2.10
	35	260	2.60	38.70	2.95	4.70	1.40	3.00
	36	280	2.60	2.70	3.25	12.10	2.90	5.94
	37	300	1.90	6.75	3.65	5.30	1.20	4.44
	38	320	3.95	1.80	3.30	9.20	2.90	4.26
	39	340	2.85	1.55	2.20	10.50	1.65	10.20
	40	360	-5.00	5.55	5.55	8.40	24.55	35.52
	41	380	-5.00	3.70	2.50	15.10	4.45	29.70
	42	400	-5.00	2.65	2.05	130.55	4.25	14.76
	43	420	-5.00	3.85	3.75	21.65	20.90	7.62
	44	440	-5.00	5.00	12.05	9.50	-5.00	7.02
ID = 66 TRT = M BRD = A	45	20	1.55	2.50	3.55	2.40	2.05	10.86
	46	40	3.60	3.05	1.35	2.15	4.85	3.66
	47	60	1.75	3.25	1.45	1.70	20.35	4.50
	48	80	6.55	2.00	1.90	1.45	5.30	2.40
	49	100	7.35	2.50	4.55	1.60	4.45	5.28
	50	120	2.95	3.70	3.30	3.80	-5.00	7.56
	51	140	1.90	4.00	1.50	1.40	-5.00	8.16
	52	160	2.25	14.60	0.90	3.50	-5.00	2.82
	53	180	4.60	17.70	2.00	3.10	-5.00	3.36
	54	200	2.30	6.25	1.40	29.85	-5.00	1.98
	55	220	72.55	7.00	21.85	10.65	-5.00	3.54

Table 5, cont.

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5	LHP6
	56	240	15.25	2.85	9.40	5.75	-5.00	5.34
	57	260	4.80	2.75	4.45	4.80	-5.00	3.24
	58	280	3.85	4.00	4.70	3.30	-5.00	7.08
	59	300	2.90	1.60	1.90	5.20	-5.00	3.42
	60	320	11.70	10.55	2.20	1.85	-5.00	44.82
	61	340	15.25	1.85	1.75	1.75	-5.00	23.58
	62	360	-5.00	2.00	3.70	1.70	-5.00	10.74
	63	380	-5.00	1.20	3.05	4.60	-5.00	9.18
	64	400	-5.00	13.85	5.75	3.15	-5.00	4.62
	65	420	-5.00	2.10	3.25	-5.00	-5.00	4.62
	66	440	-5.00	4.20	1.90	-5.00	-5.00	3.90
	67	20	3.75	3.15	2.40	19.10	6.25	4.68
	68	40	12.80	4.45	9.55	6.45	9.95	6.96
	69	60	5.70	5.60	3.90	8.80	3.70	7.14
	70	80	13.00	4.10	3.00	4.50	4.80	39.18
	71	100	5.40	3.55	5.25	3.25	2.35	5.04
	72	120	2.60	4.15	3.95	10.85	2.55	4.50
	73	140	2.55	3.65	7.15	3.75	2.75	8.76
	74	160	3.70	4.15	2.85	5.50	2.00	18.12
	75	180	4.70	6.85	3.30	7.30	2.25	58.62
	76	200	9.00	4.20	3.15	7.45	3.05	13.92
	77	220	2.80	4.25	2.20	7.85	5.95	14.64
	78	240	3.10	3.15	4.70	9.25	4.65	8.16
	79	260	5.50	3.60	3.80	5.75	49.10	4.02
	80	280	2.50	6.85	5.00	14.95	20.35	3.30
	81	300	3.15	5.70	3.85	6.60	8.40	8.34
	82	320	3.95	9.90	41.40	6.10	9.20	4.20
	83	340	3.20	3.00	20.05	3.15	4.30	10.74
	84	360	-5.00	5.20	11.95	18.70	5.00	3.96
	85	380	-5.00	6.45	6.35	9.10	3.85	4.44
	86	400	-5.00	4.30	5.30	6.55	3.20	6.12
	87	420	-5.00	9.10	2.60	2.75	4.45	84.06
	88	440	-5.00	86.95	3.80	9.45	4.25	47.10
	89	20	1.35	1.20	8.40	1.00	1.90	1.14
	90	40	1.30	8.50	4.30	1.80	1.45	2.04
	91	60	2.25	3.40	6.20	4.75	2.40	22.80
	92	80	8.05	1.70	4.65	0.70	1.95	22.74
	93	100	4.00	2.35	35.80	0.80	0.65	7.38
	94	120	3.05	1.75	14.80	1.05	1.30	7.44
	95	140	2.50	1.80	3.00	0.80	1.00	57.84
	96	160	1.60	5.45	1.85	0.85	37.55	14.46
	97	180	1.20	1.65	1.85	1.30	0.00	12.12
	98	200	0.95	0.75	5.75	1.85	2.00	31.86
	99	220	0.85	22.80	1.55	9.55	6.95	40.56
	100	240	1.10	4.85	2.90	20.25	4.75	5.46
	101	260	5.45	2.70	1.90	10.50	11.50	4.50
	102	280	2.00	4.30	1.00	4.80	3.00	2.16
	103	300	10.55	3.35	3.20	5.55	1.90	3.24
	104	320	1.65	9.50	0.80	3.55	2.15	1.32
	105	340	-5.00	5.05	0.60	1.65	2.50	2.46
	106	360	-5.00	7.05	0.70	2.25	1.15	30.30
	107	380	-5.00	3.00	0.00	0.00	7.80	2.10
	108	400	-5.00	1.35	0.00	1.10	2.15	1.44
	109	420	-5.00	2.05	0.50	2.20	35.90	0.78
	110	440	-5.00	6.45	23.60	1.10	17.30	31.02

