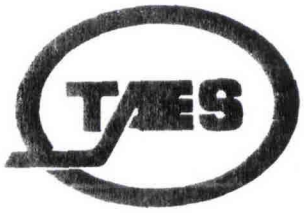

Nutritional Influence on Reproductive Efficiency in Beef Cows

Research Center

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THE TEXAS AGRICULTURAL EXPERIMENT STATION / Neville P. Clarke, Director / The Texas A&M University System / College Station, Texas

CHAPTER 3

EVIDENCE FOR SEASONAL AND NUTRITIONAL MODIFICATION OF OVARIAN AND PITUITARY FUNCTION IN CROSSBRED HEIFERS AND BRAHMAN COWS

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Summary

Alterations in endocrine response in the bovine female after consumption of monensin or exposure to changes in season were observed in two experiments. Thirty-three monensin-fed (M) or control (C) crossbred heifers in Exp. 1A were given a porcine follicle stimulating hormone (FSH-P) challenge on d 16 to 21 postestrus. Nine M heifers ovariectomized (OVX) on d 11 after the FSH-challenged estrus had a greater number of smaller corpora lutea (CL; $P<.005$) than did nine d 11 OVX C heifers. Serum progesterone (P_4) concentrations were greater in M on d 5 through 13 following the FSH-challenged estrus ($P<.001$). Seven M and seven C Brahman cows in Exp. 1B given an identical FSH challenge had similar increases in CL number and size. In Exp. 2, blood samples were obtained from 14 M and 13 C Brahman cows during winter (WI), early spring (ESp) and late spring (LSp) to characterize the preovulatory LH surge. During each seasonal period, blood samples were taken hourly from estrus through 24 h postestrus for each cow. Only one of five WI-C cows had a preovulatory LH surge compared to five of five WI-M cows ($P<.01$). No differences were found in the number of cows having an LH surge in ESp or LSp groups. Analysis of combined WI and ESp group values indicated a difference in timing of the LH surge by M or C treatment. Of those cows that had an LH surge, only three of 10C had peak LH values later than the first sample taken, compared with 10 of 15 M ($P<.10$). A heightened LH response was detected in M cows in all comparisons of LH surge profiles

(P<.005). Concurrent with changes in season from WI to ESp was an increase in number of C cows having an LH surge (one of five vs nine of 10; P<.005), and elevated LH values in ESp-C and M groups compared with WI-C and M groups (P<.05). Midluteal blood samples taken after every estrus indicated P_4 to be greatest in February M cows (P<.10) and P_4 tended to be greater in February and lower in January for all groups. Conclusions are that monensin affects the FSH-P-induced ovulation rate of bovine females of all ages. Seasonal effects occurring between the shortest and longest days of the year exert their greatest influence on the preovulatory LH surge and P_4 concentrations in Brahman cows between January and March. Seasonal effects appear to be partially modulated by nutritional factors.

Introduction

Endocrine response to alteration of the normal diet of ruminants has been the subject of considerable research in recent years. Various investigators have attempted to determine the nature of the nutrition-endocrine interaction by incorporating monensin sodium into the diet of the bovine. Monensin fed to cattle influences age at puberty (Moseley et al., 1977); McCartor et al., 1979), gross ovarian characteristics (Bushmich et al., 1980), prepartum steroid levels (Chew et al., 1978) and postpartum interval (Turner et al., 1977). Monensin appears to enhance the luteinizing hormone (LH) response in prepuberal heifers given an exogenous hormone challenge (Randel and Rhodes, 1980; Randel et al. 1982).

While it is generally accepted that environmental influences may be as important to physiological function as nutritional factors, these influences have remained more difficult to quantify. In particular,

little is known about the physiological mechanisms involved in the response to photoperiodic stimuli in the bovine. Those studies that have examined the response of specific hormones to alteration in photoperiod found no significant changes in growth hormone, progesterone and glucocorticoid concentrations in females (Peters and Tucker, 1978; Peters et al., 1979). Shortened photoperiod decreased prolactin concentrations in bull calves but had no effect on LH (Bourne and Tucker, 1975). More research of a similar nature has been conducted with sheep and horses, but distinct differences in breeding habits have made it difficult to relate those data to cattle.

Specific objectives of the current study were: (1) to quantify any gross changes in the ovaries of puberal heifers and mature cows fed monensin; (2) to determine the effect of monensin and seasonal change from winter to late spring on the ability of the bovine ovary to produce progesterone and (3) to characterize any changes in the preovulatory LH surge in response to dietary monensin and seasonal change from winter to late spring. Two experiments were conducted employing sexually mature crossbred heifers and mature Brahman cows.

