ABSTRACT

MCKEE, SARA LYNN. Evaluation of Oral Colostrum or an Organic Botanical Preparation on Reproduction in Dairy Cattle. (Under the direction of Dr. Steven P. Washburn).

The two objectives of this research project were: 1) to observe the effects of pooled oral colostrum on reproductive traits including ovarian structures, serum progesterone concentration, and days to estrus in dairy heifers and 2) to evaluate the effects of the botanical, estrogenic compound, Heat Seek on milk progesterone values, days to subsequent estrus, as well as ovarian structures in dairy cows (Rep 1). In the first objective, purebred Holstein, Jersey, and Holstein/Jersey crossbred heifers (n = 29) were ultrasounded for presence of a viable corpus luteum (CL); average CL diameter = 2.9 ± 0.17 cm. Heifers were assigned at random to 1 of 4 treatment groups: 1) untreated control (n = 7); 2) positive control, 25mg Lutalyse ($PGF_2\alpha$) i.m. (n = 7); 3) 1 L oral colostrum (n = 7); or 4) 2 L oral colostrum (n = 8). Ultrasound measurements and blood samples were collected before treatment (d 0) and at 1 and 4 d after treatment to determine changes in CL size and serum P4 concentrations. Heifers received tailhead paint and were observed for estrus twice daily. Exact stages of the estrous cycle were not known, however sizes of CL and serum concentrations of P4 were comparable across treatments at the start of the experiment (d 0). Comparisons of P4 concentrations showed significant differences between $PGF_2\alpha$ vs. all other groups at d 1 ($P < 0.01$) and d 4 ($P < 0.05$). Based on P4 and ovarian palpation, all 7 $PGF_2\alpha$ treated heifers responded with luteal regression as expected. Heifers treated with I L or 2 L of colostrum had higher P4 on d 1 and d 4 ($P < 0.05$) than the negative control group and had similar sizes of luteal structures on d 4 indicating a lack of effect of luteal regression from treatment with colostrum. All 7

heifers receiving $PGF_2\alpha$ came into estrus within 4 d after treatment (2.8 ± 0.2 d) whereas only 3 heifers from the untreated group, 2 from the 1 L group, and 3 from the 2 L group were detected in estrus within 7 d after treatment. All heifers detected in estrus had P4 >2.0 ng/ml at treatment which declined thereafter. In contrast, the 14 heifers not observed in estrus by 7 d all had concentrations of P4 >2.0 ng/ml at treatment and at 1 and 4 d after treatment. All $PGF_2\alpha$ -treated heifers responded with a decline in P4 and subsequent estrus whereas responses among the 15 heifers that received colostrum were similar to that of the 7 untreated heifers. Results of a bioassay were used to determine that there was little estrogenic activity in a sample of the pooled colostrum, which was unexpected. It is not known whether use of colostrum of higher quality or at higher doses would be more effective. In summary, it was concluded that use of oral colostrum would not be effective in facilitating luteal regression and earlier onset of estrus in dairy heifers.

For the second objective, a two-replicate experiment was conducted to determine the effects of the botanical, estrogenic compound, Heat Seek on reproductive traits. Cows in the first replicate (n = 24) and second replicate (n = 27) were purebred Holstein, Jersey, or various H/J crossbreds. Preliminary milk samples were collected in both replicates to determine positive cyclicity (P4 >2ng/ml). In each replicate, cows were separated at random into 2 treatment groups (Control or Heat Seek-treated) based on breed group. In the first replicate, ultrasound data was collected on d 1 for initial observation of ovarian structures and then again on d 5. Cows received tailhead paint and were monitored twice daily until detection of estrus. Daily milk samples of 5-8mls were also taken from cows for analysis of progesterone concentration. Heat Seek treated cows received a daily 13-g bolus capsule of Heat Seek (Rep 1: 0.10µg/kg; Rep 2: 0.11µg/kg estrogen equivalent

given per day) for 6 consecutive days (d1-d6). Lastly, post treatment milk samples were taken on d 10 from all cows for analysis of P4. In Rep 1, average CL diameters for cows in the control group were 2.0 ± 0.7cm (n = 3) on d 1. By d 5, average diameters for this group were 1.8 ± 0.3cm (n = 6). Cows in the treated group had d 1 average diameters of 1.9 ± 0.3cm (n = 8). Heat Seek treated cows had average CL diameters of 1.3 ± 0.1cm by d 5 (n = 7). For both replicates, there were no differences in milk progesterone concentrations for all 6 sampling days for both treatment groups. Post-treatment samples also showed no significant differences between the 2 groups (Rep 1 $P = 0.86$; Rep 2 $P = 0.54$).   In Rep 1, average days to estrus for control cows were 24.0 ± 10.4 d and 24.8 ± 9.2 d for treated cows. For Rep 2, average days to estrus for control cows were 19.2 ± 3.4d and 27.3 ± 8.7d for treated cows. There were no significant effects of low or high d 1 progesterone levels in regards to attainment of estrus within 25 d for the combined replicate data ($P = 0.52$ for treatment; $P = 0.35$ for P4 value on d 1). The percentage of those that conceived to their first subsequent estrus after d 1 for Rep 1 was 33% (Control) and 55% (Heat Seek). Rep 2 percentages were 50% (Control) and 69% (Heat Seek). Based on the lack of significant differences in milk progesterone for cows treated in both replicates it was concluded that estrogens present in Heat Seek capsules may not have been sufficient to elicit a response. Variation in responses may have been subject to time of treatment and stage of cycle. Low progesterone levels on d 1 and during treatment may have also been a result of cows that were not cyclic or cows with a regressed CL.

Evaluation of Oral Colostrum or an Organic Botanical Preparation on Reproduction in Dairy Cattle

by
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BIOGRAPHY

Sara Lynn McKee was born in Hahn, Germany to parents Donald and Dagmar McKee. Eventually her family moved to Etters, Pennsylvania where she grew up and graduated high school in 2002. Sara went on to pursue a Bachelor of Science degree in Animal Science, with a minor in Equine Science at The Pennsylvania State University in 2006. In the spring of 2008, she began her work to achieve a Master of Science degree at North Carolina State University in Animal Science under the direction of Dr. Steven P. Washburn.

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INTRODUCTION

Reproductive efficiency can be enhanced through use of common ovulation-control systems to minimize estrous detection and allow for a predetermined time of insemination in order to maximize conception rates (Chenault et al., 1976). Common methods for attaining estrous synchronization are through treatment combinations of prostaglandin $F_2\alpha$ ($PGF_2\alpha$), estrogens, progesterone, and gonadotropin-releasing hormone (GnRH), or analogues of these hormones. In addition, the most frequently used methods typically are $PGF_2\alpha$ or estrogenic therapies. Subsequent CL regression from these methods is followed by a new estrous cycle. This is indicated by a decrease in progesterone secretion by the CL, an increase in estradiol secretion, a preovulatory surge of luteinizing hormone (LH), ovulation and production of a new CL (Chenault et al., 1976).

In addition to these synthetic methods for manipulating reproductive physiology, natural methods have been investigated. Natural methods include endogenous estrogens and prostaglandins found in colostrum as well as naturally occurring estrogens found in plant material also known as phytoestrogens. Primary focus of this literature review will be to detail methods of controlling the estrous cycle through hormonal treatments in ruminants, provide a background on the hormonal activity of colostrum and plants, and describe research focusing on the use of colostrum and phytoestrogens for regulation of reproduction in livestock and laboratory animal species.

LITERATURE REVIEW

Control of Fertility with Estrogens

Estrogen metabolites, such as estradiol or estrone sulphate are known to be luteolytic in the cow (Brunner et al., 1969). Additionally, hysterectomy has been associated with a reduction in the luteolytic effects of estradiol indicating that the uterus is a site of estradiol action contributing to luteolysis. Luteolysis can be considered as a disruption of corpus luteum (CL) function and decrease in concentrations of progesterone to less than 1 ng/ml (Hixon et al., 1983).

Eley et al. (1979) observed the action of estrone sulphate on luteal function in 12 cyclic beef heifers. Heifers were injected subcutaneously with 28 mg estrone sulphate (n = 6) on d 10 of the estrous cycle while control heifers (n = 6) were injected with 7 ml corn oil. Injections were continued once daily until detection of estrus. After return to estrus, the 6 control animals were then injected with 56 mg estrone sulphate/d until detection of a subsequent estrus. Length of the estrous cycle was monitored for both treatment groups. Treatment with 28 or 56 mg estrone sulphate/d significantly reduced cycle length compared to that of animals injected with corn oil. Control, 28 mg, and 56 mg/day treated heifers averaged estrous cycle lengths of 21.2 ± 0.75, 18.7 ± 0.42, and 18.2 ± 0.34 d, respectively. Plasma progestins declined sooner in treated heifers, falling from 4.8 ng/ml on day 14 to estrous levels <1 ng/ml at day 18. In comparison, progestin concentrations in control heifers declined from 6.02 ng/ml on day 16 to <1 ng/ml at 21.2 d. Based on cycle length and progestin concentrations, luteal regression began earlier in the heifers treated with 28 mg estrone sulphate/d (Eley et al., 1979).

In a three-experiment study, Kittok and Britt (1977) noted a similar reduction in serum progesterone when 250 µg of estradiol-17β was given to ewes daily on d 11 and 12, or 12 and 13 of the estrous cycle, compared to non-treated control ewes. Ewes treated on d 11 and 12 versus d 12 and 13 tended to decline more rapidly; however, by d 14 there were no differences in serum progesterone between treated groups. Further both groups had lower serum progesterone concentrations on d 14 following mating to a vasectomized ram than did similarly mated control ewes. However, only 6 of 12 ewes mated to intact rams after being treated with estradiol showed a similar decline in progesterone (Kittok and Britt, 1977).

In a study by Burke et al. (2000), 24 non-lactating Friesian cows were used to determine if a small dose of estradiol benzoate (EB) during the middle stages (luteal phase) of the estrous cycle would synchronize ovarian follicular development, estrus, and ovulation. The cows received either 1mg of EB i.m. on d 13 of the estrous cycle (n = 12), or served as untreated controls (n = 12). The ovaries of each cow were examined daily by ultrasound from day 7 to the following ovulation. Data on diameter and location of each corpus luteum and follicle were collected. Those treated with 1 mg of estradiol benzoate displayed a noted rise in plasma concentrations of estradiol (12 pg/ml vs.1 pg/ml in controls) during the initial 24 h following treatment and a decline in progesterone between 24 and 48 h after treatment compared to control cows. This difference between groups was coincidental with earlier time to regression of the corpus luteum seen in the treated cows. Atresia of the dominant follicle at that time was a result of the EB treatment and resulted in emergence of a new wave of follicular development 4 to 5 d after treatment (Burke et al., 2000).

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Control of Fertility with Prostaglandin $F_2\alpha$

It has been determined that administration of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) can be used to achieve luteolysis and synchronization of estrus and ovulation in cattle (Hansel et al., 1975). In addition, hysterectomy in both cows and sheep during the estrous cycle results in maintenance of the CL indefinitely (Wiltbank and Casida, 1956; Caldwell et al., 1969). Pharris and Wyngarden (1969) were the first to demonstrate that $PGF_2\alpha$ induced luteolysis in the rat. By 1970 and 1972, it was then suggested that $PGF_2\alpha$ was luteolytic in both sheep and cows. As reported by Hafs et al. (1974), these observations demonstrated that $PGF_2\alpha$ was a uterine luteolysin, and represented a new means of regulation of the estrous cycle and fertility. Investigations have now termed $PGF_2\alpha$ as the endogenous luteolytic substance in both sheep and cattle (LaVoie et al., 1975).

Estrous synchronization using $PGF_2\alpha$ reduces labor for estrous detection, allows artificial inseminations at predetermined times, and usually involves schemes where two injections of the hormone are administered at 10 to 14 d intervals (Stevenson et al., 1984). This same efficiency can also be attained through the use of prostaglandin analogs (Cooper, 1974; Leaver et al., 1975; Roche, 1976). Milvae and Hansel (1983) also had success in achieving luteolysis with heifers receiving 10 and 25 mg of 13, 14-dihydro-$PGF_2\alpha$ injected intramuscularly on d 10 of the estrous cycle.

Lauderdale et al. (1974) found that return to estrus is typically noticed within 2 to 4 d after administration of $PGF_2\alpha$. This stimulates luteolysis in which decreased plasma progesterone and LH levels lead to further regression of the CL and a shorted estrous cycle (Milvae and Hansel, 1983). As reported by Louis et al. (1974), administration of 5 mg

PGF$_2\alpha$ caused decreased diameter of the corpus luteum within 24 h and a decline in serum progesterone from 3.6 ng/ml to 1.0 ng/ml within 48 h. In addition, an increase in serum estradiol was noticed by 12 h and standing estrus at 72 h in six Holstein cows given PGF$_2\alpha$ on day 11 of the estrous cycle.

La Voie et al. (1975) investigated the ability of PGF$_2\alpha$ to stimulate luteolysis in 6 hysterectomized beef and dairy cows. Treated cows received 30 mg of PGF$_2\alpha$ injected intramuscularly between 29 and 50 d after the last pre-hysterectomy estrus, control cows received i.m. dosages of saline. Cows treated with PGF$_2\alpha$ were observed in estrus 2 to 3 d post-treatment. These cows also had reduced blood serum progesterone concentrations from greater than 6 ng before the start of treatment to less than 1.5 ng, 1 to 5 d post-treatment. Corpora lutea were collected 5 to 7 d after treatment and showed that CL from PGF2α - treated cows appeared to be in a state of regression, while CL from control cows appeared to be active and still functional. Diameters of CL from treated cows were also smaller than the saline-treated cows (La Voie et al., 1975).

Hafs et al. (1974) noted similar results after depositing 5 mg PGF$_2\alpha$ into the uterus of 6 Holstein cows during diestrus. The treatment caused a 50% decline in progesterone within 12 h. In addition, estradiol more than doubled within 24 h, and LH peaked at 71 h with estrus occurring at 72 h and subsequent ovulation at 95 h after injection (Hafs et al., 1974).

In the same study, Holstein heifers were treated with 30mg PGF$_2\alpha$ i.m. (n = 5) during diestrus (d 8 to 14) or 30mg intravaginally (n = 6) during metestrus (3 d after estrus). Blood plasma progesterone began to fall within 10 min while estradiol concentrations increased within 1 hour after administration. Intravaginal PGF$_2\alpha$ resulted in a more variable and

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delayed effect by about 1 d compared to the i.m. treated group (Hafs et al., 1974). Burfening et al. (1978) noticed similar results in plasma progesterone in cyclic beef heifers.