ID = 27
TRT = M
BRD = A

ID = 51
TRT = C
BRD = S

Table 5, cont.

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5	LHP6
ID = 26 TRT = M BRD = A	111	20	1.95	4.15	7.00	2.00	2.60	0.84
	112	40	1.90	9.40	2.30	1.30	1.25	1.80
	113	60	18.30	4.25	2.50	3.15	3.10	1.26
	114	80	46.90	8.35	2.35	14.50	1.35	0.84
	115	100	31.15	8.00	5.50	1.70	2.55	0.84
	116	120	45.05	1.40	0.80	0.95	2.10	1.14
	117	140	4.90	8.40	2.75	1.30	1.45	1.14
	118	160	9.15	1.45	22.15	0.00	3.45	0.96
	119	180	1.05	1.05	2.80	1.10	1.95	2.28
	120	200	0.70	0.95	0.90	4.60	1.40	32.88
	121	220	0.65	1.40	0.95	2.00	3.00	15.12
	122	240	1.40	19.00	1.45	6.70	5.70	13.38
	123	260	1.30	8.25	1.75	1.85	8.05	3.84
	124	280	1.30	2.65	3.80	1.30	1.75	4.62
	125	300	3.80	2.00	43.85	0.85	3.30	4.20
	126	320	16.25	3.00	29.45	8.40	3.65	7.44
	127	340	2.70	2.75	22.30	50.05	2.10	1.26
	128	360	-5.00	1.05	8.95	4.45	2.20	3.84
	129	380	-5.00	2.90	5.55	2.20	1.15	1.20
	130	400	-5.00	4.20	9.75	4.15	0.65	8.22
	131	420	-5.00	2.95	5.55	2.00	1.45	2.04
	132	440	-5.00	2.95	2.50	0.80	1.55	13.92
ID = 40 TRT = C BRD = S	133	20	4.45	3.40	10.30	3.70	4.75	10.56
	134	40	8.90	12.55	28.45	9.10	7.65	6.36
	135	60	16.70	5.35	30.30	6.20	4.20	8.28
	136	80	8.25	4.35	17.55	5.85	3.50	5.40
	137	100	8.35	2.90	35.80	4.50	3.85	6.78
	138	120	5.90	6.05	6.10	5.00	31.95	9.18
	139	140	3.80	2.40	68.90	3.45	6.90	15.30
	140	160	5.20	2.85	4.30	2.75	7.10	5.46
	141	180	5.25	7.30	7.15	3.50	9.50	134.22
	142	200	4.50	9.90	8.25	5.10	5.30	78.30
	143	220	5.75	26.85	3.20	3.85	5.00	19.86
	144	240	5.15	14.00	3.80	13.05	11.50	18.72
	145	260	5.85	9.20	6.90	8.25	6.60	16.14
	146	280	2.95	5.55	5.50	8.40	53.00	8.22
	147	300	4.40	8.90	4.20	46.55	52.55	13.74
	148	320	4.85	5.60	3.60	26.20	29.05	12.24
	149	340	3.60	6.70	6.10	8.35	41.75	133.86
	150	360	-5.00	4.05	30.45	9.05	11.30	90.66
	151	380	-5.00	4.30	21.35	80.85	10.80	27.66
	152	400	-5.00	5.80	15.75	4.30	6.95	27.06
	153	420	-5.00	4.15	17.00	18.70	5.65	11.82
	154	440	-5.00	9.60	8.65	4.95	3.95	22.98
ID = 49 TRT = C BRD = S	155	20	6.55	5.95	8.25	4.75	5.00	8.22
	156	40	7.35	7.30	5.95	2.85	-5.00	5.88
	157	60	22.65	7.95	7.65	3.80	3.35	6.72
	158	80	7.55	4.65	5.60	8.05	5.25	3.90
	159	100	6.40	6.25	6.45	22.85	16.55	7.56
	160	120	6.80	10.65	7.80	6.65	13.25	6.96
	161	140	8.85	7.45	8.65	7.65	10.90	10.86
	162	160	8.90	10.30	4.30	5.45	13.70	8.94
	163	180	4.70	8.05	5.45	9.00	7.20	11.52
	164	200	10.40	-5.00	8.95	7.75	8.35	61.56
	165	220	5.50	-5.00	8.65	6.65	15.65	19.44

