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EFFECTS OF TRANSPORTATIONS, A HIGH LACTOSE DIET AND ACTH INJECTIONS ON THE WHITE BLOOD CELL COUNT, SERUM CORTISOL AND IMMUNOGLOBULIN G IN YOUNG CALVES

By

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SIMENSEN, E., B. LAKSESVELA, ANNE KRISTINE BLOM and Ø. V. SJAASTAD: *Effects of transportations, a high lactose diet and ACTH injections on the white blood cell count, serum cortisol and immunoglobulin G in young calves.* Acta vet. scand. 1980, *21*, 278—290. — Twelve calves were subjected to 5 different, consecutive treatments considered to induce stress. These included a) transportations for 4—8 h at temperatures just above or well below 0°C at the start of the experiment and 6 and 11 weeks later, b) feeding a high lactose diet and c) ACTH injections.

The transportations resulted in a transient suppression in the level of serum IgG, and increase of cortisol, blood neutrophils and lymphocytes. One and 2 weeks after the first transportation, the lymphocyte count was suppressed.

Intramuscular injections of ACTH also resulted in temporary increases in serum cortisol, blood neutrophils and lymphocytes, but no obvious changes in IgG. Thus, the suppressed levels of IgG which were seen after the transportations did not appear to be directly related to the increased level of cortisol.

The high lactose diet was not accompanied by a significantly higher frequency of diarrhoea and lower mean weight gain. The IgG level did not appear to be influenced by the lactose level of the diet, and calves fed a high lactose diet responded similarly to transportation as calves fed a normal type of milk replacer. Apart from some diarrhoea, no health problem occurred.

transportations; lactose levels; ACTH; cortisol; IgG; blood neutrophils; blood lymphocytes; calves.

There is evidence in the literature that stress may play an important role in the pathogenesis of infectious diseases in young animals. Thus in calves, development of diseases after transfer from their usual environment is recognized as a problem (*Sinha & Abinanti* 1962).

Several investigations have been carried out to study the stressing effect of transportation in young calves. The level of circulating glucocorticoids and the white blood cell count have frequently been used for measurement in such studies.

Transportation has been found to modify the immunologic response to antigen injections (*Hartmann et al.* 1976). Possibly, therefore, transportation stress may influence the level of circulating immunoglobulins. However, studies in order to establish such a relationship has not hitherto been reported.

Together with transfer of calves to a new environment there is usually a change of diet. The type of feed after purchase has been reported to influence death rate (*Staples & Haugse* 1974) as well as the frequency of diarrhoea (*Weiher et al.* 1975, *Slagsvold et al.* 1977), and may thus aggravate the transportation stress.

The aim of the present experiment was to study a) the stressing effect of transportation in young calves, in particular by assessing the levels of circulating immunoglobulins, b) a possible interaction of transportation and a high lactose diet tending to increase the frequency of diarrhoea, c) whether such a diet influences the immunoglobulin levels and d) whether ACTH injections influence the immunoglobulin levels.

MATERIALS AND METHODS

Experimental procedure

Twelve male calves of the Norwegian Red Cattle Breed (NRF), 17 days old on average, were subjected to a 5-stage treatment:

Stage 1 — transportation over 2 days from local farms to the experimental farm
Stage 2 — diets with different levels of lactose
Stage 3 — transportation and diets with different levels of lactose
Stage 4 — transportation and ACTH injection
Stage 5 — 4 days of ACTH injections

The 5 stages of the experiment are outlined in Table 1.

Table 1. An outline of the treatments to which the 12 calves were subjected during the 5 stages of the experiment.