Chenault et al. (1976) observed trends in both progestin and estradiol concentrations following i.m. injection of either 30 mg $PGF_2\alpha$ (intramuscularly, n = 4) or intrauterine deposition of 10 mg $PGF_2\alpha$ (n = 3). Decreases in progestins in plasma were noticed within 24 h while estradiol increased linearly from time of injection to 52 h post injection. Additionally, intervals from administration to estrus, peak of LH, and ovulation were 75, 79, and 100 h, respectively. Variability in timing of these cyclical events suggests that a single timed insemination after one injection would not result in optimal fertility (Chenault et al., 1976).

In a study by Stevenson et al. (1984), Holstein heifers were treated with $PGF_2\alpha$ at two stages of the estrous cycle. Heifers were either treated early (d 6 to 9) or later (d 14 to 15) in the cycle. Compared with heifers treated late in the estrous cycle, heifers that were treated early in the cycle produced less progesterone before $PGF_2\alpha$ treatment and had greater peak concentrations of estradiol at estrus. Heifers that were treated early in the cycle had shorter intervals from treatment to estrus, to peak estradiol, and to peak LH and to initiation of estrus after the peak in estradiol than did heifers treated later in the cycle. The use of a two-injection method is important because it allows the animals treated to respond to one of the injections as $PGF_2\alpha$ is not luteolytic during the early (d 0 to 4) or late (d 16 to 20) stages of the estrous cycle. Therefore, the majority of animals should be in early stages of the estrous cycle at the second $PGF_2\alpha$ injection (Stevenson et al., 1984).

Exogenous $PGF_2\alpha$ has also been useful with postpartum dairy cows. In research by Simmons et al. (1979), $PGF_2\alpha$ was used to induce estrus to decrease calving interval and time spent monitoring estrus. Cows were treated with 30mg $PGF_2\alpha$ i.m. on day 60 postpartum. Twenty-nine of the 51 treated cows were seen in estrus within 5 d. These treated cows had significantly fewer days to first service while control cows required more heat checks than treated cows (39 vs. 8.2 checks) (Simmons et al., 1979).

Control of Fertility with Prostaglandin $F_2\alpha$ and Estrogenic Combinations

There is evidence in the use of estrogens simultaneously with $PGF_2\alpha$ in inducing luteolysis. Louis et al. (1977) observed enhanced synthesis of $PGF_2\alpha$ in vitro by uterine tissue after treatment with estradiol in progesterone-primed ewes. Treatment of ewes with estradiol also resulted in greater concentrations of $PGF_2\alpha$ in uterine venous blood of ewes (Barcikowski et al., 1974).

Hixon et al. (1983) investigated the luteolytic interaction between $PGF_2\alpha$ and estradiol benzoate in heifers. They randomly assigned 20 heifers to one of four groups; the first group received 200 µg estradiol benzoate ($E_2\beta$) twice daily on d 10, 11, and 12 of the estrous cycle plus 7 mg $PGF_2\alpha$ given concurrently with the last injection of $E_2\beta$. The second group received control vehicles for $E_2\beta$ (sesame oil) and $PGF_2\alpha$ (saline). The third and fourth groups received $E_2\beta$ with sesame oil or $PGF_2\alpha$ with saline, respectively. All groups received their treatments intramuscularly. The treatment group receiving estradiol benzoate and $PGF_2\alpha$ resulted in luteolysis in all heifers when compared with the vehicle-treated control group, and a decline in concentration of plasma progesterone was noticed as well as a shorter mean length of the estrous cycle. The group administered $PGF_2\alpha$ was luteolytic in

only one of five animals. The estradiol benzoate plus saline treatment had no effect on the length of the estrous cycle or on concentrations of progesterone (Hixon et al., 1983).

Peters et al. (1977) conducted a five-trial study to compare the efficacy of one or two injections of $PGF_2\alpha$ with or without subsequent treatment with 400 µg estradiol benzoate (EB) intramuscularly to synchronize estrus in lactating beef cows and heifers. Cows and heifers treated with EB 48 h after an initial $PGF_2\alpha$ injection had increased precision of estrus synchronization in both the cows and heifers. For cows and heifers receiving the estrogen treatment, 23% and 15%, respectively, increased onset of estrus within a targeted time period of 56 to 86 h after $PGF_2\alpha$ injection. Conception and pregnancy rates were not altered by estradiol benzoate, however. For heifers and cows receiving a second injection of $PGF_2\alpha$ 12 d after the initial treatment gave no advantage in synchronization of estrus in the animals (Peters et al., 1977).

In a similar field study conducted by Dailey et al. (1986), 322 lactating dairy cows were used to determine if treatment with estradiol benzoate 40 to 48 h after treatment with $PGF_2\alpha$ would bring a greater proportion of cows into estrus compared to $PGF_2\alpha$ treatment alone. The cows were split into two groups, which were either injected intramuscularly with 25 mg $PGF_2\alpha$ (n = 141) or received $PGF_2\alpha$ and were then injected intramuscularly with 400 µg estradiol benzoate 40 to 48 h after $PGF_2\alpha$ treatment (n = 181). Those treated with estradiol had a higher proportion of cows in estrus within 5 d after $PGF_2\alpha$ treatment. In addition, greater proportions treated with estrogen were in estrus on d 3 than those not receiving treatment. Cows were bred at observed estrus; however there were no differences

in conception rates among groups. Welch et al. (1975) observed similar results with lactating beef cows as well as Dailey et al. (1983) in dairy heifers.

Hormonal Activity of Bovine Colostrum and Milk

Estrogenic Activity of Bovine Colostrum and Milk

Steroid hormones are normally found in both the milk of nonpregnant animals and colostrum of pregnant animals. The steroids, estrogen, progesterone and corticoids, and peptide hormones, insulin, growth hormone, and prolactin are essential for mammogenesis and onset of lactation (Erb et al., 1977). In cows, concentrations of estrogens in milk appear to increase during pregnancy, during estrus, and following administration of estradiol or diethylstilbestrol (Monk et al., 1975). Colostrum has approximately 20 to 100 times as much estrogen as milk (Wolford and Argoudelis, 1979). However, these levels are transient as the number of days postpartum increase.

In a study by Monk et al. (1975), blood, urine, and milk were collected from 13 cows during various periods of gestation. Each cow showed elevated blood plasma and urinary estrogen prepartum followed by rapid decreases by 1 to 3 d postpartum. Total estrogen in urine ranged from 3669 pg/ml 1 d before calving to 10 pg/ml 5 d postpartum. Total estrogen content in blood plasma ranged as low as 44 pg/ml 5 d postpartum to a peak of 960 pg/ml 1 day before parturition. Concentration of total estrogen (estradiol and estrone) exceeded 1000 pg/ml in colostrum and milk from cows milked prepartum, and was correlated with total estrogen in blood plasma and urine before and after calving. Total estrogens in milk ranged from 10 pg/ml 5 d postpartum to 1642 pg/ml at 1 d prepartum (Monk et al., 1975).

Hormonal changes in blood plasma, colostrum, milk, and urine through d 25 of lactation in 14 Holstein cows were also studied by Erb et al. (1977). The study compared excretion of hormones into colostrum and milk when onset of lactation was caused by normal calving (C), induced calving (IP) with 25 mg of dexamethasone and estradiol-17β (E$_2\beta$), and induced lactation (IL) with E$_2\beta$ and progesterone. Changes in concentrations of E$_2$ß, E$_2\alpha$, and estrone in blood plasma and colostrum were associated positively from d -3 to +5 and thereafter through d 17 in group IL and d 25 in groups C and IP. Total estrogens in colostrum (d -3 to +2) for group C averaged 2039 $\pm$ 202 pg/ml for d -3 to -1 and 1867 $\pm$ 438 pg/ml for d 0 to 2. Group IP colostrum averaged 2176 $\pm$ 194 pg/ml for d -3 to -1 and 1736 $\pm$ 321 pg/ml for d 0 to 2. Day 0 was considered the day of calving for the C and IP groups, or just before first milking for group IL. Total estrogens for group IL was 714 $\pm$ 292 pg/ml for d 0 to 2. Rapid decreases in the estrogens after calving were also observed. Total estrogen in these mammary secretions varied from .03 to .06% of that excreted in urine during the first 2 d after calving when concentration in blood plasma and urinary excretion rates were still high (Erb et al., 1977).

Shifts in proportions of the estrogenic compounds in colostrum from prepartum to postpartum were also noticed in the study. Before calving (d -3 to -1), E$_2\alpha$ was higher in colostrum from the IP group. In addition, groups C and IP, concentration ratios for E$_2\alpha$ increased postpartum whereas those for E$_2\beta$ and estrone decreased. However, average concentration ratios for total estrogen were about the same among all groups after d 2 of lactation. Therefore, these shifts in estrogenic proportions suggest that metabolic conversion

of $E_2\beta$ and estrone to $E_2\alpha$ may occur in the udder after lactation is established (Erb et al., 1977).

In a review of data by Erb et al. (1977), concentrations of estrogens in milk of untreated cows (3 to 25 d of lactation) averaged 28 ± 2, 13 ± 1, 160 ± 14, and 202 ± 15 pg/ml for estrone, estradiol-17β, estradiol-17α, and total estrogen, respectively. In comparison, concentrations in colostrum on day of calving were 65 to 80 times higher for estradiol-17β, and estrone, and eight-times higher for estradiol-17α. Compared to concentrations in blood plasma, concentrations of estradiol-17β in mammary secretions were higher prepartum and lower during early lactation, estrone was lower both prepartum and postpartum.

There is evidence that the cow's udder may be a minor route for excretion of estrogen. The total estrogen removed daily in milk of cows may be considerably less than 1% of the total metabolized (Monk et al., 1975). This may be due to the excretion of estrogen in urine and feces being 3-to 4-times higher than that in milk (Erb et al., 1977). The lower estrogenic activity of colostrum compared to urine and feces were also observed by Pope and Roy (1953).

A study by Turner (1958) also demonstrated that the cow's udder is a minor route for estrogen excretion in regards to biological activity. Dried milk from non-pregnant cows and dried colostrum from pregnant cows were fed ad libitum to ovariectomized test mice for 10 d. The average uterine weight of mice given dried milk of non-pregnant cows and cows fed diethylstilbestrol was 10.48 mg and 12.07 mg respectively. The average uterine weight of mice given dried milk from cows pregnant at d: 27-97, 104-193, 200-268 were 11.35 mg, 13.71 mg, and 16.45 mg, respectively. Samples of colostrum from six normal cows and from

three cows experimentally induced to lactate showed low uterine weight increases. According to Turner, "normal lactating cells are only slightly permeable to the normal circulating estrogen of the cow's blood, even in late pregnancy, when estrogen secretion is high and secretion of milk is low. Additionally, the secreting mammary gland epithelial cell either is non-permeable or is only slightly permeable, to circulating natural estrogenic hormones or to synthetic estrogens, such as diethylstilbestrol" (Turner, 1958).

Prostaglandin $F_2\alpha$ Activity in Bovine Colostrum and Milk

Research by Manns (1975) observed levels of $PGF_2\alpha$ in milk after injection of 30 mg $PGF_2\alpha$ in 4 cows. Endogenous levels of $PGF_2\alpha$ ranged from 200 to 400 pg/ml. After injection, excretion of the hormone in milk peaked during the first hour after treatment to 910 $\pm$ 120 pg/ml, with no further increase caused by treatment after 3 h (Manns, 1975). This increase was then followed by a steady decrease to pretreatment levels by 7 h post-treatment. The average excretion rate found by Manns (1975) was 2.9 µg/day following 30 mg $PGF_2\alpha$ injection.

Hansel et al. (1976) obtained slightly different results when 5 or 20 mg $PGF_2\alpha$ were administered into the uterus. Milk samples taken from 6 cows from pretreatment milkings plus eight post treatment milkings had $PGF_2\alpha$ ranges of 125-232 pg/ml. Furthermore, the measurements of $PGF_2\alpha$ in milk samples before and after treatment indicated slightly higher means following administration but these concentrations were not elevated significantly for more than about 7 h after administration of $PGF_2\alpha$. Additionally, there were no differences in $PGF_2\alpha$ concentrations in the milk of animals receiving 5 or 10 mg treatments (Hansel et al., 1976).

A study by Simmons et al. (1979) observed the amount of $PGF_2\alpha$ residue in milk after treatment in 17 postpartum dairy cows. Their primary objective was to determine if $PGF_2\alpha$ could reduce days open and number of estrus checks. Their second objective was to determine how $PGF_2\alpha$ in milk would be affected. Cows were treated with 30 mg $PGF_2\alpha$ on d 60 postpartum. Milk samples were collected at two milkings before treatment and for 10 milkings after treatment with $PGF_2\alpha$. In their investigation, levels of $PGF_2\alpha$ in milk were elevated significantly above pretreatment only for one sample, which was taken during the third milking, that was within 2 to 7 h of the single $PGF_2\alpha$ treatment (1300 pg/ml). Average $PGF_2\alpha$ concentrations during all other samples were at or near 700 pg/ml (Simmons et al., 1979).

These observations were approximately twice those reported by Manns (1975) and triple those reported by Hansel et al. (1976). These differences from other research may have been attributed to the sensitivity of extraction methods and antibodies used at those times (Simmons et al., 1979). Lastly, methods of administration may have also lead to the observed differences in mean $PGF_2\alpha$ concentrations. Manns and Simmons administered 30 mg $PGF_2\alpha$ intramuscularly while Hansel administered 5 or 20 mg into the uterus.

Additionally, Walker and Peaker (1980) documented levels of $PGF_2\alpha$ in mammary secretions of goats milked 18 to 4 d prepartum ranged from 200 to 400 pg/ml. Colostrum samples taken at parturition through d 4 ranged from 500 to 800 pg/ml, with the highest concentration occurring at parturition (d 0).

Turner and Holdsworth (1984) concluded that estrogenic metabolites in colostrum might have a luteolytic effect if given orally to mid-luteal cows. Their research, which

consisted of 13 normally cyclic Friesian cows in total; 6 cows were dosed with 900 ml colostrum orally, 5 cows received 900 ml milk, to which a known concentration of authentic estrone sulphate had been added. They also had a control group of 2 cows dosed with 900 ml milk containing a low endogenous level of estrone sulphate. Treatments were administered orally around day 9 or 10 following estrus. They assayed milk samples taken on the day of treatment and for 2-3 d thereafter. These samples were then assayed through radioimmunoassay for progesterone levels. Levels of estrone sulphate in the samples used for treatment were equal to or greater than 1000 pg/ml in both the colostrum group (n = 6) and those given milk with added estrone sulphate (n = 5).

