GENETIC VARIABILITY IN MEASURES OF BEEF CATTLE IMMUNE RESPONSE

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ABSTRACT

The objective of this study was to determine if a relationship could be found between dam and offspring immune measures. A secondary objective was to determine the relationship between dam's performance (expected progeny difference, EPD) and her immune measures. The regression between dam and offspring was significant for number of white blood cells, indicating selection for this trait would be productive. White blood cell numbers were positively correlated with maternal EPD for birth weight (r=.34), weaning weight (r=.36) and yearling weight (r=.38). Number of peripheral lymphocytes was also correlated with maternal EPD for birth weight (r=.40), weaning weight (r=.37) and yearling weight (r=.38). These data indicate that selection for leukocyte numbers would be successful and that these traits are favorably correlated with measures of growth.

Keywords: Beef cattle, Immune response, Genetics.

INTRODUCTION

While much is known about genetic sources of variation in live animal performance, body composition and reproduction, relatively little is known about underlying genetic mechanisms of immune function. If immune function of domestic animals could be improved through selection, there could be substantial economic gains.

Previous studies have concentrated primarly on genetics of immunoglobulin and antibody response (Muggli et al., 1984; Gilbert et al., 1988). These studies have found , generally, that the heritability of immunoglobulin level is low and that progress from selection for this trait might be slow, yet possible. We know of no reports of heritability estimates of numbers of metabolic activity of beef cattle leukocytes. Therefore, the objective of this work was to obtain first approximations of the level of heritability of leukocyte numbers and function and to determine if variation in these traits was correlated with measures of performance. Later studies involving greater munbers would benefit from the hypotheses generated in this study.

METHODS

General: Twenty-eight purebred Angus cows and their calves (20 females, 8 males) were used in this study. Cows were 2 to 10 years old and calves were three to four months old and nursing their dams. Cows were fed during the preceding year to meet or exceed NRC recommended nutrient requirements of beef cattle (NRC, 1984). Cows were grazing wheat pasture of a sudan-sorghum hybrid during most of the year and were fed crop residues and protein supplement during times of the year when no pasture forage was available.

Bleeding and sample preparation: Each cow and her calf were bled on day 0 and day 7 (day 0 blood samples were used only for background antibody titer determinations). Blood samples were obtained via jugular vein using 20 ml syringes containing heparin. On day 0, cow and calf were injected subcutaneously with 1 ml of 40% sheep red blood cells (SRBC).

The blood samples were centrifuged for 20 minutes to separate plasma. Plasma samples were stored frozen in glass vials until assayed.

Immune measures: Blood samples obtained on day 7 were assayed for various immune measures. Blood smears were made using whole blood. The smears were fixed in methanol and stained with LeukoStat Solution I and II for differential counts. Total white blood cells (WBC) were counted using a hemocytometer.

The lymphocyte transformation assay (LTA) was used to determine metabolic activity of the T and B cells. Fifteen ml of blood were centrifuged for 20 minutes at 2000 RPM. Plasma was aspirated and placed in glass vials for later use. The buffy coat (which contains the white blood cells was removed and placed in a 15 ml conical centrifuge tube and mixed with Roswell Park Memorial Institute (RPMI) 1640 medium (with sodium bicarbonate and gentamicin sulfate). Ten ml of the buffy coat-RPMI mixture were layered onto 4 ml of histopaque and centrifuged at 1350 RPM for 40 minutes at 25°C. The opaque interface containing the mononuclear cells was aspirated and transferred to a clean conical centrifuge tube. The opaque interface was washed in RPMI and centrifuged at 2000 RPM for 10 minutes. The supernatant was removed and the cells were resuspended in 1 ml of RPMI. The cells were counted using a hemocytometer. The samples were diluted in RPMI supplemented with 10% fetal bovine serum (FBS) and a cell concentration of $5x10^6$ cells/ml was obtained.

