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Response of milk yield, plasma cortisol, amino acids, urea and glucose to a single low-dose administration of adrenocorticotrophic hormone in lactating cows

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Summary — An experiment was conducted to determine the effects of a single low-dose administration of adrenocorticotrophic hormone (ACTH) on milk yield, plasma cortisol, free amino acids, urea and glucose in lactating cows. The animals were treated with either 6 IU synthetic ACTH or 5 ml physiological saline (control) administered intravenously via a jugular vein catheter. Blood was withdrawn 60 and 5 min pretreatment (baseline), and 10, 20, 30, 60, 120, 180, 240, 300, 360, and 420 min post-treatment. A rapid positive response ($P<0.05$) in plasma cortisol occurred within 10 min of administration of 6 IU ACTH. The maximum increase in plasma cortisol concentration occurred at 1 h post-ACTH treatment and plasma cortisol returned to baseline 4 h later. Until 7 d after ACTH administration, no effect on milk yield was recorded. In comparison with the saline-treated group, the ACTH-treated group exhibited a significant ($P < 0.05$) increase in the plasma concentrations of 3-methylhistidine, glycine, histidine, isoleucine, leucine, lysine, valine, and glucose. In contrast, the concentrations of alanine, aspartate, glutamate, glutamine and proline decreased significantly ($P < 0.05$) after ACTH treatment. Hormone administration had no effect on the plasma arginine, asparagine, methionine, phenylalanine, serine, threonine, tyrosine, and urea. These results demonstrate that the bovine species behaves like other mammals with respect to its metabolic response to stress. Thus, during stress, ACTH increases adrenal cortical activity which, in turn, stimulates protein catabolism in muscle and gluconeogenesis from some non-essential amino acids.

exogenous ACTH / milk yield / cortisol / metabolite / cattle

Résumé — Réponses de la production laitière, de la cortisolemie, de l’aminoacidémie libre, de l’urémie et de la glycémie à une administration unique d’ACTH à faible dose chez la vache en lactation. Une expérience a été menée pour déterminer les effets d’une administration unique de l’hormone adrénocorticotrope (ACTH) à faible dose sur la production laitière, le cortisol,

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l'urée et le glucose du plasma chez la vache en lactation. Les animaux ont été traités soit avec 6 UI d'ACTH de synthèse soit avec 5 ml du sérum physiologique (contrôle), administrés par voie intraveineuse par l'intermédiaire des cathéters intrajugulaires. Du sang a été prélevé 60 et 5 min avant traitement (valeurs initiales), et 10, 20, 30, 60, 120, 180, 240, 300, 360 et 420 min après traitement. Une réponse corticotrope positive et rapide ($P < 0.05$) a été observée 10 min après administration de 6 UI d'ACTH. La concentration maximale du cortisol plasmatique a été enregistrée 1 h après traitement et le retour aux valeurs initiales 4 h plus tard. Dans les 7 j qui ont suivi l'administration de l'ACTH, aucun effet sur la production laitière n'a été observé. Par comparaison avec les contrôles, les animaux traités à l'ACTH ont présenté une augmentation significative ($P < 0.05$) des concentrations plasmatiques en 3-méthylhistidine, glycine, histidine, isoleucine, leucine, lysine, valine et glucose. En revanche, les concentrations en alanine, aspartate, glutamate, glutamine et proline ont diminué significativement ($P < 0.05$) après traitement à l'ACTH. L'administration de l'hormone n'a pas eu d'effets sur les concentrations en arginine, asparagine, méthionine, phénylalanine, sérine, thréonine, tyrosine et urée. Ces résultats montrent que la réponse métabolique de la vache au stress est la même que celle connue chez les autres espèces mammifères. Ainsi, pendant le stress, l'ACTH augmente l'activité du cortex surrénalien qui, à son tour, stimule le catabolisme des protéines musculaires et la néoglucogenèse à partir de certains acides aminés non essentiels.

ACTH exogène / production laitière / cortisol / métabolite / vache

INTRODUCTION

The normal response of the body to stress is the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, which stimulates the adrenal cortex to increase the synthesis and release into the blood of large quantities of corticosteroids. The adrenal corticosteroid concentration in peripheral blood (mainly cortisol in the cow, Venkataseshu and Estergreen, 1970) has thus been used as a measure of animal response to a variety of stressors, including transport and handling (Mitchell et al, 1988), confinement (Holley et al, 1975), social disruption (Friend et al, 1977), surgery (Pearson and Mellor, 1975), and infection (Dvorak, 1971).

