PEDIGREE ANALYSIS IN CRIOLLO LIMONERO.

Análisis de Pedigrí en Criollo Limonero.

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ABSTRACT

In order to continue the genetic program for the conservation of the Criollo Limonero population, a study was carried out to evaluate the genetic variability of this local breed. For this purpose, 2552 genealogical records from the Criollo herd at the Carrasquero Local Station (INIA) located at Playa Bonita, Mara County, North of Zulia State-Venezuela, during the 1985-2003 period were used. Global inbreeding of the herd (f_H) and animal inbreeding were determined; in addition, Average of relatedness (AR), probabilities of gene origin, number of founders (NF), effective number of founders (f_e), effective ancestors number (f_a), genome founders number (N_g) and number of founders explaining 50% (F50) of the genetic variability. For the year 2003 f_H was 0.35%, whereas AR, NF, f_e, f_a, N_g and F50 were 3.4%, 386, 63.5, 38, 27.71 and 18 respectively. Even though AR showed a high relationship among the individuals in the herd, f_H did not have a significant increase. For this reason, mates should be planned carefully in order to avoid related mates. Probabilities of gene origin suggest that genetic variation has decreased in this population due to a bottleneck effect and genetic drift, without increasing inbreeding. An important genetic diversity exists in this population which should be preserved, given the importance of this genetic resource for the region and the country.

Key words: Criollo limonero, bovine, gene origin probability, inbreeding.

RESUMEN

Con el objetivo de continuar el programa de conservación genética de la raza Criollo Limonero, se realizó un estudio para evaluar la variabilidad genética del rebaño, utilizando 2552 registros genealógicos disponibles entre los años 1985 y 2003 en la Estación Local Carrasquero, ubicada en el sector playa Bonita, municipio Mara, al norte del estado Zulia-Venezuela. Se determinó la consanguinidad de cada animal y la consanguinidad global del rebaño (f_H), el promedio de relación de parentesco (AR), los parámetros de la probabilidad de origen del gen, el número de fundadores (NF), el número efectivo de fundadores (f_e), el número efectivo de ancestros (f_a), número de genomas fundadores (N_g) y el número de fundadores que explican el 50% (F50) de la variabilidad genética. La f_H en el año 2003 fue de 0,35% y el AR 3,4%, el NF 386, f_e 63,5, f_a 38, N_g 27,71; y el valor de F50 fue 18. La f_H no tuvo un incremento marcado a pesar que el AR indicó que existe una alta relación entre los individuos de la población, por lo que los apareamientos deben planificarse para evitar el apareamiento de animales emparentados. Los valores de probabilidad de origen de los genes indican que la población ha perdido variabilidad genética por un efecto de cuello de botella y por deriva genética, aunque este deterioro no se refleje en un incremento en la consanguinidad. Aun existe una cantidad importante de diversidad que debe preservarse, dada la importancia que representa este germoplasma para la región y el país.

Palabras clave: Criollo limonero, bovino, probabilidad origen del gen, consanguinidad.

INTRODUCTION

Currently, preservation of domestic species is an important strategy for contributing with animal biodiversity, breed conservation as well as utilization of genes involved in animal production. In developing countries, deterioration of bovine populations is even greater due to the lack of resources needed for planning of preservation programs where native breeds have been substituted by noble ones for production of both milk and beef.

In Latin America, there is a great deal of breeds brought up to the continent by conquerors from Europe, which have been adapted for centuries to tropical environments. These

breeds called "Criollas" are widely spread in Central as well as South America and the Caribbean Sea. In the specific case of Venezuela, the bovine named Criollo Limonero has been oriented for milk production through selection in western Zulia State. This particular breed is used in crossbreeding plans because of its great combining ability for reproduction and milk yields with exotic breeds.

Populations are highly related to genetic variation throughout time, and theoretically, the smaller the genetic variability the greater the deleterious gene expression is, which could compromise the survival of any given population. That is why some methods allowing evaluation of genetic variability are performed; such as, pedigree analysis, description of visible genes and molecular markers [1-4, 21].

In small populations, the lack of genetic variation is generally evaluated through inbreeding rate, with a high sensitivity of the parameter to pedigree quality as well as some disadvantage of the population about not remaining closed for much time [14].

Probabilities of gene origin, previously utilized in wildlife populations [6], have been recently utilized for evaluating the genetic variation in domestic animals by considering detection of some significant changes in those animals before increasing inbreeding [10-15, 25].

