

Market stress-associated changes in serum complement activity in feeder calves

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SUMMARY

Classical hemolytic complement (C) of calves was analyzed during a protocol designed to imitate the usual market handling of feeder calves from the southeastern United States. Serum C concentrations of the calves ($n = 100 \times 4$ years) were evaluated on their farm of origin, on arrival at an auction market, on arrival at a feedyard, and during their first 4 weeks in the feedyard. Complement concentrations (measured in CH_{50} units) were typically lowest at the farm of origin and highest when the calves entered the auction market 28 to 133 days later. Serum C concentrations decreased after the calves encountered the severe stresses of being in the auction market for 7 days, 24-hour truck transport (1,932 km) to the feedyard, and the first 7 days in the feedyard. The C concentrations recovered after 21 to 28 days in the feedyard. Steers had significantly ($P \leq 0.05$) lower C concentrations than did heifers in 3 of 4 years at the farm of origin, and in 2 of 4 years at the auction market. Morbid calves had significantly ($P \leq 0.05$) lower C values than did healthy calves on day 7 in the feedyard in 3 of 4 years. There were significant differences in C concentrations of calves from different farms of origin in each of the 4 years. There was no significant difference in C concentrations of calves that were vaccinated vs those not vaccinated with *Pasteurella haemolytica*.

The classical complement (C) system has an important role in many host defense mechanisms against microbial invasion.¹ This system is involved in several in vivo biologic activities including cytolysis, virus neutralization, chemotaxis, opsonin-mediated immune adherence, immediate hypersensitivity reactions, and certain autoimmune diseases.² Serum C cascade is an important factor involved in specifically acquired immunity (C1 to C9) and nonspecific immunity (C3 to C9).³ Despite its contribution to antimicrobial host defense, the effect of stress on C and the role C has in the bovine respiratory disease complex (BRDC) have not, to our knowledge, been determined.

Pasteurella haemolytica serotype 1 (Ph1) is the most important bacterium involved in BRDC.⁴⁻⁷ This bacterium

is responsible for most feeder calf pneumonic deaths and chronic pneumonias, which severely impact the feeder calf industry.⁸ In a previous study,⁹ it was determined that marketing stress had an effect on blood bactericidal activity in calves. Complement's role in bactericidal activity is well-known, and because of its role in immunity, it was of particular interest to compare C concentrations in healthy calves with those in calves affected with BRDC.

The purposes of the study reported here were to determine normal classical hemolytic C concentrations in feeder calves and to determine whether these concentrations were affected by market stress, BRDC-related morbidity, gender, experimental Ph1 vaccination, or farm of origin.

Materials and Methods

Calves and processing procedures—One-hundred calves of English breeds were purchased annually for 4 years from 3 to 7 cow-calf producers in eastern Tennessee. Calves were privately contracted to be purchased at premium fall prices. The extra profit was incentive for producers to allow us to process their calves one time on the farm of origin before the calves were transported to an auction market. Calves of both genders were estimated to weigh 56 to 158 kg on the farm of origin at initial processing. In year 4 of the study, calves were transported to a centrally located scale, individually weighed, and returned to their farm of origin.

On the farm, calves were separated from their dams, identified by ear tag, and vaccinated against *Clostridium chauvoei*, *C novyi*, *C septicum*, *C sordellii*, and *C perfringens*. Every other calf was vaccinated with an experimental Ph1 vaccine.¹⁰ Blood samples were obtained from all calves by jugular venipuncture. Nasal turbinate mucus specimens were obtained from all calves by use of swabs. Male calves were castrated, and all calves were treated for parasites with ivermectin (1 ml/50 kg of body weight, SC). After processing, the calves were returned to their dams.

Calves were transported 8 to 32 km to an auction market. At the market, calves were weighed and again processed. All calves were vaccinated against infectious bovine rhinotracheitis and parainfluenza-3. Lost ear tags were replaced, and calves previously vaccinated with Ph1 were given a second inoculation, except during the third year, when Ph1 vaccination was given only at the farm of origin. Blood samples were obtained from the jugular vein. The PCV was determined for 30 calves from various farms in the first year.

