

Milk trait heritability and correlation with heterozygosity in yak

X.P. JIANG¹, G.Q. LIU², C. WANG³, Y.J. MAO², Y.Z. XIONG¹

¹ College of Animal Science and Veterinary Medicine, Huazhong Agricultural University,
Wuhan, P.R. China

² College of Animal Science and Veterinary Medicine, Yangzhou University, Yangzhou, P.R. China

³ College of Life Science, Xiamen University, Xiamen, P.R. China

Abstract. 382 yak cows were examined for milk yield, fat, protein and lactose contents. Six polymorphic loci, α_{s1} -CN, κ -CN, β -CN, β -Lg, α -La and MUC-1, were scored by PAGE electrophoresis for each individual. The values of milk yield, fat, protein and lactose content were 247.13 kg, 5.81%, 5.18% and 4.93%, respectively. Based on the 6 polymorphism loci, the average heterozygosity of the yak population was 0.1794. Calculated by the marker-based method, heritability estimates for milk yield, fat, protein and lactose contents were 0.353 ± 0.093 , 0.316 ± 0.101 , 0.415 ± 0.098 and 0.481 ± 0.035 , respectively. The relatively high or medium heritability of these traits indicate that it is feasible to rely directly on them in breeding for the improvement in a relatively short period. The significant linear regression between heterozygosity and fat percentage with a positive slope ($R = 0.0420$) indicated that inbreeding affected milk fat content in this population.

Key words: associative overdominance, heritability, milk trait, yak.

Introduction

The yak (*Bos grunniens*) is a unique farm animal native to central Asia and well adapted to the high altitude, cold Himalayas and Tibetan Plateau environment. There are two types of yaks: the valley-type and the plateau-type. The valley-type yak was raised mainly by the *Kham* Tibetan and the plateau-type by the *Amdo* Tibetan in China (WU 1999). About 15 million yaks and yak-hybrids provide multiple products for these remote areas, including milk, meat, hair and skin. Although yak is a very important farm animal, the performance of the yak in Tibet have been degrading sharply in the recent years (JI et al. 2003, ZI 2003).

Received: February 10, 2004. Accepted: March 28, 2004.

Correspondence: X.P. JIANG, College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, P.R. China, e-mail: xpjiang@mail.hzau.edu.cn

The *Jiulong* yak is the typical valley-type raised by the *Kham* Tibetan people for many centuries in western Sichuan. Traditionally, pure breeding was the main method for selection and even today crossbreeding is rarely practiced. This may be attributed to the topography of the distribution area: high mountains with deeply incised valleys restrict the exchange of yaks. Because of the rarely exchanged genes between yak populations and the relatively small populations, the inbreeding levels of the yak population may be high (the actual situation is unknown).

At any given time, there will be variation in individual inbreeding levels in the yak population. If inbreeding mainly contributes to the depression of the population, there must be a correlation between individual milk traits and individual inbreeding. Pedigree records are useful for the assessment of this correlation. Unfortunately, there are no pedigree records in our yak populations. Thus, genetic markers might provide an alternative strategy (JIANG et al. 2002). Three methods can be used for this purpose, (1) the estimation of the inbreeding coefficient for individuals using markers to reconstruct the non-existent pedigree records, and then look for a correlation between traits and the deduced inbreeding coefficient; (2) the construction of a genotypic index that depends on the mutational dynamics of the marker locus as an indicator of inbreeding; (3) looking for a correlation between the marker heterozygosity (as an inverse indicator of inbreeding) and the traits of individuals. If significant correlations exist, inbreeding may affect the investigated traits. Such correlations are often found in the pig (JIANG et al. 2003a, LIU et al. 2003), yak (JIANG et al. 2003b), seal (COLTMAN et al. 1998) and many other species (BIERNE et al. 2000, TSITRONE et al. 2001).

