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Title: GENETIC POLYMORPHISM OF SHEEP HEMOGLOBIN

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Five breeds of sheep (Columbia, Targhee, Rambouillet, Lincoln, and Suffolk) were found to possess two hemoglobin types (Hb. A and Hb. B) when their hemoglobins were examined by disc gel electrophoresis. The hemoglobin alleles proved to be co-dominant.

Three of the breeds (Columbia, Targhee, and Rambouillet) were maintained at altitudes of 4, 800 to 7, 500 feet above sea level and the Lincoln breed investigated was maintained at altitudes of 750 to 1, 200 feet above sea level. These four breeds had a frequency of Hb. A of 16% or greater. The Suffolk flock maintained at an altitude of 250 feet above sea level had a frequency of Hb. A of approximately 5%. These data suggest an adaptive advantage for Hb. A at higher altitudes.

An increase in inbreeding was observed to be associated with an increase in the homozygosity at the hemoglobin locus. The association

was linear to a level of 35% inbreeding after which there was a noticeable drop in the amount of homozygosity as inbreeding exceeded 35%.

There were no significant differences among the means of production traits for the three classes of hemoglobin alleles when the mean values of the following production traits were tested: (1) average daily gain from birth to weaning, (2) weaning weight, (3) weaning conformation, (4) weaning condition, (5) weaning index, (6) yearling weight, (7) average daily gain from weaning to yearling, (8) yearling condition, (9) yearling conformation, (10) yearling grease fleece weight, (11) yearling fleece grade, and (12) yearling index.

Genetic Polymorphism of Sheep Hemoglobin

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INTRODUCTION

The discovery of genetic polymorphism demonstrated by proteins that differ electrophoretically furnishes information for the study of the genetics of the organism at all levels, i. e. molecular, cellular, or the entire organism. The knowledge obtained from the determination of genetic polymorphism from protein differences permits the study of protein evolution and thus the evolution of the organism.

Qualitative genetic traits enable man to examine the effects of breeding systems on the frequencies of alleles where inbreeding and selection are practiced. It is assumed that the genes for quantitative traits behave in the same manner as the qualitative genes and thereby allow a monitoring of the shifts in gene frequencies of all genes where various breeding systems are used. The discovery of a qualitative gene trait that may serve as a genetic marker for a desirable combination of quantitative genes which will make for better animal production is possible as well as desirable.

The purpose of this study was to determine the polymorphic forms of sheep hemoglobin as demonstrated by electrophoretic patterns of sheep hemoglobins and to determine the inheritance of these different hemoglobins.

Frequencies of the different hemoglobins were determined in unselected control lines, and these frequencies were compared with the frequencies of the hemoglobins in inbred lines and line crosses. By comparing the lines in which selection has been practiced with the line crosses, one should be able to determine gene frequency response to inbreeding and selection.

The frequency of hemoglobin types was compared between breeds that were maintained in different environments. Three of the breeds (Columbia, Rambouillet, and Targhee) were maintained at high altitudes of 4, 800 to 7, 500 feet above sea level; the Lincoln breed was maintained at altitudes of 750 to 1, 200 feet above sea level; and the Suffolk breed was maintained at an altitude of 250 feet above sea level. The different environments may result in a different frequency of hemoglobin alleles. Also, two of the breeds (Columbia and Targhee) were developed from a cross between the Lincoln and Rambouillet breeds.

The association of hemoglobin types with production traits of the sheep was examined to see if the hemoglobin types might be useful to predict better performing animals. The study not only adds to our store of knowledge, but it should have economic value if there is a reasonable association between hemoglobin types and productivity of the animals.

LITERATURE REVIEW

Pauling et al. (1949) discovered that sickle cell anemia in humans could be attributed to a hemoglobin (Hb. S) that differed electrophoretically from the normal adult hemoglobin (Hb. A_1). Since then, there has been an extensive search for polymorphic hemoglobins that differ electrophoretically in most all of the animals using hemoglobin as a respiratory pigment.

Lehmann and Huntsman (1966) report that 35 human hemoglobin variants have been discovered by electrophoresis, and many more are being discovered and reported. Lehmann and Huntsman (1966) point out that, while all hemoglobin variants in humans are not responsible for abnormal conditions, there are a considerable number that are responsible for abnormal conditions. It is the association of hemoglobin variants with abnormal conditions in humans that has prompted the extensive investigations of hemoglobins in farm animals.

The most extensively studied hemoglobins of farm animals are bovine (cattle) and ovine (sheep). Efremov and Braend (1965) report that five normal adult hemoglobins (Hb. A, Hb. B, Hb. C, Hb. D, and Hb. K) and one normal fetal hemoglobin (Hb. F) have been demonstrated in cattle. Beale et al. (1966) reported that two normal adult hemoglobins (Hb. A and Hb. B), one abnormal adult hemoglobin (Hb. C), and one normal fetal hemoglobin (Hb. F) have been demonstrated in sheep.

Harris and Warren (1955) were the first investigators to report the discovery of polymorphic hemoglobins in sheep. They tested 12 pregnant ewes and observed Hb. A and Hb. B in the homozygous and heterozygous state in the various individual ewes (5 AB, 3 AA, and 4 BB). Each of the fetuses had only one type of hemoglobin (Hb. F). This has since been confirmed by many investigators on a large scale, notably Evans et al. (1956); Evans, Harris, and Warren (1957); Huisman, van Vliet, and Sebens (1958) and Stormont (1968).

The discovery of Hb. C was first made by Blunt and Evans (1963) when they were observing potassium and hemoglobin type changes under anemic stress. They did not label the new hemoglobin because they thought it may be one of the two normal adult hemoglobins.

Independently Braend, Efremov, and Helle (1964) and van Vliet and Huisman (1964) discovered that a new adult hemoglobin was produced when anemia occurred naturally or when anemia was induced.

Braend, Efremov, and Helle (1964) observed a new hemoglobin in a sheep that had parasitically induced anemia. They called this new hemoglobin - hemoglobin N. [This Hb. N is the same hemoglobin as the Hb. C observed by van Vliet and Huisman (1964). See Efremov and Braend (1966).]

van Vliet and Huisman observed that a new hemoglobin that they called Hb. C was produced in a sheep that had been bled to an

anemic state. They observed that Hb. C occurred only in sheep that possessed the normal Hb. A in the homozygous or heterozygous state. They observed that Hb. C replaced only Hb. A as anemia was induced; but as the sheep was allowed to recover to normal levels of hemoglobin, the Hb. C was replaced by Hb. A. The term Hb. C will be used to refer to Hb. C and Hb. N in subsequent discussions in order to avoid confusion.