Table 5, cont.

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5	LHP6
	166	240	8.25	-5.00	8.35	9.65	15.50	13.86
	167	260	10.05	-5.00	7.65	6.70	11.90	12.42
	168	280	10.45	-5.00	8.40	6.40	10.70	10.56
	169	300	5.35	-5.00	12.50	11.85	10.45	10.20
	170	320	10.10	-5.00	8.85	7.05	6.50	6.66
	171	340	5.35	-5.00	57.80	16.65	6.20	8.58
	172	360	7.55	-5.00	24.80	29.30	3.85	7.56
	173	380	6.15	-5.00	16.75	10.45	4.75	4.26
	174	400	3.85	-5.00	14.80	7.30	3.95	11.82
	175	420	-5.00	-5.00	7.50	8.65	4.35	10.14
	176	440	-5.00	-5.00	6.40	4.50	4.30	7.14
	177	20	5.15	7.30	5.50	5.70	5.15	8.58
	178	40	14.65	6.65	8.35	6.60	4.20	7.56
	179	60	5.70	8.65	6.15	13.10	4.80	10.38
	180	80	4.15	5.80	5.95	5.15	12.05	19.02
	181	100	14.65	4.25	6.20	6.00	7.85	13.38
	182	120	6.25	9.15	6.30	6.45	17.60	12.60
	183	140	8.10	3.75	5.45	13.60	4.80	5.10
	184	160	13.65	3.85	8.90	9.55	15.30	24.48
	185	180	6.20	6.75	19.20	11.15	18.80	13.68
	186	200	5.15	5.00	8.70	8.40	8.30	6.18
	187	220	8.60	4.65	9.90	8.45	9.30	6.12
	188	240	5.60	3.65	5.55	10.75	14.45	12.36
	189	260	5.10	5.35	8.80	10.30	13.05	7.14
	190	280	8.80	5.15	7.80	9.35	12.50	7.02
	191	300	4.05	7.50	34.40	9.25	14.00	6.84
	192	320	9.95	4.40	2.85	11.55	11.45	6.54
	193	340	8.60	5.45	4.55	7.45	10.25	6.84
	194	360	7.75	16.65	5.40	14.80	8.50	7.08
	195	380	6.75	20.30	4.40	5.60	6.65	6.54
	196	400	4.40	14.20	13.85	6.85	9.50	6.66
	197	420	-5.00	8.50	4.05	5.25	10.35	6.78
	198	440	-5.00	5.55	5.75	6.00	6.05	7.14
	199	20	6.00	4.15	4.75	2.90	13.55	9.36
	200	40	8.05	6.70	5.05	3.75	6.25	4.98
	201	60	3.05	2.80	1.80	2.15	5.25	3.00
	202	80	24.55	2.40	2.90	2.10	6.75	2.22
	203	100	5.80	2.85	3.15	2.40	15.85	4.14
	204	120	4.30	4.50	26.25	112.80	13.20	3.42
	205	140	2.65	3.50	14.60	15.80	5.30	2.94
	206	160	5.45	3.90	26.85	13.35	15.25	11.52
	207	180	4.65	6.60	7.40	10.15	6.45	6.84
	208	200	2.95	8.55	17.70	11.75	6.70	2.16
	209	220	2.95	11.70	7.10	7.30	2.15	2.76
	210	240	4.25	5.35	5.15	6.20	7.25	9.84
	211	260	4.70	6.80	7.25	9.70	4.55	5.10
	212	280	2.65	5.30	5.10	3.40	3.65	3.96
	213	300	25.55	4.85	5.60	5.95	20.20	10.26
	214	320	6.05	4.00	4.35	2.35	11.05	19.86
	215	340	5.10	5.85	4.10	5.30	2.75	3.42
	216	360	4.70	4.55	2.70	2.25	10.70	3.30
	217	380	4.60	2.15	5.15	2.35	3.50	13.20
	218	400	1.85	3.80	3.60	35.80	10.10	3.78
	219	420	-5.00	8.05	36.45	16.50	14.50	4.80
	220	440	-5.00	3.65	25.65	23.30	22.15	4.26