Stage	Treatment	Day of experiment	Comments
1	Transportation 1[a]: 5 to 30 km to a market place barn	0	Outdoor temperature about 2°C
	Transportation 2[a]: 320 km for 8 h to the experimental farm[b]	1	
2	Group 1[c] (Diet 1): A regular milk replacer	3—49	Diet 1: 64 % skim milk powder, 20 % sweet whey powder, 15 % butter
	Group 2[c] (Diet 2): A high lactose milk replacer		Diet 2: 30 % lactose, 40 % skim milk powder, 14 % sweet whey powder, 15 % butter
3	Transportation[a]: 190 km for 4 h	42	Outdoor temperature about 1°C
4	Transportation[a, d]: 140 km for 4 h	77	Outdoor temperature about —7°C
	ACTH[d]: 1.0 i.u./kg i.m.		
5	ACTH[e]: 0.7 i.u./kg i.m.	84, 85, 86, 87	The experiment was terminated on day 105
	Controls: No extra treatment		

[a] Transportation in a covered truck with tight sidewalls.
[b] On the experimental farm the calves were tied up in individual stalls with metal grates in a heated building.
[c] The calves were divided evenly in Group 1 and Group 2, according to age and live weight.
[d] Three of the calves in Group 1, and 3 of the calves in Group 2 of Stage 2.
[e] The same calves as those which were given ACTH in Stage 4.

In addition to the main ingredients in Diet 1 and Diet 2 of Stage 2 (Table 1), the diets contained 0.8 % emulsifiers and 0.5 % mineral premix (*Slagsvold et al.* 1977). The milk replacers were introduced to all calves over a period of 3 days, and the solid materials were dissolved 1:10 in tepid water, mixed mechanically and administered in quantities according to metabolic live weight. The daily allowance averaged 4.9 l in the first week, 6.3 l in the second, 6.8 l in the third and 7.0 l during the remainder of the 46-day period. From day 15 and onwards, all calves were given hay and tepid water according to appetite. The hay quality, examined as described by *Laksesvela et al.* (1977) was characterized as medium to coarse. Ground barley was also fed to appetite from day 12 to 21, thereafter being rationed. Daily allowances of barley, administered according to metabolic live weight, were increased from an average of 490 g during the third week to 730 g in the last week.

Clinical and biochemical studies

Blood for obtaining serum, but not for white blood cell count, was obtained on the local farms on day 0 before the transportation. On day 1, 42 and 77 blood was drawn shortly before and after the transportations. The calves treated with ACTH were bled immediately before the injections, and on day 77 and 84 they were also bled 4 h after the injection. The pre-treatment blood samples, as well as the remaining samples were drawn at approx. 9 a.m.

Serum cortisol was determined by radio-immunoassay using the principles outlined by *Simensen et al.* (1978) for corticosterone. White blood cells were counted in an electronic cell counter (Cellescope 101, AB Lars Lundberg, Sweden). Blood smears stained with the May-Grünwald Giemsa method (*Niepage* 1974), were used for differential count of the white blood cells. Serum IgG was determined by single radial immunodiffusion (*Mancini* 1965). Furthermore, GOT, GPT, haematocrit and haemoglobin were also measured.

During Stage 2, the consistency of the faeces was graded according to a scale ranging from 1, very solid, to 5, profuse diarrhoea. Grades 4 and 5 were both considered as diarrhoea.

The results were analyzed statistically using Student's t-test. Levels of $P < 0.05$ were considered as being significant.

RESULTS

Cortisol (Fig. 1)

All the transportations resulted in a significant, but short-lasting increase in the average cortisol level. A similar increase was seen upon 1 injection of ACTH, whereas repeated ACTH injections (Stage 5) resulted in suppressed levels.