The occurrence of Hb. C in non-anemic sheep that possess Hb. A has been studied by Braend and Efremov (1965). They found that Hb. C composed one to two percent of the hemoglobin in some breeds but was not detectable in other breeds. They investigated six breeds of sheep (Spael, Iala, Rygja, Cheviot, Oxford Down, and Spanish Merino) and could detect Hb. C in the sheep that possessed Hb. A in the Spael, Iala, and Rygja (old Norwegian mountain breeds) but could not detect Hb. C in the sheep that possessed Hb. A in the Cheviot, Oxford Down and Spanish Merino. They suggest that there is a definite breed difference.

Boyer et al. (1966) have completed an extensive investigation on the three adult hemoglobins which has culminated in their determination of the primary amino acid sequence of the beta chains of each of the hemoglobins. They concluded that the sheep hemoglobin molecule is a tetramer consisting of two alpha (α) chains and two beta (β) chains. The three hemoglobins (Hb. A, Hb. B, and Hb. C)

differ only in the β chains, and the β chains of the Hb. A and Hb. B are allelic products. The β chains of Hb. A and Hb. B differ by seven amino acid residues and the β chain sequence of Hb. C differs from Hb. A by 16 amino acid residues and from Hb. B by 21 amino acid residues.

The discovery of two normal polymorphic hemoglobins in adult sheep has prompted intensive investigation into the frequency of these alleles to see if they are the same in all breeds. The investigation of hemoglobin frequencies has been done more extensively in European sheep (Evans et al., 1956; Evans, Harris, and Warren, 1957, 1958a, and 1958b; Efremov and Braend, 1966; and Braend and Efremov, 1965) with only one investigator observing the frequencies of sheep hemoglobin in the U. S. (Stormont, 1968).

The frequencies of the two normal alleles varies quite markedly from breed to breed, but the frequency of the alleles has very good association with particular environments. Evans, Harris, and Warren (1958b), after investigating the frequency of Hb. A and Hb. B in 33 breeds of British sheep, were able to show that the breeds of sheep that were common to higher altitudes had a significantly higher frequency of Hb. A; and the breeds that were common to lower altitudes had a significantly higher frequency of Hb. B. They postulate a selective advantage for Hb. A at the higher altitudes.

Evans and Blunt (1961) have added to the theory of selective

advantages for the different hemoglobin types in different environments by comparing the frequencies of the hemoglobin types in Romney Marsh and Southdown breeds of sheep in different environments. The frequencies of the hemoglobin types were compared between the Romney Marsh and Southdown breeds in England and in Australia. It was found that the frequency of Hb. A had increased significantly in Australia in both breeds (frequency of Hb. A in Great Britain was 9.0% in Southdown and 11.0% in Romney Marsh and the frequency of Hb. A in Australia was 28.0% in the Southdown and 44.0% in the Romney Marsh). The above evidence, according to Evans and Blunt, demonstrates that the selective advantages of Hb. A and Hb. B have been altered in the different environments and account for the shift in the frequency of the alleles.

The selective advantages the two different hemoglobins might give the animals have been explained by several authors on the difference in oxygen affinity (oxygen tension curves) between the hemoglobins (Huisman, van Vliet, and Sebens, 1958; van Vliet and Huisman, 1964; and Dawson and Evans, 1966). All of these investigators have found that Hb. A has a greater oxygen affinity than Hb. B; and this evidence, when coupled with the occurrence of Hb. A in higher frequency at the higher altitudes, seems to be the explanation for Hb. A having a selective advantage over Hb. B in environments where oxygen is in shorter supply.

The difference in oxygen affinity might be a plausible explanation for maintenance of the hemoglobin polymorphism, but several investigators think that the association of hemoglobin type with some critical production trait might better explain the maintenance of the hemoglobin polymorphism. This idea is still maintained, even though the association of other single loci traits with production traits in sheep is not encouraging (Stansfield et al., 1964).

Parker, Weseli, and Cartwright (1967) attempted to associate the hemoglobin types of six different breeds of cattle (Angus, Brahman, Charbray, Charolais, Hereford, and Santa Gertrudis) and breed crosses between these six breeds with production traits as an explanation for the polymorphic hemoglobins in cattle. However, they were unable to find sufficient variation of the two types of hemoglobin (Hb. A and Hb. B) in any of the six breeds and the breed crosses and they concluded that herds with more variation of hemoglobin phenotypes would have to be found in order to test association of hemoglobin types with productivity.

Stansfield et al. (1964) attempted to associate red cell antigen types in Rambouillet, Targhee, Columbia, and two flocks of cross bred sheep with several production traits: (1) condition score of lamb at weaning, (2) conformation of lamb at weaning, (3) side wool grade, (4) thigh wool grade, (5) staple length of lamb's fleece, (6) amount of face covered by wool, (7) composite selection index score (of above

traits), (8) body weight as a yearling, (9) condition score as a yearling, (10) conformation score as yearling, (11) amount of grease in yearling fleece, (12) staple length of yearling fleece, (13) yearling fleece grade, and (14) neck score (based on skin folds). They concluded there was no significant association between any of the red cell antigens and the production traits mentioned above.

King et al. (1958) determined the frequency of the class of alleles (60.7% AA, 35.1% AB, and 5.2% BB) of hemoglobin types in a flock of Scottish black face Lanark sheep and correlated the hemoglobin type classes with the following production traits: (1) Body weight of the ewe in November before mating, (2) fleece weight of the ewe at shearing in July, (3) birth weight of the lamb, (4) June weight of the lamb, (5) weaning weight of the lamb in August, (6) cannon bone length of the lamb (measured on hind cannon bone in June), (7) medulation index of the lamb's fleece, and (8) mean fiber length of the lamb's fleece. They concluded that there was no significant association between any of the above-mentioned traits and the hemoglobin types. However, they did find a tendency for those sheep possessing Hb. B, whether in homozygous or heterozygous state, to produce and to wean more lambs than sheep which were homozygous for Hb. A.

Evans and Turner (1965) investigated further the possibility of Hb. B being associated with better lamb production in the Australian

Merino sheep. They investigated four experimental flocks of Australian Merino where (1) one flock was not selected for reproductive performance, (2) one flock was selected for high twinning rate, (3) one flock was selected for low twinning rate, and (4) one flock was selected for multiple births (triplets and quadruplets were reported very common in this flock). They concluded from their evidence that sheep with Hb. B (BB or AB) did in fact produce and wean more lambs than did the sheep homozygous for Hb. A, but Hb. A seemed to confer advantages after weaning which accounted for the maintenance of the polymorphism of hemoglobins.