ID = 83
TRT = C
BRD = A

ID = 05
TRT = C
BRD = S

Table 5, cont.

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5	LHP6
ID = 02 TRT = C BRD = S	221	20	6.60	6.85	7.50	8.50	5.65	6.18
	222	40	4.70	8.35	12.65	7.55	7.40	8.28
	223	60	5.40	6.95	10.30	7.55	7.10	10.98
	224	80	5.50	7.45	7.45	9.15	6.55	6.72
	225	100	5.45	9.60	5.30	7.80	38.40	5.94
	226	120	4.95	7.50	10.20	14.05	12.30	6.30
	227	140	3.40	8.55	9.25	5.30	9.50	8.10
	228	160	11.10	8.15	8.05	6.25	8.40	7.68
	229	180	8.90	7.75	9.05	6.80	7.30	6.30
	230	200	9.35	8.80	9.80	4.80	7.25	5.94
	231	220	4.65	7.25	11.85	9.70	8.15	6.66
	232	240	5.50	7.20	40.65	7.40	5.65	5.64
	233	260	5.10	7.50	13.70	6.25	6.70	5.40
	234	280	5.25	6.30	9.90	6.10	5.45	6.30
	235	300	9.60	6.40	7.00	7.35	10.50	11.34
	236	320	-5.00	6.05	6.55	8.35	12.45	5.82
	237	340	9.40	5.60	8.25	5.45	9.60	6.78
	238	360	7.50	5.25	8.10	5.10	7.30	8.10
	239	380	8.20	4.65	6.20	5.35	7.65	7.20
	240	400	5.40	6.60	8.05	10.55	6.15	7.02
	241	420	-5.00	8.75	7.60	5.70	8.25	5.16
	242	440	-5.00	12.20	8.35	5.30	5.60	6.48
ID = 03 TRT = C BRD = A	243	20	4.15	4.45	5.30	8.95	5.65	10.08
	244	40	3.95	2.65	6.10	9.20	5.80	1.38
	245	60	3.20	3.60	6.80	5.65	5.50	8.34
	246	80	4.05	4.90	4.30	5.90	7.15	8.34
	247	100	4.95	4.45	5.75	5.30	6.05	8.76
	248	120	4.55	5.50	4.65	6.60	6.80	8.52
	249	140	4.45	4.45	5.20	6.20	6.20	6.00
	250	160	4.30	3.60	4.25	5.00	25.65	7.98
	251	180	5.25	3.90	7.30	5.10	12.40	7.74
	252	200	5.70	4.45	6.45	6.25	8.50	6.78
	253	220	4.85	6.05	4.65	4.50	8.35	10.68
	254	240	5.75	4.15	4.70	4.60	7.70	8.28
	255	260	4.90	2.95	0.00	5.70	6.50	99.42
	256	280	4.25	4.55	5.15	5.60	5.30	60.24
	257	300	6.65	4.30	12.75	4.40	4.95	38.04
	258	320	7.90	4.20	31.40	4.40	5.00	18.42
	259	340	4.50	4.45	13.15	4.20	4.85	17.46
	260	360	4.90	8.50	14.85	4.45	5.10	12.54
	261	380	5.35	5.55	8.25	4.65	5.95	12.30
	262	400	9.40	4.65	6.40	4.35	68.15	7.56
	263	420	-5.00	9.00	5.70	4.40	27.50	7.74
	264	440	-5.00	4.80	11.40	6.60	10.45	9.18
ID = 06 TRT = C BRD = S	265	20	2.40	2.85	9.75	2.45	9.20	4.08
	266	40	2.65	3.35	5.30	1.90	7.55	3.66
	267	60	3.30	1.40	3.40	2.15	4.90	4.38
	268	80	1.90	2.80	2.50	2.30	5.45	7.92
	269	100	1.90	2.25	1.60	3.55	3.10	3.42
	270	120	1.75	1.65	2.25	11.90	4.15	3.24
	271	140	4.50	1.80	1.95	11.10	3.15	37.68
	272	160	1.85	6.70	7.90	4.65	3.10	16.68
	273	180	2.30	2.30	1.90	3.65	2.70	8.76
	274	200	1.70	3.15	2.20	3.40	2.00	9.96
	275	220	2.15	16.05	3.10	2.60	13.45	6.66