Moreover, ACTH challenge may help evaluate adrenal function (Alam et al, 1986). However, to avoid possible side effects of adrenocorticotrophic hormone in cattle, such as a decrease in milk yield, one should always use the lowest ACTH dose possible (Van der Kolk et al, 1991). The decrease in milk yield was reported to be associated with an ACTH dose of at least 160 IU (Brush, 1960; Campbell et al, 1964; Bremel and Gangwer, 1978). Thus, Van der Kolk et al (1991) suggested that a test dose of 6 IU ACTH may be used as a standard test for the evaluation of adrenocortical function in lactating cows when administered intravenously and when plasma samples for cortisol assay are collected just prior to administration and 1 h later.

It is well established that glucocorticoids enhance resistance to stress by providing more glucose to the stressed organism for muscle work, tissue repair, etc (Munck et al, 1984; Hartmann, 1988). The effects of glucocorticoids when blood glucose levels increase include inhibition of glucose uptake in several peripheral tissues (Reilly and Black, 1973; Munck et al, 1984) and stimulation of hepatic gluconeogenesis (Baird and Heitzman, 1970).

However, the exact role of glucocorticoids in the regulation of protein metabolism during stress has received little attention. Although significant stress-induced changes in plasma amino-acid (AA) concentrations have been reported in sheep (Slater and Mellor, 1977) and in man (Wannemacher, 1977), no results have been published con-
cerning this phenomenon in cattle. This information is essential because of the implication of AA in glucose homeostasis in the bovine species. The aim of this paper was therefore to evaluate the possible effects of 6 IU ACTH on milk yield and plasma cortisol, AA, urea and glucose in lactating cows.

**MATERIALS AND METHODS**

**Animals**

Four non-pregnant, lactating Friesian cows 4.0–6.5 years old, weighing 472 to 560 kg (mean ± SE: 510.7 ± 26.0) were used. All cows had been through at least 2 calvings. Days of lactation ranged from 154 to 203 (184.3 ± 15.3), the average milk yield was 17.20 ± 1.7 l per 24 h, and the cows were milked twice daily between 06.15 and 07.00 am, and between 3.30 and 4.15 pm. All animals were housed in individual tie-stalls and were accustomed to frequent handling. They received approximately 9 kg meadow hay, 1 kg dried beet pulp, 1 kg barley and 7.7 kg dairy concentrates per 24 h, covering about 1.1 times their energy requirements for body maintenance and milk production. In addition, the N balance was positive in all animals. The daily feed was given in 2 equal meals, the first at 06.15 am and the second at 3.30 pm. All animals had unrestricted access to water throughout the experiment.

**Treatment and sampling schedule**

The 4 cows were used in a 2 factor crossover design. Two animals were treated at 09.00 am with 6 IU synthetic ACTH$_{1-24}$ (Synacthen®, Ciba Laboratories, Sussex, UK) suspended in 4.76 ml of 0.85% sterile saline solution, and 2 were treated at the same time with 5 ml physiological saline (control), equivalent to the volume of medium injected into the ACTH-treated group. After 2 weeks and at the same hour, each animal was given the opposite treatment. The interval of 2 weeks between treatments was used because previous observations (Brush, 1960) established that recovery of milk yield was still incomplete after 7 d, following a single intramuscular injection of 160 IU ACTH in cows. The milk yield at morning and evening milkings was recorded the day prior to ACTH or saline treatment, the day of treatment, and for 7 days following treatment. To minimize stress-induced increase in plasma cortisol concentrations from sampling procedures, an indwelling jugular-vein catheter was implanted under local anaesthesia in each cow the day before each sampling period. The end of each catheter was linked to a rubber tubing, itself connected with elastic bands to the cow’s neck. Thus, the ACTH or saline solution was administered intravenously by syringe through the rubber tubing, and blood samples were collected into heparinized tubes by syringe through the same rubber tubing. Samples were taken without any restraint on the animals other than the neck chains by which they were held in their stalls. Blood (20 ml) was withdrawn 60 and 5 min before (baseline data), and 10, 20, 30, 60, 120, 180, 240, 300, 360, and 420 after ACTH or saline treatment. The blood samples were centrifuged at 2 000 g for 15 min at 4°C and the plasma stored at −20°C.