From the preceding literature, it can be generalized that there exist two normal adult sheep hemoglobins (Hb. A and Hb. B) which differ electrophoretically. The frequencies of these hemoglobin types differ from breed to breed and they seem to be maintained in polymorphic frequencies by the environment under which the sheep are maintained.

The possible adaptive value one hemoglobin type has over the other in a particular environment might be due to the higher oxygen affinity Hb. A has over Hb. B in environments of higher altitudes or where oxygen is not as readily available. The adaptive value Hb. B seems to have over Hb. A where oxygen supply is plentiful is that it is associated in some way with better reproductive performance.

MATERIALS AND METHODS

Five different breeds of sheep--Targhee, Columbia, Rambouillet, Lincoln, and Suffolk--were used in the study. The Targhee, Columbia, and Rambouillet breeds were checked for frequency of their hemoglobin alleles, and their hemoglobin types were examined for association with their production traits; but the Suffolks and the Lincolns were checked only for the frequency of their hemoglobin alleles.

The Targhee, Columbia, and Rambouillet breeds used in this study were part of the United States Department of Agriculture flock kept at the United States Sheep Experiment Station at Dubois, Idaho. The Targhee and Columbia breeds were developed at the U. S. Sheep Breeding Station from crosses of Lincoln X Rambouillet.

The Columbia, Targhee and Rambouillet sheep used were bred in a diallel mating system in which four inbred lines of each breed were crossed in all possible ways to produce 12 line crosses. Blood samples were taken from all animals of a four-line diallel in each of the three breeds; and, in addition, blood samples were taken from unselected control populations in each of the three breeds. The sample size obtained in these three breeds was unequal as indicated in Table 3.

The Suffolk sheep are part of the Oregon State University flock

kept at Corvallis, Oregon. The Suffolks are a closed line of sheep that was started with three sires and 45 dams in 1952.

The Lincoln sheep used in this study are part of a purebred flock of Lincoln sheep owned by Mr. Don Kessi of Harlan, Oregon. The Lincolns are an open population in which Mr. Kessi purchases breeding rams each year from different sources, and keeps the better ewe lambs for breeding stock as they are needed.

Approximately 15 ml of blood were drawn from the jugular vein with a 16-gauge needle into a heparinized test tube which contained three drops of 1000 USP units per milliliter heparin solution. The test tubes were stoppered and placed in an ice chest to keep them cool until the red blood cells could be separated from the plasma and lysed. The whole blood was centrifuged at 1800 RPM for ten minutes in order to remove the plasma. The cells were then washed three times with sterile 0.9% sodium chloride solution and were centrifuged at 1800 RPM for ten minutes after each washing to remove the saline solution. After the saline solution from the last washing had been removed, the cells were lysed with a volume of cold distilled water that equaled that of the packed cells. After lysing the cells, the hemolysate was centrifuged at 2700 RPM for 20 minutes. Five milliliters of the hemolysate were decanted and placed in a 15 milliliter serum bottle. The bottle was stoppered and carbon monoxide was slowly bubbled through each bottle for one minute. After this, the

hemolysate was frozen for transfer and storage until analysis of the hemoglobin types could be made.

The determination of hemoglobin types was accomplished by disc acrylamide gel electrophoresis after the method of Ornstein (1964) and Davis (1964). The following modifications were made: The acrylamide gels were set up in glass tubes that were three inches in length with an inside diameter of 5 millimeters. The separation gel and the sample gel were each five-sixteenths of an inch in length. A sample size of two microliters was run from the cathode toward the anode with a constant amperage of three and one-half milliamperes per tube until the tracker dye (Bromophenol blue) had reached a constant distance of seven-eighths of an inch ($\pm$ one-sixteenth of an inch) from the end of the glass tube.

The gels were removed from the glass tubes and the two upper gels (the sample and the spacer gels) were removed from the separation gel so that a clean surface was obtained at the interface between the spacer gel and the separation gel. This cleaned surface was considered the origin when the distances the various bands had moved were measured. The hemoglobin bands were located by their natural color.

The gels were placed on a plexiglass block that had a groove five millimeters wide and two and one-half millimeters deep machined in it. The block had been ruled off 50 lines to the inch on both sides

of the groove making it possible to measure the distances the hemoglobin bands and the tracker dye band had moved. The distance the various bands had moved was measured in total lines the bands had moved.

After the measurement of the distance the bands had moved, the R_B was calculated. The R_B is defined as the decimal fraction obtained when the total distance the tracker dye moved is divided into the total distance the hemoglobin bands had moved:

$$R_B = \frac{\text{distance hemoglobin migrated}}{\text{distance tracker dye migrated}}.$$

After identification of the various hemoglobins by the R_B value, the frequency of each allele in each breed of sheep was calculated in the following manner:

$$\text{Frequency of allele} = \frac{\text{No. of homozygous individuals} + 1/2 \text{ No. heterozygous individuals}}{\text{Total No. of individuals observed}}$$

In the three breeds of sheep (Rambouillet, Targhee, and Columbia) that were bred in the diallel mating system, the percent homozygosity in the inbred lines was compared with the percent homozygosity of the line crosses. This was done to check the effect inbreeding has on the amount of homozygosity.

The frequencies of the alleles in each breed were used in the

Hardy-Weinburg equilibrium equation ($p^2 + 2\ pq + q^2 = 1$) in order to calculate the expected frequencies of the homozygotes and the frequency of the heterozygotes. This was compared with the frequencies obtained to examine if one combination of alleles has some type of selective advantage.

The mean values of the following production traits were compared by the Least Squares method of analysis between each combination of alleles, i.e. homozygous AA, heterozygous AB, and homozygous BB, in order to check the possibility of one of the combinations of alleles being a genetic marker for one of the production traits: (1) average daily gain from birth to weaning, (2) conformation at weaning, (3) condition at weaning, (4) weaning weight, (5) average daily gain from weaning to yearling age, (6) conformation at yearling age, (7) condition at yearling age, (8) grease fleece weight as yearling, (9) yearling fleece grade, (10) yearling body weight, (11) yearling index score, and (12) weaning index score.

The segregation of the alleles is demonstrated by showing some matings where all alleles are involved. Matings where the sire was homozygous for Hb. A were not observed in the present study, but there were several matings where the Hb. A allele in the heterozygous state was involved.

