Validation and Repeatability of Measurement of Antibody Response to Ovalbumin in Beef Cattle

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ABSTRACT

The objectives of this experiment were to evaluate the pattern of antibody response to ovalbumin as a test antigen in beef cattle, and to estimate the repeatability of the measurement of that response. Purebred Angus cows and calves were immunized with ovalbumin and later tested for antibody response. Six animals were sampled 7 d post-initial immunization to characterize primary antibody response and at 3, 5 and 7 d post-secondary immunization to characterize secondary antibody response. Increases in level of IgG specific for ovalbumin were more rapid in calves than in cows but gradually, measured levels became similar. Secondary responses were measurable on all specified dates, but primary response was not evident at d 7. Correlations between successive samples on animals indicated that the measurements were repeatable (R=.73) for secondary response. These data indicate that ovalbumin is an acceptable test antigen for eliciting a humoral immune response in beef cattle and that measurement of secondary response to ovalbumin can be evaluated in beef cattle at 5 or 7 d post-secondary immunization.

KEYWORDS: immune response, antigens

Traditionally in the livestock industry, disease has been combatted through the use of vaccines, antitoxins, antibiotics and other drugs. An alternative to these costly solutions would be to selectively breed animals for increased disease resistance (Templeton et al., 1988).

Most of the research on the genetics of immunocompetence in livestock has been done in pigs and there are relatively few beef cattle studies (Warner et al., 1987). Selecting for disease resistance in a particular species requires identifiable marker traits that characterize general immunocompetence in that species, as well as heritability estimates and genetic correlations for those traits. Because of limited research in beef cattle, reliable measurements of marker traits are still

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being developed. An important aspect of determining an animal's immunocompetence is quantifying its response when challenged with an antigen, because antibody-producing ability is controlled by multiple genes that have been postulated to correlate with resistance to disease (Warner et al., 1987). This ability is our best measurement of humoral immune response. Preliminary research at Texas Tech University (Green, unpublished data) indicated that thrice washed 40% sheep red blood cells (a common test antigen in livestock) do not elicit a satisfactory response in beef cattle. However, ovalbumin (OVA) has been shown to be a foreign and harmless antigen that produces a response in dairy cattle (Burton et al., 1989). Therefore, the objectives of this study were: 1) to evaluate the pattern of antibody response to OVA as a test antigen and 2) to estimate repeatability of measurement of antibody response to OVA in beef cattle.

MATERIALS AND METHODS

Six purebred Angus cows and their calves from the Texas Tech University teaching herd, New Deal, TX, were sampled in this study. The cows ranged in age from 2 to 9 yr and were nursing equal numbers of male and female calves that were from 3 to 5 months old. During most of the preceding year, cows were grazing wheat pasture or a sudan-sorghum hybrid. During the winter months, animals were fed crop residues and supplemented with protein. Cows were fed to meet or exceed NRC requirements.

To prepare the test antigen, crystallized OVA (Grade V,; Sigma Chemical Co., St. Louis, MO.) was dissolved 2:1 in non-sterile phosphate buffered saline and then diluted 1:1 in Freund's incomplete adjuvant in 20 ml syringes. The mixture was then emulsified and stored at 25°F (-4°C) for future immunizations.