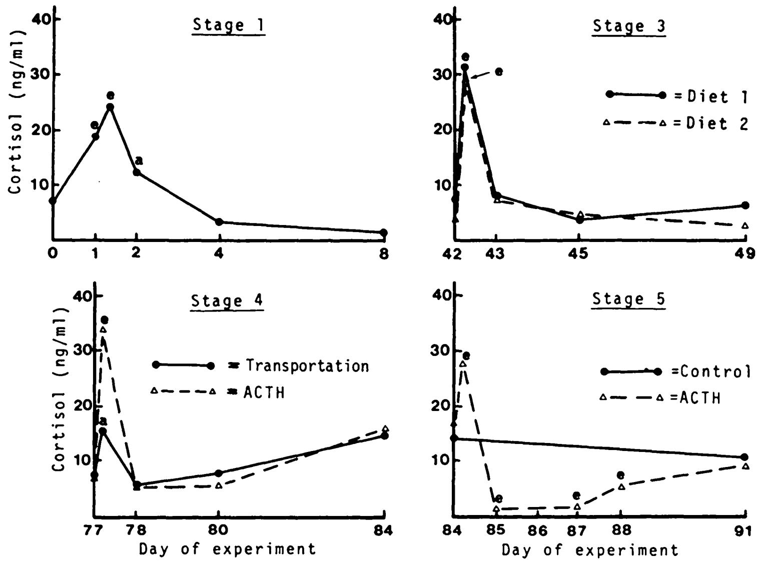
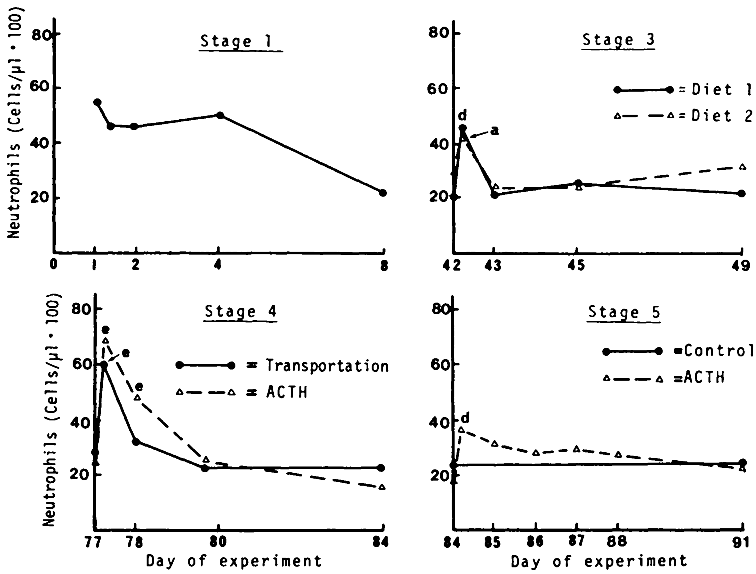


Figure 1. Effects of transportations and ACTH injections on serum cortisol level.
Stage 1, 3, 4 and 5 as outlined in Table 1. Mean values for 12 calves in Stage 1 and for 6 calves in each group in Stage 3, 4 and 5. The letters a—e indicate levels being significantly different from the pre-treatment level on the first day of the respective stage for the corresponding treatment group (a = $P < 0.05$, b = $P < 0.025$, c = $P < 0.01$, d = $P < 0.005$, e = $P < 0.001$).

White blood cell count

Neutrophils (Fig. 2)

During Stage 1 the neutrophil count was markedly elevated on day 1, 2 and 4 as compared to day 8, when the level was within the same range as the general level seen later in the experiment. The day-1 count was 253 % of the day-8 count. Following the transportations in Stage 3 and 4, significant increases in the number of neutrophils were recorded. However, the counts fell



F i g u r e 2. Effects of transportations and ACTH injections on blood neutrophil count.
Stage 1, 3, 4 and 5 as outlined in Table 1. Mean values for 12 calves in Stage 1 and for 6 calves in each group in Stage 3, 4 and 5. The letters a—e indicate levels being significantly different from the pre-treatment level on the first day of the respective stage for the corresponding treatment group (a = $P < 0.05$, b = $P < 0.025$, c = $P < 0.01$, d = $P < 0.005$, e = $P < 0.001$).

to pre-transportation levels the following day. A similar increase was seen after injection with ACTH (Stage 4 and 5), though in both these cases the levels remained high when measured on the following day.

