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Effect of dexamethasone on milk yield and composition in dairy cows

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Abstract — Dexamethasone was injected in dairy cows in order to get a better insight into the effects of corticosteroids on milk secretion and composition. A single intramuscular dose of 40 mg dexamethasone caused a 45% reduction in milk yield after 24 h; full recovery took 5 d. The secretion of fat, casein and magnesium was not affected by the treatment. Consequently, the concentration of fat, total protein, total casein, magnesium and phosphorus increased then decreased in direct proportion to the changes in milk yield. The secretion of total protein, calcium and phosphorus decreased as a result of the treatment. The concentration of lactose and the monovalent ions (sodium, potassium, and chlorine) was unaffected, and as a result their secretion decreased and returned to pretreatment level in direct inverse proportion to the changes in milk yield. Whey protein secretion decreased for 48 h and was responsible for the decrease in total protein secretion for 48 h. The most profound effect of dexamethasone is the reduction in the secretion of the osmotic components.

milk yield / dairy cows / stress / corticosteroids

Résumé — Effet de la dexaméthasone sur la production et la composition du lait chez les vaches laitières. La dexaméthasone a été injectée à des vaches laitières afin de mieux comprendre les effets des corticostéroïdes sur la sécrétion et la composition du lait. Une dose unique intramusculaire de 40 mg de dexaméthasone a réduit de 45 % la production de lait après 24 h ; le retour total à la production antérieure a pris 5 jours. La sécrétion des lipides, des caséines et du magnésium n'a pas été affectée par le traitement. En conséquence, la concentration des lipides, des protéines totales, des caséines totales, du magnésium et du phosphore a augmenté puis a diminué proportionnellement aux changements de production laitière. La sécrétion des protéines totales, du calcium et du phosphore a diminué à la suite du traitement. La concentration du lactose et des ions monovalents (sodium, potassium et chlore) est restée inchangée ; leur sécrétion a diminué et est revenue au niveau initial dans une proportion inverse aux modifications de la production laitière. La sécrétion des protéines du lactoserum a diminué pendant 48 h et a été responsable de la diminution de la sécrétion des protéines totales

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pendant 4 h. L'effet le plus important de la dexaméthasone est la réduction de la sécrétion des composés à pouvoir osmotique.

production de lait / vaches laitières / stress / corticostéroïdes

1. INTRODUCTION

Various stressful situations, whether environmental (heat stress, water deprivation [12, 16], psychological (movement in truck and reallocation; [2]), or the outcome of the trauma of surgery [2] and diseases such as mastitis [15] negatively affect the milk yield in dairy cows. The common denominator in most of these situations is activation of the hypothalamic-pituitary-adrenal axis [20]. An adrenocorticotropin hormone (ACTH)-induced elevation in corticosteroids has been reported to negatively affect milk secretion in dairy cows [2–4, 20]. The decrease in milk secretion is most likely related to the elevation of blood corticosteroids [1, 5].

Only limited data are available regarding the influence of corticosteroids on the composition of organic and inorganic constituents in the milk of dairy cows. In particular, there is no information whether corticosteroids affect differentially the secretion of proteins (whey and casein) and mineral fractions. There is also no information on how the large increase in milk yield during the last decade affected the response to corticosteroids. Our objective was to find out if treatment with synthetic corticosteroid (dexamethasone) would affect the yield and composition of the milk of dairy cows. This information may contribute to better insight into the physiological basis for the decrease in milk secretion under stress and disease states.

2. MATERIALS AND METHODS

Four non-pregnant cows were located in the experimental herd of the ARO and were used as the experimental group. The cows were multiparous and at mid or late lactation. Four control cows were selected to match the experimental cows in respect to milk yield, parity and stage in lactation. The cows were fed a typical Israeli total mixed ration that was composed of 65% concentrates and 35% forage and contained 17% protein throughout the trial. The cows were milked three times daily at 05.00, 13.00, and 20.00 h. Basal milk yield and composition were recorded for 3 d before the treatment. The experimental cows were injected once intramuscularly into the neck with 40 mg dexamethasone (dexamethsone-HCl, Vitamed, Haifa, Israel) dissolved in 40 ml of saline between 13.00 and 13.30 h after the noon milking. The control cows were injected at the same time with 40 ml of saline.

