Influence of coat colour on Chamarita sheep phenotypes, behaviour, welfare and performances

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Summary

Many rustic breeds under low levels of genetic selection maintain variations in coat colour, which local farmers link to production traits, but few quantitative analyses have been made of the effect of coat colour on several zootechnical parameters of importance. The aim of the study was to describe differences in morphology, production, behaviour and welfare of adult sheep ewes (n = 50) in a *Chamarita* breed flock in terms of coat colour. The wool and skin colour of *Chamarita* sheep are quite variable, with white, black and *galana* sheep. Morphological measurements (weight, body length, height at withers and thorax circumference) were not significantly different for different coat colours but white sheep tended to be larger. Average litter size (1.29 lambs per ewe) was also similar, but white coated sheep gave birth to larger lambs. White-mother lambs also had a higher average daily growth during the milking period, although final weaning weight was similar to black ones. Most (>70 percent) of all the aggressive interactions observed were performed by white sheep, while affiliative interactions (friendly interactions that promote group cohesion) were similar among coat colour. Regarding the welfare assessment, most indicators were similar among sheep with different coat colour except for glucose in blood plasma, which was higher in white sheep. Chamarita ewes are relatively small compared with other breeds, and well adapted to their environment, including the production system and harder climatic conditions. Their performance is within the average of local breed sand still has margins for improvement, which underline the importance of their conservation.

Résumé

Beaucoup de races considérées comme rustiques et ayant été soumises à une faible pression de sélection génétique conservent des variations dans la couleur de leur robe que certains éleveurs associent à des caractères productifs. Cependant, l'effet de la couleur de la robe sur des paramètres zootechniques d'importance n'a guère été étudié. L'objectif de cette étude a été de décrire les différences en morphologie, production, comportement et bien-être selon la couleur de la robe de 50 brebis adultes de la race Chamarita. Les mesures morphologiques (poids, longueur, hauteur au garrot, circonférence thoracique) n'ont pas différé significativement entre les couleurs de robe même si les brebis blanches ont tendu à être plus grandes. La prolificité (1.29 agneaux/brebis) a aussi été similaire mais les brebis blanches ont eu des agneaux plus grands. Les agneaux de mères blanches ont aussi eu un gain moyen quotidien plus élevé, bien que le poids final ait été similaire à celui des agneaux de mères noires. La plupart (>70 pour cent) des interactions agressives observées ont été réalisées par des brebis blanches, alors que le pourcentage d'interactions affiliatives a été similaire pour les deux couleurs de robe. En ce qui concerne l'évaluation du bien-être, aucune différence n'a été décelée entre les couleurs de robe pour la plupart des indicateurs, hormis le glucose sanguin qui a été plus élevé chez les brebis blanches. Les brebis Chamaritas sont relativement petites par rapport à d'autres races et sont bien adaptées à leur environnement productif. Leurs performances productives sont satisfaisantes et peuvent encore être améliorées, comme quoila conservation decetteraces' avère importante.

Resumen

Muchas razas consideradas rústicas y sometidas a poca presión de selección genética mantienen variaciones en el color de capa que algunos ganaderos relacionan con caracteres productivos, pero se han realizado pocos estudios respecto al efecto del color de capa en

algunos parámetros zootécnicos de importancia. El objetivo del presente estudio es describir diferencias en morfología, producción, comportamiento y bienestar de ovejas adultas (n = 50) de raza Chamarita en cuanto a color de capa. Las medidas morfológicas (peso, largura, altura a la cruz, circunferencia torácica) no resultaron significativamente diferentes entre colores de capa, aunque las ovejas blancas tendían a ser más grandes. La prolificidad (1.29 corderos/oveja) también fue similar pero las ovejas blancas tuvieron corderos más grandes. Los corderos de madres blancas también tuvieron una mayor ganancia diaria (ADG), aunque el peso final fue similar al de los corderos de madres negras. La mayoría (>70 percent) de las interacciones agresivas observadas fueron ejecutadas por ovejas blancas, mientras que las interacciones afiliativas resultaron similares en ambos colores de capa. En cuanto a la evaluación del bienestar, la mayoría de los indicadores no presentaron diferencias entre colores de capa excepto la glucosa en sangre que fue mayor en las ovejas blancas. Las ovejas Chamaritas son relativamente pequeñas comparadas con otras razas, y están bien adaptadas a su ambiente productivo. Sus caracteres productivos son razonables con un buen margen de mejoría, demostrando la importancia de su conservación.

