AN ABSTRACT OF THE THESIS OF

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Title: LACTATIONAL RESPONSE OF HOLSTEIN COWS TO ORAL ADMINISTRATION OF A SYNTHETIC GLUCOCORTICOID

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Two experiments involving 33 Holstein cows were conducted for the primary purpose of studying the effects of flumethasone (a synthetic glucocorticoid) on milk and milk constituent yields. The secondary purpose was to compare udder health, reproductive performance, and body weight changes of cows on flumethasone therapy.

Nine Holstein cows were assigned randomly to three treatments in Experiment I and received .00, .01, and .05 mg. of flumethasone daily in treatment groups I, II, and III, respectively. All cows were fed 10 to 12 pounds of alfalfa hay daily, corn silage ad libitum, and one pound of concentrates for every three pounds of milk produced per day, throughout the 120 day experiment.

Average daily milk yield, mature equivalent (ME) milk, 4% fat corrected milk (FCM), and ME, 4% FCM were significantly less ($P<.05$) for treatment III than treatment I. Cows on treatment II

produced more milk than cows on treatment I. However, the differences were not statistically significant.

Experiment II involved 24 Holstein cows initiating their second or third lactations which were allotted randomly to four treatments. Cows on treatments I, II, III, and IV received .00, .005, .01 and .02 mg. of flumethasone, respectively, each day. Flumethasone therapy was initiated four days post partum and continued until milk secretion ceased or the completion of a 305 day lactation. Cows in this experiment received the same ration as cows in Experiment I.

Milk, butterfat, and solids-not-fat (SNF) were significantly lower ($P < .05$) for treatment IV than treatment II. Controls were intermediate. During the first 70 days of lactation cows on .005 mg. of flumethasone produced significantly more ($P < .05$) butterfat (23.9%) and SNF (18.39%) than controls and cows receiving .01 mg. of flumethasone produced significantly more ($P < .05$) butterfat (21.04%) than controls. There were no significant differences between treatment means during the second 70 days of lactation. Cows receiving .01 mg. of flumethasone produced significantly more ($P < .05$) milk, butterfat, and SNF between 141 and 210 days of lactation than cows receiving .02 mg. of flumethasone.

There were no differences between treatments for measures of udder health, reproductive performance, and body weight changes. However, there was a significant difference ($P < .05$) in length of

lactation. Cows receiving .02 mg. of flumethasone had considerably shorter lactations (31 to 43 days).

These results indicate that .05 mg. of flumethasone has an inhibitory effect on lactation. Although .02 mg. of flumethasone had an initial stimulatory effect, there was an inhibitory effect on milk secretion and synthesis of butterfat and SNF later in lactation. These data suggest that .005 and .01 mg. of flumethasone may stimulate lactation in dairy cattle.

Lactational Response of Holstein Cows to Oral Administration of a Synthetic Glucocorticoid

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INTRODUCTION

Man has attempted to increase milk production by altering the genetic makeup, nutrition, management, and other factors in dairy cattle that directly influence milk yield.

Dairy cattle have been selected for high milk yields for decades. However, selection is a relatively slow means of increasing milk yield because of its moderate heritability (25%) and the long generation interval (4.5 years). Maximum progress due to selection has been shown to be a little more than 2% per year.

Economic pressures have forced dairy farmers to adopt more efficient management techniques than were possible a few years ago. This, coupled with improved nutrition has resulted in tremendous increases in lactation averages. Currently in the U.S. there are few dairy herds subjected to substandard nutrition or management.

Efficiency of milk production varies considerably between and within breeds. This is probably due to inherited differences in the activity of the endocrine glands. Some researchers have theorized that mild hypothyroidism was responsible for low production and that supplemental thyroxine may augment milk yield. Several experiments cited by Thomas, Moore, and Sykes (1949) showed that

supplemental thyroprotein resulted in a transient increase in milk secretion which was accompanied by a substantial reduction in body weight. After the initial increase in milk yield, production of all treated cows dropped below normal even after thyroprotein withdrawal. The effects of feeding supplemental thyroprotein to dairy cows during successive lactations have been investigated by Thomas and Moore (1953). They observed an immediate increase in milk yield during the first lactation that was sustained only if the total disgestible nutrients (TDN) of the diet were increased to 125% of Morrison's requirements. However, when the TDN of the control diet was increased to 125% of Morrison's requirements the control cows lactated at the same level as the cows on supplemental thyroprotein. Thyroprotein during successive lactations resulted in reduced efficiency of both milk production and feed conversion.

Cortisone has been shown to stimulate increased milk secretion in parturient rats (Hahn and Turner, 1966). Other researchers (Turkington, Juergens, and Topper, 1965) have shown that corticoids stimulate synthesis of casein phosphoproteins. However, no work has been reported on the effects of corticoids on yield or composition of milk from cows.

The primary purpose of the present study was to determine the effects of flumethasone, a synthetic glucocorticoid, on yield and composition of cows milk. A secondary purpose was to

compare udder health, reproductive performance, and body weight of cows on flumethasone therapy.

Economic returns could be increased from the development of a lactational stimulant. This is particularly true if the stimulant does not have detrimental effects on feed efficiency or other physiological processes. Such increases in milk yield would enable the dairy farmer to maintain his quota with fewer cows. An exogenous stimulant may have a greater effect on low producers and might place borderline cows in the profitable category.

REVIEW OF LITERATURE

Growth of the mammary gland and its subsequent secretory activity are known to be under hormonal control (Sud, Tucker, and Meites, 1968; Williams and Turner, 1961; Cowie et al., 1952; Lyons, Li, and Johnson, 1958; Anderson and Turner, 1962, 1963). Much of our present knowledge concerning the functional activity of the mammary gland has stemmed from research with laboratory animals. The most common approaches to the study of various hormonal effects on mammary gland development and milk secretion in laboratory species are gland removal and gland removal and hormone replacement.

Developmental and functional activities of the mammary gland are controlled by hormones secreted by the pituitary (Griffith and Turner, 1959; Grosvenor and Turner, 1959a and 1959b), thyroid (Grosvenor and Turner, 1959c; Griffith and Turner, 1961; Jacobsohn, 1960), ovaries (Day and Hammond, 1945; Cowie et al., 1952; Turner, Yamomoto, and Ruppert, 1957; and Williams and Turner, 1961); and adrenals (Anderson and Turner, 1962; Anderson and Turner, 1963; Hahn and Turner, 1966). The actions of some of the hormones influencing mammogenesis or lactogenesis are not completely understood. This review will be limited to the effects of adrenal cortex hormones and their interactions on the lactational

processes.

Effects of Adrenal Corticoids on Mammary Gland Development and Milk Secretion

Gland removal or gland removal and hormone replacement are the common methods of assessing the role of the various hormones on milk synthesis. This is also true for the hormones of the adrenal cortex. In a study by Cowie and Folley (1944a) there was no change in mammary gland structure but there was a decrease in gland area following adrenalectomy in gonadectomized rats. However, these same authors (1947c) in a similar study, observed a slight structural regression in the mammary gland following adrenalectomy.

Evidence of the need for adrenocorticoids in normal mammary gland growth was presented by Lyons, Li, and Johnson (1958). They found that deoxycorticosterone at a level of 100 mcg. per day synergized with growth hormone and estrone in the hypophysectomized, ovariectomized, adrenalectomized rat and produced growth of the mammary gland comparable to that in the intact animals. Cowie and Lyons (1959) observed a similar synergistic action of adrenocorticoids with growth hormone. Anderson and Turner (1963) attempted to determine the effect of corticoid replacement therapy in adrenalectomized-ovariectomized rats on mammary growth by measuring the glandular deoxyribonucleic acid (DNA). They

observed that corticoid replacement in the absence of gonadal hormone replacement had no effect on mammary growth according to the DNA content of the mammary glands. However, injections of estradiol benzoate and progesterone plus 100 mcg. of 1, 2 dehydroxycortisone or 100 or 200 mcg. of hydrocortisone acetate per day increased the DNA content of the mammary gland significantly above that of adrenalectomized-ovariectomized rats receiving estradiol benzoate and progesterone. Also, animals receiving 1.0 mg. of hydrocortisone acetate or 250 mcg. of deoxycorticosterone acetate per day plus estradiol benzoate and progesterone had levels of DNA similar to the adrenalectomized-ovariectomized group on estradiol benzoate and progesterone. The authors felt that this lack of response was due to the low level of deoxycorticosterone acetate replacement. Anderson and Turner (1962) using higher levels of deoxycorticosterone acetate reported a response. Kumeresan, Anderson, and Turner (1967) found that cortisone produced a significant increase in mammary gland DNA content in mature, intact, pregnant rats.