Heritability is usually estimated with animals of known relatedness reproduced using a controlled breeding program (FALCONER, MACKAY 1996). But in our yak population, pedigree information was undocumented, although genetic parameters were keenly needed for genetic improvement. To our knowledge, there were no estimates of heritability for yak milk traits up to now. Fortunately, the marker-based method could work in this situation (RITLAND 1996). Estimates of heritability for the pig (JIANG et al. 2002) and many other species (MOUSSEAU et al. 1998, RITLAND 2000, THOMAS, HILL 2000, KLAPER et al. 2001) were obtained successfully by the marker-based method.

The objectives of this study were (1) to measure the milk traits, (2) to estimate heritability based on the marker method and (3) to estimate inbreeding depression in the yak population.

Material and methods

Animals and milk traits

A total of 382 yak cows in a typical colony of classification were randomly sampled. A 30 mL milk sample was collected from each yak cow at the morning milking. The data on the daily milk yield of each yak cow was collected and

the 6-month milk yield was deduced and adjusted to the third parity by the milk index method (CAI 1992). Milk fat percentage, milk protein percentage and lactose for each sample were mensurated by a Milkscan-1340A/B (Denmark).

Genetic variation estimation

Six polymorphic loci, Casein α_{s1} (α_{s1} -CN), Casein κ (κ -CN), Casein β (β -CN), lactoglobulin β (β -Lg), alpha-lactoalbumin (α -La) and epithelial mucin (MUC-1), were scored by PAGE electrophoresis (GROSCLAUDE 1987, MEDRANO, SHARROW 1989) with slight modifications (JIANG 2003b). Heterozygosity was averaged across the six protein loci for each yak. Mean heterozygosity values were used for univariate trait regression analyses.

Heritability estimation

Heritability is estimated by methods described by JIANG et al. (2002). In brief, yaks in the population were measured for milk traits and genotyped for protein marker loci. A linear estimation procedure used by RITLAND (2000) was employed to infer the relatedness between pairs of yaks based upon the protein marker data. Here the distribution of relatedness was supposed to be near continuum. Estimates of relatedness are correlated to phenotypic similarities to infer heritabilities for quantitative traits. Pairwise relatedness is defined as the probability with which two homologous alleles from two separate individuals are identical by descent. Marker-based methods can be employed to estimate pairwise relatedness. Consider a single locus with n alleles indexed by $i, j, k, l = 1, 2 \dots n$. Relatedness (r) between pairs of individuals can be estimated via a weighted method of moment estimator. The estimator can be described as follows. Let the first individual have the alleles A_i and A_j and the second, A_k and A_l . If alleles A_i and A_k are identical (e.g., the same band), then $\delta_{ik} = 1$; if they are different, $\delta_{ik} = 0$. In total, there are four pairwise comparisons, the estimator of pairwise relatedness is

$$\hat{r} = \frac{[(\delta_{ik} + \delta_{il}) / p_i] + [(\delta_{jk} + \delta_{jl}) / p_j] - 1}{4(n - 1)}$$

where n is the number of codominant alleles at the locus and p_i is the estimated frequency of allele A_i .

Yet relatedness is often estimated from multiple loci. Under the assumption that the loci are unlinked and the locus-specific estimates are independent, an efficient estimator is the sum of locus-specific estimates, each weighted proportionally by $(m-1)$, where m is the number of the loci.

To estimate heritability, a "phenotypic similarity" (Z) is defined as,

$$Z = \frac{(Y - \mu)(Y' - \mu)}{V},$$

where μ and V are the phenotypic mean and variance, Y and Y' are the trait measures on each individual. The yaks are raised under similar environmental condi-

tions, so the traits need not be adjusted to specific environmental conditions in this study. Heritability is then estimated as the covariance of Z with relatedness r (C_{Zr}), divided by the actual variance of relatedness (V_r) estimated across all pairs of individuals

$$\hat{h}^2 = C_{Zr} / 2V_r .$$

Regression analysis

Tests for overdominance effects described by DAVID (1998) were applied to the heterozygosity-milk trait regressions. Data was analyzed using univariate regression models (SAS 1996). The univariate analysis consisted of linear regression between the various milk traits (dependent variables) and mean heterozygosity (the independent variable). All univariate relationships were visually inspected for possible nonlinear relationships using scatterplots.