Electronic milk meters (S.A.E AFIKIM, Kibbutz Afikim, Israel) automatically recorded the milk yield at each milking. Milk composition was monitored for an additional 4 d. Approximately 200 ml of milk was sampled from each cow during the noon milking. Part of each of the milk samples were analysed within 24 to 48 h for protein, fat and lactose contents, and somatic cell count in the Central Laboratory of the Israeli Dairy Breeders Association. The rest of the milk samples were defatted by centrifugation at $6\,000 \times g$ for 10 min at room temperature. Protein concentration in milk fraction was determined colorimetrically by the Bradford method with a commercial (Sigma) reagent. Part of the milk samples were acidified with HCl to pH 4.6, and then centrifuged at $22\,000 \times g$ for 3.5 min at room temperature. Protein concentration in the supernatant was defined as whey protein. The difference between the protein content

of the whole defatted milk and whey protein was defined as casein. The concentrations of sodium, potassium, calcium, magnesium and phosphorus were determined by Inductively Coupled Plasma – Atomic Emission Spectrometer (Spectro, Germany). The samples were pretreated as follow: 0.5 ml of milk were mixed with 1 ml of concentrated nitric acid, and the tube containing the mixture were placed in boiling water for 30 min, and then the mixture was diluted with 15 ml of distilled water. The concentration of chlorine was determined by automatic titration and the osmolality by freezing point depression as previously described [14]. Jugular blood samples were taken a day before treatments, just before treatment and for 4 days post-treatments. The plasma was separated and glucose concentration in the plasma was determined by enzymatic-colorimetric method (Sigma kit 315–100, Sigma, Rehohot Israel).

The variation was analysed according to the General Linear Model of SAS [13] for repeated measurements. First, we tested to see if the post-treatment results differed significantly from the pre-treatment ones, using the pre-treatment results of each cow at each sampling as co-variates. When the post-treatments results differed from the pre-treatment values only in the experimental group, the statistical significance ($P \leq 0.05$) was assessed using PROC GLM of SAS for repeated measurements [13] to compare between treatments. The interrelationship between the secretion of osmotic components pressure (the product of milk secretion $\times$ osmolality determined by freezing point depression) and milk secretion was analysed by linear regression analysis using the entire data set.

3. RESULTS

In the dexamethasone treated cows, milk yield started to decrease after two milkings (16 h, not shown), reaching its lowest value (on average, 45% below the pretreatment values) after three milking (24 h) (Fig. 1). Milk yield then started to rise, but it took an additional 5 d to return to the initial level. In the control group no reduction in milk yield was observed.

The concentrations of fat increased by 45%, of total protein by 45%, of casein by 50%, of whey protein by 9%, of magnesium by 50% and of phosphorus by 21% in the dexamethasone-treated cows, reaching a peak after 24 h (Figs. 2, 3, 4). The concentration of these metabolites remained unchanged in the control group. In both groups, calcium concentration did not differ between the post-treatment and pre-treatment samplings, although it tended ($P < 0.1$) to be higher during the first post-treatment sampling in the experimental group (Fig. 4). The concentrations of fat, total protein and casein then started to decrease toward the pre-treatment level, so they remained higher than in the control, or pre-treatment values for an additional 3 days. The concentrations of whey protein (Fig. 2) and phosphorus (Fig. 4) were higher than in the control, or the pre-treatment values, after 24 h, and the concentration of magnesium (Fig. 4) was elevated for 2 d after the dexamethasone treatment.

The secretion of fat (Fig. 3), casein (Fig. 2), and magnesium (Fig. 4) was not significantly affected by the dexamethasone treatments. After 24 h following the treatments, the secretion of total protein and whey protein was reduced by 35% in the dexamethasone group, while remaining unchanged in the control group (Fig. 2). Total protein secretion recovered within the next day, while whey protein secretion remained depressed for an additional 24 h in the dexamethasone treated cows (Fig. 2).

