Influence of Environment on Passive Immunity in Calves

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ABSTRACT
Passive immunity in neonatal calves is influenced by environment. Placing newly born Holstein calves (108 head) in three different housing environments (shade, cooled shade, hutch) during hot weather produced differences in body temperature, serum corticosteroids, immunoglobulin IgG1 concentrations, and mortality. Experimental design permitted examination of effects due to treatments, time, differences in colostrum, and climatic environment in an analysis of variance. Calves exposed to the hotter, less desirable environment responded by having a higher mortality, higher serum corticosteroid concentration, and lower serum immunoglobulin IgG1 at 2 and 10 days after birth. All of these were correlated. Calves that died had serum immunoglobulin IgG1 falling below the mean for all experimental calves.

INTRODUCTION
Passive immunity in the neonatal bovine is dependent upon intestinal absorption of colostral immunoglobulins during the first 24 h after birth (1, 15, 16). There is wide variation in efficiency of lactoglobulin absorption. Twenty to 30% of the calves fed controlled amounts of colostrum during the first 24 h after birth remain hypogammaglobulinemic (19, 27), which results in high morbidity and mortality (2, 12, 26). Corticosteroids can influence cell permeability of the small intestine in postnatal mammals rendering them incapable of absorbing macromolecules including immunoglobulins (13, 15). On this basis, it is postulated that physiological stress can activate adrenal steroid output and restrict consequently immunoglobulin absorption during the critical 24-h postnatal period in the bovine. If true, ambient environment of the neonatal calf is extremely important in determining chances for survival.

The present experiment determined the influence of certain microenvironments during hot weather on immunoglobulin absorption, serum corticosteroid concentration, and viability of neonatal calves. Consideration is given to evaporative cooling (29) and two popular types of calf housing now in common use to ameliorate heat stress.

METHODS AND PROCEDURE
The experiment was during the summer months at a large commercial dairy near Phoenix, AZ. This provided hot weather conditions and a sufficient number (108) of calves born over a short period to satisfy the experimental design.

Calves were assigned randomly at birth to one of three housing environments. In treatment T1, calves (n = 36) were housed under a corrugated metal shade 9 m wide and 3 m high with the long dimension in an east-west orientation. There were no sidewalls so natural air currents contributed to comfort of the environment. Calves were confined individually in two rows of contiguous metal pens 2 m by 1 m. Adjacent calves were separated by cyclone fencing. The two rows of pens were separated by a 3 m work alley.

Treatment T2 (n = 36) was the same as T1 with the addition of an evaporative cooling system (29). Precooled air generated along the north side helped confine the moving air to the area occupied by the calves.

Treatment T3, calves (n = 36) were confined in portable, individual hutches built of 2.5 cm square tube and corrugated steel. The hutches were 2.4 m long by 1.2 m wide with about one-half the area covered by a metal roof sloping from 1.2 to 1.0 m above ground. The near solid sidewalls restricted natural air move-
Housing types in treatments T1 and T3 are common to this geographical area. Pens in all treatments were provided with 8 cm of fresh sand for bedding immediately prior to occupancy.

In planning the experiment, a randomized block design, as described by Cochran and Cox (9), was considered most desirable. Three calves born the same day (presumably experiencing the same climatic environmental conditions) and receiving the same pooled colostrum, were assigned randomly to the three treatments making up a block. Over 6 wk, from the first of July to the first part of August, 36 blocks were completed, thus replicating each treatment 36 times with 108 calves involved. Experimental design permitted examination of effects due to treatments, time, difference in colostrum, and climatic environment in an analysis of variance.

Shortly after the calves were born and before they were able to nurse, they were placed in one of the three experimental housing units, weighed, blood sampled, and given 2 liters of pooled colostrum. The procedure was completed generally within 2 h after the calf was born. The colostrum feeding was repeated at 12 h and again at 24 h. The pooled colostrum came from the first milking only, of usually two or more cows, sufficient to feed three calves (one block) 6 liters during their three feedings. After 24 h, the calves were fed whole milk for 3 days, then a commercial milk replacer thereafter. Blood samples were taken via the jugular vein before feeding colostrum, at 48 h after birth, and again at 10 days. Daily body temperatures (rectal), morbidity, and mortality were recorded for each animal.

Hygrothermographs continuously recorded temperature and humidity in each of the housing units. A temperature humidity index (THI) has been used as a means of joint evaluation of the effects of temperature and humidity (17). It is a derived statistic computed from any of several formula depending upon the measurement used to determine atmospheric moisture. In this text, data from continuous recordings of temperature and relative humidity were used in the formula: THI = \( t_d - 0.55 (1 - RH) (t_d - 58) \); where \( t_d \) = ambient temperature in degrees Fahrenheit, and RH = ambient relative humidity. Berry et al. (1) have determined that although the THI value above which thermal stress occurs varies with individual animals, it is considered generally to occur for mature dairy cows at about 73 to 75.