Materials and Methods

Exp. 1A. Thirty-three Simmental X Brahman-Hereford heifers averaging 311 kg and approximately 17 mo. of age were randomly assigned to receive a diet containing either 0 (control) or 200 mg monensin/d. Seventeen control (C) and 16 monensin-fed (M) heifers were group fed 2.24 kg of 30% ground milo (IFN 4-04-444) and 20% cottonseed meal·head^{-1}·d^{-1} (IFN 5-01-621). The heifers were maintained in drylot with access to Coastal bermudagrass hay ad libitum. Sterile marker bulls were maintained with the heifers to aid in estrous detection. Single blood

samples were collected from each heifer on d 3, 5, 7, 9, 11 and 13 after the first estrus. On d 16 of the estrous cycle, each heifer was injected with 1 mg of porcine follicle stimulating hormone (Armour[6]; FSH-P) at 0800 and 2000 h. Injections were continued until d 21 or until the heifer displayed estrus. Nine C and nine M heifers were ovariectomized in the afternoon of d 21 or the d they exhibited estrus. Eight C and seven M heifers were allowed to ovulate, blood samples were collected on d 3, 5, 7, 9 and 11 and the heifers were ovariectomized on d 11. Ovaries of heifers not allowed to ovulate were measured for follicle number, total weight, follicular fluid and ovarian stroma weight. Ovaries of heifers allowed to ovulate were processed similarly and corpora lutea (CL) were enucleated, weighed and frozen until assayed for progesterone content spectrophometrically by the method of Armstrong et al. (1964) as modified by Irvin et al. (1978).

All blood samples were immediately refrigerated upon collection, processed to yield serum and frozen until assayed for progesterone by the radioimmunoassay procedure described by Erb et al. (1976).

Exp. 1B. Fourteen Brahman cows between 2 and 6 yr of age were maintained for 3 mo on a diet containing 0 (C) or 200 mg monensin (M). Seven C cows and seven M cows were group fed 1.35 kg of 75% ground milo and 25 % cottonseed meal·head^{-1}·d^{-1} and in addition had access to Coastal bermudagrass hay ad libitum. An FSH challenge identical to the challenge treatment given to the crossbred heifers was given starting on d 15 of the estrous cycle. A paralumbar laparotomy was performed on d 13 after the FSH-P treatment estrus to inspect ovarian structures.

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[6]Armour-Baldwin Laboratories, Omaha, NE.

Students t-test was used to analyze the ovarian indices examined and analysis of variance (ANOV) was used for serum progesterone values (Ott, 1977).

Exp. 2. Twenty-seven Brahman cows with regular estrous cycles and between 2 and 6 yr of age were randomly assigned to receive a daily concentrate containing either 0 (C) or 200 mg monensin (M). Thirteen C and 14 M cows were group fed 1.35 kg of 75% ground milo and 25% cottonseed meal·head^{-1}·d^{-1}. All cows were placed on feed on the same day (January 3, 1980) and were maintained in paddocks with access to Coastal bermudagrass hay ad libitum. To aid in estrous detection, sterile marker bulls were kept with the cows. Both groups were observed at least every 4 h, and any cow standing to be mounted was removed from the herd. Blood samples were then collected via tail vessel hourly for 24 h. Blood samples were collected in this manner in early January from five C and five M cows (winter; WI). A second set of samples (early spring; ESp) was taken from 10 C and 10 M cows in March. Monensin was discontinued after the ESp sampling and one-half of the cows from each group were combined into one control group and sampled a final time in late May (late spring; LSp). Blood samples were processed as in Exp. 1 and assayed for LH using the double antibody radioimmunoassay technique described by Niswender et al. (1969). Antibovine LH serum, B-225 was used as the first antibody. A 1:100,000 dilution of the antibody that bound 36% of the ^{131}I bovine LH was utilized with nonspecific binding, less than 4%. Immunochemical LH (LER-1072-2) was used for radioiodination and the antibody hormone complex was precipitated with bovine antirabbit gamma globulin. Bovine LH (NIH-LH-B9) was used as the reference standard and the results were expressed in terms of this preparation.

The LH measurements made were; (1) time of the onset of the LH surge; (2) peak LH concentration; (3) entire LH surge profile, as measured by magnitude of LH concentrations and duration from estrus through 24 h postestrus, (4) LH profile as measured by magnitude of LH concentrations and duration from peak LH concentration through 24 h, and area under the LH curve from the LH peak to 12 h later. Area under the LH curve was calculated by integrating LH concentration over time by a method described by Stein (1967). An LH surge was defined as an acute elevation of LH at least two standard deviations above basal LH levels as measured at 24 h after the onset of standing estrus.

Single blood samples were taken on d 10, 11 and 12 after every estrus and analyzed for progesterone to confirm that an ovulation had occurred. Average luteal phase progesterone values for each estrous cycle were used for comparison of seasonal production of progesterone. Serum samples were assayed for progesterone by the radioimmunoassay procedure described by Erb et al. (1976).

Analysis of variance (ANOV) and chi-square procedures were used to determine any significant effects of monensin or season within each of the aforementioned measurements (Ott, 1977).

Results and Discussion

Exp. 1. In both groups, animals consuming monensin displayed an altered ovarian response to the FSH challenge, Monensin-fed heifers allowed to ovulate had more CL per animal ($P<.10$) and greater average CL weight ($P<.005$; table 1). The M Brahman cows had similar tendencies in number of CL per animal and approximate CL size (table 2).