Each sample ($5x10^6$ cells/ml) was added in triplicate to the wells of microtiter plates containing 100µl of the mitogens. Conconavilin A (Con A, 20µg/ml), Phytohemagglutinin (PHA 10µg/ml)), and Pokeweed (PWM 10µg/ml) were used as mitogens to stimulate B and T cell activity . The plates were incubated at 37°C in 5% CO_2 for 66 hours. After 48 hours, 100 µl of RPMI supplemented with 10% FBS. At 66 hours MTT (5 mg MMT/ml PBS) was added to each well and mixed thoroughly by repeated pipetting with the octapipette. Within an hour the plates were read using plate reader with a 600 nm filter.

An enzyme-linked immunosorbent assay was used to determine antibody levels of bovine IgG. The frozen plasma was thawed and used to measure IgG. The plasma samples were diluted (1:3000) in 10% Tween/PBS. The diluted test samples and standards were added in duplicate to the wells of bovine IgG coated microtiter plates. Rabbit anti-bovine IgG was added to each well. The plates were incubated for two hours at room temperature. The wells were emptied and washed three times. The enzyme linked goat anti- rabbit IgG linked to alkaline phosphatase was added to each well and incubated at room temperature for 1 hour. The wells were emptied and washed three times. The substrate solution was added to each well and incubated at room temperature for 30 minutes. The reaction was stopped by adding 2M NaOH to each well. The optical density of the wells was read at 405 nm and the amount of IgG present was determined.

Hemagglutination assay was used to determine dam and calf antibody response to SRBC. The plasma samples were thawed and heat inactivated in a 57°C water bath for 30 minutes. The heat inactivated samples were placed in the first wells of round -bottom plates in duplicate. PBS was added to all wells. The plates were

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diluted by removing 100µl of each sample with an octapipette and serially diluting each well from left to right, discarding the final 100µl. To each well 1% solution of SRBC was added and the plates were agitated for 1.5 minutes. The plates were covered and incubated at room temperture for 24 hours.

The titers were determined by the sedimented cells forming a distinct pattern on the bottom of the wells. The highest dilution giving a positive reaction determined the titer.

Statistical analyses and genetic parameter estimation: Data were analyzed by analysis of variance and covariance techniques employed on the general linear models procedures of SAS (1985) with effects of sire and sex of calf investigated as potential sources of variation. In order to estimate heritability of various immune meaures, the regression of offspring performance on dam performance was estimated and doubled (Falconer, 1981). In cases involving negative regression coefficient, heritability levels were assumed to be zero. Residual correlations between measures of growth and maternal performance (in the form of expected progeny differences, i.e. breeding values from the American Angus Associations national cattle evaluation program) and those of immune fuction were also estimated using covariance analyses. While the number of observations in this study was certainly limited, the objective was to identify possible trends for future study.

RESULTS

The relationships between cow and calf immune measures are presented in Table 1. A correlation ($p<.05$) was found between cow and calf total white blood cells and lymphocyte numbers resulting in a high estimate of heritability for these traits. Offspring-dam regression coefficients were low for LTA data, with LTA for pokeweed mitogen exhibiting a trend ($p=.069$). Thus, resulting heritability estimates for LTA data were all low or zero.

Table 1. Relationship between cow and calf immune measures.

Immune Measures	b*	SE_b	h^2	r**	P
IgG, mg/ml plasma	-.13	.15	0	-.17	.403
SRBC titer	-.04	.21	0	-.04	.849
Total WBC, no/µl	.60	.28	1.20	.39	.040
Lymphocytes, no/µl	.53	.38	1.06	.27	.171
Neutrophils, no/µl	-.35	.04	0	-.16	.431
Monocytes, no/µl	-.05	.62	0	-.02	.932
Eosinophils, no/µl	-.03	.22	0	-.27	.173
Lymphocyte transformation					
Background	.00	.00	0	.11	.583
PHA mitogen	-.01	.02	0	.13	.530
Pokeweed mitogen	.03	.02	.06	.36	.069
ConA mitogen	.08	.06	.16	.24	.227

*Regression coefficient of offspring on dam.
**Correlation between offspring and dam measures.
P refers to P-value associated with b- and r-values.

Table 2. Correlations between EPD's and cow immune measures.