Serum glucocorticosteroids were determined by the modified competitive protein binding method of Johansson et al. (16). Double extraction with 100% ethanol was utilized to insure complete removal of glucocorticosteroids from the calves' serum. The corticosteroid binding globulin was obtained from pooled dog serum. Sterol contamination in the dog serum was minimized by filtering the binding globulin through a Sephadex G-25 column. Removal of unbound glucocorticosteroid from the competitive binding reaction was accomplished by incubation with Florisil (activated magnesium sulfate). Reproducibility was established by performing replicate determinations from the same serum samples within and between assays (11).

Blood samples were assayed for IgG1 concentrations by modifications of the radial immunodiffusion gel procedure of Fahey and McKelvey (10). For preparation of specific antisera to bovine IgG, ammonium sulfate precipitated bovine gammaglobulins from blood serum were further purified using DEAE-Sephadex (A-50) and eluted with a .01 M sodium phosphate buffer (pH 7.4). In order to isolate IgG1 for use as a standard, the IgG peak was recycled using a sodium phosphate (pH 7.4) density gradient (0 to .02) M (4, 6, 20). Rabbits were then immunized (23, 30). The rabbit antibovine IgG specificity was determined both immunoelectrophoretically and by Ouchterlony plates (30).

For analyzing colostrum, casein was precipitated with rennin, whey separated from the solids by centrifugation, and the radial immunodiffusion gel technique used to determine the concentration of IgG1 in the whey (10, 19).

Efficiency of immunoglobulin absorption for experimental calves was obtained by calculating the ratio (expressed as percentage) of the quantity estimated in circulation after absorption was complete to the quantity fed in colostrum. The quantity in plasma was obtained by calculating the product of peak concentration of immunoglobulin in the serum (corrected by subtracting the appropriate prefeeding concentration) and plasma volume. The procedure has been used by Husband et al. (13).
RESULTS AND DISCUSSION

Stress Conditions

The daily peak temperature-humidity index (THI) for the housing treatments during the period when newborn calves were introduced into the experiment is shown in Fig. 1. Average peak THI values for treatments T1, T2, and T3 were 82.4, 81.2, and 84.5. A THI value of 73.0 is the maximum for the comfort range of bovine (18). During the day when ambient temperatures were highest, all treatments were above the comfort zone. The THI for the cooled group, T2, was lowest in all cases except on three separate days during temporary power outages. On these days, temperatures rose above 38 C for a brief period and the daily THI peak then rose above that of T1. Low THI’s at night were similar in all treatments. Calves in hutches conceivably had a slight advantage at night through exposure to cool sky.

Differences in THI among the three treatments were reflected in body temperatures of experimental calves (Table 1) by day 2 postnatal. The cool shaded animals (T2) had the lowest average body temperature (39.2 ± .15) followed by the shaded animals (T1, 39.5 ± .08) and then the animals housed in hutches (T3, 39.8 ± .10). However, these subtle differences among treatments in body temperature were not significant (P>.05).

Mortality

Mortality reached 25% (nine deaths) for the T3 treated calves by day 20 after birth. For the shaded animals (T1), one died and for the cool shaded (T2) two succumbed. Differences among treatments are significant (P<.05). All calves which died before 20 days, except one, had IgG1 immunoglobulin of serum below the least squares mean (Table 2) for their respective treatment at 2 days.

IgG1 Concentrations in Serum

Least squares mean for treatments T1, T2, and T3, at three different postnatal periods (0 days (before colostrum feeding), 2 days, and 10 days), have been computed for IgG1 (Table 2) and corticosteroids (Table 3) in calf serum. On the day of birth and before receiving colostrum, IgG1 concentrations in serum were negligible, and no differences were detected among treatments and blocks (P>.05). The calves were not, however, totally agammaglobulinemic at birth. Other authors (14, 19, 22) have observed that small amounts of immunoglobulins are synthesized by the bovine fetus.

At 2 days of age and after being fed colostrum for three feedings (up to 24 h after birth), IgG1 in serum of experimental calves increased as expected from colostral intake (Table 2) but varied widely from calf to calf relative to efficiency of absorption of colostral IgG1 (13) (1.4 to 65.3). There was no significant difference in percent absorption of IgG1 between treatment totals. However, there were significant differences between treatments with-

| TABLE 1. Calf body temperature influenced by three housing environments at three periods after birth. |
|---------------------------------|---------|---------|---------|
|                                 | 0 days  | 2 days  | 10 days |
| Shade only                      |         |         |         |
| T1                              | 39.6    | .08b    |         |
| T2                              | 39.2    | .13     |         |
| T3                              | 39.6    | .09     |         |
| Cooled shade                    |         |         |         |
| T1                              | 39.5    | .08     |         |
| T2                              | 39.2    | .15     |         |
| T3                              | 39.8    | .10     |         |
| Hutches                         |         |         |         |
| T1                              | 39.7    | .08     |         |
| T2                              | 39.5    | .18     |         |
| T3                              | 39.8    | .10     |         |

a Differences among treatments and time periods are not significant (P>.05).
b X and SD.
TABLE 2. Least square means (mg/ml) of serum IgG1 from calves in three housing environments at three periods after birth.