Enucleated CL from C heifers contained more progesterone per CL, but M heifers tended to have more progesterone per animal because of an

increased number of CL. Concentrations of progesterone were almost equal (table 1). Heifers not allowed to ovulate showed no differences in the ovarian traits measured (table 3).

Serum progesterone concentrations in the crossbred heifers were not different at any stage of the estrous cycle before the FSH challenge. Serum progesterone concentrations were greater in M heifers at d 5 through 11 following the FSH-challenged estrus ($P<.001$; figure 1).

Exp. 2. Both monensin and season appeared to affect the nature of the preovulatory LH surge. Only one of five cows in the WI-C group showed an LH surge during the sampling period compared with five of five WI-M cows ($P<.01$). The number of cows exhibiting a surge did not differ significantly between ESp-C and ESp-M groups (nine and 10, respectively). However, analysis of combined WI and ESp values gave further evidence of an alteration in the timing of the LH surge. Of those cows that had an LH surge, only three of 10 C had a peak LH value later than the first sample taken, compared to 10 of 15 M ($P<.10$). All 10 of the LSp-C cows had an LH surge. Progesterone assay of midcycle blood samples confirmed that all cows tested for LH surge had ovulated, with the possible exception of one WI-C cow whose midcycle progesterone concentrations showed considerable variation.

Because analysis of the timing of onset of the LH surge indicated that WI and ESp control groups were exhibiting a delayed behavioral estrus or that the LH surge was initiated earlier, it was considered probable that the LH peak had occurred before the initial blood sampling in a number of cows. Because the LH peak could not be verified in these cases, analysis in which peak LH was used as a measure was omitted. Comparisons were made with peak LH only when the highest concentrations

were preceded by lower concentrations.

Analysis of variance comparisons based on the estrus through 24-h LH profile indicated a heightened LH response in the M cows from the WI groups ($P<.001$) and ESp groups ($P<.005$; figure 2). Because comparison of groups ESp-C and LSp-C indicated no significant differences, data for cows in which the LH peak was known were combined to permit comparison with ESp-M cows for which peak values were known (nine of 20 C; seven of 10 M). A monensin-induced enhancement of the LH surge was also evident for this comparison ($P<.001$; figure 3).

Comparison of group WI-C with group ESp-C gave evidence of a seasonal effect. An LH surge was seen in only one of five WI cows compared with nine of 10 ESp cows ($P<.005$). This difference did not exist between ESp-C and LSp-C groups. While not statistically significant, more WI-C and ESp-C cows had early LH surges than did LSp-C animals. A seasonal difference was further indicated by examination of the estrus to 24-h LH profile. Both ESp-C and LSp-C exhibited elevated ($P<.001$) LH values when compared with WI-C (figure 4). The ESp-M group had higher LH values ($P<.05$) than did the WI-M group. Basal LH levels as measured at 24 h tended to increase from WI to LSp.

Progesterone concentrations in serum samples taken from M cows were higher than those from C cows ($P<.001$). Values from M cows were sampled during February were greater ($P<.10$) than all other monthly C or M group comparisons. In all comparisons, M cows tended to have higher progesterone concentrations than C cows and February values tended to be highest and January values lowest (table 3).

Meterological readings taken at the Overton Research and Extension Center indicated average and extreme low temperatures for January,

February and March to be 4 and -2, 2 and -9, 5 and -4 C, respectively.

Experimental results support prior assertions that monensin exerts an effect on reproduction in bovine females (Moseley et al., 1977; McCartor et al., 1979; Bushmich et al., 1980). It appears that these endocrinological changes can be induced in bovine females of varying ages. Results complement conclusions from experiments with prepuberal heifers (Moseley et al., 1977; McCartor et al., 1979; Bushmich et al., 1980), prepartum cows (Chew et al., 1978) and postpartum cows (Turner et al., 1977).

The increased ovulation rate in monensin-fed females does not appear to occur in a uniform manner because the older cows showed less dramatic increases in ovarian traits measured than did the crossbred heifers or prepuberal heifers (Bushmich et al., 1980). These gradations of response suggest that the more mature, well developed endocrine system is less sensitive to monensin-induced physiological changes. Formation of luteal cells may also be affected as M heifers in Exp. 1A had smaller corpora lutea. Strict spacial limitations may have played a role in luteal development because of the greater number of CL formed. Serum progesterone concentrations obtained after the FSH-challenged estrus confirmed that the larger number of CL released a commensurate amount of progesterone into the blood. Ability of the luteal cells to produce progesterone appeared unaffected, as indicated by the amount of progesterone in the tissue (µg/g).

Altered LH patterns displayed by cows consuming monensin in Exp. 2 confirm suggestions by Randel and Rhodes (1980) and Randel et al. (1982) that hypophyseal release of LH is mitigated by monensin-induced physiological changes. Mature cows appear capable of displaying this

response without the aid of an exogenous hormone challenge.