Immunization of animals was accomplished by subcutaneous injections of 4 ml of OVA solution as described by Burton et al. (1989). Blood samples (20 ml) were collected via jugular venipuncture into heparinized syringes at the time of immunization (d 0) and at d 7. At d 14, animals were re-immunized with 4 ml OVA solution; then blood samples were collected at d 17, 19 and 21 to measure secondary antibody responses. Additionally, on days 19 and 21, two samples were collected per animal to estimate repeatability of the OVA antibody measurement. Plasma was obtained from each blood sample and stored at 25°F (-4°C) for later antibody content determinations. The antibody (immunoglobulin G specific for OVA, IgG_{OVA}) content of the plasma was measured by an enzyme-linked immunosorbent assay (ELISA) procedure and results were expressed as absorbance units at 405 nm. The OVA antigen (.5 mg ml^{-1} PBS) was attached to solid phase, flat bottomed, 96 well microtiter plates. Plasma samples were then diluted and plated with 3 wells/sample. Plates were incubated for 2 h to allow OVA specific antibodies in plasma to bind to OVA antigen. Plates were washed and anti-bovine IgG alkaline phosphatase (whole molecule; Sigma Chemical Co., St. Louis, MO.) was added to the wells. It should be noted that, since the bovine IgG antisera was whole molecule specific, there was a possibility that it might have cross-reacted with IgM or other immunoglobulins. However, the occurrence of such reactions should have been low because the antisera product had been company-tested for specificity. Plates were incubated for 1 h to allow enzyme-linked antibodies to bind to the "stem" (or F_c portion) of the remaining plasma antibodies. An initially colorless substrate which yields a colored

metabolic product upon enzyme degradation, p-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO.) was then added. After a 30 minute incubation, NaOH was added to stop the reaction and plates were read at 405 nm using a plate reader (Bio Rad EIA; Richmond, CA.) to obtain relative plasma contents of IgG_{OVA}.

Data were evaluated for variability within replicates of the same sample. If the within sample CV was in excess of 10% (listed as satisfactory assay repeatability; Muggli et al., 1987), the outlying replicate was deleted and the CV was recalculated. Means were then calculated from edited data for each animal, sample and time point. IgG_{OVA} values were determined to be non-normal, thus data were transformed by taking the natural logarithm of each data point. Final data were analyzed by analysis of variance. There was an interaction effect ($P < .01$) of parental status (i.e. cow versus calf) by time, thus analyses were performed on cow and calf data separately with a model including the effect of day of measurement (day). Mean separations were done with least significant difference tests. Repeatability was estimated by pooling data for d 19 and 21 and estimating variation between animals (V_B) and that within animals (V_W) from a mixed model including effects of day and animal. Standard errors of repeatabilities were calculated using the following formula (Falconer, 1989):

$$V(t) = \frac{2[1+(n-1)t]^2(1-t)^2}{n(n-1)(N-1)}$$

RESULTS AND DISCUSSION

Analyses of variance by parental status indicated an increase ($P < .01$) in IgG_{OVA} over the dates measured (Table 1), in agreement with Burton et al. (1989) who followed a similar immunization schedule using OVA as a test antigen in dairy calves. This was true for both cows and calves, with the reason for the significant interaction of parental status X day being that calves increased at a faster rate between d 0 and 17 than cows, and then plateaued (Figure 1).

Table 1. Analyses of variance for measurement of ovalbumin antibody levels by parental status.

Source of variation		df	Mean square
Cows			
	Day	4	.77**
	Error	25	.03
Calves			
	Day	4	1.08**
	Error	25	.04

