

STABILIZATION OF THE MORPHOSTRUCTURE OF A SHEEP POPULATION FROM A QUADRUPLE CROSSBREEDING SCHEME

ESTABILIZACIÓN DE LA MORFOESTRUCTURA DE UNA POBLACIÓN OVINA DESDE UN ESQUEMA DE CRUZAMIENTO CUÁDRUPLE

De la Barra R.¹, Carvajal A.¹, Bravo R.¹, Martínez M.E.^{2*}

¹Instituto de Investigaciones Agropecuarias, INIA Remehue, Osorno, Chile. *eugemartinez.inia@gmail.com.

²Instituto de Investigaciones Agropecuarias, Centro Experimental Butalcura, Chiloé, Chile.

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ABSTRACT

In 1977, the Instituto de Investigaciones Agropecuarias (INIA), Chile, started a research program aimed at generating a meat-purpose ovine biotype with high maternal ability and superior prolificacy. In 2009, a process of stabilization of this animal population was initiated to offer a more stable animal biotype in time with a lower production cost. To evaluate this process, 800 quadruple crossbred females were confined to a closed herd with a 20% replacement rate. Rams randomly mated ewes (ratio 1:8), by rivalry. The replacement animals were selected from the offspring of the herd. Two evaluations were done to the quadruple crossing sheep in 2009, prior to the stabilization process (n = 261), and in 2019, on randomly selected descending adult ewes (n = 253). Nine body measurements were recorded. For the stability analysis, data from 2-3 yr old animals (112 in 2009 and 115 in 2019) and 5-6 yr old animals (97 in 2009 and 88 in 2019) were used. It can be concluded that the stabilization process generated body modifications with a general reduction of the animal format, but increasing at the same time the stability, with the characteristics of homogeneity and morphostructural stability observed in sheep populations that constitute breeds. Therefore, these sheep could be considered as an established breed.

RESUMEN

En 1977, el Instituto de Investigaciones Agropecuarias (INIA), Chile, inició un programa de investigación destinado a generar un biotipo ovino para fines cárnicos con alta capacidad materna y prolificidad superior. En 2009 se inició un proceso de estabilización de esta población animal para ofrecer un biotipo animal más estable en el tiempo y con un menor costo de producción. Para evaluar este proceso, 800 hembras cruzadas cuádruples fueron confinadas a un rebaño cerrado con una tasa de reemplazo del 20%. Los carneros se aparearon al azar con las ovejas (relación 1:8), por competencia. Los animales de reemplazo fueron seleccionados de la descendencia del rebaño. Se realizaron dos evaluaciones: en 2009 a las ovejas de cruzamiento cuádruple, antes del proceso de estabilización (n = 261), y otra en 2019, en ovejas adultas descendientes seleccionadas al azar (n = 253). Se registraron nueve medidas corporales. Para el análisis de estabilidad, se utilizaron datos de animales de 2-3 años (112 en 2009 y 115 en 2019) y de 5-6 años (97 en 2009 y 88 en 2019). Se puede concluir que el proceso de estabilización generó modificaciones corporales con una reducción general del formato animal, pero aumentando al mismo tiempo la estabilidad, con las características de homogeneidad y estabilidad morfoestructural observadas en las poblaciones de ovejas que constituyen razas. Por lo tanto, estas ovejas podrían considerarse como una raza establecida.

INTRODUCTION

Sheep arrived in Chile from Peru during the Spanish conquest (Carvallo, 1875). However, the promotion of sheep production began more recently (from 1850 onwards). Subsequently, there have been successive importations of small endowments of different types of breeding males. Over time, this has generated a breed

structure, constituted by the introduced foreign breeds and the ovine populations descended from the first sheep, as well as several kinds of crossbreeding (Latorre *et al.*, 2011; De la Barra *et al.*, 2018). Importation has been made mostly by bringing males and taking advantage of the females present in the country for breeding. It has also happened that, by keeping breeds in small isolated contingents as is the case of Romney Marsh (De la Barra *et al.*, 2010), with a marked founder effect, with intense directional selection schemes and increasing endogamy issues (Thévenon & Couvet, 2002; Rokouei *et al.*, 2010; De la Barra *et al.*, 2014; 2016), triggering processes of differentiation from the original breeds. This diverse set of genes structured in breed populations, some differentiated from the origin and some others of high variability, offers the opportunity to package the unique genetic arrangements achieved in adaptive and differentiation terms to constitute new breeds. These new breeds can become a factor of valorization of animal genetic resources that the country has accumulated in its productive history (De la Barra *et al.*, 2018).