The concentrations of the monovalent ions (sodium, potassium, and chlorine), lactose and total osmolality were unaffected (results not shown). The secretion of lactose and these ions decreased and recovered in direct inverse proportion to the changes in milk yield (Fig. 1). No changes in the

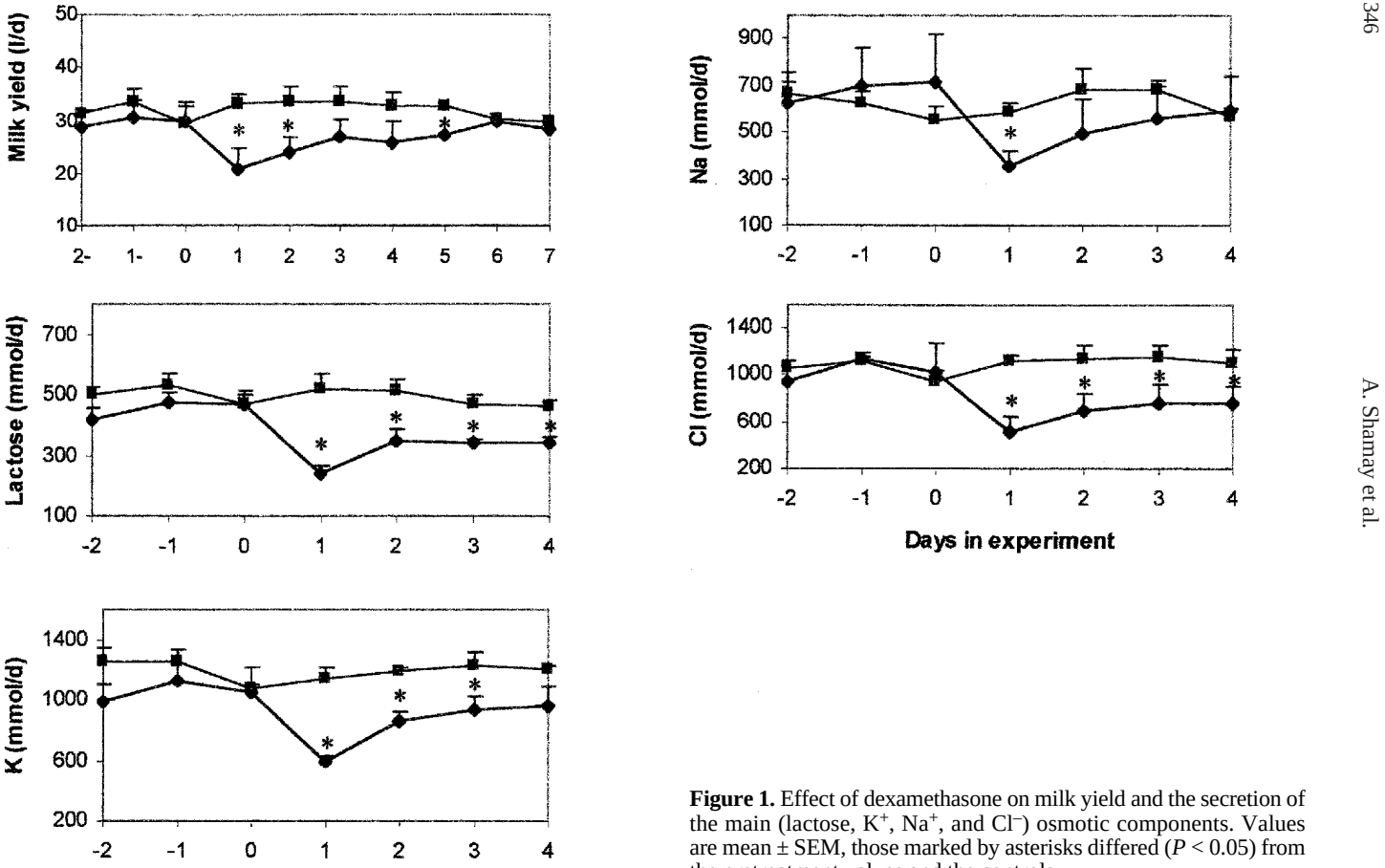

Figure 1. Effect of dexamethasone on milk yield and the secretion of the main (lactose, K^+, Na^+, and Cl^-) osmotic