Upon analyzing milk samples after administration, the first group, which received colostrum, had an immediate progesterone response. Most progesterone content in the milk samples from those animals dropped to near basal levels within 2 to3 d after treatment but only one of 5 cows actually was detected in estrus within 6 d of treatment. The second group, cows receiving treated milk containing synthetic estrone sulphate, had no apparent effect. When the treatment level was later raised to 2000 pg/ml for another group, some decline in progesterone was observed in two cows but only one reached basal levels within 2 d of treatment. Lastly, the control group, receiving the low endogenous level of estrone sulphate had a normal, cyclical pattern of progesterone and their treatment appeared to have no immediate effect (Turner and Holdsworth, 1984).

Effects observed in the colostrum-treated group may have been in part a result of the exogenous administration of estrone sulphate naturally contained in colostrum, as discussed by Turner and Holdsworth (1984). In addition to estrogens found in colostrum, $PGF_2\alpha$ and

its metabolites also present in colostrum may have been working separately or synergistically to effect apparent luteal regression in cows treated with colostrum. Certainly, from the earlier discussion, both estrogens and $PGF_2\alpha$ are involved in luteolytic processes as documented in studies cited earlier in this review.

Estrogenic Activity in Plants

Phytoestrogens are estrogenic compounds naturally present in plant materials. Their chemical structure (Figure 1), similar to estrogen metabolites, allows the chemical to bind with estrogen receptors and stimulate estrogen-like effects. There are more than 50 species of plants identified as producing phytoestrogens and common estrogenic feedstuffs including grasses, grains (oats and rye), and legumes such as alfalfa, clover, and soybeans. Plant materials that ruminants typically are exposed to include feedstuffs such as alfalfa, clover, and soybeans. These feedstuffs primarily produce two common phytoestrogens found in plant material, coumestans and isoflavones, and their metabolites.

Alfalfa is known to produce coumestans, or its metabolite, coumestrol, as well as sativol. Clover predominantly produces isoflavones that are broken down into the metabolites, genistein, formononetin, and biochanin A. Clover can also produce the coumestans, coumestrol, trifoliol, and repensol. Lastly, soybeans produce the isoflavones: genistein, daidzein, and glycetin (Adams, 1995). Soybeans can also produce coumestans.

Two common phytoestrogens, coumestans and isoflavones are metabolized similarly within ruminants. Estrogenicity in coumestans is enhanced after metabolic demethylation, where the exposed hydroxyl group can then bind to an available estrogen receptor within the blood causing estrogen-like effects. In isoflavones, estrogenicity is enhanced through

demethylation similar to coumestans, but is further reduced to equol, and then absorbed rapidly through the ruminal wall (Adams, 1995). In the rumen, demethylation of the isoflavone metabolite biochanin A leads to a potent estrogen genistein, by fermentation (Jorgensen and Freymiller, 1972). Similarly, the isoflavones daidzein and genistein are metabolized in the rumen to equol and para-ethyl-phenol, respectively (Woclawek-Potocka et al., 2005). An example of the metabolism of the phytoestrogen formononetin, an isoflavone metabolite, in sheep is depicted in Figure 2.

Phytoestrogenic content and exposure are mainly measured through chemical assays and bioassays. Accurate diagnosis can be accomplished by measuring the estrogenicity of the pasture ruminants graze on. Typical chemical assays are completed using thin layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC). Phytoestrogens are first extracted in alcohol and their fluorescence or ultra-violet (UV) absorbance is then measured to determine their concentration. Isoflavone metabolites usually take up about 5% of the dry weight within plant material whereas coumestans comprise about 1/10 of those levels. Bioassays are used to measure phytoestrogenic exposure to the animal population. Measurements can include teat length, uterine weight, weight of cervical mucus, and cervical histology (Adams, 1995). Though bioassays are simple and more accessible, they may have the tendency to be inaccurate, labor-intensive, and expensive. Therefore, chemical assays, namely HPLC are the more favorable technology used.

Factors Affecting the Estrogenicity of Plants

Factors typically responsible for differences in the estrogenic content of plants include species of plant, stage of maturity, and effects of preservation either through dehydration or fermentation for ensiling (Stob et. al., 1958). Other factors also include if the plant is suffering from a foliar disease, fertilizer deficiency, or fungal pathogen (Adams, 1995). The estrogenic activity of alfalfa also tends to increase during fermentation either in the silo or in the rumen. There is considerable difference between samples; however alfalfa silage has significantly greater estrogen activity than freshly cut alfalfa (Pieterse and Andrews, 1956). On the other hand, dehydration may lower estrogenic activity of forage (Bickoff et al., 1959). Fermentation includes preservation with molasses or sodium bisulfate when producing silage. Typically, alfalfa varies widely in its natural content of estrogenic compounds but alfalfa silage is more likely to be a function of original activity of the alfalfa than a result of the ensiling process. In addition, first growth alfalfa is higher in estrogenic activity than second growth material (Jorgensen and Freymiller, 1972).

Pieterse and Andrews (1956) reported differences in estrogenicity at different stages of maturity in alfalfa crops and other plant species. There was significant estrogen activity in early budding alfalfa crops followed by a decline until one-fourth bloom stage in which activity stayed relatively high through the seed head stage. In the three crops grown after this spring crop, estrogenic activity stayed relatively low. Significant estrogen content was also found in ladino and red clover as well as wheat, rye, and oats. When comparing potency to diethylstilbestrol per 100 g dry matter, potency levels were lower. Magee and Matrone (1958) also found similar results in green soybean forage. However, Bickoff et al. (1961)

demonstrated that subterranean clovers are very potent when comparing estrogenicity to stilbestrol in mouse models. However, in similar research, animal model responsiveness and fresh and dried samples of clovers may exhibit variability in estrogenic content (Bickoff et al., 1961).

Bickoff et al. (1959, 1960a) also observed variability in estrogenicity in earlier studies. Samples assayed at fresh and then dried stages showed no loss in estrogen activity whereas others lost 75% or higher of their original activity following dehydration. Reasons for this variability may be due to original estrogenic content either by stage of maturity or variety and as drying times and temperatures (Bickoff et al., 1960b).

Stob et al. (1957) noted differences in uterine weights in mice fed dried alfalfa extracts of 56 different varieties of the plants than those fed a control ration. In addition, differences in estrogenicity among varieties may have been due to resistance to disease, growth habits and chemical composition. Uterine weights ranged from 1.60 to 82.96 mg. However, mice fed diethylstilbestrol ranged from 20.92 to 148.46 mg meaning the estrogenic compounds present in alfalfa do not necessarily have the same degree of oral potency as diethylstilbestrol (Stob et al., 1957).

When molasses or sodium bisulfate was used as a preservative for alfalfa cut at different stages of maturity, there was no consistent difference in estrogenic activity between samples with or without preservatives (Stob et al., 1958).

Due to the well-known effects of antibiotics on fermentation, a study by Andrews and Stob (1958) investigated the influence of antibiotics on fermentation of silage and its effects on estrogenicity. Silage was preserved with either zinc bacitracin alone or in combination

with molasses and stored for 3 mo. Samples were then dried and fed to ovariectomized mice for 3 d. All silage samples showed estrogenic activity when compared to control diets given to mice. However a significant increase in estrogen activity was seen in the molasses (alone) group while the addition of zinc bacitracin did not significantly affect estrogen activity in the absence of molasses (Andrews and Stob, 1958).

Reproductive Effects of Phytoestrogens in Ruminants

Plant estrogens have widespread effects in a number of animal populations across the world. Research has been primarily focused on the harmful effects that phytoestrogens may have on reproduction in female ruminants. Furthermore, attention has focused on effects that phytoestrogens may have on mammals in the absence of any observable signs, or subclinical effects. Past research has shown that ruminants that heavily graze on estrogenic forage may display negative reproductive effects. Clover silage and alfalfa have caused effects such as estrogenism, cystic ovaries, irregular estrus, anestrus, and even temporary infertility in cattle. In sheep, estrogenic forage such as clover has caused reproductive effects, termed "clover disease" that are similar to those in cattle as well as temporary or even permanent infertility. These effects are typically considered subclinical and are hard to diagnose, as there is very little phytoestrogens present in plant material when determining the extent of animal exposure through chemical or biological assays. Methods to circumvent these harmful reproductive effects include discontinuing a pasture herd from grazing on high estrogenic forage or planting low-phytoestrogen cultivars, the most common being the latter (Adams, 1995).

Woclawek-Potocka et al. (2005) completed a two-part study to determine if phytoestrogens in soybean and/or their metabolites are detectable in the plasma of Holstein/Polish crossbred heifers (n = 10) and cows (n = 24) fed a soy diet. Secondly, the ability of a phytoestrogenic diet to influence reproductive efficiency and $PGF_2\alpha$ synthesis during the estrous cycle and early pregnancy in the endometrium was also observed. The soy-derived diet fed to cows consisted of 1900 µg/g of total phytoestrogens. Control cow diets were much lower in phytoestrogenic content (< 300 µg/g of fodder).

Serum samples of heifers fed a soybean diet had significantly higher concentrations of the phytoestrogenic metabolites para-ethyl-phenol and equol whereas control serum samples were almost undetectable. The pregnancy rates of the heifers fed the high-soy diet (3/5) and the heifers fed the standard diet (4/5) were not significantly different. However, the concentrations of PGFM (a metabolite of $PGF_2\alpha$) in the blood samples of the soy-fed heifers were significantly higher than those of the control heifers. Lastly, phytoestrogens and their metabolites from the soy diet stimulated the secretion of $PGF_2\alpha$ in different stages of the estrous cycle in the bovine endometrium samples. This stimulation predominantly occurred during the late luteal and follicular phases (Woclawek-Potocka et al., 2005).

A study by Piotrowska et al. (2006) observed whether active phytoestrogenic metabolites equol and para-ethyl-phenol could inhibit the sensitivity of the corpus luteum (CL) to Luteinizing Hormone (LH) and prostaglandin E_2 (PGE_2), a luteotropic factor and $PGF_2\alpha$ in a multiple-part in vivo and in vitro study. Initially they investigated if LH, PGE, and $PGF_2\alpha$ factors influence pulsatile progesterone secretion by the CL. Their research also examined whether the metabolites (equol and para-ethyl-phenol) could regulate pulsatile

progesterone secretion and LH-stimulated progesterone secretion. Their study was completed using normal cyclic Holstein/Polish crossbred heifers (n = 10) and cows (n = 12), whose estrous cycles were synchronized (Piotrowska et al., 2005).

Heifers fed a soy diet (2.5kg/animal/day) had higher concentrations of equol and para-ethyl-phenol in their plasma than heifers fed a standard diet (undetectable). Starting on day 12 of the estrous cycle, concentrations of progesterone in soybean fed heifers were lower than those fed the standard diet. Equol and para-ethyl-phenol were detected in the tissues of CL collected from cows fed a soybean diet. Those fed the standard diet had levels significantly lower. When the CL were perfused with LH, PGE_2, and $PGF_2\alpha$, all strongly stimulated progesterone secretion in the cows fed the standard diet. In the CL collected from cows fed the soy diet, all factors did not affect progesterone secretion. Lastly, equol and para-ethyl-phenol inhibited LH-stimulated progesterone secretion in comparison to the saline treated groups (Piotrowska et al., 2005).

Reproductive Effects of Phytoestrogens in Non-Ruminants

Similar effects as previously mentioned in ruminants, have been also reported in non-ruminants, more specifically with rat and mice models under laboratory settings. A study by McGarvey et al. (2001) observed the effects of the phytoestrogenic metabolites genistein and coumestrol on the activity of the GnRH pulse generator and the pituitary's sensitivity to GnRH stimulation and LH release in ovariectomized rats. Rats either received DMSO, a control vehicle (n = 6), estradiol (n = 6), low dose or high dose coumestrol (n = 12), and genistein (n = 6). At the end of the dosing, all animals were given GnRH to determine the pituitary's sensitivity to GnRH stimulation.

Results of this study showed that i.v. administration estradiol resulted in a reduction of LH pulse frequency and amplitude. High dose coumestrol resulted in a profound inhibition of pulsatile inhibition of pulsatile LH secretion. However, genistein, coumestrol (low dose), and the control vehicle (DMSO) had no effect on pulsatile LH secretion. Lastly, the GnRH-induced LH response was attenuated by estradiol and blocked by coumestrol (high dose) whereas the other treatments showed no response in the rats (McGarvey et al., 2001).

In another study, Whitten et al. (1995) examined the effects of coumestrol on immature rats through exposure through the milk of rat dams fed a coumestrol, control, or commercial soy-based diets. Treatment occurred during the first 10 d postnatal or throughout 21 d of lactation. The 10 d treatment did not significantly alter adult estrous cyclicity, but the 21-day treatment produced a persistent estrus state in coumestrol-treated females by 132 d of age (Whitten et al., 1995).

In other studies with ovariectomized rats, Diel et al. (2001) observed significant increases in height of the lumen epithelial cells of uterine and vaginal tissues. Other studies demonstrate changes of reproductive tract tissues in response to treatment with phytoestrogens in ovariectomized rats (Perel and Lindner, 1970; Santell et al., 1997). These studies used either genistein, a weak but prevalent estrogen found in soybeans and coumestrol, an estrogen found in many types of forage (Ford et al., 2006).

A study by Ford et al. in 2006 examined the effects of varying doses of genistein on estrogen-sensitive uterine and cervical tissues in ovariectomized gilts (n = 34). Postpubertal gilts were then ovariectomized and assigned at random to1 of 6 treatment groups. Treatment groups received vehicle, estradiol benzoate (2 mg/d), or genistein (50, 100, 200, or 400 mg/d)

via intramuscular injection at 12 h intervals for 10 d. Following the 10 d treatment period,

gilts were euthanized and uterine and cervical tissues were collected for chemical or

histological analysis. Uterine and cervical wet weights were increased by a dosage of 200

mg of genistein/d but not by 100 mg of genistein/d compared to the control gilts.   Tissue

growth was stimulated by genistein in a dosage-dependent manner; however none of the 4

dosages of genistein induced a response as great as that of estradiol benzoate (Ford et al.,

2006).

SUMMARY

This literature review has described common methods for manipulating the estrous

cycle in livestock species using synthetic hormones and their analogues. Typical methods

include the use of estrogenic metabolites, prostaglandins, namely $PGF_2\alpha$, or combination

treatments with estrogens and $PGF_2\alpha$ to induce luteolysis and shorten the interval to

subsequent estrous.

Estrogens typically work at the uterus and are known to contribute to luteolysis by

causing a disruption in CL function as evidenced by a decline in progesterone concentrations

in the blood. This further leads to a shortened estrous cycle and quicker return to subsequent

estrus.

Similar results may also be attained through the use of $PGF_2\alpha$ and its analogues. Its

use is a popular method for estrous synchronization in many livestock practices through its

known luteolytic action. Estrous synchronization using $PGF_2\alpha$ usually involves schemes

where two injections of the hormone are administered at 10 to 14 d intervals with a return to

estrus within 2 to 4 d after the second injection. The use of a two-injection method is

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important because it allows the animals treated to respond to one of the injections as $PGF_2\alpha$ is not luteolytic during the early (d 0 to 4) or late (d 16 to 20) stages of the estrous cycle.