RESULTS AND DISCUSSION

By use of the disc acrylamide electrophoretic technique according to the method of Ornstein (1964) and Davis (1964) it was found that the polymorphic hemoglobins in the five breeds of sheep (Columbia, Lincoln, Rambouillet, Suffolk, and Targhee) investigated were of two types--Hb. A and Hb. B. The presence of any other hemoglobin in the samples observed was not detected. It is believed that the electrophoretic technique employed allowed a sufficiently strict classification of the hemoglobin types observed, and thus minimum amount of error was involved in the detection of hemoglobin polymorphism.

All samples of hemolysate containing Hb. A were investigated for the presence of Hb. C, and there was no Hb. C detectable in the blood of any of the breeds. This is not conclusive proof that Hb. C does not comprise a small percentage of the hemoglobin of the sheep in these five breeds that possess the allele for Hb. A, because it is possible that the small sample size (2.0 microliters) of hemolysate used was not large enough to detect the presence of molecules in such small amounts.

The identification of the two hemoglobins found was aided by a sample of Hb. AB hemolysate from a heterozygous sheep sent by Dr. R. A. Rasmusen from the University of Illinois College of Agriculture, Urbana, Illinois.

There was an immediate problem in the determination of the hemoglobin types because of the presence of two bands in some samples and three bands in the rest of the samples instead of the one band for homozygous sheep and two bands for heterozygotes which is normally found in electrophoretic patterns of sheep hemoglobins when starch gel electrophoresis is used (Beale et al., 1966). Ornstein (1964) states, however, that the disc acrylamide electrophoretic technique will dissociate a complex molecule if the electrophoretic mobilities of the components of the complex molecule are different from one another. This seemed to be what was happening, i.e. the hemoglobin tetramer was dissociating into the α and β chains (see Figure 1).

To further test this hypothesis, the hemolysate samples were purposefully dissociated to see if there were any differences detectable. Duesberg and Rueckert (1965) and Lehmann and Huntsman (1966) report that hemoglobin dissolved in 8 m urea solution would dissociate into its α and β chain components. The technique of Duesberg and Rueckert (1965) was applied to the sheep hemoglobins, and it was found that there was no difference in the number of bands or the R_B values of the bands between the same samples when one portion of the hemolysate was dissolved in 8 m urea solution and was subjected to electrophoresis at the same time as a portion of the same hemolysate which was not dissolved in 8 m urea.

A. Sample 210 - Homozygous AA

1. α^A $R_B = 0.500$
2. β^A $R_B = 0.549$

B. Sample 210 - Homozygous AA and sample 637 - Homozygous BB - mixed in equal volume

3. β^B $R_B = 0.430$
4. α^A and α^B $R_B = 0.492$
5. β^A $R_B = 0.551$

C. Sample 637 - Homozygous BB

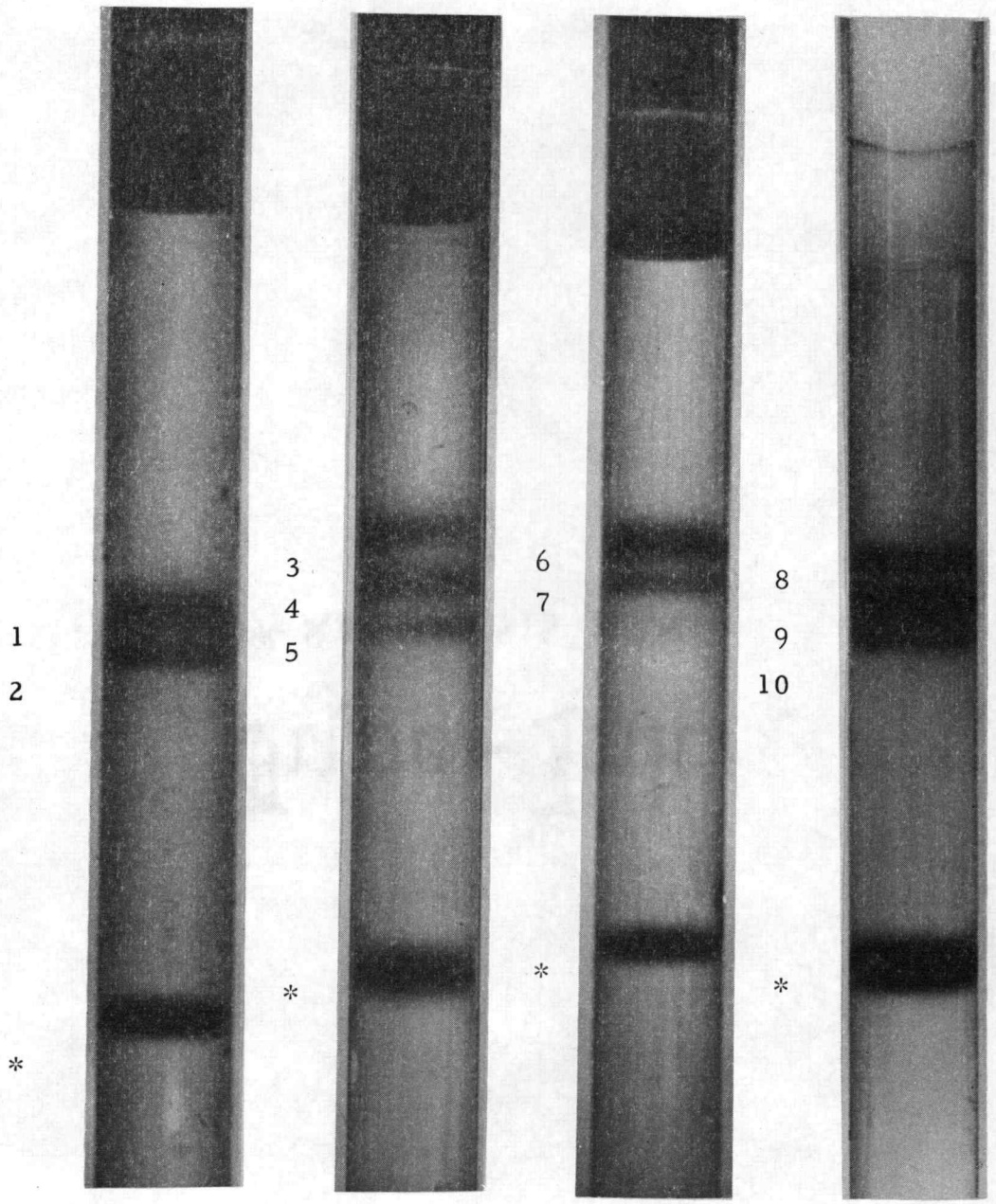
6. β^B $R_B = 0.425$
7. α^B $R_B = 0.491$

D. Sample 571 - Heterozygous AB

8. β^B $R_B = 0.429$
9. α^A and α^B $R_B = 0.489$
10. β^A $R_B = 0.562$

*Tracker dye band

Figure 1. Photographs of hemoglobin hemolysates after electrophoresis.