Table 5, cont.

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5	LHP6
	276	240	2.55	17.20	1.75	2.20	12.75	6.42
	277	260	2.65	10.85	1.10	2.60	5.55	3.36
	278	280	2.25	11.40	2.60	2.05	4.85	2.46
	279	300	2.65	5.60	1.30	2.50	5.70	5.52
	280	320	4.95	7.00	1.45	2.70	3.70	4.26
	281	340	1.90	2.30	2.25	2.35	4.00	3.18
	282	360	-5.00	2.20	1.40	2.50	1.90	3.12
	283	380	-5.00	2.65	2.60	2.65	2.35	3.72
	284	400	-5.00	2.45	18.05	1.90	2.30	3.66
	285	420	-5.00	2.35	16.95	1.85	5.45	5.88
	286	440	-5.00	2.15	10.90	3.40	8.40	42.12
	287	20	6.90	6.10	8.65	7.05	14.60	16.80
	288	40	7.40	5.85	7.40	7.80	8.55	16.80
	289	60	6.65	7.20	7.75	8.80	3.55	10.44
	290	80	6.35	7.65	6.65	10.70	6.70	10.92
	291	100	6.90	7.20	6.25	6.95	5.40	8.22
	292	120	11.90	10.50	6.80	7.15	6.05	7.50
	293	140	9.00	6.05	8.05	7.70	5.60	9.96
	294	160	5.80	6.90	6.25	7.25	9.50	11.58
	295	180	5.60	7.75	6.15	7.45	9.70	8.64
	296	200	6.30	6.45	6.70	6.25	7.75	8.46
	297	220	5.00	7.10	3.10	6.65	10.15	8.58
	298	240	5.35	7.55	6.25	6.90	8.25	8.46
	299	260	5.75	8.25	9.30	6.35	9.95	6.24
	300	280	5.15	7.60	6.00	6.25	27.70	6.00
	301	300	6.55	6.95	5.95	3.55	16.40	6.96
	302	320	6.30	8.05	6.30	5.65	12.60	7.68
	303	340	6.55	6.60	5.50	6.35	10.10	8.52
	304	360	-5.00	5.25	7.15	6.20	13.50	7.86
	305	380	-5.00	8.25	6.00	6.15	17.95	11.16
	306	400	-5.00	6.05	6.10	8.25	7.05	6.54
	307	420	-5.00	6.80	7.00	5.90	16.25	10.44
	308	440	-5.00	7.55	6.30	7.70	7.25	7.74
	309	20	3.10	2.85	2.55	3.10	2.90	3.12
	310	40	3.95	4.60	2.40	2.80	2.45	6.24
	311	60	2.80	2.50	1.90	2.65	5.30	3.48
	312	80	3.40	3.05	2.10	6.00	3.80	2.64
	313	100	2.80	3.45	3.00	6.75	4.10	2.34
	314	120	4.10	2.95	2.70	3.90	2.15	3.48
	315	140	2.65	3.50	15.55	2.70	2.25	2.64
	316	160	3.20	3.35	8.75	2.55	2.40	1.86
	317	180	3.20	3.70	5.15	2.50	27.10	2.94
	318	200	2.65	21.50	3.85	2.90	10.40	2.88
	319	220	2.85	18.10	3.60	1.60	6.70	3.18
	320	240	2.55	7.05	3.75	2.30	6.30	2.64
	321	260	3.65	5.95	3.65	2.65	4.00	2.16
	322	280	3.00	5.30	3.00	9.70	2.90	33.54
	323	300	2.10	5.30	2.30	9.05	3.90	14.64
	324	320	3.00	2.55	2.60	5.45	2.80	6.78
	325	340	3.25	2.45	2.70	5.35	3.15	5.16
	326	360	3.20	3.25	2.10	4.15	2.35	4.80
	327	380	15.45	5.30	1.80	4.40	48.20	4.62
	328	400	3.20	3.95	16.95	2.65	12.05	4.14
	329	420	-5.00	5.80	17.55	2.50	9.00	3.84
	330	440	-5.00	3.20	11.25	2.15	5.55	2.88