Research has also concluded that combinations of estrogens and $PGF_2\alpha$ may result in shortened days to estrus. Typically, these schemes have demonstrated greater success when estrogenic metabolites are administered within 48 hr after $PGF_2\alpha$ is administered and not simultaneously.

Additionally, there is growing interest in developing natural methods for controlling the estrous cycle in livestock species. Previous research has demonstrated the ability of estrogenic metabolites endogenously present in colostrum to induce luteolysis by lowering milk progesterone levels resulting in luteal regression and shortened days to subsequent estrus.   In addition to estrogens found in colostrum, $PGF_2\alpha$ and its metabolites also present in colostrum may work separately or synergistically to effect apparent luteal regression in cows treated with colostrum.

Research has demonstrated that ruminants that heavily graze on estrogenic forage may display negative reproductive effects, including cystic ovaries, irregular estrus, anestrus, temporary and even permanent infertility. These effects may be caused by phytoestrogenic metabolites naturally present in plant material, which disrupt luteal function by attenuating progesterone secretion from the CL. However, these effects are considered subclinical and are hard to diagnose, as there is very little phytoestrogens present in plant material when determining the extent of exposure through chemical or biological assays.

The objectives of this research project therefore were: 1). to examine the effects of oral colostrum which contains a variety of hormones including estrogens and prostaglandin

metabolites on luteal regression in pasture-based dairy heifers and 2). to determine possible

effects of a phytoestrogenic mixture on reproduction in postpartum pasture-based dairy cows.

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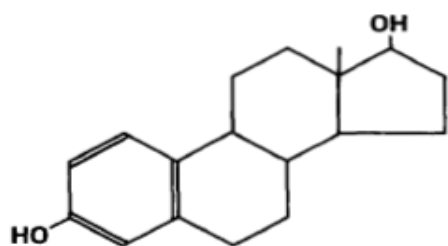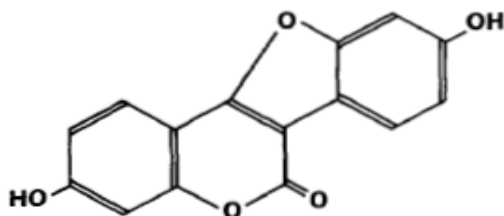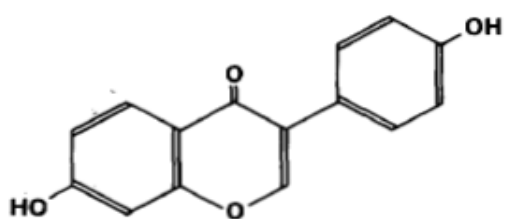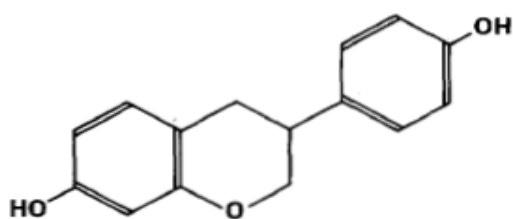
Figure 1: Chemical structures of common phytoestrogens steroidal (estradiol-17β), coumestan (coumestrol), isoflavone (daidzein), and isoflavan (equol) estrogens.
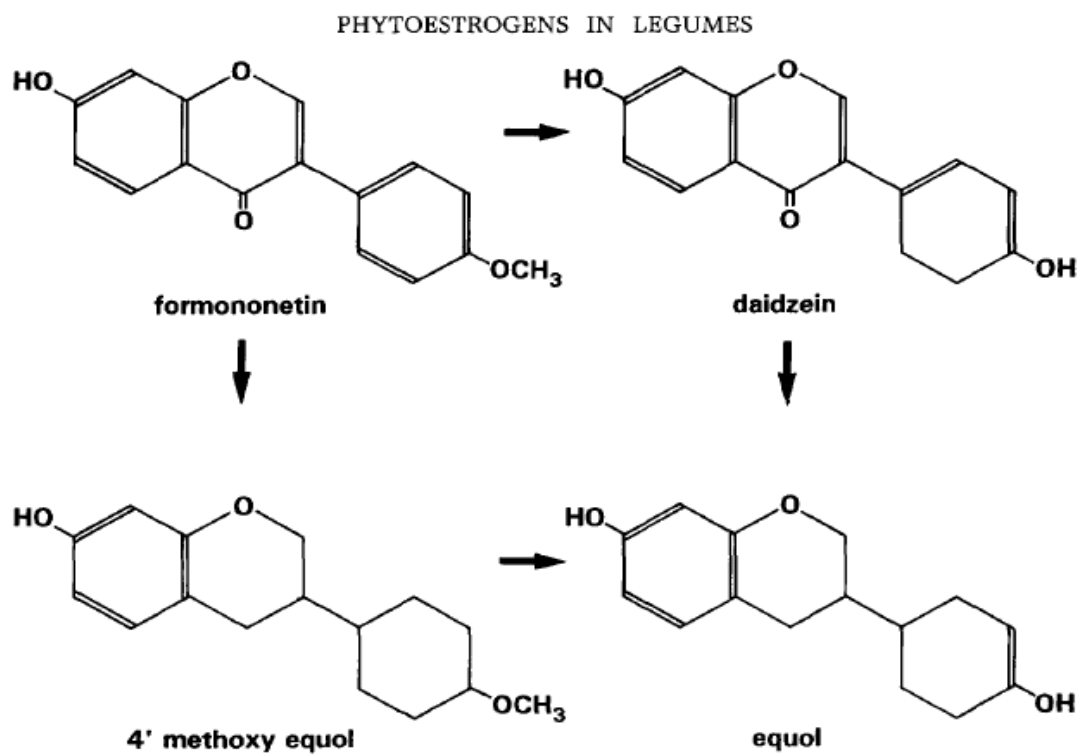
Figure 2: Suggested major patterns of metabolism of formononetin (a phytoestrogenic metabolite) in the rumen of the sheep.

CHAPTER 2

EVALUATION OF ORAL COLOSTRUM ON REPRODUCTION IN DAIRY

CATTLE

ABSTRACT

A 1984 study by Turner and Holdsworth indicated an effect of 900 ml of oral colostrum on lowering milk progesterone (P4) in cattle, presumably due to estrogens and prostaglandin metabolites in colostrum. The objective of this experiment was to examine similar responses in cyclic dairy heifers given oral colostrum in comparison to untreated heifers or to those receiving prostaglandin $F_2\alpha$. The pooled, frozen-thawed colostrum used was high (~6 L) and medium (~18 L) quality based on a colostrometer (Biogenics- Mapleton, OR). Heifers (n = 29) were Holsteins, Jerseys, or Holstein/Jersey crossbreds born between September 2006 and January 2007 (BW = 286 ± 17 kg). All heifers were housed at the Dairy Unit of the Center for Environmental Farming Systems (CEFS) in Goldsboro, NC. A corpus luteum (CL) was identified for each heifer with use of ultrasound; average CL diameter = 2.9 ± 0.17 cm. Heifers were assigned at random to 1 of 4 treatment groups: 1) untreated control (n = 7); 2) positive control, 25 mg Lutalyse ($PGF_2\alpha$) i.m. (n = 7); 3) 1 L oral colostrum (n = 7); or 4) 2 L oral colostrum (n = 8). Colostrum was administered using an esophageal tube and pump. Ultrasound measurements and blood samples were collected before treatment (d 0) and at 1 and 4 d after treatment to determine changes in CL size and serum P4 concentrations. Heifers received tailhead paint and were observed for estrus twice daily. Although exact stages of the estrous cycle were not known, sizes of corpora lutea and serum concentrations of P4 were similar across treatments at the start (d 0). Comparisons of P4 concentrations showed significant differences between $PGF_2\alpha$ vs. all other groups at d 1 ($P <$ 0.01) and d4 ($P < 0.05$). Based on P4 and ovarian palpation, all 7 $PGF_2\alpha$ -treated heifers responded with luteal regression as expected. Heifers treated with I L or 2 L of colostrum

actually had higher P4 on d 1 and d 4 ($P < 0.05$) than the negative control group and had similar sizes of luteal structures on d 4 indicating a lack of effect of luteal regression as a result of treatment with colostrum. All 7 heifers receiving $PGF_2\alpha$ came into estrus within 4 d after treatment (2.8 ± 0.2 d) whereas only 3 heifers from the untreated group, 2 from the 1 L group, and 3 from the 2 L group were detected in estrus within 7 d after treatment. All heifers detected in estrus had P4 >2.0 ng/ml at treatment which declined thereafter. In contrast, the 14 heifers not observed in estrus by 7 d continued to have P4 concentrations >2.0 ng/ml 1 and 4 d after treatment. All $PGF_2\alpha$ -treated heifers responded with a decline in P4 and subsequent estrus whereas responses among the 15 heifers that received colostrum were similar to that of the 7 untreated heifers. There was little estrogenic activity determined in a sample of the pooled colostrum, which was unexpected. It is not known whether the use of colostrum of higher quality or at higher doses would be more effective. In summary, it was concluded that use of orally administered colostrum would not be effective in facilitating luteal regression and earlier onset of estrus in dairy heifers.

INTRODUCTION

Ability to detect estrus and therefore improve detection of estrus through estrous synchronization protocols enhances managerial and economical efficiencies in cattle production systems by reducing labor costs for estrus detection and inseminations at predetermined times. This practice usually involves many schemes including $PGF_2\alpha$, progesterone, or estrogen analogues to elicit luteolysis or inducement of a subsequent estrus at a predetermined time. Manifestation of estrous behavior is believed to be due to action of estrogens, which are elevated during estrus (Helmer et al., 1985).

Pharriss and Wyngarden (1969) were the first to observe that $PGF_2\alpha$ induced luteolysis in rats. Since then, investigations have now termed prostaglandin $F_2\alpha$ as the endogenous luteolytic substance in both sheep and cattle (LaVoie et al., 1975). To acquire predetermined inseminations, $PGF_2\alpha$ is usually administered in a two-injection scheme at 10 to 12 d intervals. This is to ensure that cycling cattle at random stages of the estrous cycle will undergo luteolysis at either the first or second injection and the majority of animals will be in the early stages of the estrous cycle at the second $PGF_2\alpha$ injection (Stevenson et al., 1984).

Research has also shown that there is a possible luteolytic interaction between estradiol benzoate and prostaglandin $F_2\alpha$. Hixon et al. (1983) observed luteolysis as evidenced by a shorter mean length of the estrous cycle and decline in systemic concentrations of progesterone to less than 1 ng/ml in heifers treated with both estradiol benzoate and $PGF_2\alpha$. Treatment with estradiol benzoate following $PGF_2\alpha$ treatment tended

to increase the proportion of cows in estrus within 5 d in research conducted by Dailey et al., (1986).

Turner and Holdsworth (1984) have also suggested that the estrogen metabolites present in colostrum may have an apparent luteolytic effect as evidenced by a decline in subsequent milk progesterone samples. Because research has determined exogenous estrogens and $PGF_2\alpha$ are successful either together or in separate treatments to exhibit luteolytic effects, perhaps those same metabolites found in colostrum may also work in a similar fashion. Further, values of both progesterone and estradiol in milk are highly correlated with those found in serum (Meisterling and Dailey, 1987). Research by Hansel et al. (1975) indicates that milk normally contains small amounts (100 to 300 pg/ml) of $PGF_2\alpha$. Further, Manns (1975) found that endogenous levels of $PGF_2\alpha$ may range from 200 to 400 pg/ml. Walker and Peaker (1980) documented levels of $PGF_2\alpha$ in colostrum samples from goats milked at parturition through d 4 ranged from 500-800 pg/ml.

There has been growing attention towards finding natural methods when managing estrous in organic herds. Some possible natural methods may include using milk-derived estrogens and $PGF_2\alpha$ found in colostrum. Though there are some unknown factors to the likelihood of colostrum causing luteolytic effects in heifers and cows, results reported by Turner and Holdsworth (1984) provide evidence for inducing overt estrus in cyclic heifers and cows through administration of oral colostrum containing known luteolytic hormones (estrogens and $PGF_2\alpha$). Therefore, the objectives of the following study were to observe the reproductive effects on ovarian structures, serum progesterone concentration, and d to estrus

using 1 and 2 liters of pooled high and medium quality colostrum given orally in heifers. The hypothesis is that use of orally administered colostrum will advance luteal regression and shorten the interval to estrus compared to untreated control heifers.

MATERIALS AND METHODS

General Information

The objective of this trial was to determine if administration of colostrum could induce a decline in the levels of serum progesterone (P4) and elicit estrus in cyclic heifers as reported by Turner and Holdsworth (1984) for cows. This experiment was designed to examine the effects of luteal regression in 29 heifers studied during January 2008. Heifers were Holstein (n = 6), Jersey (n = 5), or Holstein/Jersey (n = 18) crossbreds with mean BW of 286 ± 17.3 kg. The heifers were housed at the Dairy Unit at the Center for Environmental Farming Systems (CEFS), Goldsboro, NC and were born between September 2006 and January 2007. All heifers received water and were allowed access to a grass pasture daily. Supplemental feed included daily allotments per head of 2.27 kg of ground corn plus minerals and 6.75 kg corn silage (as fed) along with ad libitum dry hay when pasture was limited or unavailable. Heifers were managed together in the same pasture.

Treatments

Heifer records including dates of birth and breed codes were collected from the farm manager. Presence of a viable corpus luteum was confirmed by ultrasound prior to the trial. Ultrasounds and CL diameter measurements were recorded on d0 (first day of treatment). Heifers were distributed into four treatment groups at random based on breed group. Negative control heifers (n = 7) and positive control heifers (n = 7) treated with Lutalyse, a

PGF$_2$α analogue were compared to responses in heifers dosed with 1 L (n = 7) and 2 L of colostrum (n = 8). Positive control heifers were treated with 25 mg Lutalyse (PGF$_2$α) i.m. at the start of the experiment (d 0). Heifers in the 1 L group were given approximately 1.05 L of pooled high/medium quality colostrum orally at the start of the experiment (d 0). Heifers in the 2 L group were given approximately 2.1 L of pooled high/medium quality colostrum orally at the start of the experiment (d 0). All colostrum treatments were administered using an esophageal tube and pump. Colostrum considered high quality (~6 L) contained >51 mg/ml immunolglobulin concentration at 72°F. Colostrum considered medium quality (~18 L) contained 30-50 mg/ml immunoglobulin concentration at 72°F. Quality of the pooled, frozen-thawed colostrum was based on a colostrometer (Biogenics, Mapleton, OR). A 100-ml sample of the pooled colostrum was kept and stored frozen until it was later analyzed for total estrogenicity.