components. Values are mean ± SEM, those marked by asterisks differed ($P < 0.05$) from the pretreatment values and the controls.
■ = control; ◆ = experiment.

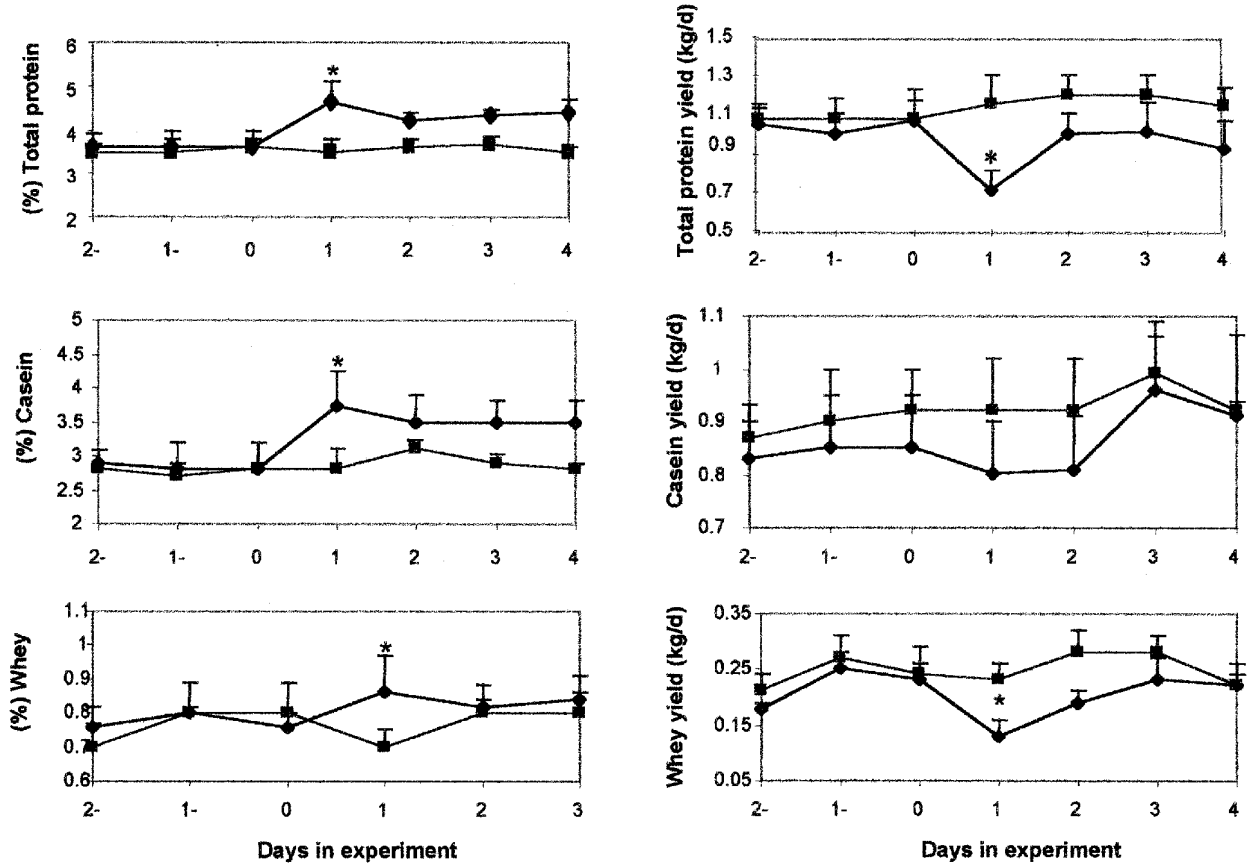

Figure 2. Effect of dexamethasone on milk concentration and milk yield of total protein, casein, and whey protein. Values are mean ± SEM, those marked by asterisks differed ($P < 0.05$) from the pretreatment values and the controls.
■ = control; ◆ = experiment.