**Chemical analysis**

Plasma from all blood samples was analyzed for cortisol, 19 AA (3-methylhistidine, MeHis; alanine, Ala; arginine, Arg; asparagine, Asn; aspartate, Asp; glutamate, Glu; glutamine, Gln; glyoxylate, Gly; histidine, His; isoleucine, Ile; leucine, Leu; lysine, Lys; methionine, Met; phenylalanine, Phe; proline, Pro; serine, Ser; threonine, Thr; tyrosine, Tyr; and valine, Val), urea and glucose. Plasma cortisol was measured by a double-antibody radioimmunoassay technique according to Massip et al (1977). The antiserum used was raised against the conjugate cortisol-3-O-carboxymethylxime BSA (Sigma, UK). The specificity of antiserum was evaluated by determining the relative cross-reaction with other steroids: cortisol 100%, cortisone 0.2%, 11-deoxycortisol 10%, corticosterone 10%, 11-deoxycorticosterone 1.8%, progesterone 1%, 17-hydroxyprogesterone 0.8% and aldosterone < 0.01%. A mixing of 0.1 ml plasma and 0.9 ml physiological saline was extracted with 10 ml distilled dichloromethane by centrifugation at 2 000 g for 10 min. After evaporation to dryness and incubation overnight at 4°C with antibody and [3H]cortisol, antibody-bound and unbound steroids were separated by addition of charcoal (0.5% suspension). A
dose–response curve for 5 pooled bovine plasma samples was parallel to the cortisol standard curve. The sensitivity of the assay was 7 pg per tube and extraction efficiency, as measured by the recovery of [3H]cortisol from plasma, averaged 96.8%. The intra- and interassay coefficients of variation were 5 and 13%, respectively. AA concentrations were determined using a rapid high-performance liquid chromatographic method, as previously described by Cunico et al (1986). Glucose was assayed in duplicate by a glucose hexokinase procedure as described by Schmidt (1961). Plasma urea was measured by the diacetyl-monoxime method (Henry, 1974).

**Statistical analysis**

Results are noted as mean ± standard error (SE). Significances of differences between means of measurements for saline and ACTH treatment, and between the values before and after saline or ACTH administration were assessed by analysis of variance for repeated measures, and with treatment and time of sampling as main effects according to SAS (1989). Cows effects were assumed to be random. In all cases, we chose to reject the null hypothesis at the 5% level ($P < 0.05$).

**RESULTS**

**Cortisolemia**

As shown in figure 1, a rapid positive response ($P < 0.05$) in plasma cortisol occurred within 10 min of ACTH administration (2.34 ± 0.26 μg/l 5 min pretreatment vs 37.82 ± 4.06 μg/l 10 min post-treatment). There were also significant differences ($P < 0.05$) between ACTH- and saline-treated groups from 10 to 240 min post-administration (fig 1). The maximum increase in plasma cortisol concentration, as a result of ACTH stimulation, occurred at 1 h after injection (64.83 ± 4.52 μg/l, fig 1). Plasma cortisol returned to the baseline within 5 h post-treatment. However, there was a significant ($P < 0.05$) elevation in plasma cortisol at the last blood sampling, which corresponded to the feeding and milking times (fig 1).

Two features of cortisol response to saline administration were noted. First, the injection of 5 ml physiological saline intra-
venously caused a slight and non-significant increase in plasma cortisol. This response was short-lived, returning to baseline by 1 h (fig 1). Secondly, plasma cortisol concentrations were relatively stable at all other blood collection times, except at the last blood sampling where there was a significant ($P < 0.05$) increase in plasma cortisol, as after ACTH treatment (fig 1).

**Milk yield**

A single injection of 6 IU ACTH given to 4 milking cows had no statistically significant effect on the milk yield. The average milk production per 24 h was 17.20 ± 1.71 the day prior to ACTH administration, 16.83 ± 2.11 the day of treatment, and 16.61 ± 1.11 the day immediately after treatment. No significant changes could be detected in daily milk yield either before and up to 7 d after ACTH administration or between ACTH- and saline-treated groups.