Monitoring for estrous behavior occurred twice daily at 12-hour intervals. All heifers received tailhead paint at the start of the experiment and were monitored for 20 to 30 min each time during the twice-daily estrous observations. Positive signs of estrus included mounting, standing to be mounted, mucus secretions, and any disruption of tail paint, or tail paint on the chest of the mounting animal. Farm staff was allowed to breed all heifers detected in estrus during the trial period regardless of treatment group. Blood samples and ultrasounds were completed 1 d after the start of the experiment to determine the extent of CL regression and decline in P4 levels (d 1). On d 4 after treatment, heifers were rectally palpated and CL diameters were recorded to observe any treatment responses. All blood samples were collected into 10 ml serum vacuum tubes (BD Vacutainer, Franklin Lakes, NJ)

and placed on ice. Samples were then centrifuged at 3000 rpm for 30 min at 4°C and stored at –20°C until analyzed for progesterone content. Weights and date of subsequent heat and breeding were recorded for each heifer.

Progesterone Concentration Analyses

Samples were first thawed to room temperature. Analysis of P4 consisted of a Coat-a-Count solid-phase Radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The standard curve was determined from seven points (0, 0.1, 0.5, 2, 10, 20, and 40 ng/ml) in duplicate. A 100-µl blood sample was pipetted into a provided Progesterone Antibody-Coated tube. A 1 ml sample of ^{125}I Progesterone was then added to every tube and vortexed. All samples were then incubated for 3 h at room temperature. Samples were then decanted and allowed to sit and dry thoroughly overnight. Samples were then counted for 1 min in a gamma counter.

Extraction of Estrogenic Metabolites Present in Pooled Colostrum

In order to determine estrogenicity of the pooled colostrum, a general extraction and purification procedure was used before a tissue culture bioassay. A 500 µl sample of pooled colostrum was added to 4 ml of diethyl ether and mixed by gentle inversion for 30 min. After mixing, the sample was then centrifuged at 3000 rpm for 5 min to separate layers. The lower aqueous layer was then frozen in liquid nitrogen in a Styrofoam bath. The organic solvent layer was collected and evaporated under a gentle stream of nitrogen and dried overnight. The sample was then reconstituted in 500 µl of 90% ethanol and separated into 2, 250 µl samples. One of these samples was sent to undergo a yeast estrogen screen. The second sample was put through a second purification process (Waters Oasis Extraction

Purification Systems, Milford, MA). After the second purification, the sample was then used in the bioassay similar to the first sample.

Yeast Estrogen Screen Bioassay

Samples underwent a yeast estrogen screen bioassay in order to determine estrogenicity of the pooled colostrum sample given in this trial. The bioassay was based on a procedure described by Arnold et al. (1996) using a yeast strain responsive to estrogens as yeast do not contain sex steroid or thyroid hormone receptors, except those introduced into the strain. For the assay, a single yeast colony was grown in SD-uracil, tryptophan medium overnight at 30°C. The following day, 50 ml of the overnight culture were diluted into 200 ml of fresh tryptophan medium and grown overnight in the presence of the colostrum sample test compounds. All compounds were initially prepared in dimethylsulfoxide (DMSO) and added such that the concentration of DMSO did not exceed 2%.

Statistical Analyses

Data were also analyzed using a model including the fixed effects of treatment group (Control, PGF$_2$α, 1 L, and 2 L) and day of ovarian structure measurement (d 0, d 1, d 4). The General Linear Model procedure in SAS (SAS Inst. Inc., Cary, NC) was utilized to generate Least Squares Means.

Data were also analyzed using a model including the fixed effects of treatment group (Control, PGF$_2$α, 1 L, and 2 L) and day of sampling (d 0, d 1, d 4). The General Linear Model procedure in SAS (SAS Inst. Inc., Cary, NC) was utilized to generate Least Squares Means. Single degree of freedom contrasts were done on comparisons of interest; 1) PGF$_2$α

vs. all other treatments, 2) Control vs. 1 L and 2 L, and 3) 1 L vs. 2 L for sampling d 0, 1, and 4.

RESULTS

Yeast Estrogen Screen Bioassay

Both extraction samples yielded very little estrogenic activity when estimated by the Yeast Estrogen Screen bioassay. Therefore, the estrogen equivalents present in the colostrum sample were too small to determine the dosage rates of estrogen actually used.

Corpus Luteum Regression Data

Heifers were ultrasounded on the day of treatment (d 0) for presence of a viable CL and then given their respective treatments. Heifers were ultrasounded again on d 1 and rectally palpated on d 4 to observe possible changes or regression of ovarian structures. Regression was noted for heifers whose CL diameter was less than 2.5 cm after treatment occurred. These data were compared with negative control and heifers given $PGF_2\alpha$. By d 4, 3 negative control heifers had CL diameters less than 2.5 cm whereas 4 of 7 and 6 of 8 1 L and 2 L heifers had CL diameters less than 2.5 cm. $PGF_2\alpha$ -treated heifers showed the greatest response, 6 had CL diameters less than 2.5 cm by d 1 and all (n = 7) had complete luteal regression by d 4.

There were significant differences observed for CL diameters among all treatment groups on d 1 only ($P < 0.05$). No differences were observed for d 4 due to the rapid response of the $PGF_2\alpha$-treated heifers, as all had regressed a CL (Table 1).

Progesterone Level Response Data

All 29 heifers in the study had serum progesterone levels >2ng/ml on d 0 (first day of treatment). On d 1, one heifer in the negative control group and 6 of 7 in the $PGF_2\alpha$ group had serum progesterone levels <2ng/ml. All heifers in both colostrum-treated groups had serum P4 >2ng on d 1. By d 4, 3 of 7 negative control heifers, 7 of 7 $PGF_2\alpha$ -treated heifers, 2 of 7 heifers receiving 1 L of colostrum, and 3 of 8 heifers treated with 2 L of colostrum had serum P4 levels <2ng/ml. Therefore, all $PGF_2\alpha$ -treated heifers responded with a decline in P4 and subsequent estrus whereas responses among the 15 heifers that received colostrum were similar to that of the 7 untreated heifers.

There were significant differences in progesterone levels among treatment groups (Table 2) on d 1 ($P < 0.01$) as well as d 4 ($P < 0.05$) mostly related to the rapid response of the $PGF_2\alpha$ treated group. Contrasts among progesterone concentrations resulted in significant differences between $PGF_2\alpha$ vs. all other groups ($P < 0.01$) and negative control vs. 1 L and 2 L ($P < 0.01$) on d 1, with the negative control P4 concentrations actually being lower. On d 4, these comparisons showed differences for $PGF_2\alpha$ vs. all other groups ($P < 0.05$). Comparisons between the 1 L and 2 L groups showed a tendency for a difference on d 1 ($P = 0.06$) with the 2 L group actually with higher progesterone. On d 4, the P4 concentrations of negative control vs. 1 L and 2 L were significantly lower ($P = 0.05$).

Estrous Response Data

Positive control heifers treated with $PGF_2\alpha$ had the fewest days to subsequent estrus (2.8 ± 0.20 d) followed by negative control heifers (4.9 ± 1.24 d), and 2 L-treated heifers (6.2

± 0.98 d). Heifers receiving 1 L of colostrum were observed in estrus 6.4 ± 1.56 d after

treatment. Proportionally, 4 of 7 negative control cows were in heat by 7 d, and 5 of 7 by 10

d. All $PGF_2\alpha$ -treated heifers were in heat within 3.5 d and 4 of 7 were within 2.5 d. Only 2

of 7 heifers from the 1 L group were in estrus by 7 d and 4 were in heat by 10 d. Lastly, the

2 L group yielded similar results in which 3 of 8 were in estrus by 7 d and 6 of 8 by 10 d

(Table 3). Two heifers in the negative control group, 3 heifers in the 1 L group, and 2 heifers

in the 2 L group were not observed in heat within 10 d of treatment.

DISCUSSION

In this study, heifers received either 1 or 2 L of pooled medium and high quality

colostrum (24 L total). However, 75% of this colostrum (~ 18 L) was of medium quality and

only 25%, (6 L) was of higher quality based on a colostrometer. Lack of response in the 1 L

and 2 L treatment groups may have been due to the quality of the colostrum used. However,

the relationship of the immunoglobulin content of colostrum to the content of estrogen and

prostaglandin metabolites and potential for luteolytic effects is not known.

Time until subsequent estrus among the other treatment groups was longer than for

those receiving $PGF_2\alpha$, although all heifers were confirmed to have a viable CL. Because

heifers were not synchronized before the start of the experiment, the exact timeline of

cyclicity for individual heifers was unknown. Therefore, some variation in responses within

and among the treatment groups may not have been due to treatment, but instead caused by

typical variation in CL regression and decrease in progesterone leading to a subsequent estrus

seen in normal, cyclic heifers. Lack of response within 10 d could have also resulted for

heifers either at the early or mid-luteal stages of the estrous cycle. Additionally, all heifers

which were detected in estrus within 7 d had P4 >2.0 ng/ml at treatment which had declined by 4 d after treatment. In contrast, the 14 heifers not observed in estrus by 7 d all had P4 concentrations >2.0 ng/ml at both 1 and 4 d after treatment, indicating a lack of response. All $PGF_2\alpha$ -treated heifers responded with a decline in P4 and subsequent estrus whereas responses among the 15 heifers that received colostrum were similar to that of the 7 untreated heifers.

Based on these results it was concluded that orally-administerered colostrum was not sufficient to cause the desired extent of luteolysis. All heifers in the $PGF_2\alpha$ -treated group responded as expected with the shortest interval to subsequent estrus, as well as faster CL regression and decline in serum progesterone concentrations. Those heifers received 25 mg of $PGF_2\alpha$ administered i.m. The manufacturer recommended dose was a significantly larger dose, which exceeds the expected amounts of prostaglandin metabolites present per L of colostrum, which may range from 200-400 pg/ml (Manns, 1975). The route of administration also differed in these studies, and there is no documentation of efficacy of oral administration of $PGF_2\alpha$ or its metabolites.

With regard to prostaglandin metabolites, Hansel et al. (1976) obtained slightly different results when 5 or 20 mg were administered into the uterus. Milk samples taken from 6 cows from pretreatment milkings plus eight post treatment milkings had $PGF_2\alpha$ ranges of 125 to 232 pg/ml (Hansel et al., 1976). Average $PGF_2\alpha$ concentrations found by Simmons et al. (1979) were at or near 700 pg/ml when cows were treated with 30 mg $PGF_2\alpha$ 60 d postpartum. In their investigation, $PGF_2\alpha$ levels in milk were elevated significantly above pretreatment only for one sample (1300 pg/ml), which was taken during the third

milking, that was within 2 to 7 h of the single $PGF_2\alpha$ treatment (Simmons et al., 1979).

These differences may have been a result of different chemical assays used for each study.

Additionally, these values are typically much lower than the exogenous amounts of $PGF_2\alpha$

given to synchronize estrus in most production systems.

Colostrum has approximately 20 to 100 times as much estrogen as milk (Wolford and

Argoudelis, 1979). According to research by Monk et al. (1975), total estrogens in milk

range from, 1642 pg/ml 1-day prepartum to 10 pg/ml 5 d postpartum. According to Erb et al.

(1977), expected amounts of total estrogen metabolites (estrone, $E_2\beta$ and $E_2\alpha$) present per L

of colostrum may range from 1.0-2.0 ng/ml, 0.3-0.8 ng/ml, and 0.6-1.0 ng/ml, respectively.

When using estrogens in synchronization schemes, Eley et. al. (1979) used daily s.c.

injections of 28 or 56 mg of estrone sulfate to shorten estrous cycles by about 3 d. However,

those doses of estrogen are much higher than the total amount of estrogens found in

colostrum as well as the expected doses in 1 L or 2L of colostrum. Estrogens can also have

synergistic effects with $PGF_2\alpha$ in luteal regression (Hixon et al., 1983). Dailey et al. (1986)

also noted this synergism when estradiol benzoate was administered 40 to 48 h after $PGF_2\alpha$.

Bioassays conducted on the pooled sample of colostrum also indicated that the typical

amounts of estrogens present in colostrum or milk are much lower than what research has

indicated to be a sufficient amount to induce luteolysis. The average uterine weight of mice

given dried milk from cows pregnant at d: 27 to 97, 104 to 193, 200 to 268 were 11.35 mg,

13.71 mg, and 16.45 mg, respectively. Samples of colostrum from six normal cows and from

three cows experimentally induced to lactate, showed low uterine weight increases (Turner,

1958).

The lack of response to the yeast assay on estrogenic activity of the pooled colostrum was unexpected given prior reports about the presence of estrogens in colostrum (Monk et al., 1975; Erb et al., 1977; Wolford and Argoudelis, 1979; Turner and Holdsworth, 1984).

The amount of prostaglandin metabolites in colostrum from the trial of Turner and Holdsworth (1984) was not known and therefore it is uncertain why they observed decreases in progesterone after treatment. In addition, amount of estrogenic activity found in the colostrum sample had very little activity and therefore would not have been sufficient to elicit an effect when given to heifers. Cows treated with 1 ng/ml or 2-ng/ml estrone sulphate in 900 ml milk did not respond similarly to those receiving 900 ml colostrum. Therefore, these results by Turner and Holdsworth (1984) do not coincide with those seen in this study. The current study used more animals and even higher doses of colostrum then the 1984 report yet failed to elicit a response either in advancing luteal regression or in initiating earlier onset of estrus compared to untreated heifers.

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Table 1. LSMeans for CL regression data (cm) by treatment group on d 0, d 1, d 4[1].

Day of Measurement	Negative Control	PGF$_2\alpha$[2]	1 Liter	2 Liters
Day 0	2.9 ± 0.24	2.7 ± 0.24	2.8 ± 0.24	3.0 ± 0.24
Day 1	2.5 ± 0.17	2.2 ± 0.19	2.6 ± 0.19	2.8 ± 0.18
Day 4	2.3 ± 0.24	0	2.1 ± 0.29	2.0 ± 0.27

1. Overall CL diameter means differed on d 1 ($P = 0.02$) only.
2. Colostrum-treated groups did not show a significant regression in diameter whereas PGF$_2\alpha$ -treated heifers came into estrus within 4 d after treatment as evidenced by their complete luteal regression (treatments were administered on d 0). Ovarian structures were ultrasounded on d 0 and d 1 but ovarian observations and estimates were completed through rectal palpation on d 4.

Table 2. LSMeans for progesterone concentrations by treatment group on d 0, d 1, d 4[1].