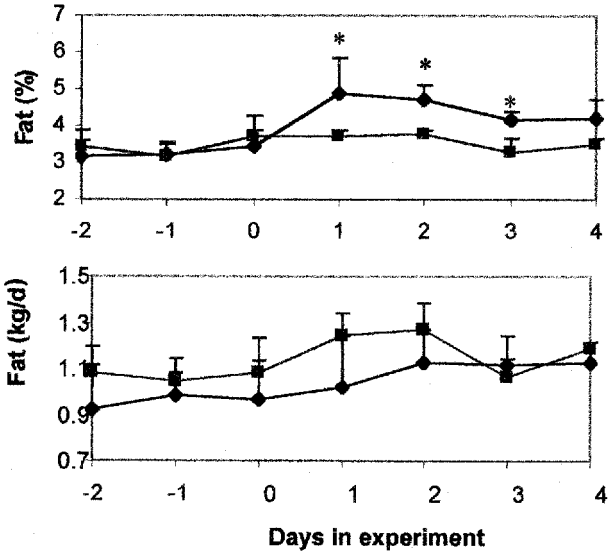

Figure 3. Effect of dexamethasone on milk concentration and milk yield of total fat. Values are mean ± SEM, those marked by asterisks differed ($P < 0.05$) from the pretreatment values and the controls. ■ = control; ◆ = experiment.

concentration and the secretion of these ions were found in the control cows. Using the entire data set, a remarkable linear relationship between the secretion of osmotic components (composed mostly of lactose, sodium, potassium and chlorine) and milk yield was found (Fig. 5). Glucose concentration in the blood was measured to find whether dexamethasone treatment affected glucose concentration, which in turn might affect lactose secretion. As shown in Figure 6, glucose concentration in the blood elevated in the treated cows to 105 mg per 100 ml one day after dexamethasone injection and was back in the normal range after 2 d.

4. DISCUSSION

The proportional decrease in milk yield, and the latency period before milk yield decreased in the present study was similar to those observed in ACTH- and dexamethasone-treated cows [5, 20]. The latency period and the rather slow recovery in milk yield following injection of material with a short biological half-live such as dexamethasone suggest that its effect was inductive and not direct. The differential negative effect of dexamethasone treatment on the secretion of aqueous-osmotic components (lactose, sodium, potassium and chlorine) and whey proteins in terms of the extent and kinetics of the response suggest that at least two mechanisms are induced by dexamethasone.

The lack of negative effect of dexamethasone on casein and fat output in combination with its marked effect on fluid secretion explains the increase in the concentrations of fat, casein and protein in the treated cows. In contrast to casein, the output of total whey proteins was depressed, which was the main reason for the decrease in total protein output. The increase in fat and protein concentration in milk and