After processing, calves from all contracted farms were commingled at the auction market, and 30 to 60 local sale

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Table 1—Serum classical complement* activity in market-stressed feeder calves across evaluation days: yearly least-square mean by gender, morbidity status, and *Pasteurella* vaccination status

Variable studied	Year 1			Year 2			Year 3			Year 4		
	Mean of 7 evaluation days			Mean of 7 evaluation days			Mean of 6 evaluation days			Mean of 6 evaluation days		
	No. of calves	CH ₅₀		No. of calves	CH ₅₀		No. of calves	CH ₅₀		No. of calves	CH ₅₀	
	LSM	Range		LSM	Range		LSM	Range		LSM	Range	
Gender												
Heifers	58	142.4	128 to 156	40	102.1	88 to 113	48	97.0	82 to 115	48	87.9	81 to 95
Steers	43	136.5	121 to 151	60	90.7	74 to 107	55	84.5	77 to 95	52	83.8	76 to 94
Morbidity status												
Healthy	59	139.7	126 to 153	87	99.0	83 to 114	67	89.2	80 to 96	91	86.2	78 to 95
Sick 3 d	29	134.3	110 to 154	9	90.5	74 to 111	17	88.9	79 to 97	7	87.3	71 to 108
Sick ≥ 6 d	13	141.8	114 to 167	4	98.1	63 to 117	19	88.7	75 to 111	2	78.1	61 to 105
Combined sick days	NA	NA	NA	NA	NA	NA	NA	NA	NA	9	84.8	67 to 101
<i>Pasteurella</i> vaccination status												
Vaccinated	51	140.6	128 to 151	50	97.1	83 to 111	52	90.7	80 to 100	49	85.1	78 to 93
Not vaccinated	50	138.4	116 to 153	50	95.7	80 to 108	51	90.8	80 to 109	51	86.6	79 to 97

* Adjusted least-square mean (LSM) of CH₅₀ U/ml, and range of LSM.
NA = not available.

Table 2—Serum classical complement* activity in market-stressed feeder calves across evaluation days: yearly least-square mean by farm of origin

Farm of origin	Year 1			Year 2			Year 3			Year 4		
	Mean of 7 evaluation days			Mean of 7 evaluation days			Mean of 6 evaluation days			Mean of 6 evaluation days		
	No. of calves	CH ₅₀		No. of calves	CH ₅₀		No. of calves	CH ₅₀		No. of calves	CH ₅₀	
	LSM	Range		LSM	Range		LSM	Range		LSM	Range	
Br	11	159.0	143 to 173	28	106.8	87 to 124	24	80.6	73 to 91	24	80.8	68 to 89
Ca	16	132.4	84 to 166
De	10	126.0	105 to 151
Gl	16	138.1	108 to 157	56	97.6	84 to 118
Or	19	135.7	117 to 147
St	29	143.9	135 to 162
Al	16	84.8	69 to 113	20	75.5	68 to 85	10	78.5	74 to 86
Wi	19	95.8	83 to 104	18	82.7	74 to 96
Wil	10	89.2	79 to 113	6	89.7	71 to 106
Ke	12	111.9	83 to 103
Lm	10	90.4	77 to 103
Ja	8	91.8	75 to 109
Hi	30	92.0	76 to 104
Ca	12	90.7	83 to 105

See Table 1 for key.
The 2 or 3 letter codes in column 1 are abbreviations for the last name of the farm owner.

barn calves were mixed with the 100 contracted calves for 2 to 3 days. Sale barn calves were removed from the group before the local sale. Calves were provided with water, alfalfa hay, and a concentrate ad libitum. Contracted calves remained at the auction market barn for 7 days, then were transported 1,932 km to the USDA-Agriculture Research Service feedyard in Bushland, Tex.