Knowing that adrenocorticoids stimulate growth of the mammary gland it seems reasonable to assume that they would also affect the rate of milk synthesis. Gaunt, Eversole, and Kendall (1942) showed that 1.0 mg. of 17-hydroxy-11-dehydrocorticosterone in the drinking water maintained lactation at approximately 80% of

the normal level in adrenalectomized rats. On the basis of standardized litter weight change, 0.5 mg. produced a less dramatic beneficial effect. They also reported a beneficial effect when adrenalectomized female rats were given graded doses of deoxycorticosterone acetate (0.1, 1.0, and 2.0 mg.). However, the results within graded dosages were quite variable. Their results differed somewhat from the findings of a similar study by Cowie and Folley (1944a). The latter authors found that lactation was partially maintained in adrenalectomized rats by deoxycorticosterone acetate, 17-hydroxy-11-dehydrocorticosterone and adrenal cortex extract. Their results indicated that 1.0 mg. deoxycorticosterone acetate was more effective than 1.0 or 0.5 mg. of 17-hydroxy-11-dehycrocorticosterone or 1.0 or 2.0 ml. of adrenal cortex extract. They also noted that 1.0 mg. of 11-dehydrocorticosterone had deleterious effects on lactational performance. In a follow-up study, Cowie and Folley (1947) injected 0.1, 1.0 or 3.0 mg. of deoxycorticosterone acetate subcutaneously into female rats adrenalectomized four days post partum and observed that 3.0 mg. daily restored lactation to the control level. On a log dose basis, smaller dosages showed a linear relationship in the degree of restored lactation. Contrary to the results of their previous study (1944a) Cowie and Folley (1947b) found that daily subcutaneous injections of 0.5 mg. of 11-dehydrocorticosterone

gave a positive lactational response in adrenalectomized rats. In another experiment, Cowie (1952) attempted to determine the effects of dietary sodium on the replacement value of adrenal cortex steroids and synergistic effects of adrenocortical steroids in lactating adrenalectomized rats. He found that high dietary sodium had no significant effects on lactational response to corticoid replacement. His data also showed that lactation was partially restored when daily injections of 0.56 mg. of deoxycorticosterone acetate or 0.5 mg. of 11-dehydrocorticosterone were administered to adrenalectomized rats. This is in agreement with earlier work by Cowie and Folley (1944a, 1947a and 1947b). It was further observed (Cowie, 1952), that one or three 15 mg. implants of cortisone stimulated lactational response, and synergistic effects of two 11 mg. implants of cortisone with one 50 mg. implant of dehydroxycorticosterone acetate restored normal lactation. However, he was unable to show any significant beneficial effects on lactation due to daily injections of 0.56 mg. of 11-dehydrocorticosterone acetate in adrenalectomized rats.

Wettstein and Anner (1954) observed that aldosterone exerts considerable cortisone and hydrocortisone-like activity. Based upon this observation, Cowie and Tindell (1955) decided to test the effects of aldosterone on lactation in adrenalectomized rats. They also tested the effects of 9α-halo derivatives of hydrocortisone on

lactation since Borman, Singer, and Numerof (1954) and Leatham and Wolf (1954) had observed that certain 9α-halo derivatives of hydrocortisone were more effective in maintaining life after adrenalectomy than deoxycorticosterone acetate. Results of their experiment showed that, on the basis of litter weight, 100 mcg. of chlorohydrocortisone acetate maintained lactation at the control level. Also, daily injections of 1.0 mg. of hydrocortisone, 100 mcg. of fluorohydrocortisone acetate, and 50 mcg. of aldosterone each partially maintained lactation to the same degree. Weight gains were not significantly different. Kon and Cowie (1961) showed that 200 mcg. of 9α-fluorocortisone per day maintained normal lactation in rats adrenalectomized on the fourth day of lactation.

Previous work by Cowie (1952) showed that implants of cortisone and dehydrocorticosterone acted synergistically to maintain normal lactation in adrenalectomized rats. Anderson and Turner (1962) studied the synergistic effects of injections of hydrocortisone acetate and deoxycorticosterone acetate on lactational response. They found that combinations of 100 mcg. of hydrocortisone acetate and 50 mcg. of deoxycorticosterone acetate supported low levels of lactation. Also, 250 mcg. of hydrocortisone acetate and 100 mcg. deoxycorticosterone acetate improved lactation but total yield was significantly lower than that of controls. When 500 mcg. of hydrocortisone acetate and 250 mcg. of deoxycorticosterone acetate

were injected daily, normal lactations were maintained. Two treatments produced marked body weight changes. Lactating females receiving 250 mcg. of deoxycorticosterone acetate gained 2.9 gm. daily while those on 500 mcg. of hydrocortisone acetate lost 3.8 gm. per day. Based on the observation that these hormones used alone were unable to promote normal lactation they suggested that 250 to 500 mcg. of hydrocortisone acetate or an equivalent in glucocorticoid activity and 250 mcg. of deoxycorticosterone acetate or an equivalent of mineralocorticoid activity are required for maintenance of normal lactation in adrenalectomized rats.

In view of the findings of Peron (1960) that the two predominant secretions of the rat adrenal gland were aldosterone and corticosterone, Anderson and Turner (1963) tested the effects of these hormones on maintenance of lactation in adrenalectomized rats. Their data showed that 25 mcg. of aldosterone-21-acetate plus 1.0 mg. corticosterone daily maintained lactation at approximately 80% of the normal level. Each of the two hormones injected singly into adrenalectomized rats prompted a somewhat lesser lactational stimulus.

Due to the finding of Anderson and Turner (1963) that corticosterone and aldosterone in combination would partially maintain lactation in adrenalectomized rats, Hahn and Turner (1966) decided to test the effects of these two hormones singly and

in combination in intact rats. Milk yield was measured by taking the difference between pre- and post-nursing litter weights after a 10 hour separation of the pups from their dams. Their results showed no beneficial effects due to .75 mg. of cortisone but 1.0 mg. elicited a 12 to 27% increase in milk yield between the 14th and 20th day of lactation. The increase due to daily injections of 1.25 mg. of corticosterone was less pronounced than injections of 1.0 mg. of corticosterone. The injection of 25 mcg. of aldosterone failed to increase milk yield. The combination of 25 mcg. of aldosterone and 1 mg. of corticosterone increased milk yield 34.8, 24.0, 12.6 and 12.7% on days 14, 16, 18, and 20 of lactation, respectively. However, the combination of 25 mcg. of aldosterone and 1.0 mg. of corticosterone compared to 1.0 mg. of corticosterone alone showed no significant difference. This led the authors to the assumption that increased milk yield was primarily due to corticosterone.

Effects of Adrenal Hormones on Milk Composition

Effects of various hormones upon milk composition are not fully understood. Campbell et al. (1964) tested the influence of corticoids on milk composition by injecting adrenocorticotropic hormone (ACTH) into dairy cows. They found that injections of 200, 300, and 400 international units (I.U.) of long-acting ACTH

caused a reduction in yields of milk, butterfat, and solids-not-fat (SNF). However, because of the negative correlation between milk yield and the percentages of milk constituents they observed an increase in percentages of butterfat and SNF.