The objectives of this study were: to analyze individual pedigrees within herd with the purpose of characterizing the genetic variability under the hypothesis of existence of a correlation between variability and preservation of the breed; and to suggest new strategies that allows to continue to work on the preservation program of the Criollo Limonero breed.

MATERIALS AND METHODS

According to The National Institute for Agricultural Research in Venezuela (INIA), the Criollo Limonero herd is nearly 500 pure heads of cattle [8]. This reduced population of bovines, as stated by Barker [5] is becoming in danger of extinction. The Laral Research Unit, located at Carrasquero town, within Mara County, in Zulia State, western Venezuela, has a tropical dry forest climate with 27.4°C air temperature and 1,000 mm rainfall, annually. For three decades, the Criollo herd bas been mated among 5 families to avoid inbreeding. Mating plans for Bulls from a family sire to heifers and cows of the preceding family are depicted in (FIG. 1).

Data for pedigree analyses included 2630 records of bulls and cows born between the years 1985 to 2003, including 554 animals born before 1985, which were added to the base population. Pedigree files were edited using SAS [22] and data file included animal, sire, dam, birth date, sex, and pertaining family. Every animal was coded using year of birth, animal identification and sex, in order to assure a unique identification number. All animals were recoded by using the Pedigree
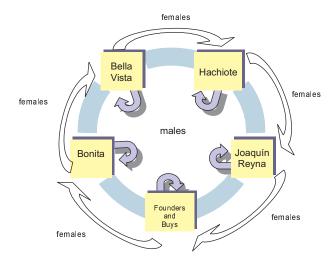

FIGURE 1. FAMILY DISTRIBUTION AND MATING DESIGN AT CARRASQUERO LOCAL STATION/ DISTRIBUCIÓN DE LAS FAMILIAS Y DISEÑO DE LOS APAREAMIENTOS EN LA ESTACIÓN LOCAL CARRASQUERO.

Viewer Program [16]. Inbreeding, probability of gene origin and relationship average were estimated using the Fortran Pedigree Package of Boichard [7].

The inbreeding coefficient F, defined as the probability for an individual having two identical alleles by descent [28] was estimated by the method of Meuwissen and Luo [18] and Van Raden [23]. Average of Relatedness for each animal was estimated as the row average of the numerator relation matrix (A) for each animal.

Number of individual founders was calculated assigning to each individual a value of 1.0 for those belonging to that population, a value of 0.5 for every offspring and 0.25 for the case of grand sons, and so forth. The effective size of a population (N_e) was defined as the size of an ideal population capable of producing an inbreeding increase similar to that expected [28] and the following mathematical expression was used for its calculation:

$$N_e = \frac{4 * N_m * N_h}{N_m + N_h}$$

Where both, N_m and N_h is the number of sires and dams in the population, respectively. Mainly, this parameter has been utilized as a predictor of the breed or population capability for maintaining through time; although it may be utilized for evaluation of the genealogical variability status [9]. When there is some lack of pedigree information, this value could be overestimated [6].

The parameters (f_e) and (f_a) are the effective number of founders and ancestors which can determine a significantly swift on the mating strategy before its consequences appear in some inbreeding increase. A founder is some ancestor with unknown parents. An unknown ancestor of an animal is consid-

ered to be a founder. Cumulative probabilities are added for every founder k and characterized for its expected contribution q_k to the gene pool of the population. Contributions of all founders measure the preservation of genetic variability.
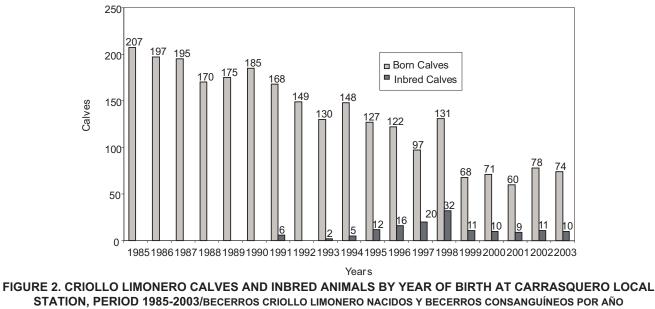
Rochambeau and Chevalet [20] and Lacy [17], proposed the effective number of founders (f_e), as the number of founders expected to produce the same genetic diversity as the one existing in the studied population. When each founder has the same expected contribution (1/f), the current number of founders is similar to the effective number. As founder contribution balance increases, the equivalent founder increases. On intensive selection programs, the equivalent founder is overestimated when genes belonging to a small herd are widely used. Boichard [6] proposed to find the effective number of ancestors (f_a), which is the minimum number of ancestors explaining the genetic diversity of the studied population. In order to estimate the number of founder genomes (N_g) it is necessary to estimate the number of founder's population genes, which is the probability for a gene to be in the studied population.