L y m p h o c y t e s (Fig. 3)

In Stage 1 the lymphocyte count on day 1 was within the same range as the general level seen later on in the experiment. A significant increase was seen after transportation on day 1, while the day-8 and day-16 counts were suppressed as compared to levels seen later. Also in Stage 3, the transportation was followed by an increase in the lymphocyte count, but only in the Diet-1 group this increase was significant. Repeated injections of ACTH in Stage 5 resulted in significantly elevated levels of lymphocytes.

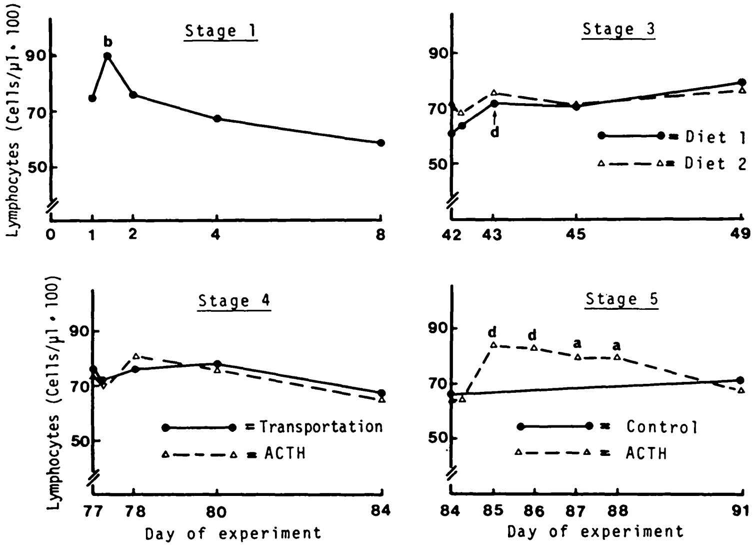


Figure 3. Effects of transportations and ACTH injections on blood lymphocyte count.
Stage 1, 3, 4 and 5 as outlined in Table 1. Mean values for 12 calves in Stage 1 and for 6 calves in each group in Stage 3, 4 and 5. The letters a—e indicate levels being significantly different from the pretreatment level on the first day of the respective stage for the corresponding treatment group (a = $P < 0.05$, b = $P < 0.025$, c = $P < 0.01$, d = $P < 0.005$, e = $P < 0.001$).

Immunoglobulin G (Fig. 4)

All transportations resulted in significant, but temporary suppressions of the average serum IgG, whereas ACTH injection did not appear to be followed by any obvious changes in the IgG levels. Diet did not appear to influence IgG levels (Table 2).

Weight gain and clinical findings (Table 2)

During Stage 2, the average weight gain was higher in Group-1 calves than in Group-2 calves. The differences between the groups were, however, not significant. Diarrhoea was somewhat more frequent in Group-2 calves. On average, the number of days with diarrhoea was 5.6 in Group 2 as compared to 4.3 in Group 1. In both groups, diarrhoea occurred most frequently during the second week of the feeding test. Apart from diarrhoea, no health problems were encountered during the experiment.