Introduction

Many local breed sunder low selection pressure maintain large variations in coat colour, which the farmers link to production traits, but a few quantitative analyses have been made to substantiate the effect of coat colour on differences in morphology, production, behavior and welfare. Chamarita sheep are a breed from La Rioja (Spain), included in the Official Catalogue of Spanish Livestock Breeds under Endangered Native Breeds. The Chamarita population sizes about 10 000 sheep and it is officially recognized as an endangered breed to be preserved. The herd book was created in 2007 by the Chamarita Sheep Association of La Rioja (or AROCHA as it is known by its Spanish acronym), which has an official recognition to promote and control the breed. Most herds are found in the mountains of lower La Rioja and in the Cidacos and Linares valleys (Warren, 2011). Animals are small in size (adult rams 55–65 kg, ewes 35–40 kg; Barrio, Falceto and Doménech, 1991), probably as an adaptive response totheir hard living conditions and in their extensive and sustainable production system, which uses local resources (Álvarezand Arruga,2007). The wool and skin colour of Chamarita sheep are quite variable, with white, black (roya) and rarely galana (a combination of white, black and brown skin with white and black/roya wool) coats (Figure 1). The roya sheep is born black but their wool turns in red dish black with age, looking like brown. This type of coat is locally called roya. The black skin is mainly located in the face. It is likely that, as with other breeds in the region, breeders have selected for hardiness (through fertility and morphological characteristics) and adaptation to the environment and less for pure white wool. Nowadays there are many black sheep in herds, since it is associated with positive zoo technical traits (Doménech etal., 1992). Black animals usually have a white spot on the nape (crowned), and another at the distal end of the tail (puntiblancos). Sometimes, the spot of the neck extends over the head, forehead and nasal area (caretos).

Currently, Chamarita sheep are mostly used for meat production, both for suckling lamb(12–14kgliveweight) and a weaned lamb with a short indoor fattening period (20–22 kg live weight; Doménech et al., 1992). The Chamarito lamb was recognized as a quality brandin April 2010 (PROCORCHA). Thus, farmers now used a production system that ensures compliance with strict animal welfare health programmes, respecting the origin of sheep fed on extensive pastures and lambs produced in La Rioja on their mother's milk. In this study, we describe the morphological, productive and welfare traits of adult female Chamarita sheep and the influence of coat colour on these traits.

Materials and methods

A flock of 50 Chamarita adult ewes were transported from La Rioja to the Animal Experimentation Support Service (SAEA) of the University of Zaragoza, Aragón (41◦41_N). All sheep used were raised, transported and slaughtered according to cur- rent regulations of the European Community Commission (1986) for Scientific Procedure Establishments. All the protocols were approved by the Animal Experimentation Ethics Committee of the University of Zaragoza. This study was the collaboration between the Autonomous Regions of Aragon and La Rioja (Spain).

Animals

The adult multiparous ewes were housed in pens during pregnancy and lactation (2 m2 per ewe), fed twice a day (at 08.00 and 15.00) with pellet concentrate (11.5 MJ ME per kg DM and 15.5 percent crude protein; 0.3 kg per ewe) and ad libitum lucerne chaff (Medicagosativa). The pen was equipped with a metallic water trough (1.5 m × 0.60 m) and two metallic feeders (4.5 m × 0.80 m, 27 cm per ewe) and a lick stone for minerals.

Most ewes were inseminated by controlled natural mating before leaving the source farm and lambed at the University a few weeks after arrival. The lambs were kept together in the same pen with their mothers (2 m2 per sheep and lamb). Flock prolificacy was calculated as the total number of lambs born per total ewes lambing. Lambs were weighed at birth (BW) and at weaning (WW). Pre-weaning average daily gain (ADG) was estimated by the difference WW BW divided by the total milking period (30 days).