Treatment Day	Negative Control	$PGF_2\alpha$ [2]	1 Liter	2 Liters
Day 0	7.6 ± 1.16	7.5 ± 1.16	6.7 ± 1.16	8.5 ± 1.08
Day 1	5.2 ± 0.79	1.5 ± 0.79	7.1 ± 0.79	9.3 ± 0.74
Day 4	2.4 ± 1.59	0.2 ± 1.59	5.7 ± 1.59	6.9 ± 1.48

1. Overall P4 means differed on d 1 ($P < 0.01$) and d 4 ($P = 0.02$).

2. $PGF_2\alpha$ -treated heifers had a greater decline in progesterone values by d 4 followed by the negative control group. The 1 L and 2 L groups showed variable trends in their progesterone concentration values. Orthogonal contrasts for $PGF_2\alpha$ vs. all other groups ($P < 0.01$) and negative control vs. 1 L and 2 L ($P < 0.01$) showed significance on d 1. On d 4, these comparisons showed significant differences for $PGF_2\alpha$ vs. all other groups only ($P < 0.05$). Orthogonal contrasts for 1L vs. 2 L were not significant for all days.

Table 3. Estrous responses among treatment groups. [1,2]

Treatment Group	Proportion in Estrus in 7 Days	Proportion in Estrus in 10 Days	Average Days to Estrus [3]
1. Negative Control	4 of 7 (57%)	5 of 7 (71%)	4.9 ± 1.2
2. $PGF_2\alpha$	7 of 7 (100%)	7 of 7 (100%)	2.8 ± 0.2
3. 1 L Colostrum	2 of 7 (29%)	4 of 7 (57%)	6.4 ± 1.6
4. 2 L Colostrum	3 of 8 (38%)	6 of 8 (75%)	6.2 ± 1.0

1. Exact date of cycle when treatments were administered was not known.
2. Presence of a viable corpus luteum (CL) was determined for all heifers used.
3. Average number of days until first observed estrus for those responding within 10 d.

CHAPTER 3

EVALUATION OF AN ORGANIC BOTANICAL PREPARATION ON

REPRODUCTION IN DAIRY CATTLE

ABSTRACT

A two-replicate experiment was conducted to estimate effects of the botanical, estrogenic preparation, Heat Seek on reproductive traits. Variables examined included milk progesterone, days to subsequent estrus, and ovarian structures (first replicate).   Cows were housed at the Dairy Unit of the Center for Environmental Farming Systems (CEFS) in Goldsboro, North Carolina. The first replicate used a group of cows (n = 24) born between the months of September 2001 and February 2006, who had recently calved during February 2008-December 2008 (BW = 438 ± 30 kg). Cows in the second replicate (n = 27), were born between October 2001 and January 2007 and had previous calving date ranges between October 2008 and February 2009 (BW = 407 ± 37 kg). Preliminary milk samples were collected in both replicates to determine if cows had resumed estrous cycles (P4 >2ng/ml). Cows were randomly allotted into 2 treatment groups (Control or Heat Seek-treated) in each replicate based on breed groups. Cows were Jersey, Holstein, or Holstein/Jersey crosses. In the first replicate, ultrasound images were taken on d 1 for initial observation of ovarian structures and then again on d 5 for any changes. Cows in both replicates received tailhead paint and were monitored twice daily until estrus was detected in all cows. Daily milk samples of 5 to 8 mL were also taken from these cows for later analysis of progesterone concentration. Heat Seek treated cows received a daily 13-g bolus capsule of Heat Seek for 6 consecutive days (d 1 through d 6) after their morning milking time (Rep 1: 0.10µg/kg; Rep 2: 0.11µg/kg estrogen equivalent given per day). Post treatment milk samples were also taken on d 10 from all cows in both replicates. These samples were centrifuged and frozen for later progesterone analysis. In Rep 1, only 3 cows in the control group had a CL and

average diameters of CL for those cows were 2.0 ± 0.7 cm on d 1. By d 5 average diameters

for this group were 1.8 ± 0.3cm for 6 cows that had a CL. Cows in the treated group (Heat

Seek) had d 1 average CL diameters of 1.9 ± 0.3 cm for 8 cows. Heat Seek-treated cows had

average CL diameters of 1.3 ± 0.1 cm by d 5 ($n = 7$). For both replicates, there were no

differences between treatment groups in milk progesterone concentrations across all 6

sampling days. Post-treatment samples also showed no significant differences between the 2

groups (Rep 1 $P = 0.86$; Rep 2 $P = 0.54$).   In Rep 1, average days to estrus for control cows

were 24.0 ± 10.4 d vs. 24.8 ± 9.2 d (n.s.) for treated cows. For Rep 2, average days to estrus

for control cows were 19.2 ± 3.4 d vs. 27.3 ± 8.7 d (n.s.) for treated cows. In addition,

progesterone level on d 1 (high vs. low) did not affect estrous responses within 25 d for the

data combined over both replicates. Percentages of cows that conceived to the first estrus

after start of treatment for Rep 1 were 33% (Control) and 55% (Heat Seek). Respective

percentages in Rep 2 were 50% (Control) and 69% (Heat Seek). Chi square analysis of

estrual and conception responses indicated no significant differences. The lack of significant

differences in milk progesterone data for both treatment groups in the 2 replicates suggests

that the estrogens present in the Heat Seek capsules may not have been sufficient to elicit a

response in those treated. The variation in responses may have been subject to the timing of

treatment and stage of cycle during treatment. Low progesterone levels on d 1 and during

treatment may have also been a result of cows that were either not cyclic or because they had

a recently regressed CL.

INTRODUCTION

Estrogen and its metabolites such as estradiol benzoate and estrone sulphate have been shown to be luteolytic in cattle. Additionally, hysterectomy has been associated with a reduction in the luteolytic effects of estradiol. Therefore, the uterus is a site of estradiol action contributing to luteolysis, which we now know, involves the production of prostaglandin $F_{2\alpha}$ ($PGF_2\alpha$) from the uterus (La Voie et al., 1975). Luteolysis can be considered as a reduced length of estrous cycles following treatment and decrease in concentrations of progesterone to less than 1 ng/ml (Hixon et al., 1983).

Eley et al. (1979) also demonstrated that a once daily injection of 28 or 56 mg of estrone sulphate on d 10 of the estrous cycle until detection of estrus had a significant luteolytic effect on cyclic beef heifers. Heifers were injected subcutaneously with 28 mg estrone sulphate (n = 6) on d 10 of the estrous cycle while control heifers (n = 6) were injected with 7 ml corn oil. Injections were continued once daily until detection of estrus. After return to estrus, the 6 control animals were then injected with 56 mg estrone sulphate/day until detection of a subsequent estrus. Control, 28 mg, and 56 mg/day treated heifers averaged estrous cycle lengths of 21.2 ± 0.75, 18.7 ± 0.42, and 18.2 ± 0.34 d, respectively. Plasma progestins declined sooner in treated heifers, falling from 4.8 ng/ml on d 14 to estrous levels <1 ng/ml at day 18. In comparison, progestin concentrations in control heifers declined from 6.02 ng/ml on day 16 to <1 ng/ml at 21.2 d (Eley et al., 1979).

In a study by Burke et al. (2000), 24 non-lactating Friesian cows were used to determine if a small dose of estradiol benzoate (EB) during the middle stages (luteal phase)

of the estrous cycle would synchronize ovarian follicular development, estrus, and ovulation. The cows received either 1 mg of EB i.m. on d 13 of the estrous cycle (n = 12), or served as untreated controls (n = 12). Data on diameter and location of each corpus luteum and follicle were collected. Those treated with 1 mg of estradiol benzoate displayed a marked rise in plasma concentrations of estradiol (12 pg/ml) during the initial 24 h following treatment and a decline in progesterone between 24 and 48 h after treatment compared to control cows. This difference between groups was coincidental with earlier time to regression of the corpus luteum seen in the treated cows. Atresia of the dominant follicle at that time was a result of the EB treatment and resulted in emergence of a new wave of follicular development 4 to 5 d after treatment (Burke et al., 2000).

Similar in function to estrogens, phytoestrogens are natural plant-derived estrogens. Research has linked phytoestrogens present in common feedstuffs, such as alfalfa, clover, or soybean to negative reproductive effects such as temporary and permanent infertility in sheep and cattle. However, these are effects observed in ruminants that graze on estrogenic forages consistently. If phytoestrogens are used in a small, controlled manner, the exogenous phytoestrogenic metabolites present may be used to induce a disruption in CL function by inhibiting its luteotropic factors such as progesterone as observed by Piotrowska et al., (2005).

Research by Piotrowska et al. (2005) determined that heifers fed a soy diet (2.5kg/animal/day) had higher concentrations of phytoestrogenic metabolites equol and para-ethyl-phenol in their plasma than heifers fed a standard diet. Starting on d 12 of the estrous cycle, concentrations of progesterone in the soybean fed heifers were lower than those fed

the standard diet. Equol and para-ethyl-phenol were detected in the tissues of CL collected from cows fed a soybean diet whereas CL from the cows fed a standard diet was undetectable. Equol and para-ethyl-phenol inhibited LH-stimulated progesterone secretion in comparison to the saline treated groups (Piotrowska et al., 2005).

Estrous cyclicity may also be affected as reported by Whitten et al. (1995) who examined the effects of a 21 d treatment of coumestrol on immature rats through exposure through the milk of rat dams fed a coumestrol, control, or commercial soy-based diets. The 21 d treatment produced a persistent estrus state in coumestrol-treated females by 132 d of age.

In other studies with ovariectomized rats, Diel et al. (2001) observed significant increases in height of the lumen epithelial cells of uterine and vaginal tissues. Other studies reported changes of reproductive tract tissues in response to treatment with phytoestrogens in ovariectomized rats (Perel and Lindner, 1970; Santell et al., 1997).

These mechanisms will be examined throughout the following study in order to evaluate potential of an organically acceptable method of reproductive management in cattle, similar to the investigation using oral colostrum. The project will examine the effect of a commercially available botanical mixture, Heat Seek, with potential estrogenic activity on ovarian structures, milk progesterone levels, days to estrus, breeding, and subsequent pregnancy in postpartum dairy cows. The ingredients of this preparation include: Damiana Leaf, wild yam, Partridge berry, squaw vine, Black Cohosh, European Cranberry bush, Evening primrose, Dong quai root, Flaxseed or linseed, and vitamin B6. The hypothesis is

that the botanical preparation administered orally over 6 d will potentially shorten the intervals from treatment to estrus and from treatment to pregnancy compared to untreated control cows.

MATERIALS AND METHODS

General Information

The objective of this study was to investigate the effects of the phytoestrogenic product Heat Seek on the estrous cycle of cyclic cows in two separate replicates. Cows were housed at the Dairy Unit of the Center for Environmental Farming Systems (CEFS) in Goldsboro, North Carolina. They were maintained together on a sacrifice pasture with water provided ad libitum. Cows were also provided round bales of alfalfa and fescue haylage at least every other day and fed to appetite. Additionally, cows received supplemental feed before morning and evening milking times. Supplemental feed included 6.35 kg ground corn, 2.27 kg cottonseed, 17.24 kg corn silage (as fed), and 1.36 kg soybean meal per day.

The first replicate used a group of cows (assigned 33 cows and used 24) born between the months of September 2001 and February 2006, who had recently calved during October, 2008 to December, 2008 (Average DIM = 101.5 d). Cows in this replicate underwent 3 preliminary milk samplings to determine postpartum cyclicity by milk progesterone analysis. These samples were taken at 15 d intervals (December 16, December 31, 2008, and January 15, 2009) during evening milking times. A volume of at least 5 to 8 mL were collected from each cow and placed on ice. These samples were then centrifuged at 3000 rpm for 15 min at 4°C and then frozen at 20°C until they were analyzed for progesterone concentration.

Cows in the second replicate (assigned 29 cows and used 27), were born between October 2001 and January 2007 and had previous calving dates ranging between October 2008 and February 2009 (Average DIM = 69.5 d). In order to determine their cyclicity, a single set of preliminary milk samples were taken on March 12 during the evening milking time, (5 to 8 mL total milk sample per animal) and kept on ice for further analysis for progesterone content. As in Rep 1, these samples were later centrifuged at 3000 rpm for 15 min at 4°C and then frozen at -20°C pending P4 analysis.

Cyclicity was determined as preliminary samples with progesterone levels >2ng/ml for cows used in both replicates. Cows (n = 11) that were assigned but not used in the study were later determined to have been inseminated before the experiment was initiated.

Treatments

After preliminary milk sampling for cows in Rep 1, all 24 cows (BW = 437.9 ± 29.8 kg) were assigned to one of two groups at random based within breed group. Cows were Jersey (n = 3), Holstein (n = 1), or crosses of Holstein and Jersey (n = 20). The first group (n = 11) was the control group, while the second group of cows (n = 13) was the treatment group, which received Heat Seek. Body weights were collected on all cows during preliminary milk sampling and again on d 3. Using ultrasonography, sizes and locations of ovarian follicles and corpora lutea were noted on d 1 and again on d 5 in order to detect changes during treatment. It should be noted one cow died before the replicate was completed and therefore her data were not used for the analyses.

In Rep 2, 27 cows (BW = 406.9 ± 37.2 kg) were separated into two groups; negative control (n = 14) and Heat Seek treated cows (n = 13). The groups were chosen at random by breed code (Holstein, n = 4; Jersey, n = 4; crosses of Holstein and Jersey, n = 19). Cows from Rep 1 were used in this replicate (n = 4; control = 2, treated = 2) and were kept in their respective previous treatment group. Body weights were collected on the first day of the replicate (d 1).

Records were collected on all cows. This included date of birth, breed group, and last calving date. During each replicate, date of estrus, breeding, and later confirmation of pregnancy were recorded. Estrus was monitored in all cows beginning at the start of the replicate. Cows in both replicates received tailhead paint and were monitored twice daily, or every 12 h until estrus was detected in all cows. Positive signs of estrus included mounting, standing to be mounted, mucus secretions, and any disruption of tail paint or tail paint on the chest of the respective animal. Farm staff was allowed to breed all cows detected in heat during the replicate. Daily milk samples of 5 to 8 mL were also taken from these cows for later analysis of progesterone concentration. Milk samples were collected either after morning or evening milking times. Samples were centrifuged at 3000 rpm for 15 min at 4°C for their skim portion. Samples were kept frozen at –20°C until analyzed for progesterone content. Heat Seek treated cows received a daily 13-g bolus capsule of Heat Seek for 6 consecutive days (d 1 to d 6) after their morning milking time. Lastly, post treatment milk samples were also taken on d 10 (final day of Heat Seek treatment) from all cows in both replicates. These samples were centrifuged and frozen for later progesterone analysis.