On arrival at the feedyard, the calves were placed in 4 pens. They were given a 60% concentrate diet, alfalfa hay, and water, and were allowed to rest overnight. The next day, each calf was weighed and processed. Processing consisted of recording rectal temperatures, jugular venipuncture for blood samples, obtaining nasal turbinate mucus swab specimens, replacing lost ear tags, injecting calves with ivermectin, and treating sick calves with a 3-day course of antibiotics.

The scoring system for selecting sick calves and the treatment regimen have been described.¹¹ Briefly, sick calves were identified by scoring points on the bases of clinical signs of nasal and ophthalmic exudate, signs of depression or anorexia, and fever ≥ 40 C. Sick calves were treated with erythromycin (22 mg/kg) for at least 3 days.

Calves were in the feedyard for approximately 4 weeks

during each experiment. The calves were weighed, and blood samples were obtained weekly.

Blood processing for complement—Blood was obtained from each calf in 10-ml or 25-ml plastic syringes^a that were convertible for use as centrifuge tubes. Samples were allowed to clot at ambient temperature for approximately 20 minutes and then were placed on ice to cool thoroughly. They remained on ice for 2 hours before serum removal. Blood samples obtained at the farm of origin and those obtained at the auction market were centrifuged under refrigerated conditions for 10 minutes at maximal speed in portable, clinical centrifuges^b fitted with fixed-angle heads. Blood obtained at the feedyard was centrifuged for 20 minutes at 3,500 × g at 4 C. Serum was decanted into 4-ml glass vials^c, placed on dry ice, and stored at -85 C. All serum samples were assayed within 6 months of collection.

Classical hemolytic C assay—The procedure used was that of Renshaw et al¹² with some modifications of Barta

^a Sarstedt Inc, Newton, NC.

^b Model CL, Damon/IEC, Needham Hts, Mass.

^c No. 224-882 Wheaton, PGC Scientific, Gaithersburg, Md.

Table 3—Serum classical complement* activity in heifers and steers on indicated evaluation days

Year	Gender	No. of calves	Evaluation days						
			FO	AM	FY1†	FY2	FY3	FY4	FY5
1	Heifers	58	130.8±2.1	156.1±2.5	122.7±2.4	127.5±2.4	150.7±2.7	153.7±2.7	155.7±2.3
	Steers	43	123.4±2.3	150.5±2.7	120.6±2.6	126.7±2.2	141.5±2.6	147.9±2.4	145.2±2.1
2	Heifers	40	88.3±2.4 ^a	108.6±2.8 ^a	91.5±2.7 ^b	94.4±2.5	110.6±2.5	108.4±2.5	112.7±2.1
	Steers	60	74.0±1.9	87.9±2.4	78.4±2.2	88.4±2.0	100.8±2.1	99.3±2.1	106.5±1.8
3	Heifers	48	88.9±1.6 ^{b,†}	114.5±1.7 ^a	102.1±1.8 ^a	82.3±1.6	93.4±2.1	ND	100.9±2.3 ^a
	Steers	55	80.1±3.0	94.6±3.3	81.7±3.2	77.2±1.5	91.4±4.1	ND	81.8±4.2
4	Heifers	48	94.2±1.6 ^{a,†}	82.9±1.5	80.5±1.4	81.8±1.9	93.3±1.9	ND	94.8±1.7
	Steers	52	80.9±1.5	75.9±1.4	77.6±1.3	82.7±1.8	93.6±1.8	ND	92.0±1.6

* Adjusted CH₅₀ units/ml serum as least-square mean ± SEM. † feedyard (FY) evaluation number. ‡ Gender by farm and gender by vaccine status interactions existed.
FO = farm of origin; AM = auction market. ^{a,b} = Significant gender effect for indicated year; a = P ≤ 0.05 and b = P ≤ 0.01.
See Table 1 for evaluation days.