Immune Measures	EPD Birth Weight	EPD Weaning Weight	EPD Weaning Maternal	EPD Yearling Weight
IgG, mg/ml plasma	-.09	.03	-.05	-.02
SRBC titer	.11	.01	-.17	.20
Total WBC, no/µl	.34+	.36+	-.17	.38+
Lymphocytes, no/µl	.40*	.37+	-.19	.38+
Neutrophils, no/µl	.10	.15	-.02	.18
Monocytes, no/µl	-.01	.04	-.22	.09
Eosinophils, no/µl	-.17	-.02	-.07	-.03
Lymphocyte transformation				
Background	-.25	-.20	.20	-.26
PHA mitogen	-.15	-.06	.27	-.17
Pokeweed mitogen	-.18	-.07	.23	-.17
ConA mitogen	-.12	-.09	-.01	-.06

+ $P < .10$

* $P < .05$

EPD = Expected progency difference estimated by National Cattle Evaluation program of American Angus Association, St. Joseph, MO (1988).

Correlations between EPD and cow immune measures are presented in Table 2. Cow's total WBC and number of lymphocytes were favorably correlated with EPD for birth, weaning and yearling weights ($p<.10$ or $p<.05$).

Sire effects on calf immune measures are presented in Table 3. Sire had an effect on calves' response to PHA mitogen ($p\leq.05$) in the lymphocyte transformation assay. Sires A, B, and D showed greater LTA under PHA mitogen than sire C.

Table 3. Sire effects on immune measures.

Immune measures	Sire A	Sire B	Sire C	Sire D
IgG, mg/ml plasma	1.20	1.83	1.07	1.21
SRBC titer	3.18	2.71	3.33	3.50
Total WBC, no/µl	12255	14407	11200	12343
Lymphocytes, no/µl	8888	9650	8696	9472
Neutrophils, no/µl	2885	4282	2426	2851
Monocytes, no/µl	166	151	32	429
Eosinophils, no/µl	315	325	46	83
Lymphocyte transformation				
Background	.19	.18	.11	.17
PHA mitogen	.35[a]	.38[a]	.23[b]	.32[a]
Pokeweed mitogen	.33	.32	.22	.27
ConA mitogen	.29	.28	.24	.25

a,b Means with same superscript are not significantly different, ($P < .05$).

Table 4. Sex effects on immune measures.

Immune measures	Male	Female	P*
IgG, mg/ml plasma	.99	1.49	.12
SRBC titer	4.29	2.75	.03
Total WBC, no/μl	11656	13120	.28
Lymphocytes, no/μl	8763	9375	.66
Neutrophils, no/μl	2361	3536	.12
Monocytes, no/μl	368	138	.11
Eosinophils, no/μl	153	271	.55
Lymphocyte transformation			
Background	.18	.17	.69
PHA mitogen	.27	.36	.002
Pokeweed mitogen	.28	.31	.34
ConA mitogen	.21	.29	.02

*Significance level of effect of sex of calf.

Sex effect on immune measures are presented in Table 4. SRBC titers were effected by sex of the calf. Male calves had higher titers ($p<.05$) than the heifer calves. Heifer calves had a higher lymphocyte response to PHA ($p<.01$) and ConA ($p<.05$) than bull calves.

DISCUSSION

These data represent a first attempt at understanding genetic variability in leukocyte numbers and function in beef cattle. The primary objective was to generate hypotheses to be tested in later studies with larger numbers. Immune measures which appear to offer the greatest genetic variability are numbers of WBC and lymphocytes. On the other hand, lymphoyte metabolic function (LTA in particular) seems to have a lower degree of heritability in these cattle.

It is not possible to say, at this point, which direction would be desirable (selecting for a greater or lower number of cells). Given a positive correlation between leukocytes numbers and EPD values, the authors predict that more leukocyte are preferred. However, a large-scale selection study should be addressed to this question.

A negative, but non-significant, correlation existed for WBC and lymphocyte numbers with EPD weaning weight maternal. This potential negative relationship between general maternal ability (primarily milk production) and leukocyte numbers should be viewed with caution at this stage, due to marginal significance and low numbers.

General lack of significant differences among sires may simply be a reflection of this particular sample of sires. A larger sample of sires would be desirable before firm conclusions should be drawn.

Sex effects in immune measures were not consistent. In absence of other evidence, differences due to sex were probably random and not biologically meaningful.

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