More recent studies (Elias and Rivera, 1959; Elias, 1959; Rivera and Bern, 1961; Juergens et al., 1965; Turkington, Juergens, and Topper, 1965; and Turkington and Topper, 1966) on the effects of corticoids on milk composition have been performed with mouse mammary gland explants. Mammary gland explants were induced to synthesize milk (Elias and Rivera, 1959; Elias, 1959; and Rivera and Bern, 1961) when maintained on synthetic medium 199 (developed by Morgan, Morton, and Parker, 1950) when insulin, cortisone, and prolactin were added to the medium. It has been established (Rivera and Bern, 1961) that alveolar breakdown occurs when synthetic medium 199 is not fortified with insulin or cortisone. Juergens et al., (1965) investigated the effects of insulin, hydrocortisone, and prolactin on the secretory activity of mouse mammary gland explants. At a concentration of 5.0 mcg. per ml. of medium, any of the three hormones alone or in pairs elicited minimal or no stimulation of casein phosphoprotein synthesis. Based on ^{32}P incorporation into the casein phosphoproteins, a combination of all three hormones effected a phosphoprotein synthesis several times greater than the initial rate. This result

was confirmed by Turkington, Juergens, and Topper (1965). In their experiment a combination of insulin, hydrocortisone, and prolactin resulted in a three to six-fold augmentation in the rate of casein phosphoprotein synthesis. When Turkington and Topper (1966) added ovine pituitary prolactin or human placental lactogen along with hydrocortisone and insulin to medium 199, biosynthesis of ^{32}P labeled casein was stimulated in mouse mammary gland explants. Further observations revealed that the stimulation involved augmentation of all the casein components.

At least one adrenal hormone, hydrocortisone, is required for casein synthesis. Additional experimentation could disclose a role of other adrenocorticoids in milk protein synthesis.

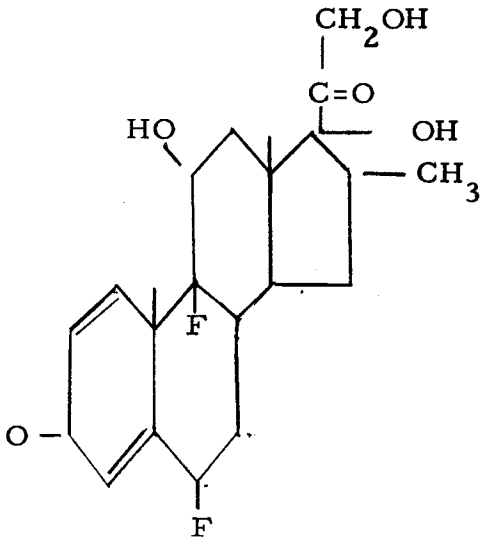
Effects of 40 I. U. of prolactin and 5.0 mg. of cortisol acetate on epimerase and pyrophosphorylase, the enzymes responsible for lactose synthesis, were studied by Heitzman (1967) in psuedo-pregnant rabbits. His investigation disclosed a three-fold and a four to six-fold increase in activity of these two enzymes after three and five daily injections, respectively. Further research on the effects of adrenal cortex hormones on lactose synthesis is needed.

As in the case of lactose, very little experimental work has been reported on the influence of corticoids on butterfat and protein yields. Campbell et al. (1964) found that 200, 300, and 400 I. U. of

a long-acting ACTH preparation injected into dairy cows decreased milk fat yield. From Campbell et al. (1964) we cannot conclude that all of the adrenal steroids depress milk fat yield because ACTH stimulates all of the corticoids and some corticoids could stimulate a response which is antagonistic to the response stimulated by others. Apart from the work of Campbell et al. (1964), the best indication of the effects of adrenocorticoids on butterfat yield is the correlation which is known to exist between butterfat and SNF yields. Work at the Virginia Station (Loganathan and Thompson, 1967) showed that there is a positive correlation of .87 between butterfat yield and SNF yield. A subsequent study (Thompson and Loganathan, 1968) revealed a .85 correlation between the yields of butterfat and SNF. Juergens et al. (1965), Turkington, Juergens, and Topper (1965) and Turkington and Topper (1966) have established that hydrocortisone stimulates casein synthesis. Since casein is a major component of milk protein and milk protein is a large portion of SNF, it is possible that the high correlation between butterfat and SNF is due to adrenocorticoids. More experimentation needs to be conducted to establish the role of adrenocorticoids in the synthesis of butterfat, protein, and other major milk components.

METHODS AND MATERIALS

Thirty-three Holstein cows from the Oregon State University herd were used in two experiments to determine the effects of oral administration of flumethasone on lactation. Flumethasone, a chemical modification of prednisolone occurs as a white to creamy white, ororless, crystalline powder. Chemically it is a 6α, 9α-difloro-16α methylprednisolone. Its chemical structure is given below.



Experiment I was a randomized block design (see Table 1) involving nine Holstein cows, six in their first and three in their second lactations. Cows were divided into three groups of three each according to freshening date, level of milk yield, and lactation number prior to being assigned randomly to the three treatments. Age at calving and days post partum at the onset of flumethasone therapy were analyzed to determine whether or not

there were any biases in the pre-treatment data. Treatment I, the control group, received one ounce of propylene glycol and treatments II and III received .01 and .05 mg. flumethasone, respectively, dissolved in one ounce of propylene glycol. The appropriate solution was administered daily on pelleted concentrates fed individually during the afternoon milking.

Oral administration of flumethasone was initiated 60 or more days (average of 100 days) post partum for all treatment groups. The cows were maintained in one group and fed 10 to 12 pounds of alfalfa hay daily and corn silage ad libitum. Pelleted concentrates [14% crude protein (CP) and 74% total digestible nutrients (TDN)] were fed at the rate of one pound for every three pounds of milk produced.

Milk weights were recorded twice daily during the 120 day experiment. Milk production was adjusted to a mature equivalent (ME) basis using the current USDA factors (McDaniel et al., 1967). In addition, milk production was adjusted to four percent fat corrected milk (4% FCM) and ME, 4% FCM. Actual milk yields and adjusted milk yields (ME, 4% FCM, and ME, 4% FCM) were analyzed by analysis of variance (Snedecor, 1962). All cows were observed closely for abnormal treatment effects.

Table 1. Design of Experiment I.

Producing ability	Daily levels of flumethasone		
	.00 mg. (Cow No.)	.01 mg. (Cow No.)	.05 mg. (Cow No.)
High	854	801	849
Medium	863	861	823
Low	847	856	818

The second experiment was also a randomized block experiment (see Table 2) involving 16 cows initiating their second and 8 cows initiating their third lactation.

Cows were divided into six groups of four each according to lactation number, milk merit index (McGilliard, 1962) and expected freshening date before being assigned randomly to the four treatments. Treatment I (controls)and treatments II, III, and IV received .00, .005, .01 and .02 mg. of flumethasone, respectively, dissolved in one ounce of propylene glycol. One ounce of the appropriate solution was top dressed on a mixture of one pound of soaked beet pulp and one pound of pelleted concentrate and was fed during the afternoon milking each day.

Daily oral administration of flumethasone was initiated on the fourth day post partum and continued until milk secretion ceased or the completion of 305 days on therapy. All treatment cows were fed and managed in one group. Alfalfa hay was fed at the rate of

10 to 12 pounds per day and grass or corn silage was fed ad libitum. A pelleted concentrate (14% CP and 74% TDN) was fed at approximately one pound for every three pounds of milk produced. In the afternoon, cows were locked away from the feeding area for two and one half hours prior to milking to assure complete consumption of the top dressed concentrate. During the course of this experiment it was necessary to move the cows to three different corrals while the new dairy barn was being constructed. The effects of this should be random across all treatment groups because the cows were grouped according to freshening dates before being allotted to treatment.

Table 2. Design of Experiment II.