The physiological mechanism responsible for the monensin-induced reproductive changes remains obscure. The altered volatile fatty acid (VFA) pattern caused by monensin (Richardson et al., 1976; Prange et al., 1978) implies a possible role of glucose and (or) tropic hormones as intermediary affectors of reproductive processes. Volatile fatty acids have been investigated to determine their effect on glucose (Leng et al., 1967; Herbein et al., 1978), insulin (Manns and Boda, 1967; McAtee and Trenkle, 1971, growth hormone (Trenkle, 1971; Randel et al., 1977; Burrell et al., 1977) and prolactin (McAtee and Trenkle, 1971). No definite conclusions can be drawn from these studies that could explain how monensin-altered VFA patterns might cause the reproductive responses noted.

Seasonal comparisons indicate that environmental phenomena occurring in the period between the shortest and longest day of the year exert their greatest effects on endogenous release of LH and progesterone between January and March. The number of cows having an LH surge, the overall LH profile and luteal phase progesterone concentrations all show significant changes during that time, and further changes between March and June appeared to be minimal. The differences in timing of the LH surge between treatment groups in WI and ESp of Exp. 2 appeared to be the results of seasonal effects on the control groups, as the late spring control group had less tendency to have a delayed behavioral estrus or an early LH surge.

Research by other investigators with the equine (Oxender et al. 1977; Turner et al., 1979) and ovine (Schanbacher and Lunstra, 1976; Howland et al., 1978) implies that LH appears to be near maximal levels

shortly before and during the period of greatest ovulatory activity. Reduction of LH activity during winter months in Brahman cows may be analagous to the response in ewes, wherein the absence of an LH surge is a major factor in provoking the seasonal anovulatory condition (Legan and Karsch, 1979).

Interaction between season and nutrition is somewhat difficult to assess because of a lack of recent data regarding this relationship. The most perceptible evidence of a nutritional mediation of a seasonal-endocrinological association is the greater number of cows that exhibited an LH surge in early WI while consuming monensin. Dietary monensin seems to display a capacity to "over-ride" seasonal effects. Further studies are indicated to determine whether observed differences are due to an overall higher plane of nutrition evoked by monensin at a critical time of year, or whether altered volatile fatty acid concentration in the rumen, and subsequent energy pathways, are exerting an effect on the endocrine system above and beyond an improved nutritional balance.

TABLE 1. MEAN OVARIAN VARIABLES OF CONTROL AND MONENSIN-FED SEXUALLY MATURE HEIFERS FOLLOWING EXOGENOUS FSH-P CHALLENGE AS MEASURED AT ESTROUS CYCLE DAY 11 OVARIECTOMY.

Ovarian variable	Treatment Group	
	Control	Monensin
Ovarian weight, g	12.5	14.5
Stroma weight, g	5.0	4.0
Follicular fluid weight, g	3.5	2.6
Number follicles >8 mm	33.2	25.0
Number follicles ≤ 8 mm	1.0	1.1
Largest follicle	10.8*	8.4
CL/animal	3.7**	8.0
CL weight, g	2.5	1.9
Progesterone/ animal, µg	1,012	1,805
Progesterone/ CL, µg	272	226
Progesterone conc., µg/g	145	145

*P<.10.

**P<.005.

TABLE 2. MEAN OVARIAN VARIABLES OF CONTROL AND MONENSIN-FED BRAHMAN COWS FOLLOWING EXOGENOUS FSH-P CHALLENGE AS MEASURED ESTROUS CYCLE DAY 13 LAPAROTOMY.

Ovarian variable	Treatment group	
	Control	Monensin
CL/animal	2.3	3.1
CL size, mm	15.4	16.3

TABLE 3. MEAN OVARIAN VARIABLES OF CONTROL AND MONENSIN-FED SEXUALLY MATURE HEIFERS FOLLOWING EXOGENOUS FSH-P CHALLENGE AS MEASURED BY OVARIECTOMY AT ESTRUS.

Ovarian variable	Treatment Group	
	Control	Monensin
Ovarian weight, g	7.7	8.3
Stroma weight, g	4.6	4.7
Follicular fluid weight, g	3.0	3.5
Number follicles > 8 mm	22.7	19.4
Number follicles $\leq$ 8 mm	3.4	4.2
Largest follicle, mm	12.6	14.3

Figure 1. Effect of monensin on serum progesterone in FSH-challenged sexually mature beef heifers.