At present, 46 sheep breeds are present in Chile, but only 10 are officially registered in the Servicio Agrícola y Ganadero (SAG), the official Chilean livestock control authority. The declaration and registration of breeds in Chile it is voluntary. In the last 10 yr, four Chilean breeds have been registered: the Chilota sheep breed (De la Barra *et al.*, 2011), the Marin Magellan Meat Merino breed (De la Barra *et al.*, 2013), the Künko sheep breed (De la Barra *et al.*, 2016) and the Patagonian Robertson Merino sheep breed (De la Barra *et al.*, 2014). On the other hand, of the total breeds present in Chile, 34 have a census of less than 500 heads.

Showing inbreeding and low selection pressure issues, which added to the fact that all are part of incomplete absorption processes, generates a high phenotypic variability in the different racial groups (Notter, 1999; De la Barra *et al.*, 2015; 2016). On the other hand, attempts have been made to create new breeds from multiple crossbreedings. One case is the R1 crossbreeding initiated by Instituto de Investigaciones Agropecuarias (INIA) in 1977 (Crempien, 1990), also called the Hidango breed, or the Austral crossbreeding (Finnish Landrace × Romney Marsh) started at the Austral University in 1983 (Farías, 2009).

One of the problems derived from the incomplete absorption of many populations and multiple crossbreedings is the instability showed by the morphostructural format of the population in the descendant generations, given that this has a direct impact on the homogeneity of the type of animal and its productive characteristics (Mueller *et al.*, 2003). Thus, it is necessary to evaluate the morphostructural stability of the populations before carrying out registration processes for new sheep breeds, since intergenerational stability is understood as a characteristic of the breed definition (Sierra, 2001; De la Barra *et al.*, 2013). This requires considering the need to develop knowledge about the stabilization dynamics of hybridized animal populations, in order to take advantage of the advantages of crossbreeding to improve populations without resulting in a loss of homogeneity of offspring, and to continue advancing in the development of new breeds by crossbreeding and in multibreed genetic evaluation systems (Elzo, 2006; Latorre *et al.*, 2011). The objective of the present work was to evaluate the results of a stabilization protocol carried out with a population of female sheep resulting from quadruple crossbreeding in order to generate information about this process, its duration and the levels of homogeneity and stability that can be achieved.

MATERIAL AND METHODS

The quadruple crossbreeding

In 1977, the Instituto de Investigaciones Agropecuarias (INIA), Chile, started a research program at Hidango Experimental Center (Libertador General Bernardo O'Higgins Region), aimed at generating an ovine biotype with a high reproductive capacity, what is measured through the prolificacy rate and where a value greater than 150% was used as a selection criterion, which would support an intensive production of sheep meat (Crempien, 1990). The construction of this biotype was made by two sequential crossbreedings using four breeds. Four racial nuclei were established with Finnish Landrace (FL), Dorset Horn (DO), Border Leicester (BO) and Merino precoz (ME). Each breed contributed 25%. Two breeds were crossbred to create a paternal hybrid (FL rams on DO ewes) and the other two for a maternal hybrid (BO rams on ME ewes). Subsequently, the resulting hybrids were crossbred, which allowed producing the quadruple-type sheep FIBODOME (Mujica, 2005; Gómez, 2010; Vargas, 2011). Throughout the process, the selection criteria was based on the prolificacy rate and birth weight.

The FIBODOME crossbreed was evaluated and showed an average prolificacy between 140% and 165%, average weight of fleece from 3.0 to 3.4 kg, average fineness of wool from 20 to 22 microns, dairy production between 180 and 240 L in 115 d, 7% to 8% milk fat and 30-35 kg per lamb at 115 d of age. These productive levels would support a high production system that could reach 800 kg lamb meat per hectare, with wool of good value and good hardiness (Crempien, 1990; Avendaño *et al.*, 2005; Mujica, 2005; Vargas, 2011).

Stabilization methodology

Although the productive characteristics reached in the crossing were attractive, they can only be repeated holding the double crossbreeding scheme under the criteria of selection and controlled reproduction. Otherwise, subsequent generations will tend to exhibit unpredictable productive characteristics. For this reason, in 2009 a stabilization process that would allow establishing some genetic characteristics of the hybrid, but in a stable manner over time and at a lower production cost was started. First, an evaluation of the morphostructure of the quadruple population was carried out. In the following season, 800 quadruple crossbreeding females were confined to a single herd, which remained closed until 2019, with a replacement rate of 20%. A ratio of one ram per 8 sheep was used. In this herd, 50-d mating periods were carried out every season. Mating was totally random when the 800 sheep met the 100 rams in a single mating place. The replacement males and females incorporated each season were selected from the offspring of the herd, considering the birth weight as selection value. In 2015, a stabilizing selection was initiated, defining an ethnotype that was the reflection of a morphostructural population average of the quadruple crossbreed (Vargas, 2011). Modal selection was made according to the ethnotype, eliminating the outlier animals.