Producing ability	Daily levels of flumethasone			
	.00 mg. (Cow No.)	.005 mg. (Cow No.)	.01 mg. (Cow No.)	.02 mg. (Cow No.)
High	858	849	806	865
	863	864	854	870
Medium	833	838	831	813
	881	869	878	868
Low	823	835	815	826
	856	866	846	848

Milk from all cows was weighed and recorded twice daily. Actual butterfat and SNF yields were calculated on the basis of the percentages of these two components which were determined

by the Dairy Herd Improvement Association (DHIA) supervisor. Percentages of butterfat and SNF were determined by the Babcock and Golding bead (Erb and Manus, 1963) methods, respectively.

Milk yield data were adjusted for days open according to the adjustment factors of Smith and Legates (1962). The actual records for milk were further adjusted to an ME basis. Cow 881 was removed from the experiment because she failed to conceive after milking 250 days and Cow 849 became severely lame and ceased to lactate following hoof trimming. Lactation records on these two cows were projected according to the USDA factors (McDaniel, Miller and Corley, 1965) because persistency of their lactations indicated that they would normally have completed 305 day lactations. Percentages of butterfat and SNF as determined by the DHIA supervisor were used to calculate the ME, adjusted for days open, yields of butterfat and SNF.

The data on yields of milk and milk components were treated statistically by analysis of variance for a randomized block design (Snedecor, 1962). Where no significant interaction occurred, sums of squares for interaction were included in the sums of squares for error to obtain the error mean square. Duncan's new multiple range test for means (Duncan, 1955) was applied to the data when the analysis of variance indicated statistical significance. Percentage comparisons and the analysis of variance for the

randomized block were performed on data for actual milk, butterfat, and SNF yields for the first three 70-day segments of lactation in an attempt to establish whether or not flumethasone had an effect on a particular stage of lactation. Seventy-day lactation segments were used because all cows reached peak milk flow during the first 70 days. Several cows in different treatment groups ceased to lactate after 220 days post partum so the last part of the lactation was not analyzed independently.

Incidence of clinical mastitis was recorded and the California Mastitis Test (CMT) was conducted monthly. The CMT data were analyzed by analysis of variance as described previously.

Cows were closely observed at calving time and records kept on difficult calvings, incidence of parturient paresis, and ketosis as these conditions could affect the ensuing lactation. Reproductive records included age at parturition, dates of observed estrus, services to conception, abnormal estrous cycles, abnormal reproductive tracts, days open, and gestation length. Cows in this experiment were weighed four days post partum and every 30 days thereafter. Reproduction data and body weight changes were analyzed by analysis of variance.

RESULTS AND DISCUSSION

General Considerations

Age of cow at parturition has been shown (Bailey, 1952; and Sargent, Butcher, and Legates, 1967) to affect the yield of milk and milk components. As a result of these observations an analysis was carried out to determine whether there were any significant differences in age of the cows in the treatment groups in both experiments. The differences in age at freshening were not statistically significant ($P < .01$). F-ratios calculated in a one factor analysis of variance were $F = 1.006$ (2, 6, d.f.) and $F = 0.48$ (3, 20, d.f.) for Experiments I and II, respectively.

Lactation studies by Lamb and McGilliard (1960) and VanVleck and Henderson (1961) showed that cows produce heavier in the early months of lactation than in the latter part of lactation. Because of these observations cows involved in an experiment, where milk yield is the main criterion for measuring differences, should be at the same stage of lactation. One of the requirements for cows used in Experiment I was that they must have been 60 or more days post partum. The F-ratio ($F = 0.43$ with 2, 6 d.f.) resulting from a one factor analysis of variance showed that differences in the number of days post partum for controls and treatment groups were statistically insignificant ($P < .01$).

Data on effects of body weight on lactation yield (Clark and Touchberry, 1962) show that each additional 100 pounds of body weight gained prior to freshening results in an increase of 134 pounds of milk and 7.8 pounds of butterfat produced in the ensuing lactation. In second and later lactations there is a sudden loss of body weight due to the mobilization of body reserves to meet the energy requirement for milk synthesis (Miller, Hooven, and Creegan, 1969). Many dairy cows have the inherited capacity to lactate at a level beyond what their daily dietary energy intakes would maintain. Consequently, they must mobilize body reserves if a high level of production is to be maintained. The observations of Clark and Touchberry (1962) and Miller, Hooven, and Creegan (1969) show that differences in body weight at parturition can have a pronounced effect on lactation yield.

Body weight was not recorded for Experiment I. Milk production during the 120-day trial should not have been affected by weight at parturition or at initiation of the trial because all cows were past peak yield (averaged 100 days post partum) and should have been in an increasing state of flesh. A one factor analysis of variance showed that differences in initial body weight were not statistically significant ($P < .01$) among treatment groups in Experiment II ($F = 1.06$ with 3, 20 d.f.).

Milk Production

Average daily actual, 4% FCM, age adjusted milk, and age adjusted 4% FCM for Experiment I were analyzed to determine whether there were any differences between treatment groups due to daily oral administration of flumethasone. From the analysis of variance (Table 3) statistically significant differences ($P < .05$) between treatments for actual milk and 4% FCM and highly significant differences ($P < .01$) between treatments for age adjusted milk and age adjusted 4% FCM were obtained.

Data presented in Table 4 show that the means for Treatment II (0.01 mg. flumethasone daily) were higher than the means for Treatment I (controls) for all measures of milk yield. Although the differences between means were not statistically significant ($P < .05$), a larger sample size may have detected a stimulatory effect due to .01 mg. of flumethasone. Control means for average daily actual milk, age adjusted milk, and age adjusted 4% FCM were significantly higher ($P < .05$) than the means for Treatment III. Also, the yield of 4% FCM was higher for the controls than for Treatment III; however, the difference was not statistically significant. Compared to Treatment II the means for all four measures of milk yield were significantly lower ($P < .05$) for Treatment III. These results clearly indicate that daily oral

Table 3. Analyses of Mean Performance of Cows Receiving Flumethasone During Experiment I.

Item	Source of variation	Degrees of freedom	Mean squares	F-ratios	Differences between means [c] Treatment	Difference
Milk	Treatment	2	196.28	8.95[a]	Control & .01 mg.	5.20
	Block	2	457.08	20.85[b]	Control & .05 mg.	10.66[b]
	Error	4	21.92		.01 mg. & .05 mg.	15.86[b]
4% FCM	Treatment	2	147.44	7.06[a]	Control & .01 mg.	4.27
	Block	2	401.61	19.24[b]	Control & .05 mg.	9.43
	Error	4	20.87		.01 mg. & .05 mg.	13.70[b]
Milk (Age adjusted)	Treatment	2	299.16	20.10[b]	Control & .01 mg.	3.00
	Block	2	569.05	38.24[b]	Control & .05 mg.	15.60[b]
	Error	4	14.88		.01 mg. & .05 mg.	18.60[b]
4% FCM (Age adjusted)	Treatment	2	231.21	20.62[b]	Control & .01 mg.	2.07
	Block	2	507.62	45.30[b]	Control & .05 mg.	14.06[b]
	Error	4	11.21		.01 mg & .05 mg.	16.13

a/ Statistically significant ($P < .10$).
b/ Statistically significant ($P < .05$).
c/ Differences between treatment means were tested for statistical significance (Duncan, 1955).

administration of .05 mg. of flumethasone has an inhibitory effect on milk production. The data further suggest that low levels may have a stimulatory effect on the rate of milk synthesis. If daily oral administration of flumethasone does have a stimulatory effect on milk yield the optimum dose is less than .05 mg. daily.

Analysis of variance (Table 3) for all measures of milk indicated highly significant differences ($P < .01$) due to blocking. This was to be expected because the cows were grouped according to production (high, medium, and low) and then assigned randomly to treatments.

The effects of flumethasone appeared to be independent of yield because there were no significant ($P < .05$) treatment x block interactions.