** significant at $P < 0.01$.

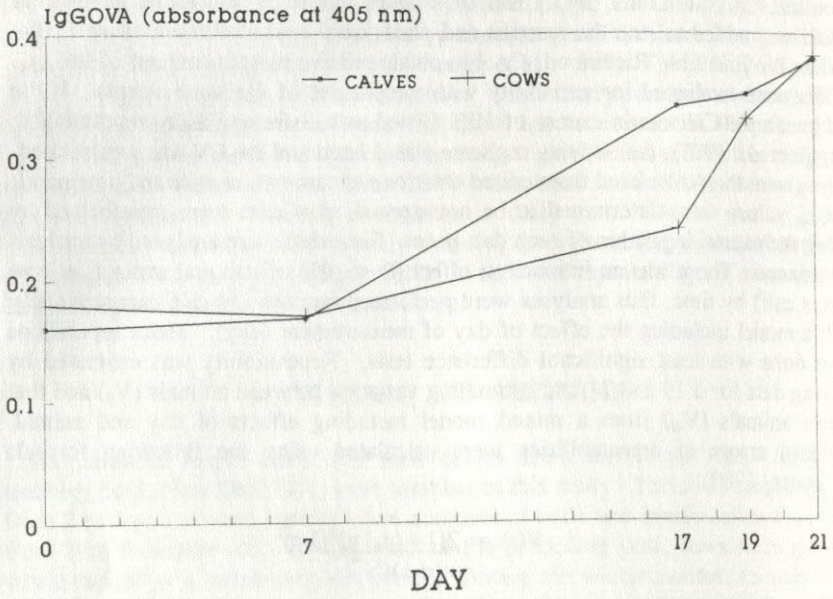

Figure 1. Ovalbumin antibody content by day of measure and parental status. (Standard error of mean = .02).

The changes in antibody content between dates of measurement are presented in Table 2. Cows experienced a larger percentage change after d 17 than calves and appeared to be still increasing at d 21, but the percent changes between d 19 and d 21 for both cows and calves were non-significant. Final differences between cows and calves at d 19 and 21 were small. Since d 19 and d 21 correspond to d 5 and d 7 of secondary response, these data indicate that secondary response to ovalbumin as a test antigen can be measured effectively at either 5 or 7 d post-secondary exposure.

Table 2. Percentage response between days of measurement by parental status.

	Days†						
	0-7	0-17	0-19	0-21	17-19	17-21	19-21
Parental Status							
Cows	0	35.2*	84.0**	116.7**	36.1*	60.3**	17.8
Calves	2.5	113.7**	118.5**	137.7**	2.1	11.2	9.0

†Percentage change in antibody level between two dates of measurement (eg., % change from d 0 to d 7). * $P < .05$, ** $P < .01$.

Differences between d 0 and d 7 as measures of primary response were small and non-significant. This was expected because the ELISA procedure measured IgG specific to ovalbumin and that IgM antibodies make up a major proportion of the primary response, while the secondary response consists almost entirely of IgG (Roitt et al., 1989).

Measures of accuracy and repeatability of IgG_{OVA} are presented in Table 3. Correlations between successive samples on the same animal were relatively high ($r=.69$ and $P<.01$ on day 19; $r=.74$ and $P<.01$ on day 21). Repeatability of the measure was estimated to be $.73 \pm .14$. Comparisons of sample means within date indicated close agreement (2.2% and 0.4% differences between means on d 19 and 21, respectively).

Table 3. Measures of accuracy of ovalbumin antibody levels.

Correlations between Samples within day
$r_{19} = .69^{**}$
$r_{21} = .74^{**}$
Variance component (V) estimates and degree of repeatability
V (between animals) = .026
V (within animal) = .009
Repeatability = $.73 \pm .14$
Means and Standard errors of IgG_{OVA} by sample and day
Day 19 (sample 1) = $.35 \pm .07$
Day 19 (sample 2) = $.34 \pm .05$
Day 21 (sample 1) = $.39 \pm .08$
Day 21 (sample 2) = $.39 \pm .07$

** $P < .01$

OVA was used in this study because it has proven useful as a test antigen in experiments with mice, pigs, sheep and dairy cattle (Biozzi et al., 1989; Babinszky et al., 1991; Berggren-Thomas et al., 1987; Burton et al., 1989). Furthermore, Burton et al. (1989) list moderate to high heritabilities for secondary response to ovalbumin in dairy calves, which will be important if response to OVA is to be incorporated into any type of selection program for immune response in beef cattle.

Collectively, the results of this study indicate that ovalbumin can effectively be used as a test antigen to elicit repeatable responses of the humoral immune system in beef cattle. Using this procedure, it appears that the best sampling time is 5 or 7 d post-secondary exposure to the antigen. This latter conclusion agrees with data on dairy calves that peaked at 7 d post-secondary exposure (Burton et al., 1989).

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