Sampling and evaluation

Population sample size was estimated following the method proposed by Cannon and Roe (1982).

$$\text{Sample Size} = \frac{1}{\left(\frac{1}{n_1} + \frac{1}{Pop}\right)}$$

where Pop is the population size:

$$n_1 = \frac{4pq}{L^2}$$

Being p the proportion of individuals in the population which are expected to be of the new incomplete absorbed crossbreeding, q is 1 – p, and L is accepted error.

It was assumed that 90% of the animals in the population were a specimen of the new animal format; accepted error was 5%, and therefore sample size was estimated at 250 ewes. In 2009, an evaluation was conducted on the quadruple sheep, and another one in 2019 on the adult descending sheep. All measurements were done on adult randomly selected ewes. Nine body measurements in each ewe were recorded (Bravo and Sepúlveda, 2010; Latorre *et al.*, 2011): head length (HL), head width (HW), rump width (RW), rump length (RL), body length (BL), body width (BW), body depth (BD), withers height (WH) and rump height (RH). Two types of analysis were carried out. The morphostructure variability analysis was carried out using all the data obtained (261 sheep in 2009 and 253 in 2019). The stability analysis was carried out using only the measurements made to 2-3 yr old (112 in 2009 and 115 in 2019) and 5-6 yr old (97 in 2009 and 88 in 2019) animals. The age was determined by dental chronometry. The normality of data was analyzed by the Lillie test (Kolmogorov Smirnov). Comparisons of means between age groups and years were performed using the Wilcoxon test. The statistical analyses were carried out using the program and the normtest and plyr packages from the R Project for Statistical Computing.

RESULTS AND DISCUSSION

Table I shows that eight of the nine biometric variables evaluated changed significantly between the two measurement moments. The only variable that did not show significant changes was the rump length (RL). The stabilization process occurs naturally in wild populations by reducing the genetic distances between individuals and increasing the frequency of certain phenotypic expressions, that acquire modal value. In the process, there is an increase in consanguinity since the mating is not producer-directed, but by rivalry between

males instead (Charlesworth, 2001; Perfectti, 2002; Matthews and Domjan, 2011), with which the predominant criteria are not racial, but of sexual aptitude and attitude. This implies a change in the participation of each breed in the genetic composition of the offspring, different from that produced with controlled crossbreeding. Increased inbreeding by reducing genetic distances, together with the effects of heterosis and specific genetic arrangements (Sánchez *et al.*, 2005; De la Barra *et al.*, 2012) are the driving forces behind the offspring differentiation process, which generates a readjustment of the animal architecture that can be seen in table I, where between 2009 and 2019 there was a statistically significant variation in the population means on the nine body architecture measurements evaluated in the descendant population.

Table I. Evolution of the body dimensions of the quadruple crossbreeding sheep prior (2009) and after (2019) the stabilization process (*Evolución de las dimensiones corporales de las ovejas cruzadas cuádruples antes (2009) y después (2019) del proceso de estabilización*).

Biometric variables	Population mean (cm) 2009 (a)	Standard deviation	Population mean (cm) 2019 (b)	Standard deviation	Difference (a - b)
HL	33.85	6.168	29.93	1.499	3.92*
HW	13.27	2.701	9.49	0.430	3.78*
RW	32.56	5.300	28.99	2.353	3.57*
RL	19.80	3.534	18.85	1.867	0.95
BL	80.06	10.738	77.05	5.891	3.01*
BW	28.00	4.336	24.67	2.632	3.33*
BD	34.27	6.735	31.23	2.927	3.04*
WH	68.52	13.402	66.32	3.136	2.20*
RH	69.44	11.625	67.46	3.057	1.98*

*Significant differences ($P \leq 0.05$). HL: Head length; HW: head width; RW: rump width; RL: rump length; BL: body length; BW: body width; BD: body depth; WH: withers height; RH: rump height.

There was a general reduction of the body format: BL -3.01 cm, BW -3.33 cm, BD -3.04 cm and height (WH and RH with -2.20 and -1.98 cm, respectively). HL decreased by -3.92 cm and HW by -3.78 cm. In the rump area, RW was reduced -3.57 cm. The morphostructure of the original population changed thus significantly in all the evaluated dimensions, which indicates that the descriptive characteristics of the population were reconfigured. This could imply that a stabilization status of the population has been achieved, which should be confirmed by analyzing the intergenerational variation (tables II and III). If so, the population with its new phenotypic characteristics could meet the required attributes to be recognized as a breed (Sierra, 2001; Latorre *et al.*, 2011).