ID = 36
TRT = M
BRD = A

ID = 78
TRT = M
BRD = S

Table 5, cont.

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5	LHP6
ID = 87 TRT = C BRD = A	331	20	9.35	3.50	18.95	9.50	7.25	13.14
	332	40	6.20	0.55	5.80	6.35	3.90	14.64
	333	60	5.40	11.40	3.60	5.00	13.30	13.92
	334	80	7.00	5.50	6.85	7.30	4.00	9.48
	335	100	6.35	8.00	35.40	87.45	5.35	8.22
	336	120	11.70	5.60	9.05	17.10	3.65	15.42
	337	140	8.10	5.15	4.60	12.60	5.85	9.00
	338	160	8.40	7.60	9.25	9.25	10.65	9.30
	339	180	7.35	14.00	5.45	9.65	6.65	11.94
	340	200	7.75	11.25	8.00	7.35	5.25	11.94
	341	220	12.30	0.00	5.60	6.15	5.00	7.50
	342	240	9.85	8.00	5.95	4.25	6.85	9.06
	343	260	8.35	6.25	5.20	4.70	7.90	9.78
	344	280	7.25	9.05	4.85	7.15	5.80	8.58
	345	300	7.05	7.20	5.65	0.00	9.20	10.86
	346	320	21.90	6.25	6.75	15.95	4.40	12.06
	347	340	15.25	5.55	5.00	8.40	4.05	9.66
	348	360	9.10	5.95	6.35	13.30	4.65	17.82
	349	380	8.15	5.80	4.60	6.70	5.45	13.68
	350	400	4.85	4.50	4.70	8.80	3.20	11.16
ID = 46 TRT = M BRD = A	351	420	-5.00	24.00	8.70	5.80	4.55	11.64
	352	440	-5.00	16.10	5.90	6.20	5.10	16.86
	353	20	5.75	5.95	3.75	3.95	5.15	3.12
	354	40	3.65	11.30	5.75	2.75	3.45	5.82
	355	60	9.00	11.80	4.35	5.40	3.25	5.22
	356	80	5.90	7.60	4.40	3.00	3.40	4.80
	357	100	2.70	7.75	4.95	6.20	3.75	4.50
	358	120	10.00	3.45	4.40	72.15	4.80	2.88
	359	140	5.15	3.30	3.90	52.15	3.50	4.20
	360	160	2.85	4.45	5.05	10.45	2.35	3.36
	361	180	2.85	-5.00	3.65	9.00	3.15	40.92
	362	200	2.90	-5.00	5.95	12.40	3.15	30.78
	363	220	7.15	-5.00	7.85	6.25	4.75	19.86
	364	240	4.25	-5.00	4.00	5.15	2.90	14.10
	365	260	4.00	-5.00	7.80	3.55	3.75	9.90
	366	280	3.25	-5.00	61.50	3.55	2.60	7.74
	367	300	1.60	-5.00	26.70	2.60	4.00	7.08
	368	320	2.90	-5.00	14.30	4.95	3.40	5.82
	369	340	3.20	-5.00	9.45	3.70	24.75	6.54
	370	360	-5.00	-5.00	7.05	2.90	15.45	6.00
	371	380	-5.00	-5.00	8.05	3.40	6.95	3.42
	372	400	-5.00	-5.00	7.05	2.30	6.20	3.72
	373	420	-5.00	-5.00	5.40	3.30	4.55	5.28
	374	440	-5.00	-5.00	7.55	4.85	4.90	146.40

Table 5, cont.

	OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5
ID = 22 TRT = M BRD = S	1	20	6.25	6.60	4.45	95.10	4.25
	2	40	15.30	10.20	5.80	32.40	6.45
	3	60	11.30	7.55	7.15	13.25	5.75
	4	80	10.45	7.00	17.65	9.20	10.15
	5	100	4.40	22.35	6.50	12.50	4.15
	6	120	4.95	19.65	6.85	23.80	10.65
	7	140	8.65	104.00	7.55	14.00	3.10
	8	160	16.50	10.00	10.15	18.35	3.65
	9	180	9.20	7.75	13.70	3.70	5.75
	10	200	8.70	9.40	28.40	6.90	3.95
	11	220	10.50	5.15	20.60	5.90	5.15
	12	240	5.95	6.05	11.35	11.50	2.65
	13	260	6.05	6.55	7.25	11.35	4.30
	14	280	6.75	4.30	11.15	12.35	4.85
	15	300	36.05	3.40	6.10	8.75	6.20
	16	320	20.90	7.85	18.15	7.50	8.40
	17	340	14.20	9.30	4.70	22.50	4.25
	18	360	7.65	-5.00	3.50	6.20	14.75
	19	380	5.00	-5.00	15.90	5.50	5.30
	20	400	9.10	-5.00	7.10	7.85	3.65
	21	420	-5.00	-5.00	9.55	5.20	4.05
	22	440	-5.00	-5.00	7.75	14.60	95.85
ID = 19 TRT = C BRD = A	23	20	1.90	4.65	2.55	6.80	2.30
	24	40	2.55	3.50	2.60	5.50	9.85
	25	60	3.10	4.45	4.50	5.45	5.15
	26	80	4.05	2.30	9.50	8.95	5.55
	27	100	1.20	1.55	13.65	23.65	45.15
	28	120	3.05	2.95	42.00	3.90	7.50
	29	140	3.15	1.75	4.55	6.75	16.90
	30	160	8.15	2.30	7.30	9.80	4.05
	31	180	3.95	4.45	6.90	17.25	11.15
	32	200	2.80	21.70	3.35	5.95	5.55
	33	220	7.70	11.80	2.45	4.40	8.95
	34	240	5.55	6.15	6.95	4.45	20.15
	35	260	2.95	13.50	2.60	3.65	7.55
	36	280	2.30	3.20	23.40	4.25	6.50
	37	300	5.25	10.55	16.85	8.70	2.35
	38	320	6.45	5.50	5.90	6.90	19.00
	39	340	2.60	42.10	5.90	7.85	14.30
	40	360	4.10	2.65	2.55	2.75	26.05
	41	380	9.95	2.75	2.50	5.30	3.05
	42	400	3.45	3.55	4.40	7.95	23.55
	43	420	13.50	7.50	2.35	9.05	30.10
	44	440	4.60	3.90	3.70	2.30	8.25
ID = 29 TRT = C BRD = A	45	20	3.60	3.00	4.00	4.95	5.50
	46	40	5.15	3.75	4.80	4.10	4.40
	47	60	3.75	2.90	4.25	3.65	6.55
	48	80	3.90	3.75	2.65	3.30	3.55
	49	100	3.65	4.00	4.35	2.95	6.65
	50	120	4.15	2.75	2.90	3.05	2.65
	51	140	3.45	2.85	3.80	5.20	2.60
	52	160	4.30	2.85	3.15	3.30	2.35
	53	180	6.00	4.10	3.25	3.95	4.85
	54	200	4.25	3.15	2.65	2.90	3.20
	55	220	2.45	3.25	3.20	4.50	2.20

Table 5, cont.

OBS	TIME	LHP1	LHP2	LHP3	LHP4	LHP5
56	240	3.10	3.55	3.05	3.55	3.80
57	260	3.35	3.30	6.45	3.40	2.35
58	280	4.55	3.10	-5.00	2.70	-5.00
59	300	2.70	3.65	-5.00	3.50	-5.00
60	320	3.05	2.30	-5.00	4.70	-5.00
61	340	2.70	2.80	-5.00	3.35	-5.00
62	360	2.35	2.75	-5.00	3.50	-5.00
63	380	2.55	6.10	-5.00	5.45	-5.00
64	400	2.20	4.50	-5.00	3.20	-5.00
65	420	3.30	2.75	-5.00	7.05	-5.00
66	440	3.10	3.55	-5.00	7.95	-5.00