<table>
<thead>
<tr>
<th>Time-days</th>
<th>Housing treatments</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>0</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>25.5</td>
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<tr>
<td>10</td>
<td>19.4</td>
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L.S.D. (.05) = 4.38.

in blocks, thus further validating a major objective of the experimental model, to separate the colostrum effects (including the immunoglobulin concentration) from ability of calves to absorb immunoglobulins. The least squares means for treatments at day 2 indicated a significant difference (P<.05) between IgG1 concentrations in serum in calves housed with the shaded housing (T2, 25.5 mg/ml) over those in calf hutch (T3, 18.6 mg/ml). Calves in the cooled shade (T1, 22.0 mg/ml) also had a higher concentration of IgG1 than the calves in T3. The difference was not significant (P>.05) although the inverse trend of heat stress to IgG1 concentration in serum was evident. No differences in serum IgG1 were detected among calves at the three different bleedings in the cooled shade (T2) versus the shade only (T1).

Serum IgG1 at 10 days after birth (Table 2) indicated a significant difference (P<.05) between IgG1 concentrations in serum in calves housed with the shaded housing (T2, 25.5 mg/ml) over those in calf hutch (T3, 18.6 mg/ml). Calves in the cooled shade (T1, 22.0 mg/ml) also had a higher concentration of IgG1 than the calves in T3. The difference was not significant (P>.05) although the inverse trend of heat stress to IgG1 concentration in serum was evident. No differences in serum IgG1 were detected among calves at the three different bleedings in the cooled shade (T2) versus the shade only (T1). Serum IgG1 at 10 days after birth (Table 2) for all treatments was proportionately less (23 to 31%) than at 2 days. Klaus et al. (19) and Husband et al. (13) found a similar reduction of passive immunoglobulins in calf serum. The rapid decline in IgG1 concentrations of serum over 8 days is of considerable interest because of the expected related loss in passive immunity and the role stress may have in decreasing the concentration of passive immunoglobulins or suppressing endogenous synthesis of new immunoglobulins (7, 8). Until active immunity begins in the calf at 20 to 30 days of age (12, 14, 20), a further decline in immunoglobulins of serum could be expected. In animals which are hypogammaglobulinemic from limited transmission of lactoglobulins, further depression for 3 or 4 wk could place them in serious jeopardy to challenging pathological organisms (2, 3, 12, 26).

Serum Corticosteroids

Concentrations of corticosteroids in serum at day 0 were higher in T3 calves (Table 3) (T3, 56.2 ng/ml versus T1, 41.6 ng/ml, and T2, 41.6 ng/ml). Blood samples were taken within an hour after calves were placed in the experimental microenvironment before feeding colostrum. Even so, the hyperadrenocorticism in T3 reflects the immediate sensitivity of calves to the differences in environment, presumably the higher ambient temperature. Calves in all treatments (Table 3) had high concentrations of serum corticosteroid on the day of birth, then seemed to stabilize lower as they became older. At 10 days postnatal, calves in the T3 environment were still in a state of hyperadrenia as judged by the high concentration (31.2 ng/ml) of corticosteroids in serum compared to the other two treatments (T1, 16.4 ng/ml and T2, 14.4 ng/ml).

SUMMARY

Experimental calves being exposed to higher ambient temperatures (T3) have slightly higher body temperatures, higher corticosteroids in serum, lower IgG1 concentrations in serum, and higher mortality rate. These results and others (13, 15) indicate that T3 treated calves, which were under a greater stress due to a hotter and less desirable environment, responded with a higher secretion of adrenal steroid during neonatal stages. Elevated peripheral corticosteroids may suppress permeability of the small intestine to absorption of colostral IgG1.

Significant intrablock and intratreatment correlations (28) (P<.05) are percent IgG1.
absorption vs. IgG₁ serum concentration (.62) and corticosteroid serum concentration (.56); IgG₁ serum concentration vs. corticosteroid serum concentration (.73) and body temperature (.39); corticosteroid serum concentration vs. body temperature (.41); body temperature vs. peak daily temperature (.75) and THI (.31); and THI vs. peak daily temperatures (.83).

Results of this experiment provide evidence that environment may influence passive immunity of the neonatal calf. Though ambient temperature and humidity are the adverse environment factors involved, other conditions which could stimulate hyperadrenalemia, i.e., pain, fear, and apprehension during the first 24 h of life, could be as effective. Optimal environmental conditions for the neonatal calf should be explored further.

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