When analyzing the stability of the population (table II), it can be seen in 2009, prior to the stabilization process, both age groups presented coefficients of variation greater than 10%, which is the variability that authors such as Sierra (2001), Herrera and Luque (2009), Bravo and Sepúlveda (2010), Latorre *et al.* (2011) and De la Barra *et al.* (2015) indicate as superior range for animal populations with breed characteristics. In this regard, it is interesting to note that prior to the stabilization process, the 5-6 year-old sheep showed a coefficient of variation for the evaluated body variables of 16.09% whereas in 2-3 year-old sheep was 18.03%. This reveals the high variability of the hybrid's morphostructural format, with a high dispersion of the values around the means of the body variables. In this sense, it can be stated that prior to the stabilization process, a high morphostructural heterogeneity was declared within the population. At the same time, it was observed (table II) that the group of 2-3 year-old animals presented higher coefficients of variation than the group 5-6 year-old group for all the variables evaluated. This is indicative that the morphostructural format increases its dispersion from one generation to another. In theory, the controlled crossbreeding of the quadruple would allow maintaining a certain body format with some homogeneity, but the evidence indicates that it became disordered as the offspring moved away from the foundational racial nuclei, a phenomenon in which the loss of heterosis could be involved. Heterosis occurs through repetitive crossbreeding with related females or by

increasing inbreeding in the foundational racial nuclei (Thévenon and Couvet. 2002; Rokouei *et al.*, 2010; De la Barra *et al.*, 2014).

Table II. Variation coefficient in body measurements of sheep of two successive generations descendent from a quadruple crossbreeding before the stabilization process (year 2009) and after the stabilization process (year 2019) (*Coeficiente de variación de las medidas corporales de las dos generaciones sucesivas de descendientes de las ovejas cruzadas cuádruples antes (2009) y después (2019) del proceso de estabilización*).

Biometric variables	2009	2009	2019	2019
	5-6 year-old sheep (%)	2-3 year-old sheep (%)	5-6 year-old sheep (%)	2-3 year-old sheep (%)
HL	14,33	19,77	4,87	4,95
HW	18,47	21,73	3,93	4,86
RW	14,03	16,76	9,09	6,71
RL	16,10	17,69	9,18	9,79
BL	10,91	13,19	5,17	9,49
BW	11,67	15,57	10,77	9,09
BD	17,59	20,17	9,73	10,03
WH	17,20	20,44	4,99	4,49
RH	15,54	16,95	4,45	4,62
n	97	112	88	115
Media	16.09	18.03	6,90	7,11

HL: Head length; HW: head width; RW: rump width; RL: rump length; BL: body length; BW: body width; BD: body depth; WH: withers height; RH: rump height.

It can also be seen in table II that after ten years of the stabilization process, the quadruple population shows a similar variability between the two age groups (5-6 years 6.9%, and 2-3 years 7.11 %) in the mean of the variables considered. In other words, both age groups showed a lower variability than the upper range of 10% which various authors assign to populations with racial characteristics (Sierra, 2001; Herrera and Luque, 2009; Bravo and Sepúlveda, 2010; Latorre *et al.*, 2011 and De la Barra *et al.*, 2015). This implies that of the quadruple animal population after stabilization it exhibits intergenerational stability in the morphostructural format. In other words, population stability increased, with no increase in the dispersion of the values of each variable being observed in the descendant generations. In this sense, the stabilization process to which the quadruple sheep population was subjected generated modifications of the body format, a general reduction in its dimensions, but at the same time increased the stability of said format, endowing it with the characteristics of homogeneity and morphostructural stability, typical in sheep populations that constitute breeds (Sierra, 2001; Herrera and Luque, 2009; Bravo and Sepúlveda, 2010; Latorre *et al.*, 2011 and De la Barra *et al.*, 2015). In other processes of crossbreeding stabilization such as the case of Merino with Corriedale (that derived in the 4M sheep breed) and the polyhyride based on Australian Merino that gave rise to the PRM sheep breed, changes in morphostructural variability of similar tendency have been observed (Latorre *et al.*, 2011; De la Barra *et al.*, 2013; De la Barra *et al.*, 2014).

CONCLUSIONS

The analysis of the morphostructural variations showed that the stabilization process undergone by the quadruple sheep population generated modifications of the body format that determined a general reduction of its dimensions, increasing the format stability and endowing it with the characteristics of homogeneity that are usually found in the sheep populations that constitute breeds. In this sense, the multiple crossbreeding sheep could be considered as a breed population.

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