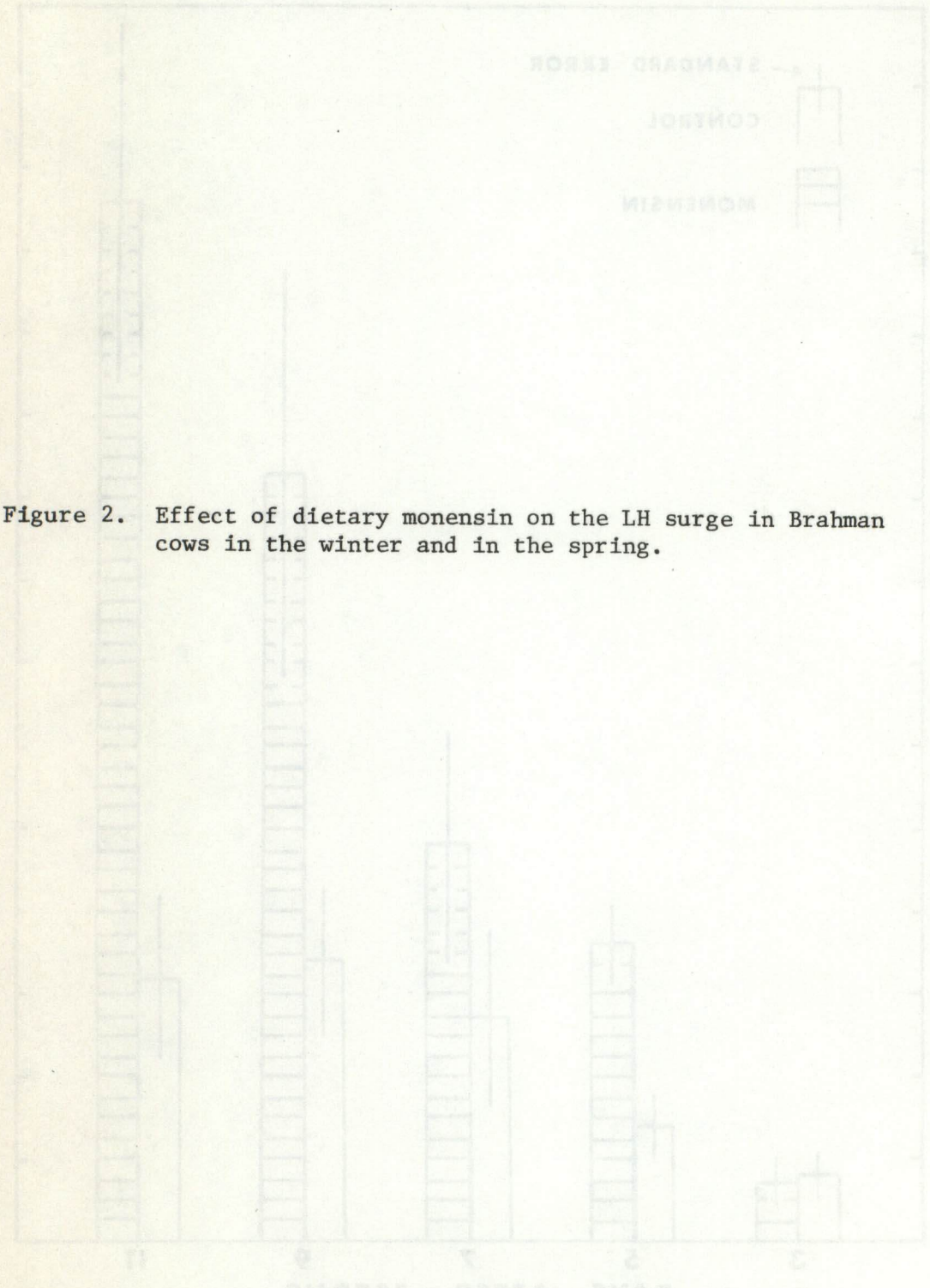


Figure 2. Effect of dietary monensin on the LH surge in Brahman cows in the winter and in the spring.