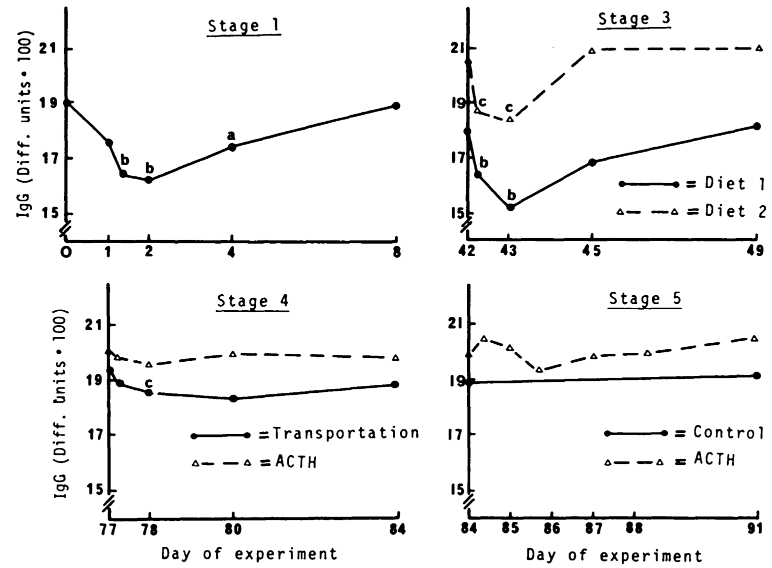


Figure 4. Effects of transportations and ACTH injections on serum immunoglobulin G level.
Stage 1, 3, 4 and 5 as outlined in Table 1. Mean values for 12 calves in Stage 1 and for 6 calves in each group in Stage 3, 4 and 5. The letters a—e indicate levels being significantly different from the pretreatment level on the first day of the respective stage for the corresponding treatment group ($a = P < 0.05$, $b = P < 0.025$, $c = P < 0.01$, $d = P < 0.005$, $e = P < 0.001$).

Table 2. Effects of diets with different levels of lactose (Stage 2). The main ingredients in Diet 1 were 64 % skim milk powder, 20 % sweet whey powder and 15 % butter. In Diet 2, 30 % lactose was substituted for 24 % of the skim milk and 6 % of the whey powders. Mean values ± s for 6 calves in each group.

		Day of experiment					
	Group	2	8	16	22	35	49
Body weight (kg)	Diet 1	42.0±10.0	46.3±9.8	50.8±11.1	55.8±13.1	66.8±15.4	75.8±17.6
	Diet 2	42.1±7.9	45.2±8.6	48.6±9.4	52.8±10.1	60.8±11.9	70.8±13.7
IgG (diffusion units)	Diet 1	1223±289	1655±293	1893±367	1967±255	1890±505	1808±375
	Diet 2	2013±365	2123±220	2218±173	1942±285	2072±548	2093±114
Accumulated number of days with diarrhoea	Diet 1	—	2	18	20	25	26
	Diet 2	—	3	20	27	32	34

DISCUSSION

The transportations in the present study were performed at temperatures around or below 0°C. As covered trucks with tight sidewalls were used, the calves were not exposed to massive draughts, and in no case they did appear to suffer from the cold. Cooling as a stress factor is therefore thought to be of minor importance in the present study.

All transportations caused a significant but short-lasting increase in the serum cortisol level. Thus the present study confirms the results of previous investigations (*Hartmann et al.* 1973, *Völker et al.* 1973, *Dvorak* 1975, *Stephens & Toner* 1975, *Johnston & Buckland* 1976). The observed increase in the level of blood neutrophil counts as a response to transportation is also in agreement with previous results (*Hartmann et al.* 1973, *Völker et al., Boss* 1974, *Verter et al.* 1975, *Ruppanner et al.* 1978). A similar response subsequent to ACTH injections has been reported (*Wegner & Stott* 1972, *Patka & Dvorak* 1975 and *Paape et al.* 1977).

Hartmann et al. (1973) registered increased blood lymphocyte counts after transportations lasting 4.5 and 6 h, whereas a suppression was observed when the transportation lasted 2.5 h. A similar suppression has been reported to follow a transportation of only ½ h (*Verter et al.*). In the present study, an increased lymphocyte count was seen following the transportation in Stage 1 and 1 day afterwards in Stage 3, but not in Stage 4. In Stage 1, suppressed levels were seen 1 and 2 weeks afterwards. In the studies of *Wegner & Stott, Patka & Dvorak* and *Paape et al.*, no significant changes in the number of blood lymphocytes after ACTH injections were demonstrated. In the present study, this was also the case in Stage 4, whereas in Stage 5, significantly elevated levels were recorded following the repeated injections of ACTH. Fluctuations in lymphocyte count as a response to transportation or ACTH were small compared to the fluctuations seen in the neutrophil count.

Besides the changes in serum cortisol and white blood cells, a significant but short-lasting suppression of serum IgG was recorded as a response to transportation. This suppression was most pronounced on the first day following the transportations. These observations indicate that changes in the level of circulating immunoglobulins may be used as a quantitative measure of the stressing effect of transportation.