1
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8
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10
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A
B
C
D

It was observed that length of storage and carbon monoxide affecting the hemolysate also influenced the dissociation of the hemoglobin tetramer. It was observed that when hemoglobin samples were electrophoresed the same day the blood was collected and carbon monoxide was not bubbled through the hemolysate the dissociation of the tetramer was not as complete. This was determined by the density of the bands.

The β chain of Hb. A (β^{A}) was the most rapidly moving band; the α chain which is common to both hemoglobin types was the intermediate moving band (this was determined by the middle band in heterozygotes and the middle band when homozygous AA and homozygous BB samples were mixed being the heaviest of the three bands); and the β chain of Hb. B (β^{B}) was the slowest moving band (see Table 1).

The inheritance of the hemoglobin is by co-dominant alleles since the allele is expressed if the sheep possess it, i. e. in the heterozygous Hb. A and Hb. B are readily detected in the hemoglobin phenotype of the sheep. The alleles segregate freely from one another as is shown by the matings shown in Table 2.

Allele frequency

The frequencies of the hemoglobin types found in the five breeds of sheep examined are indicated in Table 3. The expected number of

Table 1. The mean values of the measured distances individual bands migrate and the mean values of the R_B value of each hemoglobin band.

Bands[1]	Distance Moved[2]	R_b[3]
Bromophenol Blue	75 ± 2	---
β^A	42.0 ± 0.5	0.573 ± 0.020
α^A and α^B	37.0 ± 0.5	0.496 ± 0.020
β^B	33 ± 0.5	0.440 ± 0.020

[1] The bands are arranged in order from fastest to slowest migrating band.

[2] The distance moved is measured in total lines moved and measurement made on a surface rule fifty lines to the inch.

[3] The error of the R_B values is determined by running one sample of each class of alleles forty times and calculting the error from the distribution of R_B values.

Table 2. Matings showing the segregation of the hemoglobin alleles.

SIRES		DAMS			OFFSPRING	
Eartag No.	Hemoglobin Phenotype	Eartag No.	Hemoglobin Phenotype	Sex	Eartag No.	Hemoglobin Phenotype
7788	AB X	570	BB	♀	1554	BB
7788	AB X	9789	BB	♀	1605	AB
7788	AB X	623	AB	♂	1679	AA
7788	AB X	9811	AA	♀	1630	AB
7633	AB X	8071	AB	♂	3713	AB
7633	AB X	8071	AB	♂	3714	BB
7633	AB X	7974	AB	♀	3929	AA
8623	BB X	9817	AA	♀	1599	AB
8623	BB X	202	AB	♂	1609	AB
8623	BB X	202	AB	♀	1610	BB
8623	BB X	9770	AB	♀	2096	BB

Table 3. The number of sheep sampled in each breed and the observed and expected numbers of individuals in each class of alleles.

Breed	Total No. of Animals		AA	AB	BB
Rambouillet	156	Observed	12	47	97
		*Expected	8.07	54.85	93.07
Targhee	274	Observed	24	98	152
		*Expected	19.45	107.11	147.47
Columbia	328	Observed	14	79	235
		*Expected	8.73	89.54	229.73
Suffolk	95	Observed	2	6	87
		*Expected	2.63	9.47	85.27
Lincoln	56	Observed	2	23	27
		*Expected	3.26	20.49	32.23

*Determined by Hardy-Weinburg equilibrium equation.

individuals in each class of alleles was calculated using the Hardy-Weinburg equilibrium equation. Differences between the observed number and expected number of individuals was tested for significance by the chi-square method. There were no significant chi-square values obtained when the differences between observed and expected number of individuals in any class of alleles in any of the breeds were examined. This indicates that the breeds examined are in equilibrium for the hemoglobin alleles and that there is no natural selection favoring any class of the alleles.

The fact that natural selection is not favoring one class of the alleles does not determine if one of the alleles is better adapted to a particular environment. It is expected that offspring of even a selected population would be in Hardy-Weinburg equilibrium until selection is practiced on them with the qualification that the parents are mated at random. The adaptive advantage of an allele in different environments can be determined by examining the frequency of the allele in different environments and testing for significant difference between the frequency of the allele in the two environments.

The Rambouillet, Columbia, and Targhee flocks that are maintained at altitudes of 4, 800 to 7, 500 feet above sea level and the Lincoln flock maintained at altitudes of 750 to 1, 200 feet above sea level had gene frequencies of the Hb. A allele of 16% or more as indicated in Table 4. The frequency of Hb. A allele in the Suffolk

flock that is maintained at 250 feet above sea level was 5.26% as indicated in Table 4. There is a highly significant difference between the frequency of Hb. A in the flocks maintained at higher altitudes and the one flock maintained at the relatively low altitude when the difference between the frequencies of Hb. A at the different altitudes is tested by the chi-square method. The Hb. A seems to give the animals that possess it an adaptive advantage in higher altitudes. This conclusion agrees with the data of several investigators, principally Evans, Harris, and Warren (1958b).

Table 4. The frequency of Hb. A at the different altitudes.

Breed	Frequency of A allele	Altitude*
Rambouillet	22.76%	4,800-7,500
Targhee	26.64%	4,800-7,500
Columbia	16.31%	4,800-7,500
Lincoln	24.11%	750-1,200
Suffolk	5.26%	250

*Altitude in feet above sea level.

The adaptive advantage Hb. A gives the animals is believed to be the result of Hb. A having a greater oxygen affinity than Hb. B. This possibility has been investigated by Dawson and Evans (1966) and they concluded that Hb. A did deliver more oxygen to the tissue

when the animals were exercised or when placed in an atmosphere with reduced oxygen content. They concluded from the results of their investigation that oxygen affinity differences between Hb. A and Hb. B were great enough to give Hb. A an adaptive advantage in higher altitudes.

The data in the present study are in agreement with the theory of selective advantage for Hb. A in higher altitudes. It is especially in agreement when the frequency of Hb. A is compared between the Rambouillets, Columbias, and Targhees that are maintained at the high altitudes around Dubois, Idaho, and the Suffolks which are maintained at the low altitudes of Corvallis, Oregon (see Table 4).