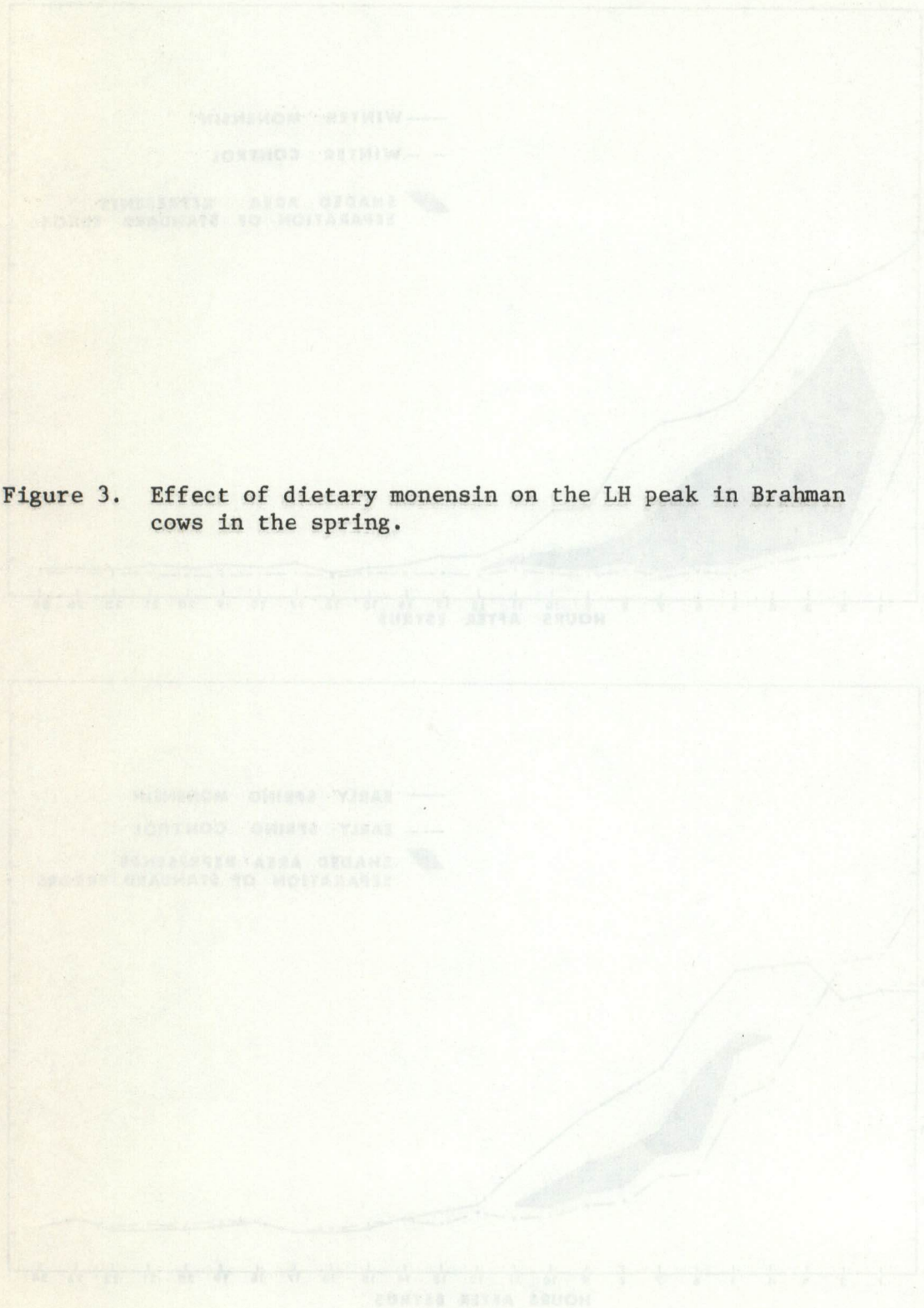
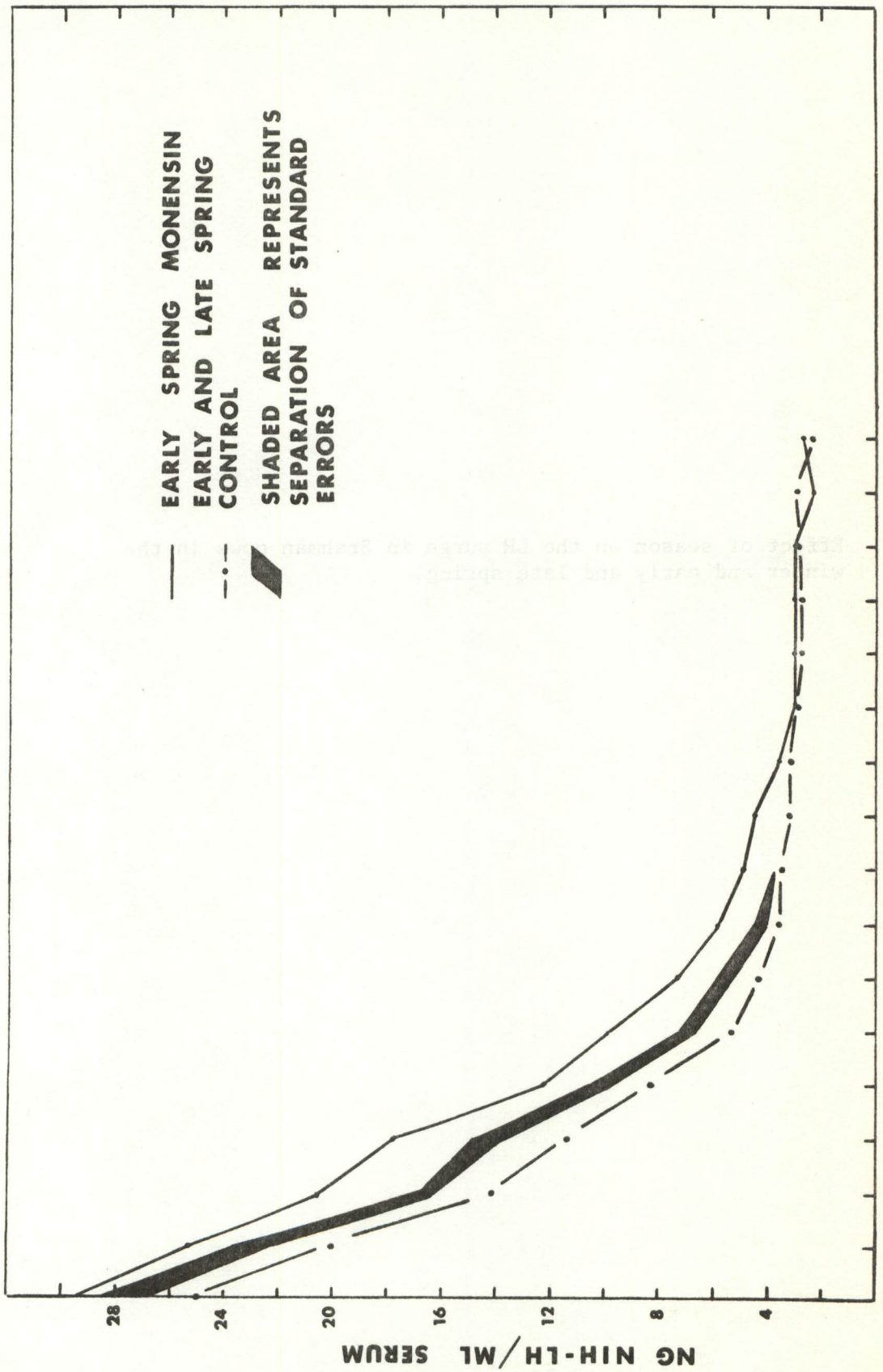