Progesterone Concentration Analyses

Milk samples were allowed to thaw to room temperature before the start of the progesterone analysis. Analysis of P4 consisted of a Coat-a-Count solid-phase Radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The standard curve was determined from seven points (0, 0.1, 0.5, 2, 10, 20, and 40 ng/ml) in duplicate. A 100 µl skim milk sample was pipetted into the provided progesterone Antibody-coated tubes, from every milk sample taken. Then, a 1 ml sample of ^{125}I Progesterone was added to all tubes. Tubes were vortexed and then incubated at 25°C for 3 h at room temperature. After the incubation period, the supernatant was decanted from the Antibody-coated tubes, blotted and dried overnight. Finally, the antibody-bound fraction of the ^{125}I Progesterone was quantified in a gamma counter for 1 min.

Extraction of Estrogenic Metabolites Present in Heat Seek Capsules

A general procedure was used to extract and purify estrogen metabolites from a 13-g Heat Seek bolus capsule. The procedure was based on a method described by Seguin et al. (2004). Three 2-g samples were taken from a Heat Seek capsule. Each sample was mixed with 2 ml of distilled H_2O and incubated for 30 min at 37°C. To reconstitute the samples, 2 ml of HCl and 16 ml of ethanol were added to each sample and mixed, heated to boiling and then cooled. Once cooled, samples were then centrifuged at 1500 g for 10 min. Samples were then cleaned by running them through cartridges by pre-wetting cartridges with 5 ml methanol and 5 ml H_2O (Waters Oasis Extraction Purification System, Milford, MA). A 1ml ethanol extract was diluted with 3 ml of H_2O and then injected through the cartridges. Cartridges were then washed with 2 ml of 20% methanol in water and eluted with 2 ml of

80% methanol in water. These eluates were then placed through a yeast estrogen screen similar to the colostrum sample. Each of the 3 samples was split into two 150 µl subsamples. One subsample from each pair was sent to undergo a bioassay to determine their respective estrogenicity. The second 150 µl aliquot subsamples were then put through a second extraction process (Waters Oasis Extraction Purification System, Milford, MA) and placed through the bioassay similar to the first sample.

Yeast Estrogen Screen Bioassay

Samples underwent a yeast estrogen screen bioassay, similar to the colostrum sample in order to determine estrogenicity of the capsules given in both replicates. The bioassay was based on a procedure described by Arnold et al. (1996) using a yeast strain responsive to estrogens as yeast do not contain sex steroid or thyroid hormone receptors, except those introduced into the strain. For the assay, a single yeast colony was grown in SD-uracil, tryptophan medium overnight at 30° C. The following day, 50 ml of the overnight culture was diluted into 200 ml of fresh tryptophan medium and grown again overnight in the presence of the Heat Seek capsule test compounds. All compounds were prepared in dimethylsulfoxide (DMSO) and added such that the concentration of DMSO did not exceed 2%.

Statistical Analyses

Data were analyzed using a model including the fixed effects of treatment group (Control and Heat Seek) and day of sampling (d 1, d 2, d 3, d 4, d 5 d 6, and post-treatment). The General Linear Model procedure in SAS (SAS Inst. Inc., Cary, NC) was utilized to generate Least Squares Means.

Data were also analyzed using a model including the fixed effects of treatment groups (Control and Heat Seek) and d 1 progesterone values (low: P4 < 2ng/ml or high: P4 > 2ng/ml) for estrous responses within 25 d. Additionally, a single degree of freedom interaction was conducted to determine the effect of treatment (control or treated) and progesterone concentration on d 1 for subsequent estrous responses.

Chi-square analyses were conducted to determine significance of estrual and conception responses for the proportional data yielded.

RESULTS

Yeast Estrogen Screen Bioassay

Both extraction samples yielded significant estrogenicity as determined by the YES bioassay. The first sample contained 3.44µg/g estrogen equivalents. The second sample, which underwent a second purification process, yielded 3.55 µg/g estrogen equivalents. Mean estrogen equivalents for both samples therefore were 3.50 µg/g. Calculations for daily dosages using this mean were 45µg/g estrogen equivalents per Heat Seek capsule (13 g capsule size). Daily dosages given per cow were determined using the 45 µg daily dose, per kg body weight. Replicate body weight averages were used to calculate this daily dose per cow of estrogen equivalents. Therefore, Rep 1 daily dose given per cow would have been 0.10µg/kg body weight (Rep 1 average BW = 437.9 kg). Rep 2 daily doses per cow would have been 0.11µg/kg body weight (Rep 2 average BW = 406.9 kg).

Replicate 1 Ultrasound Data

Cows in Rep 1 were ultrasounded on the first day of treatment (d 1) and again on d 5 for observations of any forming or regressing ovarian structures. Those with viable CL,

diameter measurements were recorded. Average CL diameters for cows in the control group were 2.0 ± 0.7 cm (n = 3) for d 1. By d 5 average diameters for this group were 1.8 ± 0.3 cm (n = 6). Cows in the treated group had d 1 average diameters of 1.9 ± 0.3 cm (n = 8). Treated cows (Heat Seek) had average CL diameters of 1.3 ± 0.1 cm by d 5 (n = 7).

Cyclicity Data

Milk samples were used to determine cyclicity status on all cows in both replicates during the 6 d of treatment. The milk progesterone data for Rep 1 (n = 24) determined 5 cows used were not cyclic (P4 <2ng/ml; Control: n = 2 Control, Treated: n = 3).

According to the milk progesterone data for the cows in Rep 2 (n = 27), 6 cows in the second replicate were not cyclic at the start of the experiment (P4 <2ng/ml; Control: n = 3, Treated: n = 3).

Additionally, in Rep 1, nine cows were inadvertently used in the experiment that had actually been inseminated before the start of the experiment, three of which were treated with Heat Seek. Those cows were inseminated 16 (n = 4), 15 (n = 3), 14 (n = 1), or 1 (n = 1) d before the start of the experiment. Overall, 8 of the 9 cows were confirmed pregnant and 3 of those 8 pregnant cows had been given Heat Seek.

Similarly in Rep 2, two cows were used that had been previously inseminated before the beginning of that replicate. One cow was inseminated 15 d before to the start of the experiment and the other 7 d before the beginning of the replicate. Both were treated with Heat Seek and were later confirmed pregnant. Therefore, considering both replicates, 5 of 5 cows receiving Heat Seek and 5 of 6 control cows which were inseminated before the onset

of treatment conceived. Therefore, estrogens present in Heat Seek did not adversely affect the ability of those cows to maintain pregnancy.

The data from the 11 cows that had been inseminated before the start of treatment were not used for analyses and summary of results reported below.

Progesterone Response Data

Milk samples were collected from all cows daily to determine progesterone response to treatment, compared to no treatment. Post-treatment milk samples were also observed.

On d 6, (last day of Heat Seek treatment), 8 control cows had progesterone levels less than 2 ng/ml while 7 treated cows had similar progesterone levels. Post-treatment milk samples showed similar trends where 7 control and 5 treated cows had P4 levels below 2 ng/ml (Figure 3).

In Rep 2, 7 control cows and 7 treated cows had progesterone levels less than 2 ng/ml on d 6. Post-treatment milk samples determined 9 control cows and 10 treated cows had P4 levels below 1 ng/ml (Figure 4). Combined progesterone data for both replicates are depicted in Figure 5.

For both replicates, there were no differences in milk progesterone concentrations for all 6 sampling days between the two treatment groups. Post-treatment samples also showed no significant differences between groups (Rep 1 $P = 0.13$; Rep 2 $P = 0.75$).

Estrous Response Data

Proportions were determined for cows from both treatment groups of both replicates observed in heat within 25 and 60 d. By 25 d after treatment in Rep 1, 6 of 11 control cows and 7 of 13 Heat Seek-treated cows were observed in estrus. By 60 d, 9 of 11 control cows

and 12 of 13 treated cows were observed in estrus (Table 5). One control cow was in heat on d 65, and one treated cow was observed in heat on d 66. Lastly, one control cow from the replicate was never bred and subsequently left the farm. Average days to estrus did not differ significantly between control cows (24.0 ± 10.4 d) and treated cows (24.8 ± 9.2 d).

In Rep 2, 9 of 14 control cows and 8 of 13 Heat Seek-treated cows were observed in heat within 25 d of treatment. Twelve control cows and all 13 treated cows were observed in estrus by 60 d (Table 5). Two control cows were not observed in heat within the 60 d time period. Average days to estrus were not significantly different: control cows were 19.2 ± 3.4 d vs. 27.3 ± 8.7 d for treated cows.

On d 1, 11 of 25 total control cows across replicates and 17 of 26 total treated cows across replicates had milk P4 under 2ng/ml. Therefore 14 of 25 (56%) control cows but only 9 of 26 (35%) treated cows across replicates had milk P4 above 2ng/ml (Table 4). For cows with either low (P4 < 2ng/ml) or high (P4 > 2ng/ml) progesterone values on d1, there were no significant differences in estrous responses within 25 d. However, the interaction between treatment and progesterone level approached significance for estrous responses ($P = 0.06$). Control cows with lower milk progesterone values on d 1 were more likely (81% Control vs. 65% Heat Seek) to have been observed in estrus by 25 d whereas treated cows with higher milk progesterone on d 1 were more likely (78% Heat Seek vs. 43% Control) to have been observed in estrus by d 25 (Table 4).

Fertility data in Table 5 exhibits the proportion of those cows that were monitored for estrus for this project and conceived to their first service. In Rep 1, 3 out of the 9 control cows observed in estrus and subsequently bred conceived to a first service within 60 d. Six

out of 11 treated cows in this replicate, which were observed in estrus and bred, were later

confirmed pregnant. In Rep 2, 6 out of 12 control cows were confirmed pregnant and 9 of 13

cows treated cows conceived to their first service within 60 d (Table 5). Combined, there

were 9 of 21 (43%) control cows and 15 of 24 (62.5%) of cows receiving Heat Seek that

conceived to a first service within 60 d after start of treatment.

DISCUSSION

In this experiment, cows were given a daily bolus capsule of the botanical estrogenic

product Heat Seek, for 6 consecutive days to determine if the phytoestrogens present in the

product could elicit a reproductive response, compared to negative control cows that received

no treatment. Presumably cows that were at or near the mid-luteal stages of the estrous cycle

would exhibit luteolytic responses as a result of the exogenous estrogenic treatment.

Additionally, milk samples collected during 6 d of treatment were assayed for progesterone

content. Observation expected in response to estrogens present in the Heat Seek capsules

included decline in milk progesterone concentrations during the sampling process, indicative

of luteolysis with a subsequent estrus thereafter.

Progesterone concentration between treated and untreated cows did not differ among

treatments in either trial. This was observed however in research by Piotrowska et al. (2005).

Heifers in their research received a standard or a 2.5kg/d soy diet. By d 12 of the estrous

cycle, those fed the soy diet exhibited lower plasma levels of progesterone compared to the

standard diet heifers. Heifers fed the soy diet also had higher concentrations of equol and

para-ethyl-phenol (phytoestrogenic metabolites) in their plasma than heifers fed the standard

diet (undetectable) (Piotrowska et al., 2005). Although the presence of phytoestrogenic

metabolites was not quantified in the animals used in this experiment as the previously

mentioned study, the estrogenicity of the capsules was assayed. Though there were

significant estrogen equivalents present in the Heat Seek capsules, it was not sufficient

enough to elicit a reproductive response in the cows used in this study.

Bioassays conducted on the samples of the Heat Seek capsules indicated that the

typical amounts of estrogens present in the product are much lower than what research has

indicated to be a sufficient amount to induce a reproductive effect. Stob et al. (1957) found

differences in uterine weights in mice fed dried alfalfa extracts of 56 different varieties of

plants than those fed a control ration.   In addition, differences in estrogenicity among

varieties may have been due to resistance to disease, growth habits, and chemical

composition. Uterine weights ranged from 1.60 to 82.96 mg. However, mice fed

diethylstilbestrol ranged from 20.92 to 148.46 mg meaning the estrogenic compounds present

in alfalfa do not necessarily have the same degree of oral potency as diethylstilbestrol (Stob

et al., 1957).

Attainment of subsequent estrus among treated and untreated groups was variable

between the two replicates. Because all cows in both experiments were not synchronized

before the start of the experiment, therefore timing of treatment and day of cycle may have

been variable among cows. Responses seen in the treatment groups may have been due to

natural late-luteal CL regression and decrease in progesterone leading to a subsequent estrus.

On the other hand, lack of response may have signified a cow was either at the early stage of

the estrous cycle when the CL is not responsive to $PGF_2\alpha$. Additionally, because the

majority of cows were still considered post-partum, cyclicity status may have been

responsible for the lack of sensitivity to treatment these cows displayed in both experiments. There were cows with low progesterone values during the duration of the experiment (P4 <2ng/ml) and therefore may have been anestrual.

When treatment and progesterone values were compared to estrous responses within 25 d, no significant differences were observed however there was a moderate interaction between treatment and progesterone concentration. Based on the statistical analysis, it is not certain why those with lower progesterone at the start of the experiment (P4 < 2ng/ml) were more likely to have been observed in estrus. It was believed that those with higher progesterone at the beginning of the experiment were cyclic and would have responded to the estrogens present in the Heat Seek capsules. The estrogenicity in the capsules may have been one explanation on these observed responses. In addition, the cyclicity of the cows in relation to their progesterone values during the experiment may also have reduced their responses. More control cows also had higher milk P4 at the start of the experiment than did treated cows, which was reflected when observing the effects of both treatment and d 1 progesterone on estrous response data.

Lastly, maturity and preservation methods comprising the ingredients found in each Heat Seek capsule were unknown. Because the ingredients are not common feedstuffs, it is not known if estrogenicity could be increased by harvesting at different stages of growth or long-term preservation such as fermentation or dehydration, which is known to increase estrogenicity in common forages (Stob et al., 1958). In addition, different species of the plant ingredients may also alter the estrogenic content of the capsules. The estrogenic activity of forages tends to increase during fermentation either in the silo or in the rumen;

however, there are significant differences between plant samples (Pieterse and Andrews, 1956). On the other hand, dehydration may lower estrogenic activity of forage (Bickoff et al., 1959). Other factors affecting the estrogenic content of plant material includes if the plant is suffering from a foliar disease, fertilizer deficiency, or fungal pathogen (Adams, 1995).

The current study used a botanical estrogenic product containing known amounts of estrogenic equivalents, however, failed to elicit a response either in advancing luteal regression or in initiating earlier onset of estrus compared to untreated cows.