Four criteria (ME milk adjusted for days open and actual milk produced during the first, second, and third 70-day segments of lactation) were used in Experiment II for determining differences between treatment groups due to daily oral administration of flumethasone. Analysis of variance (Table 4) indicated differences among treatments at the 10% level of probability for ME milk adjusted for days open and actual milk produced during the second or third 70-day segments of lactation. There were no differences between treatments for actual milk during the first 70 days of lactation.

A comparison of the means (Table 4) showed that ME milk

Table 4. Analyses of Milk Yield Data of Cows Receiving Flumethasone During Experiment II.

Item	Source of variation	Degrees of freedom	Mean squares	F-ratios	Differences between means [c] Treatment	Difference
ME milk adjusted for days open	Treatment	3	23664313.88	2.82[a]	Control & .005 mg.	2925.30
	Block	2	4988184.31		Control & .01 mg.	2348.80
	Error	18	8387231.39		Control & .02 mg.	1303.59
					.005 mg. & .01 mg.	576.50
					.005 mg. & .02 mg.	4228.80[b]
					.01 mg. & .02 mg.	3652.30
Actual milk days 1-70 of lactation	Treatment	3	791332.67	1.86	Control & .005 mg.	820.66
	Block	2	29975.80		Control & .01 mg.	703.66
	Error	18	426222.65		Control & .02 mg.	553.66
					.005 mg. & .01 mg.	117.00
					.005 mg. & .02 mg.	267.00
					.01 mg. & .02 mg.	150.00
Actual milk days 71-140 of lactation	Treatment	3	925839.82	2.65[a]	Control & .005 mg.	613.34
	Block	2	57374.04		Control & .01 mg.	767.17
	Error	18	348950.19		Control & .02 mg.	38.34
					.005 mg. & .01 mg.	153.83
					.005 mg. & .02 mg.	575.00
					.01 mg. & .02 mg.	728.83

Continued

Table 4--Continued.

Actual milk days 141-210 of lactation	Treatment	3	1251889.44	2.44	Control & .005 mg.		359.33
	Block	2	371639.62		Control & .01 mg.		663.17
	Error	18	513796.91		Control & .02 mg.		394.50
					.005 mg. & .01 mg.		303.84
					.005 mg. & .02 mg.		753.83 b/
					.01 mg. & .02 mg.		1057.67 b/

a/ Statistically significant ($P < .10$).
b/ Statistically significant ($P < .05$).
c/ Differences between treatment means tested for statistical significance (Duncan, 1955).

adjusted for days open was significantly lower ($P < .05$) for Treatment IV (.02 mg. flumethasone) than Treatment II (.005 mg. flumethasone). Means for Treatment I (controls) and Treatment III (.01 mg. flumethasone) did not differ significantly from one another or from the other two treatment means. There were no significant differences at the 5% level of probability between the means for actual milk produced during the first and second 70-day segments of lactation. However, during the second 70 days of lactation the controls and cows receiving .02 mg. levels of flumethasone produced 15.6% and 14.8% less milk, respectively, than cows on the .01 mg. level. These differences approached statistical significance ($P < .05$). Actual milk yield of cows during the third 70 days of lactation for the .02 mg. level of flumethasone was significantly lower ($P < .05$) than for cows on the .01 mg. level. No other treatment means for milk yield during this segment of lactation were significantly different.

When means for 70-day lactation segments were compared by percentage differences from controls (Fig. 1), mean milk yield for the .02 mg. level of flumethasone was 10.5% and .91% above and 12.6% below the controls for the first, second, and third 70-day lactation segments. The cows receiving the .005 mg. level produced 14.8, 12.95, and 10.2% and cows on the .01 mg. level of flumethasone produced 12.9, 15.8, and 17.3% more milk than

controls for the same three 70-day lactation segments, respectively.

F-ratios were not calculated for block effect in Experiment II because the cows were grouped according to the Milk Merit Index before being randomized to treatment. The relative efficiency of blocking (Ostle, 1966) ranges from 82 to 95% of the expected block effect in a completely randomized block design. Because of the observation that the efficiency of the inherent block was relatively low, the interaction error term was pooled with the sums of squares for error. The inefficiency of blocking was further verified by the observation that interaction means for controls did not follow the expected ranking.

The data from Table 4 and Figures 1, 2, and 3 suggest that low levels (.005 mg. and .01 mg.) of flumethasone have a stimulatory effect on milk synthesis. Although there appears to be an initial stimulation, the data indicate that .02 mg. of flumethasone has an overall inhibitory effect on milk secretion. There are no reports in the literature on the effects of corticoids on milk synthesis in the bovine. However, Hahn and Turner (1966) observed that daily injections of 1.0 mg. of corticosterone from Day 7 through Day 9 increased milk yield from 12 to 27% above controls between Days 14 and 20 of lactation in the rat.

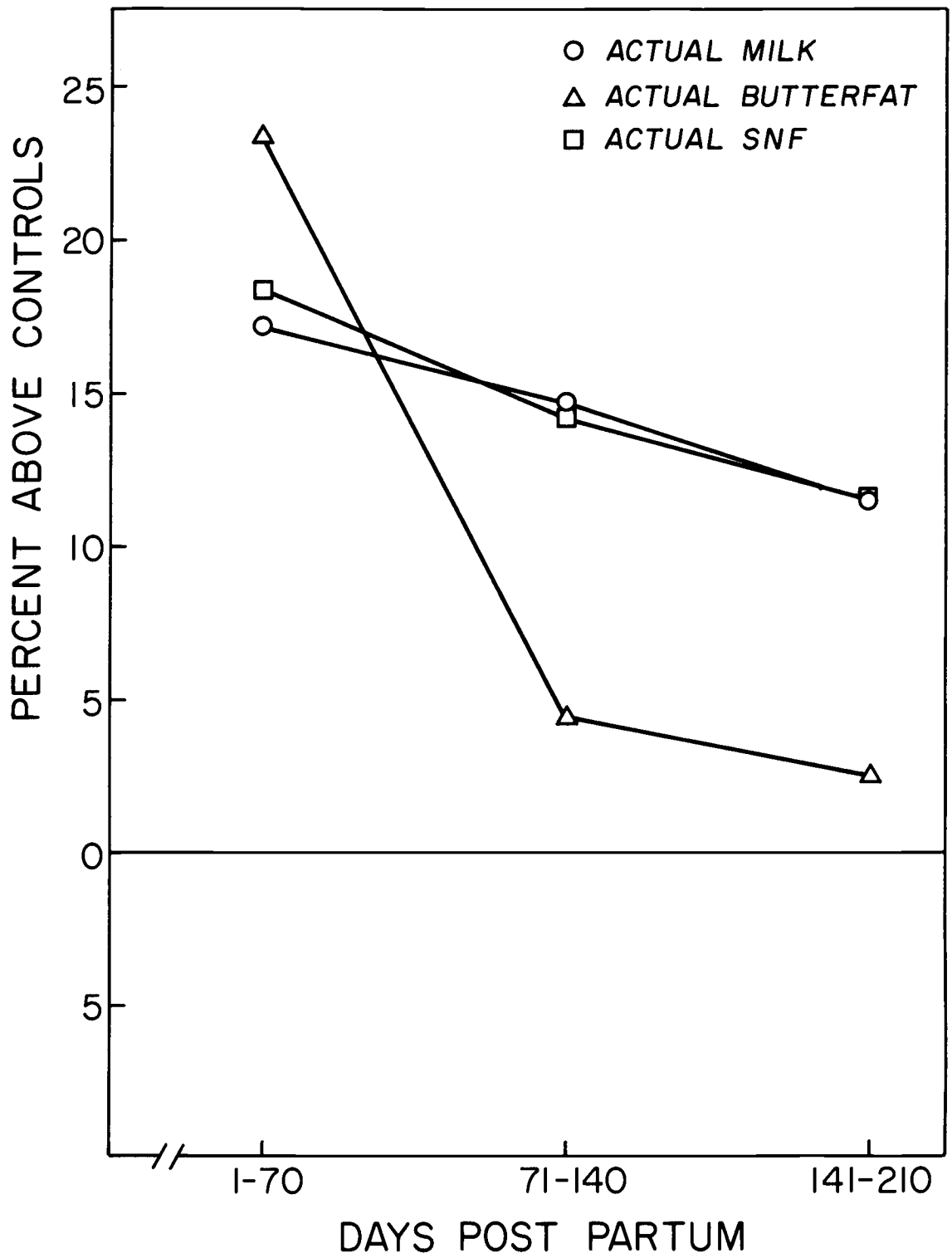


Figure 1. Lactational response due to daily oral administration of .005 mg. of flumethasone