RESULTS AND DISCUSSION

A total of 3,184 animals were included in this study, which 80.2% corresponded to those born in the period 1985-2003 and the rest before year 1985 as the base population (FIG. 2). The number of bulls evaluated was 112. Forty four had only one offspring and six bulls had more than 100 calves. On the other hand, the number of dams evaluated was 837 which had 2,552 calves during the period. A 63% reduction in number of births occurred when comparing years 1985 and 2003, with the year 2001 having the smallest number of births. This trend to reduce births in the herd was made noticeable in the last 10 years, which considerably affected the capacity to produce replace-

ments. The causes that originated such reduction are not clear, management problems, diseases, reproductive elimination of females can be acting single or altogether to produce such effect. The evaluation of the reproductive function in the herd must be studied, although the elimination of females by sanitary problems and senility was an important reason of diminution of the number of cows from year 1997 to 2003 with a total of 229 cows being culled. Efforts must be made to increase the number of births in the herd that allow to increase the census of the Criollo Limonero breed and to leave the population level of risk in which it is. A long term goal would be to reach at least 1,000 pure bred females in different associated herds, by including reproductive technologies, such as, super ovulation, *in vitro* fertilization and embryo transfer programs, which well organized could be implemented in order to asses genetic progress without reduction in the genetic variability.

Pedigree quality was excellent, the tendency integrity as well as ancestor's average increased throughout the years, determining a high degree of confidence on the parameter's estimates. The number of known ancestors per year increased gradually in the course of the period of study by indicating a well organized record keeping in the experiment station. The level of knowledge of the generations was 100; 69 and 40% for the first, second and third generation, greater than values reported by Gutiérrez on Spanish beef breeds, who reported the highest value for the Pirenaica breed with a 90% and the lowest value of 56% for the Bruna of the Pirineus in the first generation [15]. However, in the fourth generation, we had only knowledge of nearly 16% of the parents, which is considerably lower than de 68% reported for the Breton equine breed [26]. An important aspect to stand out in this study is the noticeable reduction of the knowledge of the pedigree when considering the first and second generation, where it diminished from 100% to 69% due mainly to the use of imported Criollo bulls without

FIGURE 2. CRIOLLO LIMONERO CALVES AND INBRED ANIMALS BY YEAR OF BIRTH AT CARRASQUERO LOCAL STATION, PERIOD 1985-2003/BECERROS CRIOLLO LIMONERO NACIDOS Y BECERROS CONSANGUÍNEOS POR AÑO EN LA ESTACIÓN LOCAL CARRASQUERO, PERÍODO 1985-2003.

genealogical antecedents, which should be used only in critical situations and avoided.

The number of individuals by family depicted nearly similar values, which determined the existence of a relative equal contribution of the five families to the total individuals in the population. However, mates should be planned carefully in order to level family contribution to the whole population. With five families, the expected proportion of offspring from each family should be near 20%. Results indicated that families Hachiote and Bella Vista contributed with 51% of the calves born annually. On the other hand, the Bonita families (21%), Founders and buys (13%) and Joaquin Reyna (15%) had a smaller representation of the total of the herd. As far as the use of the bulls is concerned, there were important differences in the number of calves by bull. Some bulls had a single descendant whereas 6 bulls procreated more than one hundred animals which indicated that there was an excessive use of those bulls. A similar situation was observed in the beef breed Japanese Black, where 43% of the animals born in a year descended from only 5 bulls, in a population that surpassed the 55,000 cows utilizing elite sires, exclusively [19].