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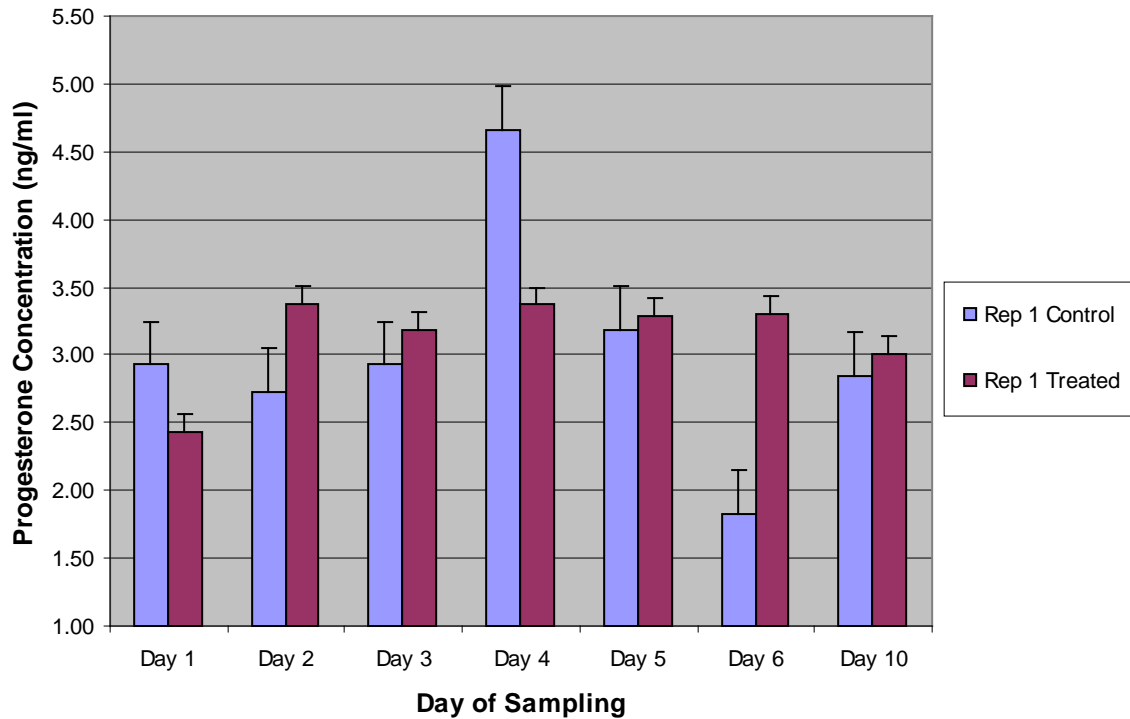**Replicate 1: Average Progesterone Levels Between Groups**

Figure 3. Average progesterone levels per day (ng/ml) of both treatment groups during treatment in Rep 1. There is variability in progesterone concentrations between both treatment groups as observations were subject to the exact date of cycle and treatment for cows. Trends in the control group show a sharp increase in milk progesterone on d 4 with a moderate decline through the post-treatment sampling.

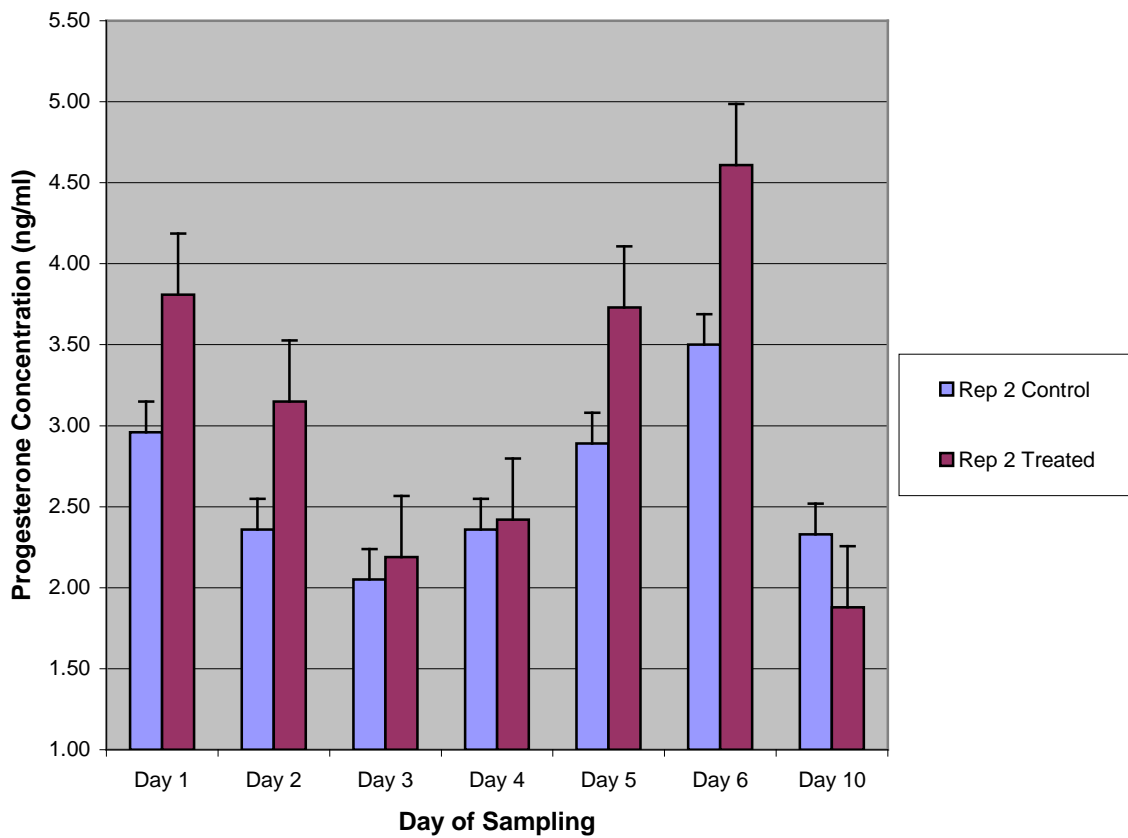## Replicate 2: Average Progesterone Levels Between Groups

Figure 4. Average progesterone levels per day (ng/ml) of both treatment groups during treatment in Rep 2. There is variability in progesterone concentrations between both treatment groups as observations were subject to the exact timing cycle and treatment for all cows. Both treatment groups show a decline in milk progesterone through d 4 followed by increases on d 5 and d 6. Subsequent post-treatment milk progesterone samplings declined for both groups.

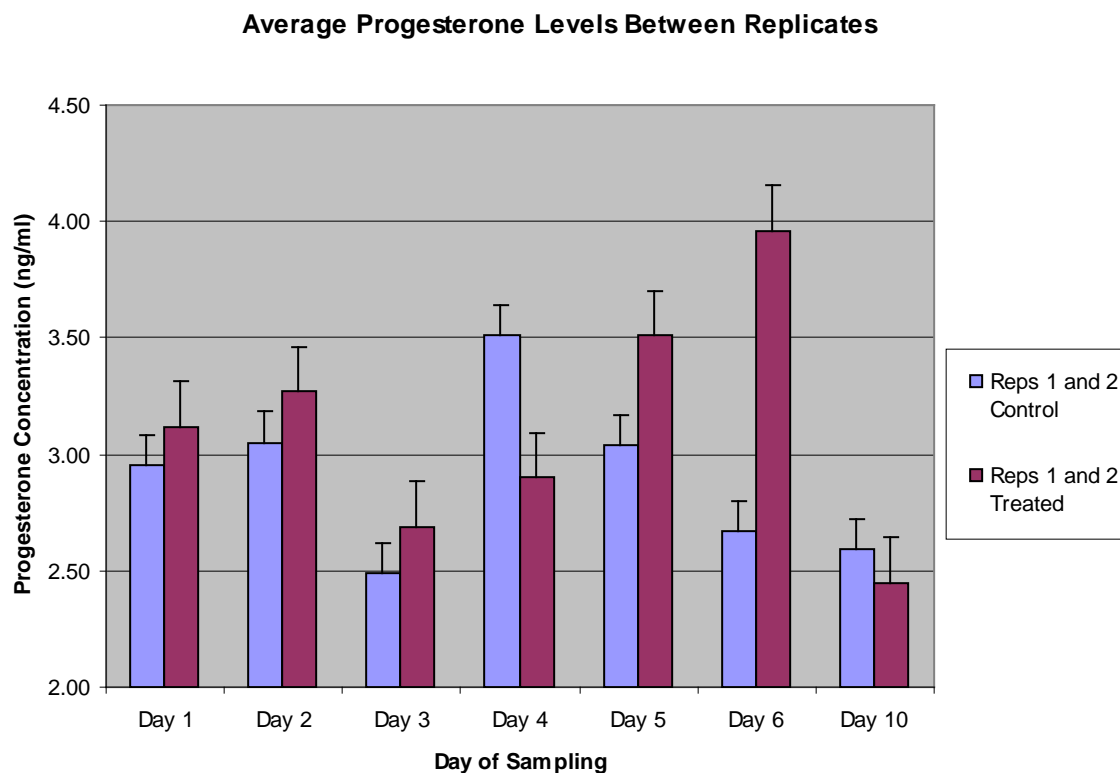**Average Progesterone Levels Between Replicates**

Figure 5. Average progesterone levels per day (ng/ml) of both treatment groups during treatment in Replicates 1 and 2. Rep 1 and 2 control cows displayed variability in their respective progesterone levels during the sampling process followed by a steady decrease by d 5 through the post-treatment samplings. Rep 1 and 2 treated cows had a slight decrease in milk progesterone through d 3 followed by an increase through d 6. Post-treatment samples were lower, similar to the control groups.

Table 4. LSMeans for effect of treatment and d 1 P4[1] on estrous responses.[2]

Treatment	Control	Control	Heat Seek	Heat Seek
P4 Value on Day 1	< 2.0 ng/ml (n = 11)	> 2.0 ng/ml (n = 14)	< 2.0 ng/ml (n = 17)	> 2.0 ng/ml (n = 9)
Estrous Response Mean	0.81 ± 0.14	0.43 ± 0.13	0.65 ± 0.11	0.78 ± 0.16

1. Effect of treatment on estrous responses ($P = 0.52$) was not significant.
2. There was a tendency towards a significant interaction between treatment and P4 on d 1 ($P = 0.06$).
3. *P*-values are included for P4 concentration means among groups. Data are pooled from both reps.

Table 5. Estrous responses between treatment groups for Replicates 1 and 2. [1,2]

Treatment Group	Rep 1 Control	Rep 2 Control	Control Total	Rep 1 Heat Seek	Rep 2 Heat Seek	Heat Seek Total
Proportion in Estrus in 25 Days	6 of 11 (55%)	9 of 14 (64%)	15 of 25 (60%)	7 of 13 (54%)	8 of 13 (62%)	15 of 26 (58%)
Proportion in Estrus in 60 Days	9 of 11 (82%)	12 of 14 (86%)	21 of 25 (84%)	12 of 13 (92%)	13 of 13 (100%)	25 of 26 (96%)
Average Days to Estrus[3]	24.0 ± 10.4	19.2 ± 3.4	21.4 ± 7.3	24.8 ± 9.2	27.3 ± 8.7	26.0 ± 8.8
Proportion Conceived to First Service (w/in 25 d)	2 of 6 (33%)	6 of 8 (75%)	8 of 14 (57%)	5 of 7 (71%)	4 of 8 (50%)	9 of 15 (60%)
Proportion Conceived to First Service (w/in 60 d)	3 of 9 (33%)	6 of 12 (50%)	9 of 21 (43%)	6 of 11 (55%)	9 of 13 (69%)	15 of 24 (63%)
Proportion Conceived (w/in 60 d)	9 of 11 (82%)	9 of 14 (64%)	18 of 25 (72%)	11 of 13 (85%)	12 of 13 (92%)	23 of 26 (88%)

1. Treatments were administered at random stages of the estrous cycle so responses were expected to be variable.
2. Ovarian structures were examined in Rep 1 on d 1 (Control CL diameter: 2.0 ± 0.7 cm, n = 3 of 11; Treated CL diameter: 1.9 ± 0.3 cm, n = 8 of 13) and on d 5 (Control CL diameter: 1.8 ± 0.3 cm, n = 6 of 11; Treated CL diameter: 1.3 ± 0.1 cm, n = 7 of 13).
3. Notes average days until first observed estrus after treatment (d 1).

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CHAPTER 4

CONCLUSIONS

Effects on reproductive traits including serum progesterone, ovarian luteal regression, and estrus monitoring were observed as a response of treatment with oral colostrum in cyclic heifers. From these data, the use of 1 or 2 L of colostrum did not effect luteal regression and advance onset of estrous behavior, unlike those treated with $PGF_2\alpha$. Luteal regression was evident in those heifers given $PGF_2\alpha$ while those given colostrum were not affected by treatment. Comparisons of progesterone concentrations showed significant differences between the $PGF_2\alpha$ treated and all other groups as well as the negative control group and 1 L and 2 L groups on d 1. Comparisons between the $PGF_2\alpha$ group and others also showed significance on d 4 of the experiment. Positive control heifers treated with $PGF_2\alpha$ showed the fewest days to new estrus followed by negative control heifers, with the colostrum treated groups thereafter.

Similar reproductive traits were observed in a second study using the botanical estrogenic compound Heat Seek on cows in a two-replicate study. There were no significant differences observed between the control and treated groups regarding milk progesterone data. Estrous responses across both replicates also yielded little significance during the 25 and 60 d monitoring period. When treatment and progesterone values were compared to estrous responses within 25 d, no significant differences were observed. However, there was a moderate interaction between treatment and progesterone concentration on attainment of estrus within 25 d. Based on the statistical analysis, it is not certain why those with lower progesterone at the start of the experiment were more likely to have been observed in estrus, as it was believed that those with higher progesterone at the beginning of the experiment

were cyclic and would have responded to the estrogens present in the Heat Seek capsules. Lack of responses seen during the experiment may have been a result of day of cycle and treatment. If cows were not mid-luteal they would not have been at a proper stage to respond to the estrogens present in the capsules. In addition, those with low progesterone values during the experiment may have not been cyclic (P4 <2ng/ml) or were late luteal and regressing their CL, therefore causing them to not respond to the phytoestrogenic treatment.

Although $PGF_2\alpha$ content in the colostrum sample was not quantified, the content of estrogens present in the sample seemed to be insufficient in inducing a reproductive response. There were higher concentrations of estrogens present in the Heat Seek capsules, however they too were unable to elicit any responses. These outcomes however, may have been due to the method of estrogen extractions, sensitivity of the Yeast Estrogen Screen bioassay, or preservation of the colostrum and Heat Seek samples.

Lastly, both colostrum and Heat Seek projects did not show any negative effects in regards to health and fertility measures. Cows that were inseminated prior to the start of the Heat Seek replicates were able to conceive after being given the capsules, regardless of the estrogens present in the capsules. In addition, conception did not seem to be harmfully effected by Heat Seek treatments for the cows monitored during this study.