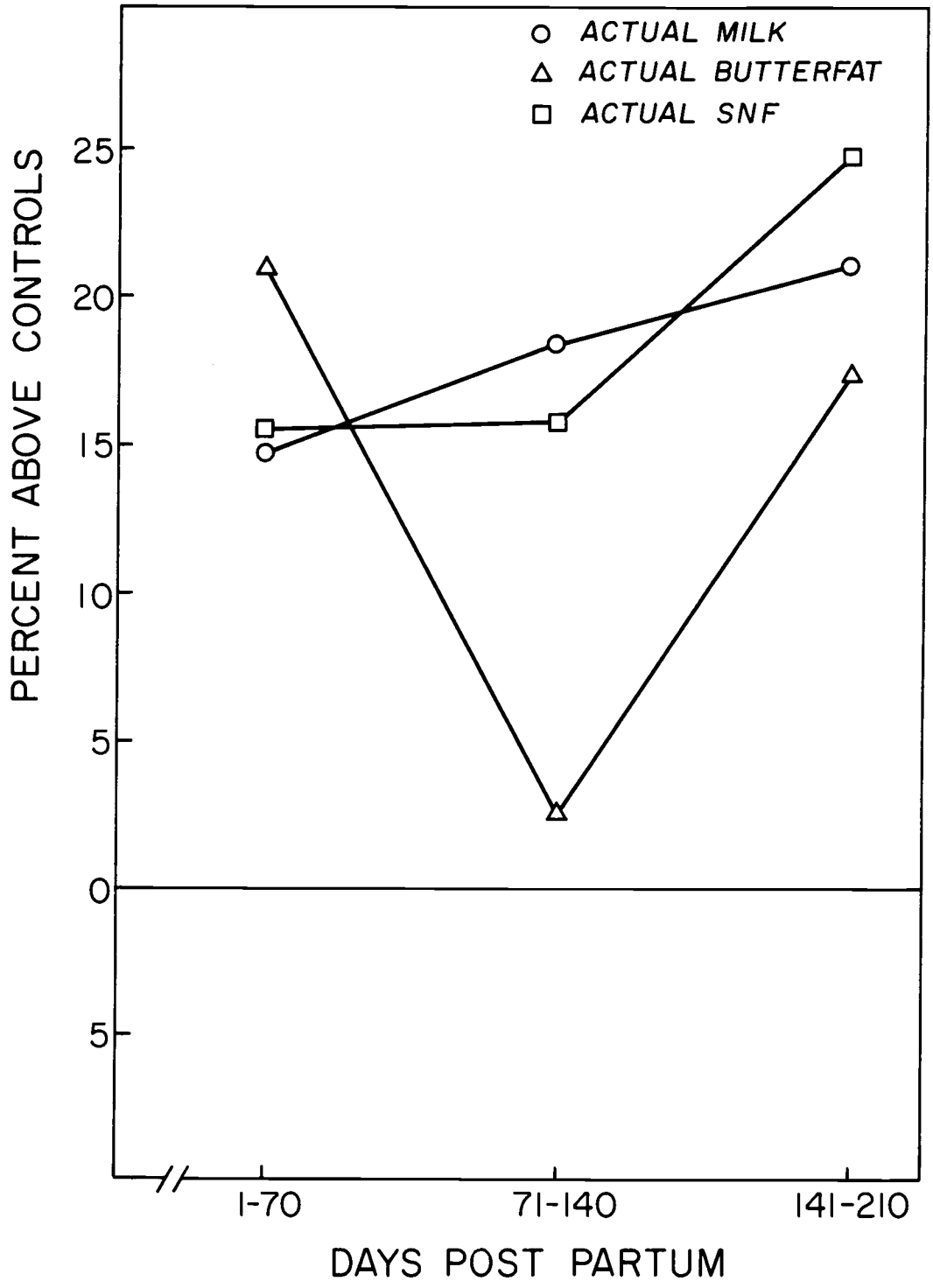


Figure 2. Lactational response due to daily oral administration of .01 mg. of flumethasone

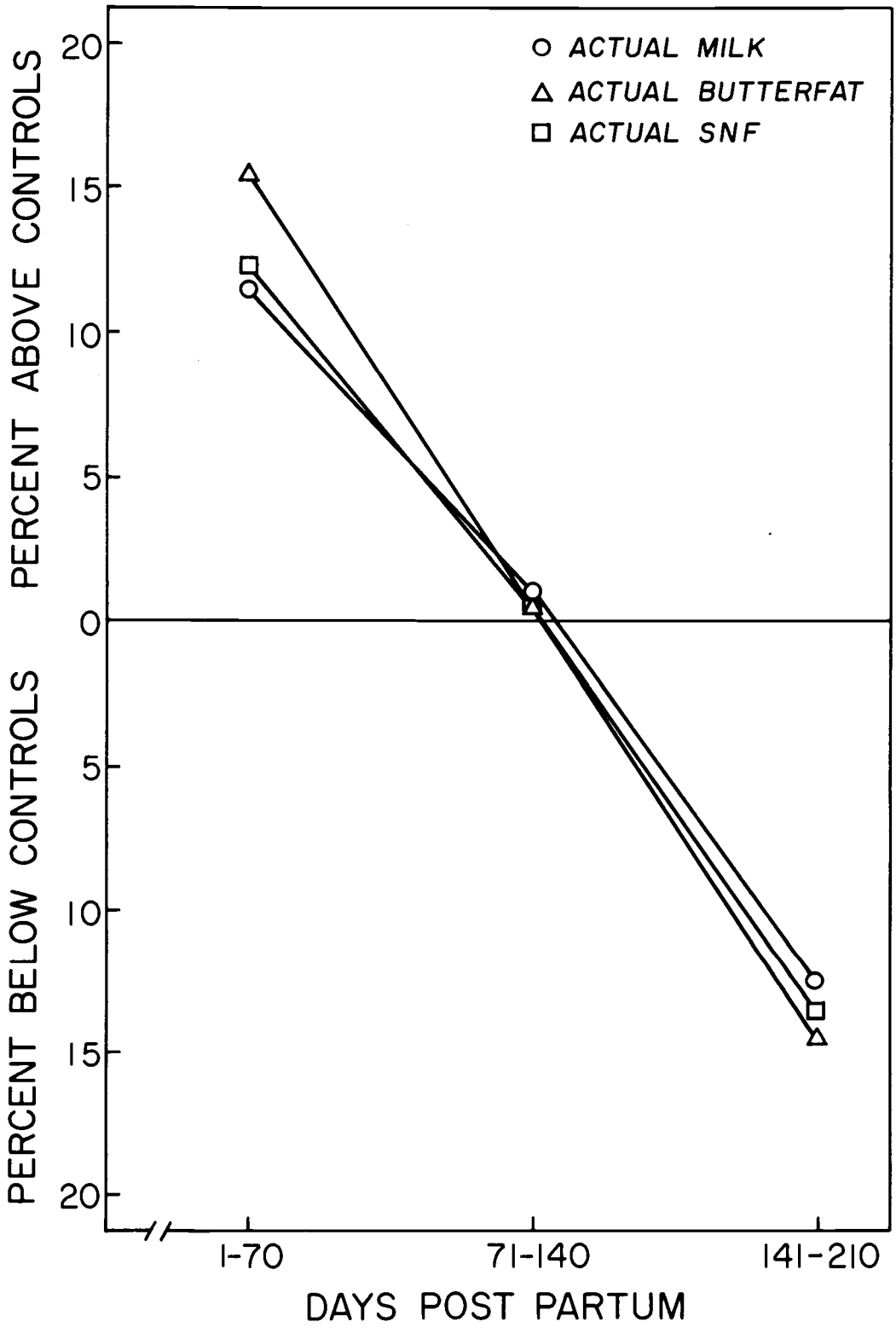


Figure 3. Lactational response due to daily oral administration of .02 mg. of flumethasone