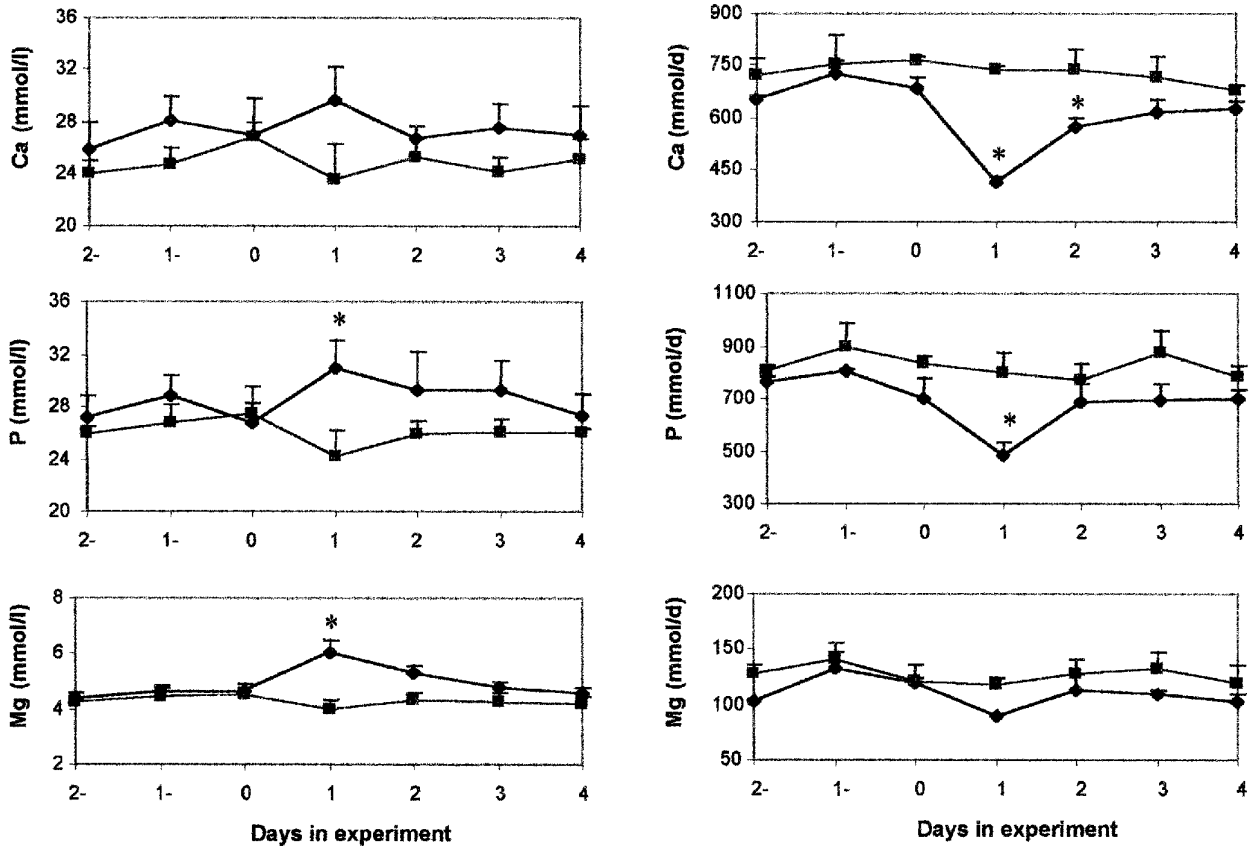

Figure 4. Effect of dexamethasone on milk concentration and milk secretion of divalent ions (Ca^{2+}, Mg^{2+}) and phosphorus (P). Values are mean ± SEM, those marked by asterisks differed ($P < 0.05$) from the pretreatment values and the controls.
■ = control; ◆ = experiment.

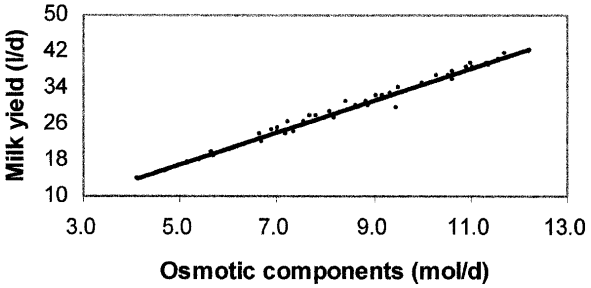

Figure 5. Interrelationship between milk yield and the secretion of osmotic components (the product of milk secretion × osmolality determined by freezing point depression) in milk.
$Y = 3.52\ X - 0.49$; $n = 54$; $r^2 = 0.99$.