**Plasma amino acids**

Plasma AA concentrations following ACTH administration are presented in table I. In comparison with the baseline values, the most common pattern was a decrease in aminoacidemia, significant ($P < 0.05$) for Pro, within the first 30 min after treatment. Thus, of the 19 AA examined, only Asn, Lys, Met and the branched-chain amino acids (BCAA: Ile, Leu, Val) did not show such a trend. In comparison with the isotime values obtained after saline treatment, ACTH administration brought about a significant ($P < 0.05$) decrease in the plasma concentrations of Ala (at 2 and 5 h post-treatment), Asp (at 4 and 5 h), Glu (at 1 and 3 h), Gln (at 5 h) and Pro (at 4 h) (table I). In contrast the plasma concentrations of Gly (at 4 h post-treatment), His (at 5 and 6 h), Ile (at 2, 4, 5 and 6 h), Leu (at 3 and 5 h), Lys (at 6 h) and Val (at 3, 5 and 6 h) increased ($P < 0.05$) after hormone administration (table I). There was also a significant ($P < 0.05$) increase in plasma MeHis concentrations at 1, 2, 3 and 5 h after ACTH treatment (fig 2). Only Arg, Asn, Met, Phe, Ser, Thr and Tyr failed to demonstrate any significant changes between pretreatment and post-treatment values or between ACTH- and saline-treated groups (table I).

**Plasma glucose and urea**

As figure 3 shows, and when compared with baseline values, there was a tendency to a decrease in glucose levels within 30 min post-ACTH treatment. After this, glycemia started to increase, with maximum concentration (4.38 ± 0.14 mM) occurring 4 h after hormone administration. Moreover, this glucose peak value was significantly ($P < 0.05$) higher than the isotime value recorded after saline administration (fig 3). However, there were no changes in plasma glucose levels after ACTH or saline treatments, due to the feeding time (fig 3).

ACTH had no or little effect on plasma urea concentrations, although a trend to an increase within the first 60 min following treatment was observed.

**DISCUSSION**

Our investigation indicated that an ACTH dose as small as 6 IU elicited a rapid increase in plasma cortisol of lactating cow, with the mean plasma cortisol rising from a resting level of 2.34 ± 0.26 to 61.22 ± 4.79 μg/l at 60 min post-treatment. This agrees with results reported by Van der Kolk et al (1991) and Ndibualonji et al (1994) in lactating and nonlactating cows, respectively.

Alam et al (1986) tested different doses of ACTH in nonlactating cows and observed maximum cortisol values of 20.4 and 25.3 μg/l, following injection of 6 and 100 IU,
Table I. Evolution of plasma amino-acid concentrations (μM) after ACTH treatment in lactating cows.

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<th>Non-essential amino acids</th>
<th>-60 a</th>
<th>-5 b</th>
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<th>+20</th>
<th>+30</th>
<th>+60</th>
<th>+120</th>
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<th>+240</th>
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<td>175 ± 13†</td>
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<td>182 ± 13*</td>
<td>206 ± 14†</td>
<td>200 ± 17</td>
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<td>13 ± 1†</td>
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<td>91 ± 5†</td>
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<td>Gly</td>
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<tr>
<td>Ile</td>
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<td>96 ± 5</td>
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<td>189 ± 9</td>
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<td>250 ± 14</td>
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</table>

All enumerative data in the table are presented as means ± SE, with n = 4. a,b Control samples. * In the same line, significantly different from both control values (P < 0.05); † For the same amino acid, significantly different from isotime value recorded after saline treatment (P < 0.05).
respectively. These workers also observed that 6 IU ACTH caused the release of a slightly higher concentration of cortisol than 5.0–10.0 μg/l released by other stimuli such as the approach of a stranger. They concluded that 6 IU ACTH may be useful for testing various physiological states of the adrenal glands in cows. These results indicate that in cows the plasma corticosteroid response is no greater during severe stress than the response following administration of 6 IU ACTH. Therefore, the use of doses of ACTH larger than 6 IU, in attempts to simulate stress in dairy cows, is not justified.

The short-lived response of plasma cortisol observed in this study when cows were treated with 5 ml physiological saline agrees with other reports (Van der Kolk et al, 1991; Ndibualonji et al, 1994). Interestingly, Alam et al (1986) observed that an intramuscular injection of saline in cattle releases similar amounts of cortisol as a 3 IU ACTH dose. This provides a reason not to use ACTH doses lower than 6 IU in cattle.