The Lincoln flock investigated also possess a frequency of Hb. A that is approximately the same as that of the breeds maintained at higher altitudes (see Table 4). This is not readily explainable because it is doubtful that the difference in 500 to 950 feet in altitude between the Lincoln and Suffolk flocks would exert the same selection pressure as the difference of 4, 550 to 7, 250 feet in altitude between the flocks at Dubois and the flock at Corvallis.

There are several explanations that are plausible: (1) This could possibly be an example of genetic drift by a small population (there are few Lincoln sheep in the area) where the frequencies determined are not representative of a population of sheep maintained at this altitude. (2) The sample number was small and this could result

in a very gross sampling error which might make the sample investigated not representative of a population maintained at this altitude. (3) It is possible that the altitude alone does not fully indicate the selection pressure of the environment in which these sheep are maintained. (4) It is possible that there is some selection for Hb. B in the flocks at Dubois which would lower the frequency of Hb. A in these flocks and thereby account for the frequency of Hb. A being approximately the same even though there is a big difference in altitude.

All of these things could explain the frequency of Hb. A being approximately the same in the Rambouillet, Columbia, Targhee, and Lincoln flocks even though they are in different environments. However, the possibility of the altitude not being fully indicative of the environment in which the Lincoln flock is maintained seems to be the most plausible. This is most evident when the pasture in which this Lincoln flock was maintained is observed. The pasture on which the sheep are grazed most of the time consists of very steep slopes and rugged terrain. The rugged terrain, when coupled with the wet and cold winters the sheep experience, constitutes an environment which might increase oxygen demands and thereby give Hb. A an adaptive advantage.

Additional evidence for the adaptive advantage of Hb. A in the Rambouillet, Columbia, and Targhee breeds is obtained when the difference in frequency of Hb. A between the unselected control lines

and the selected lines in these three breeds is examined.

In two breeds (Rambouillet and Targhee) there was a highly significant difference in the frequency of Hb. A between the unselected control lines and the selected lines (see Table 5) when tested by

Table 5. The frequency of Hb. A in the unselected control line and selected lines in the Rambouillet, Targhee, and Columbia flocks.

Breed	Line	Number of Animals	Frequency of Hb. A Allele
Rambouillet	Unselected Control	51	3.92%
	*Selected lines	105	31.90%
Targhee	Unselected Control	44	6.82%
	*Selected lines	230	30.44%
Columbia	Unselected Control	48	19.80%
	*Selected lines	280	15.36%

*These selected lines include the four inbred lines and their line crosses in the diallel mating system.

chi-square method. These two breeds showed a measurable increase in the frequency of Hb. A in the lines where selection was practiced. This seems to indicate that the Hb. A is being favored in the selection of animals for breeding. In the unselected control lines where the adaptive advantage of Hb. A would be neutral, the frequency of Hb. A remains low in these two breeds whereas the frequency of Hb. A has increased in the lines where selection has been practiced.

The exception to this situation is noticed in the Columbia breed

where there is no significant difference in the frequency of Hb. A between the unselected control line and the selected lines (see Table 5). The explanation for this deviation seems to be that there has been a sampling error in the unselected control line of the Columbia breed resulting from small sample size. There is a highly significant difference between the observed and expected numbers of individuals in each class of alleles in the Columbia unselected control line when tested with the chi-square method. In the Targhee and Rambouillet unselected control lines there is no significant difference between the expected and observed numbers of individuals in each class of alleles (see Table 6). This indicates that the sample group of the unselected control line of Columbias is not representative of the unselected control population as a whole.

Table 6. The numbers of observed and expected individuals in the unselected control lines of the Rambouillet, Targhee, and Columbia flocks.

Breed		AA	AB	BB
Rambouillet	Observed	1	2	48
	*Expected	0.01	1.92	47.10
Targhee	Observed	1	4	39
	*Expected	2.05	5.60	38.22
Columbia	Observed	5	9	34
	*Expected	1.88	15.30	30.87

*Determined by Hardy-Weinburg equilibrium equation.

In addition, when the frequency of Hb. A in the unselected control line of the Columbia breed is compared with the frequency of Hb. A that was previously determined in this control line by Stormont (1968), it is found that the frequency determined in the present study is significantly higher (see Table 7). There is no significant difference between the figures of the present study and those of Stormont for the frequency of Hb. A in the control lines of the Targhee and Rambouillet sheep.

Table 7. The frequency of Hb. A allele in the unselected control lines of the Rambouillet, Targhee, and Columbia flocks as determined in this study and by Stormont (1968).

Breed		Frequency of Hb. A Allele
Rambouillet	a	3.92%
	b	1.50%
Targhee	a	6.82%
	b	13.64%
Columbia	a	19.80%
	b	7.92%

[a]Frequencies of Hb. A in the unselected control lines.

[b]Frequencies of Hb. A in unselected control lines as determined by Stormont (1968).

These data substantiate the theory that Hb. A does have an adaptive advantage over Hb. B in the higher altitudes.

Inbreeding

It is expected that inbreeding an unselected population would increase homozygosity in a linear order, i. e. as the calculated coefficient of inbreeding increases one unit, the percent homozygosity should increase one unit. The hemoglobin locus provides an opportunity to examine the response of an uncomplicated two allele genetic system to inbreeding. The Columbia, Targhee, and Rambouillet breeds provide the opportunity to examine the homozygosity at different levels of inbreeding by investigating the four inbred lines in the diallel system in each breed. In this portion of the study the four inbred lines in each breed and the three breeds were all combined in order to provide meaningful numbers of individuals in each class of inbreeding percentage.