Table 4—Serum classical complement activity in healthy and morbid calves on indicated evaluation days

Year	Morbidity status	No. of calves	Evaluation days						
			FO	AM	FY1†	FY2	FY3	FY4	FY5
1	Healthy	59	125.5±1.8	153.3±2.1	125.9±2.1	133.6±2.3 ^a	146.1±2.3	146.9±2.0	146.2±1.8
	Sick 3 d	29	130.2±2.6	150.8±3.0	110.0±3.1	115.8±3.2 ^b	135.4±3.2	144.4±2.7	153.9±2.6
	Sick ≥ 6 d	13	139.3±3.7	154.6±4.3	122.3±4.6	114.3±5.0 ^b	143.7±5.1	151.5±4.4	166.7±4.0
2	Healthy	87	83.3±1.4	98.6±1.8	85.2±1.7	94.9±1.5 ^a	110.1±1.6	107.4±1.5	113.6±1.3
	Sick 3 d	9	76.0±4.4	89.1±5.4	79.6±5.2	73.5±4.6 ^b	102.8±4.8	101.8±4.7	110.5±4.1
	Sick ≥ 6 d	4	62.7±6.6	111.6±8.1	91.6±7.8	75.9±6.7 ^b	114.8±7.1	113.4±7.0	116.8±6.2
3	Healthy	67	85.7±1.3	94.9±1.7	87.0±1.6	80.1±1.3	96.1±1.6	ND	91.6±1.8
	Sick 3 d	17	85.1±2.6	96.7±3.4	88.4±3.1	79.3±2.6	89.6±3.0	ND	94.7±3.6
	Sick ≥ 6 d	19	84.2±2.5	111.4±3.2	94.0±2.9	74.7±2.6	82.3±2.9	ND	85.5±3.6
4	Healthy	91	84.1±1.1	79.3±3.6	78.0±0.9	86.6±1.2 ^a	94.7±1.3	ND	94.2±1.2
	Sick* ≥ 3 d	9	95.2±3.5	79.6±1.1	78.7±3.2	67.3±4.1 ^b	86.7±4.1	ND	101.3±4.2

All sick calves were combined because of insufficient animals in sick ≥ 6-day class. Means with different superscripts are significantly (P ≤ 0.02 to 0.05) different for indicated year.
See Table 1 for evaluation days.
See Table 3 for key.

and Barta.^{13,14} Rabbit erythrocyte suspensions were standardized by adjusting to an optical density of 0.41 ± 0.005 at 541 nm after lysing 0.1 ml of packed cells in 1.9 ml of distilled water. The erythrocyte-antibody mixture was prepared by adding standardized erythrocyte suspension to an equal volume of sheep anti-rabbit erythrocyte antibody at an optimal antibody dilution.¹³ The rabbit erythrocyte sheep anti-rabbit erythrocyte antibody suspension was incubated 30 minutes at 37 C, equilibrated for 45 minutes on ice, then added to dilutions of serum samples prepared in 10- × 75-mm glass culture tubes. Each sample was tested at 3 dilutions. Serum samples were incubated for 60 minutes in a 37 C water bath, centrifuged, and the optical density of the supernatant was read spectrophotometrically at 541 nm. A standard control serum was assayed with samples on each day to determine daily assay variations. Dilutions of standard serum were made each day for slope determination. The serum sample dilution nearest 50% hemolysis was used in calculating sample CH₅₀ values. The CH₅₀ U/ml of serum were calculated from the mean daily slope and von Krogh equation conversion factor.¹⁵

Statistical analysis—Least-square mean, SE of least-square mean, and probability were determined for each experiment by analysis of variance as a split plot in time, using the general linear models procedure of the statistical analysis system.¹⁶ Differences in least-square mean values between treatments within day were compared by use of Duncan multiple-range test if a significant F-test

result was obtained. Differences were considered significant at P ≤ 0.05. The sample least-square means are reported as adjusted CH₅₀ U/ml of serum on the basis of daily assay serum controls.