One hundred and forty seven inbred animals were found, three in the base population and one hundred forty four in the 1985-2003 period, representing 5.6% of the total, with maximum inbreeding coefficient of 25% (TABLE I); out of these, 83% showed inbreeding values lower than 10%, whereas 17% inbred individuals had an inbreeding coefficient greater than 10%. Likewise, only 27 bulls were parentally mated and four of them sired 84 inbred offspring for a total of 58.3% inbred individuals. Figure 3 depicts the inbreeding trend, appraising that the values previous to 1991 could not be considered due to the nature of the data, allowing single to detect consanguineous animals as of that year, when a gradual and slow increase of the consanguinity begins until reaching in year 2003 a value of 0.35%. Also, in FIG. 3, it is observed additionally the number of animals born consanguineous with respect to the total of births every year, by being able to appreciate that an accelerated increase took place from year 1992 to 1998 where the maximum value of 24.4% of the individuals was reached, soon to be diminished gradually in the subsequent years until arriving 13.5% from consanguineous animals in year 2003.

TABLE I

INBRED ANIMAL DISTRIBUTION BY LEVEL OF F/ DISTRIBUCIÓN DE ANIMALES CONSANGUÍNEOS DE ACUERDO AL NIVEL DE F.

F level, %	Individuals, (n)	Individuals, %
0- 5	54	36.73
5-10	68	46.26
10-15	18	12.24
25-30	7	4.76
Total	147	100

The proportion of inbred calves that appear in FIG. 2 reached their higher value in 1998, where 32 born animals were inbred as product of the badly reproductive management of the families between years 1995 and 1998; standing out the inadequate use of the bulls, which increased the number of inbred animals in the Hachiote, Bella Vista and Joaquin Reyna families in that period. For years 1991 and 1993, the average inbreeding of the affected animals surpassed 15% (20.83% and 15.82%, respectively). In other years, the average did not surpass 10%, from which the levels of global inbreeding in the herd did not surpass 1%. Therefore, the increasing rate of inbreeding was reduced by generation as well as by year. These results contrast with huge populations of specialized dairy breeds in the United States, where they reach values near 0.11% by year and from 0.21 to 0.89% by generation [27]. On the other hand, the increase of the relationship average reduces the probability of mating unrelated animals.

The relationship average considered for the population was 3.4%, indicating the necessity to make more emphasis in the selection of bulls at the time of planning the mating strategy, due to the fact that exists a high relationship among the animals of the herd. Similar results, where the relationship average is greater than the inbreeding of the herd was reported for the ovine of Xalda breed [14]. This situation contrasts with other populations where the excessive use of reproductive bulls allows for the value of inbreeding to be superior to the relationship average [15]. As an additional strategy for the rotational mating system, full pedigree information should be used in order to avoid matings of known related animals which would complement the strategy for maintaining inbreeding at the minimum levels.

The generational interval corresponded to 5.8 years. The values of intervals calculated by the route, father-son and father-daughter, turned out to be the minors with a minimum difference of 2.5% among them. The lowest value obtained was 4.04 in 1991, considered by the route father-son, and the maximum value was 7.5 during the years 2000 and 2002 by the route father-daughter. The generational interval, as expected, was smaller when estimated by the route father-son, since the males started earlier their reproductive activity, which it is most relevant if artificial insemination is used. The average value obtained for the generational interval (5.86 years) is in the same rank as reported in Spanish breeds [15], and far below for the value reported for the Black Japanese beef breed, which it is highly selected for carcass quality traits [19].

In TABLE II, the value of the increase of the annual generational consanguinity, the effective size of the population and the average of relation of the herd are shown. The parameters obtained for the probabilities of origin of the gene appear in TABLE III. A number of 386 founders was obtained, and values of 63.5; 38 and 27.71 for the equivalent number of founders, equivalent number of ancestors and number of genomes surpluses for all the period of study; also the values of probability of origin for periods 1985-1990 and 1991-2003 appear to verify since it has been the behavior in the last years.

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TABLE II

INCREASE OF ANNUAL AND GENERATIONAL CONSANGUINITY, GENERATION INTERVAL, EFFECTIVE POPULATION SIZE AND RELATIONSHIP AVERAGE OF CRIOLLO LIMONERO/ INCREMENTO DE LA CONSANGUINIDAD ANUAL Y GENERACIONAL, INTERVALO GENERACIONAL, TAMAÑO EFECTIVO DE LA POBLACIÓN Y PROMEDIO DE RELACIÓN DE LA RAZA CRIOLLO LIMONERO.