After both saline and ACTH administration, there was a significant increase in plasma cortisol at the final blood collection, which corresponded to the feeding and milking times. Since it has been shown in sheep (Symonds et al, 1989) and cattle (Abele et al, 1992) that feeding has no effect on plasma cortisol concentration, the slight hypercortisolemia observed in this work may be attributed to the milking stimulus. This is in accordance with previous observations in lactating cows (Koprowski and Tucker, 1973; Paape et al, 1977) showing that machine milking is associated with a peak in plasma cortisol. In addition, such phenomena may be assimilated to suckling stimuli which have been reported to induce the release of corticosteroids in rat (Voogt et al, 1969). It has thus been suggested that both suckling (Voogt et al, 1969) and milking (Koprowski and Tucker, 1976) stimulate nerve endings in the nipples which send impulses to the brain via the spinal cord, resulting in the sequential release of ACTH-releasing factor, pituitary ACTH, and finally adrenal corticosteroids.

Until 7 d after administration of ACTH, no significant changes were found in milk

![Fig 2. Effect of 6 IU ACTH administration on plasma 3-methylhistidine in 4 lactating cows (◊ saline; □ ACTH). For the sake of clarity, some standard errors have been omitted in this and the subsequent figure. See figure 1 for key.](image-url)
production. This confirms results from Van der Kolk et al (1991) using the same ACTH dose. Since it has been demonstrated above that plasma cortisol response is not greater during severe stress than following administration of 6 IU ACTH, it seems more likely that the decrease in milk yield noted by Brush (1960), Campbell et al (1964), and Bremel and Gangwer (1978), following injection of at least 160 IU ACTH in lactating cows, was due to a longer duration of ACTH action, resulting in longer exposure of the animal to adrenal corticosteroids. The results of the present study support previous findings showing that a test dose of 6 IU ACTH can be regarded as useful in a standard test for adrenocortical function in dairy cow as no effect on milk production was observed and the plasma cortisol reached a peak value 60 min following administration.

Compared to the saline-treated group, administration of 6 IU ACTH significantly increased the plasma concentrations of Gly and the essential amino acids (EAA) His, Ile, Leu, Lys and Val, and decreased those of the non-essential amino acids (NEAA) Ala, Asp, Gli, Gln and Pro. The data reported here constitute the first description of individual AA metabolism after ACTH administration in cattle and is comparable in many respects to the work from Slater and Mellor (1977) in sheep receiving an ACTH infusion at a rate of 0.25 IU/h for 2 d. Since these authors’ attention has been directed to changes taking place within a relatively long time, they reported much larger changes in plasma AA than those observed in the present study.

The elevation of plasma BCAA, Gly, His and Lys suggests an increased rate of body protein mobilization. This is supported by a significant increase in plasma MeHis in the ACTH-treated group. This AA is closely associated with skeletal muscle metabolism. In cattle, more than 93% of the MeHis formed in the body is present in skeletal muscle (Nishizawa et al, 1979). The methylation of His occurs after its incorporation into the peptide chains of actine and myosine, and, after catabolism of these myofibrillar proteins, the liberated MeHis is not recycled but quantitatively excreted into urine. In plasma, MeHis is an intermediate between myofibrillar protein-bound MeHis.
and urinary MeHis. Recently it has been shown in fasted goat that, like urinary MeHis, plasma MeHis also reflects the increased rate of myofibrillar protein degradation (Nagasawa et al, 1993).

The apparent reason for the significant decrease in plasma Ala, Asp, Glu, Gln and Pro might be that those compounds are readily removed by the liver and are used, at least in part, as glucose precursors since all these NEAA are gluconeogenic in cattle (Ndibualonji and Godeau, 1993).

Most of the changes in plasma AA concentrations after ACTH administration might be attributed to the increase in plasma cortisol. This agrees with the accepted notion that glucocorticoids stimulate protein catabolism and gluconeogenesis in response to a decrease in blood glucose (Bassett, 1968; Munck et al, 1984). Klasing (1985) summarized data showing the effects of stress and glucocorticoid administration on protein metabolism. He concluded that both stress and administration of glucocorticoids result in a net catabolism of muscle protein.