Results

Steers tended to have lower C concentrations than did heifers (Table 1). The C concentrations of Ph1 vaccinates vs nonvaccinates were not significantly different in any years. Significant differences in C concentrations were observed among farms over the 4-year study (Table 2).

There were significant gender effects on C concentrations within day in years 2, 3, and 4 (Table 3). There were significant differences in C concentrations for the morbidity groups on feedyard day 7 of years 1, 2, and 4 (Table 4). In year 3, the same trend in C concentrations was observed. The daily C concentrations of Ph1 vaccinates and nonvaccinates were not significantly different in any of the 4 years (data not shown). There were significant differences in daily C concentrations among farms in years 1 to 4 (data not shown); however, conclusions cannot be drawn about any given farm, because they were different each year (Table 2). We were unable to contract for calves from the same farms each year, so differences may be attributable to management systems and genetics.

Classical C profiles for market-stressed feeder calves (Fig 1) had a similar pattern in 3 of the 4 years. The exception was year 4, in which the calves did not have a C concentration peak at the auction market. The C pro-

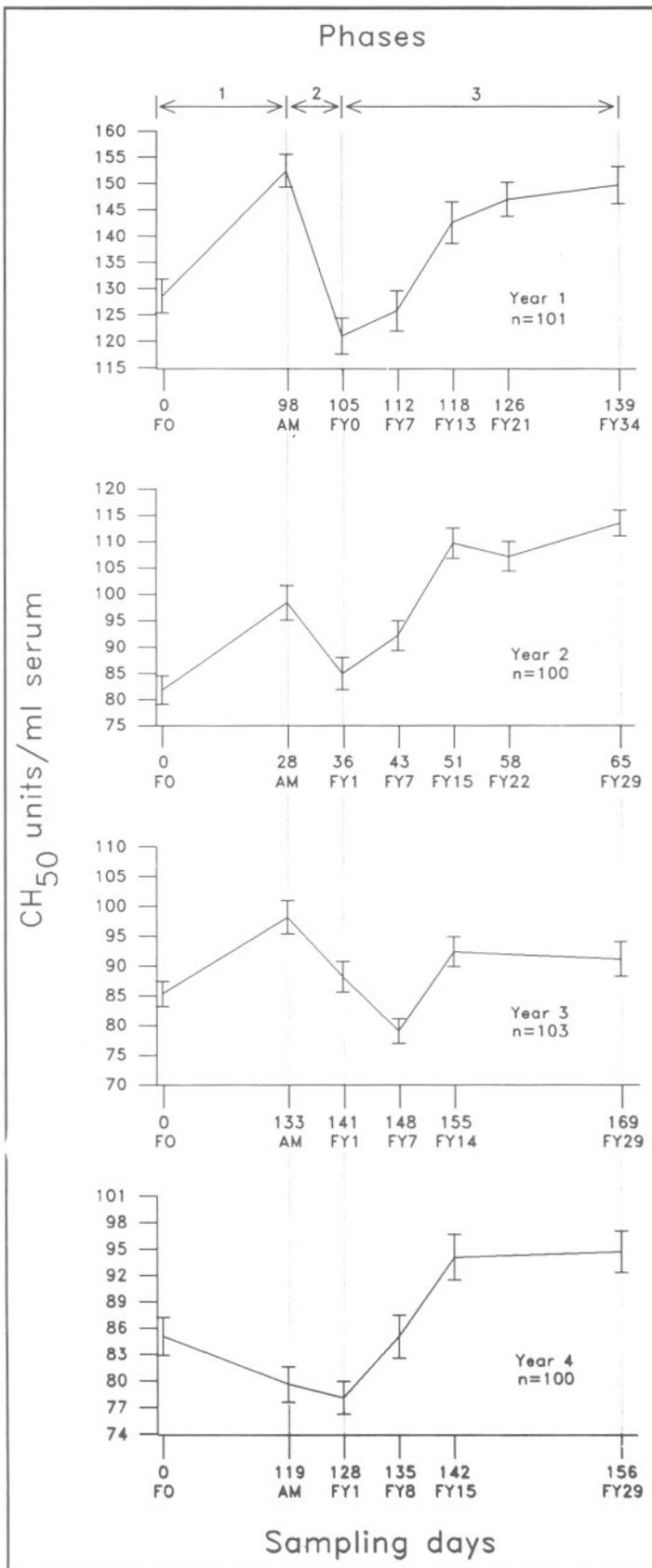


Figure 1—The classical complement profile of ≥ 100 calves illustrating the effect of marketing stress in each year of a 4-year study. Data are expressed as least-square mean \pm SEM on each sampling day. FO = farm of origin, AM = auction market, FY1 = feedyard day 1, FY7 = feedyard day 7, etc. The figure is divided into 3 phases. 1 = farm of origin to auction market; 2 = auction market to feedyard entry; and 3 = feedyard entry through feedyard exit.

files are divided into 3 phases: farm of origin to auction market, auction market to feedyard entry, and feedyard entry through feedyard exit.

Mean PCV of 30 calves tested in year 1 was 39, with a range of 26 to 45. Farm weights of 100 calves in the fourth year were 61.2 to 213.2 kg (mean, 117.9) for steers, and 59.0 to 231.3 kg (mean, 123.4 kg) for heifers. A Pearson correlation coefficient calculated for the calf weights and C concentrations was $r = +0.21$ ($P < 0.03$).

Discussion

The C profiles, with the exception of year 4, revealed low C concentrations on the farm, a C concentration peak at the auction market, a decrease on entering the feedyard, and a subsequent recovery in the feedyard. The reason for the exception in year 4 is not known.