Results and discussions

Parameters of milk yield, fat, protein and lactose contents are listed in Table 1. The four traits were in correspondence with those of previous reports (LONG et al. 1999, JIANG et al. 2003b). The standard deviation for milk yield was relatively high and the range was very wide (from 63 to 529.50 kg). The standard deviation and range for fat, protein and lactose contents were much similar to those of milk yield, which suggested that the yak population lacks artificial selection and contains sufficient genetic variability. This result was highly convergent with the actual situation.

Table 1. Sample size, means (with standard deviation and ranges) of milk trait values

Trait	Sample size	Mean	Std	Min. value	Max. Value
Milk yield (kg)	382	247.13	84.94	63.00	529.50
Fat (%)	380	5.81	1.32	2.08	9.25
Protein (%)	380	5.18	0.91	3.22	8.72
Lactose (%)	378	4.93	0.72	3.52	12.10

Std = standard deviation of the mean; Min. = minimum; Max. =maximum.

Gene frequencies of the six loci are listed in Table 2. The α_{s1} -CN locus was highly polymorphic, with 5 alleles and 8 genotypes. The MUC-1 locus was another highly polymorphic locus, with 4 alleles and 10 genotypes. There were 3 genotypes for κ -CN, β -CN, β -Lg and α -La locus, respectively. The average heterozygosity of the yak population was 0.1794. The expected heterozygosity (H_E) in 5 loci was higher than the observed heterozygosity (H_O).

Table 2. The gene frequency (P), observed heterozygosity (H_O), and expected heterozygosity (H_E) of protein loci in the yak population

Locus	Alleles	P	H_O	H_E	D
α_{s1} -CN			0.3158	0.4715	-0.3302
	A	0.0048			
	B	0.0144			
	C	0.1268			
	D	0.6986			
κ -CN	E	0.1555			
			0.2153	0.2142	0.0050
β -CN	A	0.1220			
	B	0.8780			
β -Lg			0.0150	0.1840	-0.9185
	A	0.8975			
α -La	B	0.1025			
			0.0383	0.0467	-0.1791
	B	0.0239			
α -La	D	0.9761			
			0.0900	0.1040	-0.1342
	A	0.055			
MUC-1	B	0.945			
			0.5072	0.6348	-0.2009
	A	0.4617			
	B	0.1005			
	C	0.0670			
	D	0.3708			

Note: $D = (H_o - H_E)/H_E$.

Table 3. Estimates of heritabilities (SE*) and genetic correlations in the yak based upon the marker method

Trait	Milk yield	Fat	Protein	Lactose
Milk yield (kg)	0.353 (0.093)			
Fat (%)	0.125	0.316 (0.101)		
Protein (%)	0.254	0.211	0.415 (0.098)	
Lactose (%)	0.118	0.101	0.201	0.481(0.035)

* SEs in parentheses, based on 100 bootstraps, calculated as a mean over the range of possible fractions of full-sibs by using marker data, from $\alpha = 0.07$ to $\alpha = 0.10$. Data on diagonal were heritability (SE); data below diagonal were genetic correlations.

Polymorphism information contents (*PIC*) for α_{s1} -CN, κ -CN, β -CN, β -Lg, α -La and MUC-1 were 0.4312, 0.1913, 0.1838, 0.0456, 0.0985 and 0.5658, respectively.

Heritabilities estimated using the marker-based approach are presented in Table 3. As a whole, estimates of heritability for milk traits were significantly higher than zero (ranging from 0.316 to 0.481), which was highly in accordance with previous estimates for black and white cows, obtained using sib analyses in a conventional quantitative genetic design (WEIGEL et al. 2001). These estimates belong to the medium or high heritability. It suggests that improving yak milk traits by direct selection is possible. There were positive genetic correlations among all the traits (from 0.101 to 0.254).