In these data, it is observed that an increase of inbreeding does increase the percent homozygosity regularly (see Figure 1). It is surprising that the response in the increase of homozygosity is as rapid as it apparently is at such relatively low levels of inbreeding. The class with the highest level of inbreeding may represent a sampling error since there are only ten animals in that class; however, it may represent a leveling off of the increase in homozygosity. The leveling off of an increase in homozygosity is what is expected with higher levels of inbreeding because the higher inbreeding might be

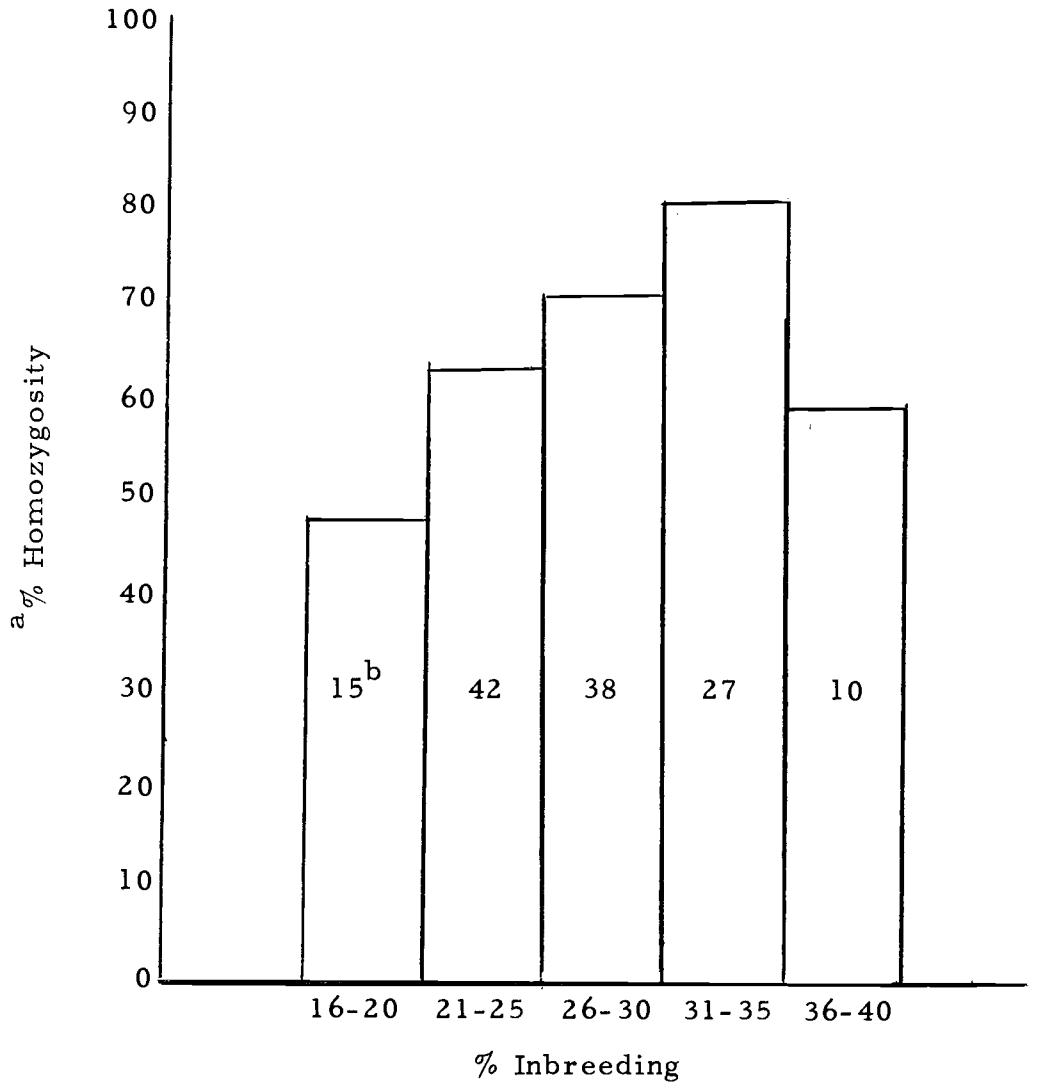

Figure 2. The response of increase in homozygosity to increased inbreeding.

[a]% homozygosity is calculated by dividing the number of homozygous individuals (AA and BB) by the total number of individuals in each class of inbreeding.

[b]Total number of individuals in each class of inbreeding.

expected to depress vigor and productivity of the animals. This depression of vigor is believed to be the result of the increase in homozygosity which accompanies increased inbreeding, creating a situation where the heterozygous individuals have a selective advantage; consequently homozygosity would not increase proportionally to the calculated coefficient of inbreeding. This may be what is occurring in the class of 36-40% inbreeding, i.e. the lower percentage of homozygosity may be indicating that the percentage homozygosity is lower than the calculated coefficient of inbreeding as a result of selecting for the heterozygous state.

The hemoglobin locus does not give a fair estimate of the amount of homozygosity in the entire genotype but it may be a reasonable monitor of the entire genotype.

Hemoglobin Types and Production Traits

The association of the hemoglobin alleles (AA, AB, or BB) with several production traits was examined in the Columbia, Targhee, and Rambouillet breeds. In the analysis all three breeds were combined to provide greater numbers. The mean values of the following production traits were determined for each of the hemoglobin alleles: (1) average daily gain from weaning to yearling age, (2) conformation at weaning, (3) condition at weaning, (4) weaning weight, (5) average daily gain from weaning to yearling age, (6) conformation at yearling

age, (7) condition at yearling age, (8) grease fleece weight as yearling, (9) yearling fleece grade, (10) yearling body weight, (11) yearling index score, and (12) weaning index score. The effects of sex, breed, year of birth, age of dam, and type of birth (twin or single) were removed by the Least Squares method of analysis, and the adjusted means of the production traits were tested for significant differences between class of alleles by F ratio values (see Table 8 and Table 9).

It was observed from the mean square values in Table 8 and Table 9 that none of the hemoglobin alleles serves as a genetic marker for the production traits which were examined. It was also observed from Table 8 and Table 9 that the effects of sex, breed, year of birth, age of dam, and type of birth influenced the production traits examined in this study.

The fact that one of the hemoglobin phenotypes does not serve as a genetic marker for at least one of the production traits is surprising since there has been an increase in the frequency of Hb. A in the selected populations (see Table 5). There are at least two explanations that are plausible for Hb. A not being associated significantly with any of the production traits examined. First, it can be observed from Table 10 that in most of the mean values of the production traits examined the heterozygous sheep for the Hb. alleles have a tendency to have higher values than either of the homozygous classes, although

Table 8. Mean square values of pre-weaning and weaning production traits.

	Degrees Freedom	Avg. daily gain - birth to weaning	Weaning Weight	Weaning Condition	Weaning Conformation	Weaning Index
Error	622	0.006	110.82	1.88	1.90	133.41
Hemoglobin Types	2	0.009	171.63	2.71	1.42	195.38
Breed-year	8	0.061**	1,826.14**	33.22**	68.27**	14,295.65**
Age of Dam	3	0.090**	1,480.37**	11.03**	5.11*	76.60
Type of Birth	2	0.725**	11,750.15**	166.58**	101.00**	120.60
Sex	1	0.504**	7,913.99**	47.69**	2.47	776.74**
Total	638					

*Significant F value at 5% level

**Significant F value at 1% level

Table 9. Mean square values of yearling production traits.