It is not known why the C concentration of the calves was consistently lower at the farms. Previously, we reported¹⁷ that calves in the southeastern United States that were grazing fescue pastures infested with an endophyte (*Acremonium cephalum*) tended to have lower C concentrations than did calves grazing Bermuda grass pastures in the same geographic location. The calves in this 4-year study came from predominantly fescue pastures. Another possible explanation is that young calves have lower C concentrations than do older calves at the farm of origin^{18,19}; however, the farms did not maintain breeding records, so age of the calves was difficult to determine. Calf body weights on the farm of origin are a crude measure of age, but calves could not be weighed on the farm. An exception developed in year 4 of the study, when calves were transported to a central location and were weighed individually. Correlation between C concentrations and calf weights ($n = 100$) in year 4 was weak ($r = +0.21$, $P < 0.03$). Therefore, other factors appear to be more important than weight in predicting relative serum C concentrations at the farm.

The higher C concentrations at the auction market are probably normal values for calves of this age group.¹⁹ The hypothesis that dehydration of the calves might have affected their C concentration at the farm or at the auction market was suggested; however, blood samples were always collected immediately after the calves were gathered on the farm and immediately after an 8- to 32-km truck transport to the auction market. The PCV values for 30 calves from 5 farms (year 1) at the auction market were normal²⁰ in mean (PCV = 39%) and range (26 to 45%) and did not suggest a problem with dehydration. Calves are apparently minimally stressed by being transported a short distance from the farm of origin to the auction market. Thus, the high C values obtained at the auction did not appear to be caused by stress.

The calves were stressed severely during their auction market stay (7 days), during their crowded, 24-hour truck transport to the research feedyard in Texas, and during their first 14 days in the feedyard.^{4,6} Serum C concentration decreased to the lowest value between auction barn arrival and feedyard arrival (or 1 day after feedyard arrival; Fig 1), except in year 3. In year 3, the lowest C concentrations were on day 7 in the feedyard. Feedyard day 7 was the only sample day in which serum was obtained during the maximum BRDC stress period. The highest morbidity developed during the first 10 days in

the feedyard. On day 7 in the feedyard, there were significant differences in C values among morbidity groups (Table 4) in years 1, 2, and 4. A similar, although not significant, trend was observed in year 3. During this time, morbid calves were apparently using more C in antigen-antibody-C reactions than they were synthesizing.