At five loci the mean milk fat content of the heterozygote was higher than that of the homozygote (see Table 4). Partial *F* tests indicated that one positive difference (α_{s1} -CN) was statistically significant (6.42 vs 5.55, $P < 0.01$). The sign test

Table 4. Means (\pm Std) of milk yield, fat, protein and lactose contents for locus heterozygosity and homozygosity

Locus	Trait	Heterozygosity	Homozygosity
α_{s1} -CN	Milk yield (kg)	266.41 \pm 94.44	238.85 \pm 79.51
	Fat %	6.42 \pm 1.48**	5.55 \pm 1.16
	Protein %	5.34 \pm 1.13	5.11 \pm 0.79
	Lactose %	4.85 \pm 0.58	4.97 \pm 0.77
κ -CN	Milk yield (kg)	258.97 \pm 84.10	244.13 \pm 85.20
	Fat %	6.08 \pm 1.23	5.73 \pm 1.34
	Protein %	5.22 \pm 1.03	5.17 \pm 0.87
	Lactose %	4.98 \pm 1.29	4.92 \pm 0.42
β -CN	Milk yield (kg)	258.12 \pm 84.10	244.77 \pm 85.20
	Fat %	6.11 \pm 1.23	5.62 \pm 1.34
	Protein %	5.12 \pm 1.03	5.10 \pm 0.87
	Lactose %	4.91 \pm 1.29	4.85 \pm 0.42
β -Lg	Milk yield (kg)	224.60 \pm 52.54	247.94 \pm 85.87
	Fat %	6.17 \pm 0.76	5.80 \pm 1.34
	Protein %	5.24 \pm 0.56	5.18 \pm 0.92
	Lactose %	4.43 \pm 0.43	4.95 \pm 0.72
α -La	Milk yield (kg)	258.44 \pm 84.10	243.83 \pm 85.20
	Fat %	6.11 \pm 1.23	5.83 \pm 1.34
	Protein %	5.29 \pm 1.03	5.14 \pm 0.87
	Lactose %	4.97 \pm 1.29	4.94 \pm 0.42
MUC-1	Milk yield (kg)	245.62 \pm 86.73	249.13 \pm 84.52
	Fat %	5.71 \pm 1.27	5.92 \pm 1.39
	Protein %	5.18 \pm 0.86	5.18 \pm 0.97
	Lactose %	4.99 \pm 0.86	4.87 \pm 0.53

In the same line means with superscript: **, $P < 0.01$

Table 5. Results of the univariate regression analysis for milk traits with individual heterozygosity

Trait	Interception	Slope	R^2	P
Milk yield (kg)	235.2031	11.5909	0.0122	0.1477
Fat %	5.4613	0.3271	0.0420	0.0050
Protein %	5.0799	0.0944	0.0067	0.2730
Lactose %	4.9351	-0.0024	0.0000	0.9702

Boldfaced values are those significant at $P < 0.01$.

and the sequential Bonferroni test (RICE 1989) suggest that there is an overall trend for the heterozygote to exceed the homozygote in terms of all the four milk traits.

The univariate regression analyses yielded one significant regression: heterozygosity with fat percentage (Table 5). The significant linear regression between heterozygosity and fat percentage (Figure 1) had a positive slope with

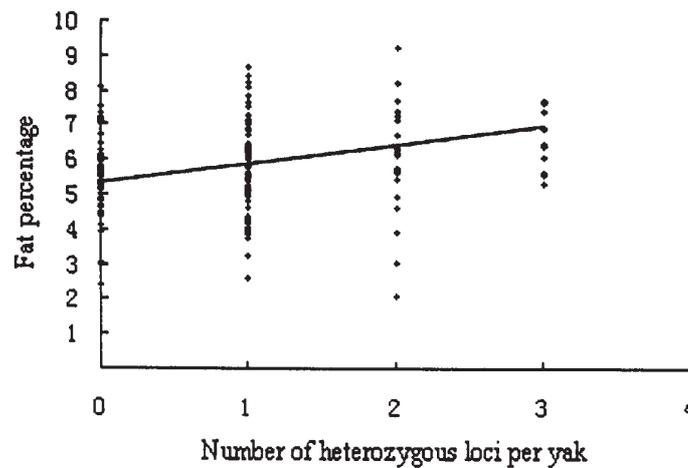


Figure 1. Milk fat content regressed against multiple locus heterozygosity at six polymorphic enzyme loci