	D. F. [1]	A. D. G. [2] weaning to yearling	Yearling Body Weight	Yearling Condition	Yearling Conformation	Grease Fleece Weight	Yearling Fleece Grade	Yearling Condition
Breed-year	5	0.0142**	2,882.88*	75.70**	81.13**	74.51**	83.80**	388,595.25**
Age of dam	3	0.0036	851.82**	6.14*	5.45*	21.22**	0.38	41.02
Type of birth	3	0.0214**	3,055.37**	14.95**	7.078*	72.31**	0.31	404.95
Sex	1	0.0462**	22,808.86**	90.02**	34.07**	383.63**	2.25	1,444,325.18**
Hemoglobin Types	2	0.0004	78.36	1.63	0.89	1.26	0.39	406.83
Error	413	0.0016	199.75	2.14	1.98	3.78	0.99	570.29
Total	426							

[1] Degrees freedom

[2] Average daily gain

* Significant F value at 1% level

** Significant F value at 5% level

Table 10. Least-square means for production traits examined in each class of hemoglobin alleles.

	AA	AB	BB
Avg. daily gain birth to weaning	0.53 lbs.	0.55 lbs.	0.54 lbs.
Weaning Weight	72.47 lbs.	75.72 lbs.	74.31 lbs.
Weaning Condition	8.12	7.72	7.90
Weaning Conformation	8.65	8.35	8.48
Weaning Index	140.98	144.55	143.20
Yearling Body Weight	100.97	103.59	102.69
Avg. Daily Gain weaning to yearling	0.10 lbs.	0.10 lbs.	0.10 lbs.
Yearling Condition	7.92	8.15	8.01
Yearling Type	8.39	8.42	8.36
Yearling Grease Fleece Weight	10.56	10.90	10.76
Yearling Fleece Grade	3.46	3.34	3.41
Yearling Index	219.78	223.48	222.91

not significantly higher. This may result in the favoring of heterozygous individuals when selection is practiced. This would mean that the hemoglobin alleles serve as a monitor for the rest of the genotype but are neutral otherwise and do not furnish the animal any adaptive value. In this instance if the heterozygous individuals are favored in selection and the hemoglobin alleles are also in the heterozygous state, then the increase in frequency of hemoglobin A may be explained.

Second, the hemoglobin alleles may be directly influencing some other trait such as more lambs being produced by sheep possessing Hb. A; or the sheep that possess Hb. A may have longer production life spans which would result in sheep that possess Hb. A remaining in the population longer and thereby cause an increase in the frequency of Hb. A. No information could be obtained from the data to determine if either one of these explanations are feasible.

SUMMARY AND CONCLUSIONS

The examination of five breeds of sheep (Columbia, Targhee, Rambouillet, Lincoln, and Suffolk) for hemoglobin polymorphism by disc gel electrophoresis revealed that in the individuals sampled there exist two hemoglobin types--Hb. A and Hb. B. These two hemoglobin types were observed in the homozygous and heterozygous state and the alleles were found to segregate freely from one another. The hemoglobin alleles were found to be co-dominant.

It was observed that the frequency of Hb. A was influenced by the altitude at which the sheep were maintained. The Columbias, Targhees, and Rambouillets were maintained at altitudes of 4, 800 to 7, 500 feet above sea level; the Lincolns were maintained at altitudes of 750 to 1, 200 feet above sea level; and the Suffolks were maintained at 250 feet above sea level. The four breeds maintained at the relatively high altitudes had frequencies of Hb. A of 16% or greater while the Suffolks maintained at the relatively low altitude had a frequency of 5.26%. This is one indication that Hb. A has an adaptive advantage over Hb. B at high altitudes.

The discovery that the frequency of Hb. A is approximately the same in the Lincoln flock and the Columbia, Targhee, and Rambouillet flocks although the latter are maintained at much higher altitudes could be explained in several ways: (1) The Lincoln flock could

possibly be an example of genetic drift, (2) the small sample size of the Lincoln flock could have resulted in a sampling error, (3) it is possible that the altitude alone does not fully indicate the extreme environment in which these sheep are maintained, and (4) it is possible that there is some reason for Hb. A not being in as high a frequency as it would in natural conditions at the Dubois station. The possibility of the Lincoln flock being maintained in a more rigorous environment than indicated by altitude alone seems to be the most plausible explanation for the above results.

In addition, the increase of the frequency of Hb. A in the selected populations of the Rambouillet and Targhee breeds over the frequency of Hb. A in the unselected control populations of these two breeds seems to indicate that Hb. A has increased where selection is practiced. It would seem from these data that Hb. A has an adaptive advantage in the higher altitudes and has increased by selection, but in the unselected control population where the adaptive advantage of Hb. A would be neutral, it has remained low.

It was discovered that an increase in homozygosity, at least in the hemoglobin locus, was produced by increasing the inbreeding. The increase in homozygosity was approximately in a straight line with an increase in inbreeding until inbreeding reached a level of 35% after which there was a drop in the homozygosity at the hemoglobin locus. This drop in homozygosity could be explained by a sampling

error since there were only ten animals in the 36-40% level of inbreeding or it could represent a favoring of the heterozygous individuals due to inbreeding depression.

The mean values of the following production traits adjusted for sex, breed, year of birth, age of dam, and type of birth were compared to determine if significant differences in production traits were associated with allelic differences at the hemoglobin locus (Hb. AA, AB, and BB): (1) average daily gain from birth to weaning, (2) weaning weight, (3) weaning condition, (4) weaning conformation, (5) weaning index, (6) average daily gain from weaning to yearling, (7) yearling condition, (8) yearling conformation, (9) yearling body weight, (10) yearling grease fleece weight, (11) yearling fleece grade, and (12) yearling trait. There were no significant differences between the adjusted mean values of the production traits associated with class of alleles, however, there was a tendency for the animals heterozygous at the hemoglobin locus to have higher mean production values. This could possibly explain the increase in the frequency of Hb. A in selected populations, i. e. the hemoglobin locus may be an indicator of the heterozygosity for the rest of the genotype. Thus, the individuals heterozygous for the hemoglobin locus would be favored in selection, and an increase in the frequency of Hb. A would result. Other possibilities which would explain the increase in frequency of Hb. A in the selected lines is that Hb. A could endow those sheep that

possess it with a longer production life span so that they are less likely to be replaced; or those sheep that possess Hb. A might produce more lambs which would make more individuals which possess Hb. A available for selection as replacements. The information to support these possibilities is not available in the present study.

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