During this time of severe stress induced partly by infectious agents, many biological, nutritional, and hormonal events are taking place that may decrease total hemolytic C. The alternate C pathway could be activated by carbohydrate antigens, in acute bacterial infections,³ or by other complex polysaccharides such as lipopolysaccharides, teichoic acids from bacteria walls, inulin, dextrans, fungal cell walls, and aggregated globulins that are high in carbohydrate content.² All of these substances can activate the alternate C pathway in the absence of specific antibody. Also, a number of acidic molecules such as heparin, DNA, lipopolysaccharides, or lipid A can bind to C1 and activate the classic pathway without the intervention of immunoglobulin.² Viruses commonly associated with BRDC may be immunosuppressive and have an effect on C. Infectious bovine rhinotracheitis virus causes a decrease in hemolytic C concentrations 7 to 10 days after viral challenge of susceptible calves that have normal C concentrations.⁴ The viremia associated with infectious bovine rhinotracheitis, parainfluenza-3, and bovine viral diarrhea creates a situation in which antigen-antibody-C complexes could cause vascular injury. It has been postulated²¹ that soluble antigens forming antigen-antibody complexes could activate C, possibly C3. This could act as a trigger for vasoactive amine release, which increases vascular permeability and results in inflammatory reactions commonly seen in calves with BRDC. Kim²¹ cautioned that vascular immune-complex injury may result from multiple vaccinations given to prevent BRDC. Bovine respiratory syncytial virus infection may activate C3a and C5a, which may have a part in the pathogenesis of this infection.²²

Pasteurella haemolytica is lysed in the presence of specific antibody and C^{9,23,24}; however, Ph1 alone will not activate the alternate C pathway.²³ It is probable that the C pathways contribute to the pathogenesis and to the suppression of Ph1 infections. It has been shown that specific bovine anti-Ph1-antibody isotype IgG, IgG1, IgG2, IgM, and IgA were capable of causing Ph1 to undergo bacteriolysis in the presence of an intact C system.^{24,25} It is unusual for IgA to induce bacteriolysis, unless the molecules are aggregated.³

During phase 2, the C profile in calves may be influenced by nutritional stress, which may develop as short periods of fasting (24-hour truck transport), weaning, or abrupt changes in diet. It has been reported²⁶ that cows given low-energy rations for 2 months had significantly lower serum concentrations of total C than did those fed adequate energy rations. This decrease in serum C resolved after 4 months on the low-energy ration. Results of that study also revealed a 20- to 40-unit decrease in C values at parturition in beef heifers. This was presumably caused by hormonal changes. Plasma glucocorticoids increase at parturition.²⁷

The phase-3 decrease in C concentration resolved after

⁴ Purdy CW, Cole NA, Foster GS. Infectious bovine rhinotracheitis viral and crude protein stress effects on bovine classical complement (abstr 198), in *Proceedings*. 69th Annu Meet Conf Res Workshop Animal Dis, 1988;35.

28 days in the feedyard to values similar to those observed at the auction market immediately before severe stress in 3 of 4 years. The higher C concentrations recorded in the first year, compared with those obtained during years 2, 3, and 4, are probably attributable to laboratory technique and a different source of rabbit RBC. We do not believe the difference in values was attributable to difference in the calves (Table 2).

The finding that steers had significantly lower C concentrations than did heifers was unexpected. Even when differences were not significant, the trend was usually similar. The same finding was recently reported in lambs.²⁸ Significant differences in C concentrations among farms of origin were not unusual and may have been influenced by management practices or genetics.

Similar trends in serum C concentrations were observed in calves in 3 of the 4 years. Thus, the C profiles induced in stressed feeder calves during marketing were reproducible. It appeared that the C profiles were affected by stress in its broadest definition. Also, the C profiles appeared to have trends similar to those reported for blood bactericidal activity curves in stressed feeder calves.⁹

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