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ΔF annual	Generation interval (years)	ΔF Generation	$N_e = \dfrac{4 * N_m * N_h}{N_m + N_h}$	Relationship Avg.
0.0187	5.86	0.1094	45	3.4%

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TABLE III

GENE ORIGIN PROBABILITY IN CRIOLLO LIMONERO/ PROBABILIDAD DEL ORIGEN DE LOS GENES EN LA RAZA CRIOLLO LIMONERO.

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Period	Founders	f_e	f_a	N_g	f_e/f_a	N_g/f_e	F 50%
1985-1990	326	48.7	30	25.34	1.62	0.52	12
1991-2003	228	55.9	32	22.23	1.75	0.40	11
1985-2003	386	63.5	38	27.71	1.67	0.44	18

There were used up to 60 sires to produce 2,552 off-spring, which demonstrates that a rotational mating system between families, indeed, allows to use great amount of bulls and to reduce the probabilities of mating related animals. In this rotational scheme with 5 families after 5 generations, it would be expected to mate related animals, even though inbreeding co-efficients should be smaller than 5%.

Taking into account the number of mature males and females (12 and 185, respectively) the N_e value was 45 for Li-monero breed and that is slightly similar or even smaller than that observed in other breeds with stronger selection pressure. Thus, Gutiérrez et al. reported low N_e values in eight Spanish beef breeds [15], which ranged in the interval from 21 to 123. Goyache et al. reported a N_e in the Xalda ovine breed of 28.4 [14], and Weigel estimated values for N_e of 161; 61; 65; 39 and 30 on Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey, respectively, in the United States [27]. These values are related to the great genetic progress reached and a massive use of elite bulls through artificial insemination.

The values of origin probability indicate that there has been a considerable loss of genetic variability in the population, the proportion between the number of equivalent founder and the equivalent ancestors gives us a value of f_e/f_a = 1.67, which corresponds to the magnitude of loss as a result of the bottlenecks. This value is above those reported for Tabapua and Polled Nellore, both with a value of 1.44 [12, 24] and the 1.48 in Guzera [11]. However, it is below to the 3.0 obtained for Normanda and 1.77 for Red Sindhi [6,10]. The value of N_g obtained in all the period was of 27.71, which it is inferior to the published for Polled Nellore (64), Guzerat (98) and Nellore (39.4), and the N_g/f_e relation was 0.43, which it is a proportion similar to the observed for the populations of Polled Nellore (0.44), Guzerat (0.39), Nellore (0.45) and Sindhi (0.43) [10-12, 25]. The smaller is the N_g/f_e ratio, the bigger is the genetic drift effect. These values indicate that loss due to genetic drift in these populations is an important factor for reducing the genetic variability. As observed in Sindi and Guzerat breeds, the N_g/f_e relation has been reduced considerably in the Criollo Li-monero breed, suggesting that the genetic drift along with the bottleneck effect is a fundamental factor gene loss in the population. Increasing the number of males to improve the balance male/female would be an important strategy to incorporate more individuals to the reproductive establishment of the herd. As a summary, it has been observed that inbreeding has stayed small during the period of study as a result of the mating system utilized; the values of origin probability indicated that this population is sufficiently small as to be affected by the bottle neck effect and the genetic drift. Thus, maintenance of the herd must be analyzed in detail to avoid a possible progress of genetic deterioration.

CONCLUSIONS

The number of births in the last 15 years has been reduced to a half, as a result of different causes for achieving a good genetic improvement. Quality of the pedigree was excellent and its integrity improved through the period of study.

Several bulls have been used in the genetic project of the research unit, which together with the rotational system of families favored the maintenance of the inbreeding at low levels, although the number of inbred animals per year increased at the end of the period.

Parameters for probabilities of origin of the genes indicated some loss of variability as a result of an effect of bottle neck as well as some genetic drift effect. Therefore, an increase in the number of sires could improve the proportional relation male/female in the herd. The effective size of the Li-monero population was somehow small as compared to other breeds with higher selection intensities.

RECOMMENDATIONS

To establish a unique and reliable system for identification of the animals that allows evaluation of the related genetic parameters of the herd through years.

To evaluate the effect of inbreeding on traits related to production as well as on those affecting survival rate and, in general, the stayability of the population.

To continue to work on the study of genetic variability using molecular marking of DNA, as a more specific and reliable tool to asses the genetic variation. This is due to the fact that inbreeding might be underestimated by means of genealogies studies.

To initiate a cooperative program for diffusion of the Criollo Limonero breed along with all possible associations of cattlemen of the region.

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