Studies on the relationship between transportation and the level of circulating immunoglobulins in calves have previously not been reported. However, the influence of transportation on other aspects of the immune system has been reported by *Hartmann et al.* (1976), who found that antibody titers against Salmonella dublin were higher when calves were injected with antigen shortly after transportation than when injected 3 days later. He also found that injections of ACTH at weekly intervals, beginning simultaneously with the antigen injection, resulted in lower antibody levels at the end of a 4-week period. In newborn calves, placed in a hot, unfavourable environment, serum IgG was lower and cortisol higher at 2 and 10 days after birth than in control calves (*Stott et al.* 1976). Depressive effects on the immune system have often been associated with increased secretion of adrenal hormones. However, the data from the present study do not support a hypothesis of a direct relationship between the glucocorticoids and the immune system, since no obvious changes in IgG were seen following the ACTH injections in Stage 4 and Stage 5.

Slagsvold et al. (1977) found that the addition of lactose to the diet resulted in an increased frequency of diarrhoea in young calves. In the present study the frequency of diarrhoea was not significantly higher in the group given a high lactose diet than in the control group.

There is evidence in the literature that diets low in protein may result in decreased levels of circulating immunoglobulins (*Hudson et al.* 1974, *Slagsvold et al.*). In addition to having a high lactose content, Diet 2 in the present study was lower in protein and energy than Diet 1. However, Diet 2 did not result in any significantly lower mean body weight gain. It is therefore not surprising that no different trends were observed within the 2 groups with respect to serum IgG during Stage 2. Nor was it unexpected that no differences in the response to transportation in Stage 3 were seen.

In the present study, transportation resulted in significant changes in serum cortisol, white blood cell count and serum IgG, factors which are all thought to play an important role in the body's defence against infections. Disease problems which tend to arise after transportation or transfer to a new environment (*Sinha & Abinanti* 1962) may possibly be related to the abovementioned or other changes in the defence mechanisms.

There seems to be a complex network of interactions between the factors involved in the body's defence against infections (*Hudson et al., Besedovsky & Sorkin* 1977). Increase in ACTH and adrenal hormones as well as stress stimuli can aggravate susceptibility to many infectious diseases, whereas they seem to protect against others (*Siegel* 1974). The significance of the responses to transportation stress recorded in the present experiment is therefore difficult to interpret.

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SAMMENDRAG

Effekter av transporter, en diett med høyt laktoseinnhold og ACTH-injeksjoner på antall hvite blodlegemer, serum cortisol og immunoglobulin G hos spedkalver.

Tolv kalver ble utsatt for 5 forskjellige, påfølgende behandlinger for å påføre dem stress. Disse inkluderte a) transporter med 4—8 timers varighet ved temperaturer litt over eller noe under 0°C ved forsøkets begynnelse, 6 og 11 uker senere, b) melkediett med høyt innhold av laktose og c) ACTH-injeksjoner.

Transportene førte til en forbigående reduksjon i seruminnholdet av IgG, og en forøkelse av cortisol, neutrofile leukocytter og lymfocytter. En og to uker etter den første transporten var innholdet av lymfocytter nedsatt.

Intramuskulære injeksjoner av ACTH førte også til en forbigående forøkelse i seruminnholdet av cortisol samt antall neutrofile leukocytter og lymfocytter, men ingen entydige forandringer i innholdet av IgG. Det nedsatte nivået av IgG som følge af transportene syntes derfor ikke å være direkte relatert til forøkelsen av cortisolnivået som ble observert.

Fóring med en melkediett med høyt laktoseinnhold førte til en høyere frekvens av diarré og lavere gjennomsnittlig vektøkning, men forskjellen til gruppen som ble gitt en mer normal melkeerstatning var ikke signifikant. Det ble ikke funnet noen sammenheng mellom ulik laktosemengde i dietten og serumnivået av IgG, og de målte effekter av transport ble ikke funnet å være påvirket av diettene. Bortsett fra diarré ble det ikke registrert helseproblemer.

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