Figure 3. Effect of dietary monensin on the LH peak in Brahman cows in the spring.


EARLY SPRING MONENSIN
EARLY AND LATE SPRING CONTROL
SHADED AREA REPRESENTS SEPARATION OF STANDARD ERRORS
NG NIH-LH/ML SERUM
HOURS AFTER PEAK LH
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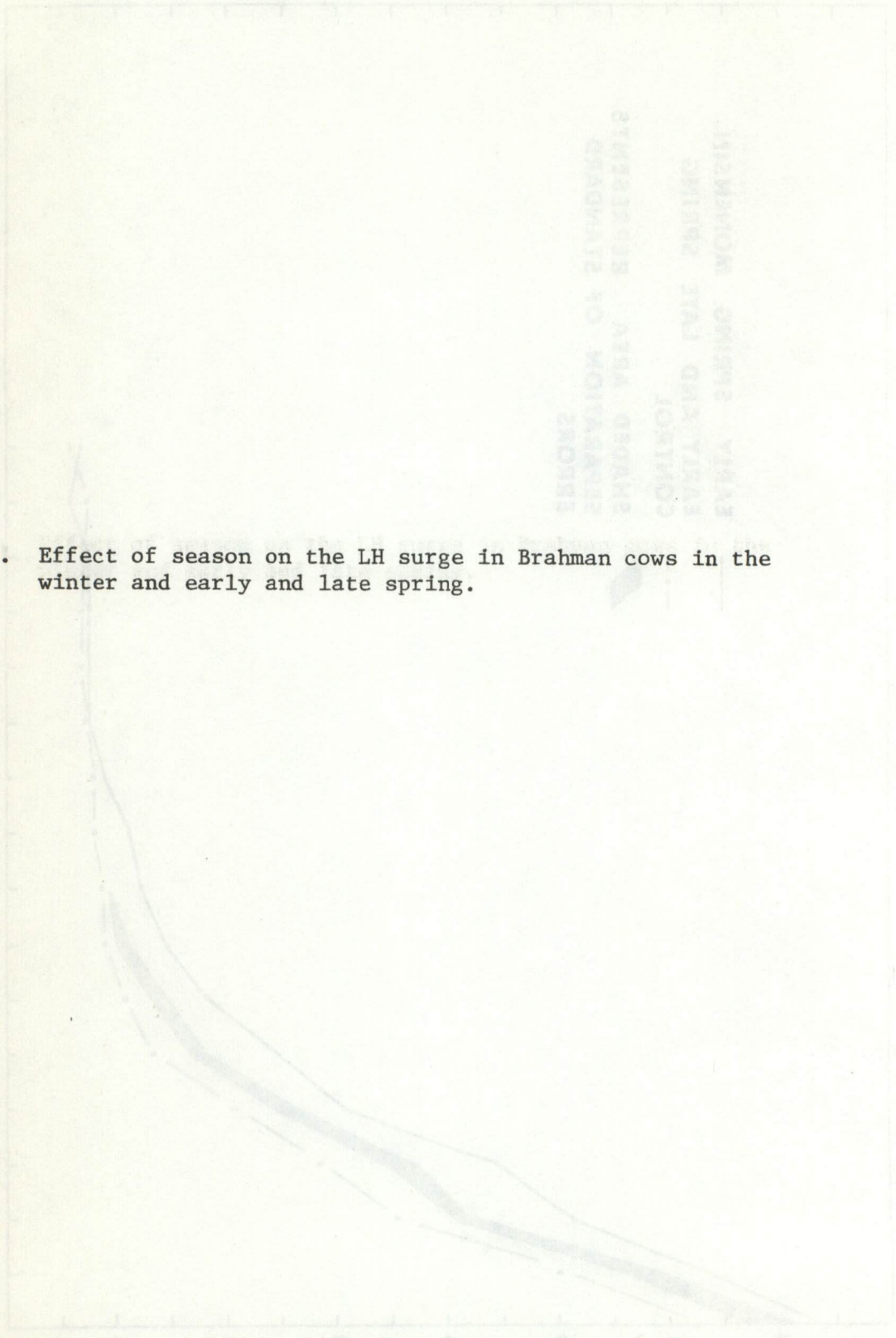
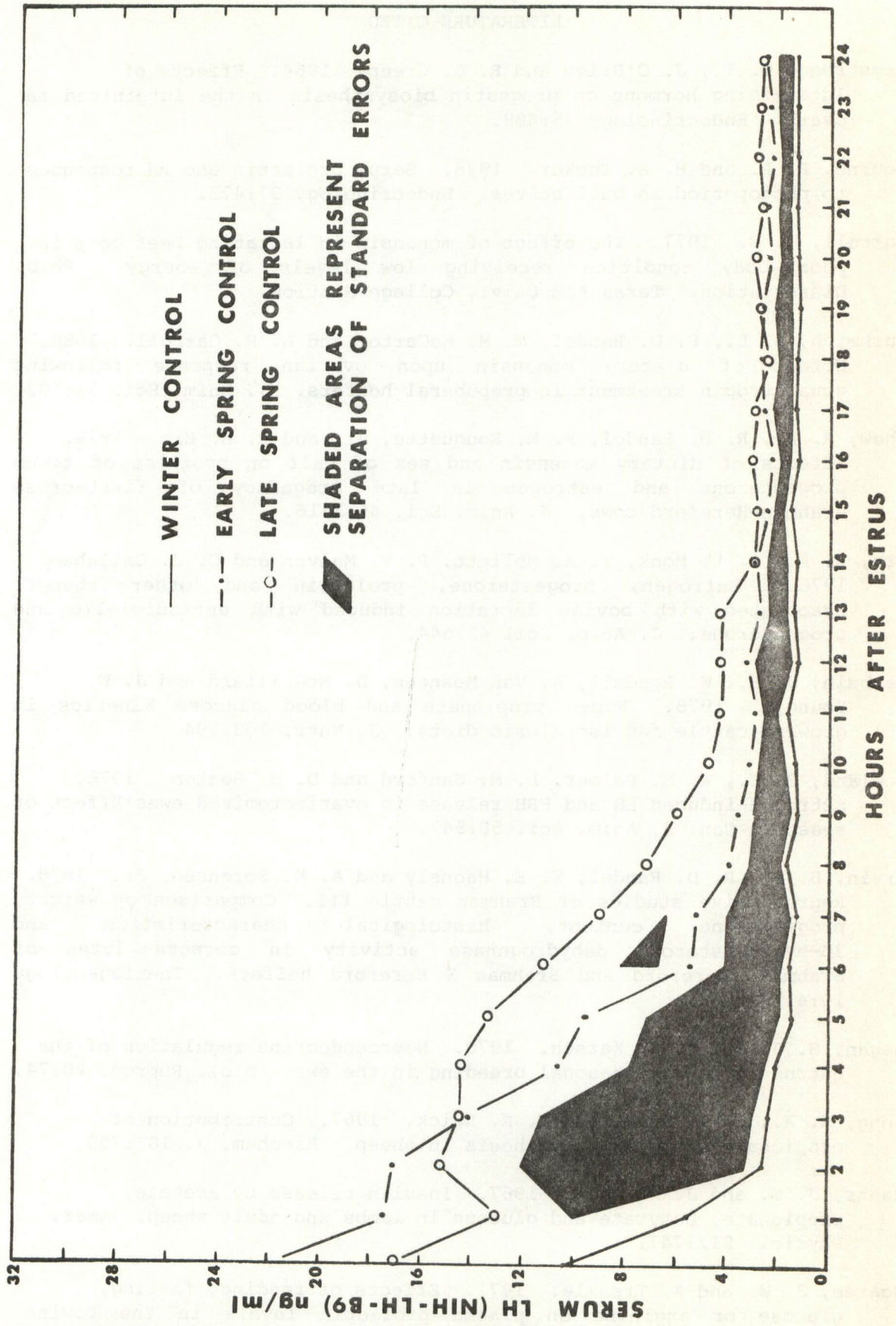


Figure 4. Effect of season on the LH surge in Brahman cows in the winter and early and late spring.


WINTER CONTROL
EARLY SPRING CONTROL
LATE SPRING CONTROL
SHADED AREAS REPRESENT SEPARATION OF STANDARD ERRORS
SERUM LH (NIH-LH-B9) ng/ml
HOURS AFTER ESTRUS
0
4
8
12
16
20
24
28
32
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