an R -value of 0.0420 ($P < 0.01$). This result indicated that inbreeding significantly affected milk fat content in the yak population. No significant regression relationships were found between heterozygosity and milk yield, protein or lactose contents. Visual inspection of all the univariate scatterplots showed no evidence of nonlinear relationships for any of the traits, although most univariate relationships had positive slope estimates (Table 5). To our knowledge, this is the first report on

using genetic markers to examine heterozygosity–milk traits correlations in the yak, although many literature sources showed a positive correlation between individual heterozygosity at neutral allozyme loci and some fitness traits in other species (DAVID 1998, BIERNE et al. 2000).

Two alternative hypotheses with opposite effects could be put forward to explain the phenomenon, i.e. that of background selection (CHARLESWORTH et al. 1993), and associative overdominance (FRYDENBERG 1963). When homologous chromosomes carry harmful mutations at different loci, the individuals that are chromosomal heterozygotes are at the same time heterozygous at these selected loci and at other loci in the same linkage group. If the harmful mutations are completely or partially recessive, selection on them leads to associative overdominance, which is “due to the nonrandom linkage between the observed pair of allelic units and the entire remainder of genetic alternatives present in the same chromosome” (FRYDENBERG 1963). Associative overdominance depends on linkage disequilibrium between selected and neutral loci, and the effect is expected to be strongest in small populations and in genomes with restricted recombination. There is a large amount of literature data on the positive correlation between individual heterozygosity at allozyme loci and some fitness traits (DAVID 1998, GRANT, ELEFTHERIOS 1994). In our study, whether the correlation is the result of intrinsic functional differences between gene variants at the electrophoretic loci scored, or whether it arises from non-random genotypic associations between these loci and others segregating for deleterious recessive genes (the associative overdominance hypothesis) should be confirmed in the future research.

Implication

The expected genetic improvement in an animal population is determined by the heritability of target traits, selection differential and genetic correlations among traits. Heritability estimates for yak milk traits, ranging from 0.215 to 0.353, indicate that it is feasible to rely directly on them in breeding for the improvement of milk traits in a relatively short period.

There is an overall trend for heterozygote means to exceed homozygote means for all the four milk traits in the yak population. A significant effect of heterozygosity on the fat content was found in the yak population. The univariate regressions explained the observed variance in the milk fat of about 4%. It was a strong relationship between heterozygosity and milk fat. That is to say, in our yak populations, inbreeding has a significant effect on fat, although milk yield, protein and lactose contents are not affected by inbreeding. So more attention should be paid to this problem and crossing between these yak colonies should be encouraged to improve heterozygosity, which could improve significantly milk performance.

Acknowledgement. This work was supported by a grant from the National Nature Science Foundation of China (NSFC, 30300253). The kind cooperation of the workers and farmers in the sampling area is greatly acknowledged.