Production of Butterfat and Solids-Not-Fat

Data presented in Tables 5 and 6 show that daily oral administration of flumethasone had similar effects on yields of butterfat and SNF. Production of ME butterfat and SNF, adjusted for days open were significantly different ($P < .05$) and ($P < .10$), respectively, between treatment groups. F-ratios show that the differences between treatment groups for actual butterfat production during the first and third 70-day segments of lactation were significant at the 5 and 10% levels of probability, respectively. The differences in actual butterfat yield among treatments during the second 70 days did not approach significance. Analysis of variance showed that differences between treatments in yield of SNF was significant ($P < .10$) during the third 70 days of lactation.

Comparison of means revealed that the production of ME butterfat and SNF, adjusted for days open, was significantly less ($P < .05$) for cows receiving .02 mg. of flumethasone daily than for cows on the .005 mg. level of flumethasone. Controls produced significantly less ($P < .05$) SNF than cows on the .005 mg. level of flumethasone and significantly less ($P < .05$) butterfat than cows on both the .005 and .01 mg. levels of flumethasone during the first 70 days of lactation. During the second 70 days of lactation there were no significant differences among treatment

Table 5. Analyses of Butterfat Yield Data of Cows Receiving Flumethasone During Experiment II.

Item	Source of variation	Degrees of freedom	Mean squares	F-ratios	Differences between means[c/] Treatment	Difference
ME butterfat adjusted for days open	Treatment	3	25700.44	3.12[a/]	Control & .005 mg.	103.33
	Block	2	5513.04		Control & .01 mg.	80.00
	Error	18	8225.88		Control & .02 mg.	35.34
					.005 mg. & .01 mg.	23.33
					.005 mg. & .02 mg.	138.67[b/]
					.01 mg. & .02 mg.	115.34
Actual butterfat days 1-70 of lactation	Treatment	3	1815.56	4.05[b/]	Control & .005 mg.	39.00[b/]
	Block	2	217.79		Control & .01 mg.	34.33[b/]
	Error	18	449.39		Control & .02 mg.	25.33
					.005 mg. & .01 mg.	4.67
					.005 mg. & .02 mg.	13.67
					.01 mg. & .02 mg.	9.00
Actual butterfat days 71-140 of lactation	Treatment	3	52.93	.136	Control & .005 mg.	6.50
	Block	2	165.38		Control & .01 mg.	3.84
	Error	18	390.73		Control & .02 mg.	.84
					.005 mg. & .01 mg.	2.66
					.005 mg. & .02 mg.	5.66
					.01 mg. & .02 mg.	3.00

Continued

Table 5--Continued.

Actual butterfat days 141-210 of lactation	Treatment	3	1316.11	2.51[a/]	Control & .005 mg.		2.83
	Block	2	313.54		Control & .01 mg.		19.66
	Error	18	524.66		Control & .02 mg.		16.50
					.005 mg. & .01 mg.		16.83
					.005 mg. & .02 mg.		19.33[b/]
					.01 mg. & .02 mg.		31.16

a/ Statistically significant ($P < .10$).
b/ Statistically significant ($P < .05$).
c/ Differences between treatment means were tested for statistical significance (Duncan, 1955).

Table 6. Analyses of SNF Yield Data of Cows Receiving Flumethasone During Experiment II.

Item	Source of variation	Degrees of freedom	Mean squares	F-ratios	Difference between means [c] Treatment	Difference
ME SNF adjusted for days open	Treatment	3	175397.82	2.87[a]	Control & .005 mg.	265.83
	Block	2	29408.67		Control & .01 mg.	205.83
	Error	18	63114.68		Control & .02 mg.	105.83
					.005 mg. & .01 mg.	51.00
					.005 mg. & .02 mg.	362.66[b]
					.01 mg. & .02 mg.	311.66
Actual SNF days 1-70 of lactation	Treatment	3	6140.93	1.88	Control & .005 mg.	72.50[b]
	Block	2	578.67		Control & .01 mg.	61.67
	Error	18	3264.94		Control & .02 mg.	48.67
					.005 mg. & .01 mg.	10.83
					.005 mg. & .02 mg.	23.83
					.01 mg. & .02 mg.	13.00
Actual SNF days 71-140 of lactation	Treatment	3	5372.78	2.10	Control & .005 mg.	49.50
	Block	2	125.04		Control & .01 mg.	55.16
	Error	18	2555.83		Control & .02 mg.	1.33
					.005 mg. & .01 mg.	5.66
					.005 mg. & .02 mg.	48.17
					.01 mg. & .02 mg.	53.83

Continued

Table 6--Continued.

Actual SNF days						
141-210 of lactation	Treatment	3	10681.33	2.64[a/]	Control & .005 mg.	29.66
	Block	2	2335.54		Control & .01 mg.	64.00
	Error	18	4050.68		Control & .02 mg.	35.00
					.005 mg. & .01 mg.	34.34
					.005 mg. & .02 mg.	64.66[b/]
					.01 mg. & .02 mg.	99.00[b/]

a/ Statistically significant ($P < .10$).
b/ Statistically significant ($P < .05$).
c/ Differences between treatment means were tested for statistical significance (Duncan, 1955).

means for butterfat or SNF yields. However, mean yields of butterfat and SNF were significantly less ($P < .05$) for cows receiving .02 mg. of flumethasone than cows on the .005 mg. level of flumethasone during the third 70 days of lactation.

As shown in Tables 5 and 6 and Figures 1, 2, and 3, flumethasone appears to stimulate the production of butterfat and SNF during early lactation. This could be due to the fact that flumethasone has gluconeogenic activity (Boland, 1961) and could have resulted in an increased mobilization of body reserves which were subsequently utilized in the synthesis of butterfat and SNF. Low levels of flumethasone (.005 and .01 mg. daily) appeared to have a stimulatory effect on both butterfat and SNF synthesis throughout lactation (Fig. 1 and 2) but the .02 mg. level of flumethasone appeared to inhibit synthesis of SNF and butterfat during later lactation (Fig. 3).

During comparable stages of lactation, flumethasone appeared to exert a similar influence on the actual yields of milk, butterfat, and SNF. This might be expected because of the high correlations between milk yield, SNF yield, and butterfat yield.

Blanchard, Freeman, and Spike (1966) reported correlations of .98 and .88 for milk yield with SNF yield and milk yield with butterfat yield, respectively. Correlations of .99 and .87 for milk yield with SNF yield and milk yield with butterfat yield

which were reported by Butcher, Sargent, and Legates (1967) confirm further the results of Blanchard et al. (1966).

Block x treatment interaction was significant ($P < .05$) for yield of SNF during the second 70 days and approached significance during the third 70 days of lactation. However, the interaction was considered to be error because interaction means for the controls did not follow the expected pattern.

California Mastitis Test (CMT)

When CMT data (Table 7) were subjected to statistical analysis the resulting F-ratio indicated that there were no significant differences ($P < .10$) among treatments. However, cow 826 had clinical mastitis for approximately one month and the right front quarter ceased to secrete milk. During this time she maintained a normal level of production.