The tendency to a decrease in plasma glucose and many AA within the first 30 min after ACTH administration is another effect of ACTH that has not been previously reported in any species. In comparing the patterns of metabolite response to ACTH treatment, it seemed that the magnitude of response was greater from 2 to 6 h post-treatment in terms of the number of parameters differing significantly from baseline values and from isotime values recorded after saline treatment. These data suggest that, after a common early decrease, a shift occurred between 2 and 6 h post-hormone administration, ACTH-treated cows generally demonstrating higher plasma concentrations for EAA and glucose, and lower ones for NEAA. In view of several considerations, it is possible that different levels of the pancreatic hormones insulin (hypoglycemic hormone) and glucagon (hyperglycemic hormone), which are dependent on an increased plasma cortisol concentration, may account for the different responses of plasma metabolites to ACTH administration.

Unfortunately, plasma insulin and glucagon concentrations were not measured in this experiment. However, some support of this view is given by the study from Bassett and Wallace (1967) who observed that an intramuscular injection of 75 mg cortisol prior to feeding of sheep considerably altered the postprandial pattern of plasma insulin and glucose. Thus, feeding in the control group resulted in little variation of plasma glucose within the first 8 h. However, in the cortisol-treated group, there was a decline in glycemia within 1 h of treatment while an elevation in plasma insulin concentrations, larger in magnitude than that observed in control group, occurred at the same time. Afterwards, plasma glucose increased during the next 6 h, while insulinemia returned to pretreatment levels, and there was no subsequent increase in this last parameter until 8 h after treatment, despite the steady increase in glucose concentration between 2 and 8 h post-cortisol administration. Furthermore, Bassett and Wallace (1967) also observed a rapid increase in plasma insulin concentration after cortisol administration in fasted sheep. Consequently, it might be that in the present study with cows, an early increase in plasma insulin occurred within the first 30 min post-ACTH administration, which could explain the observed tendency to a decrease of plasma metabolites. On the other hand, the increase in some EAA and glucose and the decrease in some NEAA between the second and the sixth hours post-ACTH administration are consistent with a decline in circulating insulin concentration such as would be expected to occur. Another work (Barseghian and Levine, 1980), using isolated perfused rat pancreas, showed that corticosterone, the main glucocorticoid in rat, inhibits the secretion of insulin while it stimulates that of glucagon in this species, demonstrating that gluco-


Corticoids have direct effects on pancreatic islet cells. Therefore, Munck et al. (1984) suggested that the basic antagonism of glucocorticoids to insulin underlies their metabolic role in stress.

Consistent with earlier studies on stress (Pearson and Mellor, 1975; Bird et al., 1981; Mitchell et al., 1988) and ACTH administration (Phillips et al., 1991), plasma glucose started to rise 1 h post-treatment when cortisol levels had maximized and reached the peak value about 3 h later, indicating that the glucose concentration is related to the plasma cortisol concentration.

Other known effects of cortisol administration in the ruminant that would have increased glycemia are a decrease in peripheral utilization of glucose (Braun et al., 1970; Reilly and Black, 1973), and an increased utilization of free fatty acids and ketone bodies for caloric needs (Bassett, 1968; Bird et al., 1981). These responses are consistent with the animals sparing glucose by utilizing alternative substrates for oxidative purposes.

As observed in stressed sheep (Slater and Mellor, 1977), the mean urea concentration was not altered after ACTH administration in this experiment. It is known that the kidney possesses specific mechanisms to modify excretion or retention of urea according to metabolic needs of the animal (Harmeyer and Martens, 1980). The cows used in the present study were fed beyond their energy and nitrogen requirements for body maintenance and milk yield. It is thus possible that the extra urea resulting from an increased AA degradation in the intermediate metabolism was excreted with urine rather than transferred into the rumen. This is supported by an increased rate in urinary nitrogen excretion observed following cortisol administration in fed sheep (Bassett and Wallace, 1967).

In conclusion, the results of the present experiment on individual AA metabolism in the lactating cow demonstrate that the bovine species behaves like other mammals with respect to its metabolic response to stress. Thus, during stress-induced increase in plasma cortisol concentration, protein catabolism in skeletal muscle is increased and AA such as Ala, Asp, Glu, Gin and Pro are rapidly taken up by the liver and utilized as substrates for gluconeogenesis in response to an early decrease in plasma glucose.

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