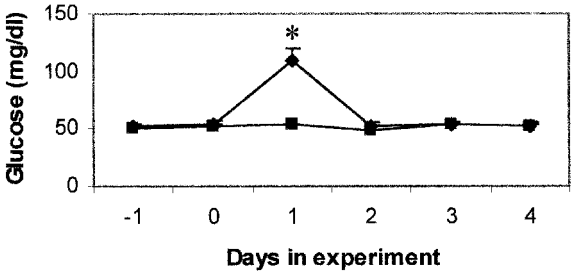

Figure 6. Glucose concentration in the blood of dexamethasone treated and control cows. Values are mean ± SEM, those marked by asterisks differed ($P < 0.05$) from the pre-treatment values and the controls. ■ = control; ◆ = experiment.

reduction in total milk protein output is consistent with those observed in ACTH-treated cows [20].

Roughly, 2/3 of the calcium, magnesium and phosphorus that are secreted in the milk of bovine are secreted in close association with the casein micelles [7–9]. In the aqueous phase, calcium, and most likely magnesium, appears in association with phosphorus and citrate, and in free ionized form [9]. The elevations in the concentration of magnesium and phosphorus in the dexamethasone treated cows are therefore consistent with the parallel elevation in the concentration of casein. Divalent ions and phosphorus were less affected by the treatment than the output of the monovalent ions in consistence with the maintenance of casein output. The output of calcium and, to a lesser extent of phosphorus, in the aqueous phase was most likely decreased, explaining the overall reduction in the output of these ions. Our results regarding the differential effects of dexamethasone on the output of the whey and casein fractions, and the elevations in the concentration of magnesium and phosphorus are to the best of our knowledge novel observations.

It was suggested that stress (ACTH-induced corticoid secretion) may exert an adverse influence on milk yield by decreasing glucose availability [5]. The amount of glucose taken by the mammary gland has been found to closely correlate with milk yield in cows [7]. Mammary uptake of glucose was lowered by dexamethasone treatment [5], which may explain the increase in blood glucose concentration following ACTH and dexamethasone treatments (Fig. 6, [5, 19]). Maximal blood glucose concentration coincided with minimum milk yield, in agreement with the above suggestion (Fig. 6, [5, 20]). However, milk yield remained lower 4 days after the recovery in blood glucose (Fig. 6, and [20]). Our results agree with the suggestion of Varner and Johnson [20] that factors other than mammary uptake of glucose may be involved with stress related decreased milk production. It is possible that the relatively short duration in reduction of the output of whey protein found in the present experiment

relates to the reduced glucose uptake by the mammary gland.

Disruption of the integrity of mammary epithelial cell tight junction depressed milk yield in goats [18]. Opening of mammary tight junctions was associated with increase in milk sodium and chlorine, due to their leakage from the blood into the lumen, and decrease in potassium concentration, due to its leakage from milk to blood [19]. Our data on monovalent ion concentration in the milk suggest that depressed milk yield following dexamethasone treatment cows was not associated with the disruption of the integrity of the mammary cell tight junctions. Furthermore, dexamethasone decreased tight junction permeability in vitro and cortisol appears to be associated with closure of tight junction in the late pregnant mammary gland of goats [10].

One of the most basic principle of milk secretion is that the total osmotic pressure of milk remains approximately constant and equal to the blood [6]. The output of osmotic components, of which 60% are contributed by lactose and 40% by the monovalent ions, determines the volume of milk [11]. The reciprocal of the slope of the linear regression between milk volume and the secretion of osmotic components in milk in the present experiment was 0.284 M (Fig. 5). This value is similar to the expected total osmotic pressure of the blood, indicating that the principle of maintaining equal gradient between milk and plasma osmolality was kept in the present experiment. Thus, the reduction in milk volume following dexamethasone treatment can be fully explained by reduction in the secretion of osmotic components (Fig. 5). As lactose is the main osmotic component, it is suggested that reduction in lactose secretion induced a coordinated reduction in the output of sodium, potassium and chlorine. We recently proposed a mechanism that connects between activation of the hypothalamic-pituitary-adrenal axis and reduction in the output of osmotic components from the alveoli into the gland lumen through production of active biological substance from β-casein by the plasmin system in milk [17].

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