Body Weight

From Table 8 it can be observed that there were no significant differences in body weights among treatment groups at the initiation of Experiment II. The same table shows that the differences between initial and terminal weights were not significantly different ($P < .05$) for treatment groups. Significant differences between initial and terminal body weight would be

Table 7. Analysis of CMT Data.

Source	Degrees of freedom	Mean squares	F-ratios
Treatment	3	1940.56	1.12
Block	2	200.37	
Error	18	1737.64	

$F_{3,\ 18,\ .05} = 3.16$

Table 8. Analyses of Body Weight Data.*

Item	Source	Degrees of freedom	Mean squares	F-ratios
Initial weight	Treatment	3	26888.00	1.06
	Error	20	25318.40	
305 day or less Change in weight	Treatment	3	2850.00	.179
	Error	20	15901.50	

*881 was weighed just prior to being culled for infertility. Her last 30-day weight was used as terminal weight.

difficult to interpret due to differences in the number of days carried a calf.

Days Milked

During the course of this experiment two cows (849 and 881) were dried off early due to management decisions. Persistency of their respective lactations indicated that they would have completed 305 day lactations. Therefore, it seemed reasonable to

extend their lactations to 305 days. After crediting 849 and 881 with milking 305 days, the F-ratio for number of days milked indicated a significant difference ($P < .05$) among treatments in lactation length. Comparison of means by Duncan's method (1955) indicated that the length of lactation for the .02 mg. level of therapy was considerably shorter than lactation length for all other treatment groups. However, these differences were insignificant. This indicates that .02 mg. of flumethasone had an apparent inhibitory effect on milk synthesis.

Reproduction

At the initiation of Experiment II it was felt that daily administration of flumethasone may have an effect on gestation length and birth weight of calves at the subsequent parturition. Statistical analysis (Table 9) showed that there were no differences ($P < .05$) in gestation lengths due to flumethasone therapy. Data on the birth weights of calves were not analyzed statistically because of missing weights on still births and a high rate of twinning. One cow in each of the control, .005, .01, and .02 mg. levels of flumethasone gave births to twins at the subsequent parturition. The high rate of twinning was not attributed to flumethasone therapy because there was one set of twins in each treatment group and the last 105 calvings in the Oregon State

Table 9. Analyses of Reproductive Performance.

Item	Source of variation	Degree of freedom	Mean squares	F-ratio
Gestation length	Treatment	3	7.00	.15
	Error	19	44.00	
Days open	Treatment	3	2075.44	.79
	Error	20	2618.05	
Services to conception	Treatment	3	.046	.06
	Error	19	.752	
Lactation length b/	Treatment	3	28175.00	5.88 a/
	Error	20	4789.60	

a/ Significant at the 1% level of probability.
b/ Cows 849 and 881 were credited with 305-day lactations.

University Holstein herd had produced nine sets of twins. Mean birth weights of single births were 97.0 pound, 99.4 pound, 103 pound, and 95 pound for the control, .005, .01, and .02 mg. levels of flumethasone, respectively. One cow (831) on the .01 mg. level gave birth to an unusually large (132 pound) still-born calf. This calf was born during the night and the probable cause of death was contributed to suffocation. Also, there were two still births from cows on .02 mg. of flumethasone. These two calves were judged to be normal in size; however, they were not weighed. Cow 813 had trouble calving so the cause of death of her calf was attributed to suffocation. Cause of death of 849's calf is not known.

Statistical treatment of data on services to conception and days open post partum (Table 9) indicated no significant effects due to oral administration of flumethasone. Cow 881 was excluded from the controls in calculating services to conception as she was culled due to infertility. However, her data were included in calculating days open post partum.

CONCLUSIONS

The following conclusions can be drawn from the results of the two experiments involving the daily oral administration of flumethasone.

No statistically significant differences were observed among treatment groups in Experiment I for age at calving or number of days post partum at the onset of flumethasone therapy. The high F-ratios for blocking indicated that blocking on level of milk yield was effective. These observations indicate that there were no biases in assigning cows to treatments.

Statistical treatment of milk yield data from Experiment I revealed that the means for average daily milk, age adjusted milk, 4% FCM and age adjusted 4% FCM were significantly ($P < .05$) lower for cows on the .05 mg. level of flumethasone than for controls. Means for all four measures of milk yield were higher for cows on the .01 mg. level of therapy than for the controls. However, the differences were not statistically significant. From these results it can be concluded that daily oral administration of .05 mg. of flumethasone has an inhibitory effect on milk production. The data suggest that during the declining phase of lactation, .01 mg. of flumethasone may exert a small stimulatory effect on milk secretion. Further investigations with larger sample sizes may

reveal the true effects of lactational response due to low levels of flumethesone therapy. It was also concluded from the results of Experiment I that the oral administration of low levels of flumethasone throughout an entire lactation should be investigated to establish whether or not flumethasone has a stimulatory effect on milk synthesis and whether the effect varies with respect to stage of lactation.

Experiment II was designed to test the effects of daily oral administration of low levels (.005, .01, and .02 mg.) of flumethasone on the 305-day lactations of 24 Holstein cows.

Comparisons of means showed that milk, butterfat, and SNF (adjusted for age and days open) were significantly lower ($P < .05$) for cows on the .02 mg. level than for cows on .005 mg. of flumethasone. Analyzing lactations by 70-day segments revealed that cows on .005 mg. of flumethasone produced significantly ($P < .05$) more butterfat and SNF, and cows on .01 mg. produced significantly more ($P < .05$) butterfat during the first 70 days than controls. During the second 70 days of lactation, cows receiving .01 mg. of flumethasone produced considerably more milk than controls and the cows on the .02 mg. level of therapy. Cows receiving .01 mg. of flumethasone produced significantly more ($P < .05$) milk, butterfat, and SNF between days 141 and 210 of lactation than cows on .02 mg. of flumethasone.

Cows on the .01 mg. level of flumethasone produced 11.7 to 21.0% more butterfat, and 15.6 to 24.9% more SNF than controls during the first three 70-day segments of lactation, respectively. During this same period cows on .005 mg. of flumethasone produced 11.4 to 17.3% more milk, 2.5 to 23.9% more butterfat, and 11.5 to 18.4% more SNF than controls. Cows on .02 mg. of flumethasone produced 11.7, 15.5, and 12.3% more milk, butterfat, and SNF, respectively, than controls during the first 70 days of lactation. Yields of milk, butterfat, and SNF for the .02 mg. level of therapy were .9, .6, and .4% above controls during the second 70 days of lactation. However, the production of milk, butterfat, and SNF for the .02 mg. level of flumethasone was 12.5, 14.5 and 13.6% lower than that of the controls during the third 70 days of lactation. These results indicate that low levels of flumethasone (.005 and .01 mg.) may have a stimulatory effect throughout lactation, but the .02 mg. level of flumethasone appears to inhibit the secretion of milk and the synthesis of butterfat and SNF during the latter part of lactation. This conclusion is further supported by the observation that there was a significant difference ($P < .05$) among treatments in length of lactation. Although a comparison of the means for length of lactation did not reveal a difference, the average length of lactation was considerably shorter for cows on the .02 mg. level of flumethasone

than for cows in all other treatment groups.

Statistical analyses of data on CMT, body weight changes, services to conception, days open post partum, and length of gestation showed that there were no differences among treatment groups. These data indicate that flumethasone did not cause any detectable side effects.

Tests of the relative efficiency of blocking indicated that blocking was only 82 to 95% as efficient as a completely randomized block design. From this observation it can be concluded that in experiments where lactational responses are used for determining treatment differences, the milk merit index is not a particularly strong criterion for blocking. This could be due to the fact that performance of the close relatives of a cow are considered in establishing the cow's breeding value for milk.

The results of these preliminary experiments may not be applicable to cows with lower producing abilities. However, the results herein reported indicate that low levels of flumethasone may stimulate milk and milk component yields; but additional studies with larger numbers of cows are required before flumethasone can be recommended generally as a feed additive for enhancing milk yield.

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