REFERENCES

- BIERNE N., BEUZART I., VONAU V., BONHOMME F., BÉDIER E. (2000). Microsatellite-associated heterosis in hatchery-propagated stocks of the shrimp *Penaeus stylirostris*. *Aquaculture* 184: 203-219.
- CAI L. (1992). Yak of China (in Chinese). Agriculture Publishing House, Beijing.
- CHARLESWORTH B., MORGAN M.T., CHARLESWORTH D. (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics* 134(4): 1289-1303.
- COLTMAN D.W., BOWEN W.D., WRIGHT J.M. (1998). Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 265: 803-809.
- DAVID P. (1998). Heterozygosity-fitness correlations: new perspectives on old problems. *Heredity* 80: 531-537.
- FALCONER D.S., MACKAY T.F.C. (1996). *Introduction to Quantitative Genetics* (Fourth edition). Longman Group Limited, Essex.
- FRYDENBERG O. (1963). Population studies of a lethal mutant in *Drosophila melanogaster*. I. Behaviour in populations with discrete generations. *Hereditas* 48: 89-116.
- GRANT H.P., ELEFATHERIOS Z. (1994). Allozyme and RFLP heterozygosities as correlates of growth rate in the Scallop *Placopecten magellanicus*: a test of the associative overdominance hypothesis. *Genetics* 137: 221-231.
- GROSCLAUDE F. (1987). Note on genetic polymorphism of milk proteins in Mongolian cattle and yaks. *ABA*. 55: 535.
- JIM J., PU C., DAWA Y., TSEREN D., DAWA C., ZHANG Y.Q., LOBSANG (2003). The situation of resources and productivity degradation of yak in Tibet. *Chin. J. Anim. Sci. Vet. Med.* 34(4): 368-371.
- JIANG X.P., LIU G.Q., XIONG Y.Z., DENG C.Y., QU Y.C. (2002). Heritability of pig growth traits estimated using molecular marker-based method. *Development and Reproductive Biology* 11(3): 161-166.
- JIANG X.P., XIONG Y.Z., LIU G.Q., DENG C.Y., QU Y.C. (2003a). Effects of individual gene heterozygosity on growth traits in swine. *Acta Genet. Sin.* 30(5): 431-436.
- JIANG X.P., MAO Y.J., XIONG Y.Z., LIU G.Q. (2003b). Association between individual gene heterozygosity and milking traits for yak. *J. Nanjing Agricultural Univ.* 26(3): 68-72.
- KLAPER R., RITLAND K., MOUSSEAU T.A., HUNTER M.D. (2001). Heritability of Phenolics in *Quercus laevis* inferred Using Molecular Markers. *The Journal of Heredity* 92: 421-426.
- LIU G.Q., JIANG X.P., XIONG Y.Z., DENG C.Y., QU Y.C. (2003). Effects of gene heterozygosity on meat quality traits in swine. *J. Nanjing Agricultural Univ.* 26(1): 56-60.

- LONG R.J., ZHANG D.G., WANG X., HU Z.Z., DONG S.K. (1999). Effect of strategic feed supplementation on productive and reproductive performance in yak cows. *Prev. Vet. Med.* 38(2-3): 195-206.
- MEDRANO J.F., SHARROW L. (1989). Milk protein typing of bovine mammary gland tissue used to generate a cDNA library. *J. Dairy Sci.* 72: 3190-3196.
- MOUSSEAU T.A., RITLAND K., HEATH D.D. (1998). A novel method for estimating heritability using molecular markers. *Heredity* 80: 218-224.
- RICE W.R. (1989). Analyzing tables of statistical tests. *Evolution* 43: 223-225.
- RITLAND K. (1996). A marker-based method for inferences about quantitative inheritance in natural populations. *Evolution* 50: 1062-1073.
- RITLAND K. (2000). Marker-inferred relatedness as a tool for detecting heritability in nature. *Mol. Ecol.* 9: 1195-1204.
- SAS (1996). SAS/STAT Software: Changes and enhancements through release 6.11. SAS Institute Inc., Cary, N.C., USA.
- THOMAS S.C., HILL W.G. (2000). Estimating quantitative genetic parameters using sibships reconstructed from marker data. *Genetics* 155: 1961-1972.
- TSITRONE A., ROUSSET F., DAVID P. (2001). Heterosis, marker mutational processes and population inbreeding history. *Genetics* 159: 1845-1859.
- WEIGEL K.A., REKAYA R., ZWALD N.R., FIKSE W.F. (2001). International genetic evaluation of dairy sires using a multiple-trait model with individual animal performance records. *J. Dairy Sci.* 84(12): 2789-2795.
- WU N. (1999). Pattern of high altitude vegetation on the eastern Tibetan plateau and its changes under human impacts during the historical period. In: *Environmental Changes in High Asia*. (G. Miede, ed.), Proc. Int. Symp. Marburg, Germany. Marburg University, Marburger Geographische Schriften.
- ZI X.D. (2003). Reproduction in female yaks (*Bos grunniens*) and opportunities for improvement. *Theriogenology* 59(5-6): 1303-1312.