Morphological measurements of ewes

One day before blood sampling, body length (BL), height at withers (HW) and thorax circumference (TC) were measured as in Miranda-de la Lama et al. (2011), and each ewe was weighed using a portable digital weighing scale. Social behaviour was evaluated by direct observation. All ewes were individually identified and marked with 30 cm-high numbers and letters painted on the sides and rump with washable paint for sheep marking (Peinture Marquage Mouton®). A platform with a seat 3 m above the ground was used to observe the flock from a distance. The ewes were observed 6 h daily, from 8:00 to 10:00 h, 12:00 to 14:00 h and 16:00 to 18:00 h for 16 consecutive days (96 h of observation) by the same trained observer. A behaviour sampling technique was used to record all social interactions (agonistic and non-agonistic behaviour). Agonistic interactions with contact included butts (when the ewe used the front of her head to make contact with another ewe), pushes (when a ewe used other parts of her body to make contact with another ewe) and bites (when one ewe bit another ewe's body using her teeth). Agonistic interactions without contact included threats (when a ewe turned towards or approached another individual with her head down and then lunged without making contact), and chase (when a ewe actively moved towards another individual, causing the latter to walk or run away). Non-agonistic interactions with contact included licking (when a ewe passed her tongue over the body of another individual) and grooming (when a ewe groomed another ewe's body using her teeth). Non-agonistic interactions without contact included sniffing (when a ewe sniffed another ewe's body) and the flehmen response (when a ewe retracted the upper lip, wrinkled the nose and bared the gums in the presence of another ewe).

Physiological welfare indicators

Blood samples were taken by jugular venipuncture with vacuum tubes during the dry period to evaluate physiologic- al responses to stress (two 10 ml tubes per animal, with and without anticoagulant, Ethylenediaminetetraacetic acid EDTA-K3). Blood was sampled using the necessary precautions to avoid sampling error on stress indicators. Samples were kept on ice for a maximum of 2 h and taken to the laboratory for routine haematological measurements. EDTA plasma and serum were centrifuged at 3 000 rpm for 10 min and aliquots were frozen and kept at −30 °C until analysed.

An automatic particle counter (Microcell counter F-800 and auto dilutor AD-260, SysmexTM both) was used to count red blood cells (RBC) and white blood cells (WBC) (number per mm3), haemoglobin (g/dl) and haematocrit (percent). The leukocyte formula was estimated from blood swabs on clean slides. Staining was performed by the rapid panoptic method using dyes from Química Clinica Aplicada Inc. With an optic immersion microscope, we counted and identified 100 leucocytes per sample (neutrophils, lymphocytes, eosinophils, basophils and monocytes). The neutrophil/lymphocyte ratio (N/L) was used an indicator of chronic stress (Lawrence and Rushen, 1993). Serum samples were used to determine the concentration of glucose (mg/dl, Ref. Glucose AE2-17), and the activity of creatine kinase (CK) (UI/L) (Ref. CK. NAC AE1-13) with a multianalyser ACE® (Clinical Chemistry System) and reagents from Alfa Wasserman. Serum concentration of non-esterified fatty acid (NEFA) levels was analysed by a multianalyser ACE® (Clinical Chemistry System of the Alfa Wasserman), with commercial kits (NEFA C Ref. 994-75409 of the Wako). The concentration of

cortisol was determined from plasma (EDTA-K3) by enzyme immunoassay using an “in home-kit” (validated by Chacón et al., 2004). Each sample was determined in duplicate from 50 µl of plasma and the results were expressed in nmol/l, with the corresponding controls. Variation coefficients of the analysis, inter- and intra-assay, were 7 and 8 percent, respectively. The concentration of lactate was deter- mined using a Sigma Diagnostic kit (lactate no. 735-10) and spectrophotometer (Lambda 5, Perkin Elmer).

Data analysis

Data were analysed using the least squares methods of the GLM procedure using SAS/STAT (9.1 SAS Inst. Inc., Cary, NC, USA) by SAS (1998), fitting a one-way model with a fixed effect of coat colour (two levels) within the ewe data. The general representation of the model used was: $y = Xb+ e$, where y was an $N \times 1$ vector of records, b denoted the fixed effect in the model with the association matrix X and e was the vector of residual effects. A prob- ability of $P < 0.05$ values was considered statistically significant.

Results and discussion

The proportion of coat colours frequencies, according to the association of breeders’ statistics, are 45 percent white coat and 55 percent non-white coat (including the minority galana coat). According with the genetic study performed by Álvarez and Arruga (2007), the Chamarita phenotypes are considered to be as a unique population.

Morphological measurements

Coat colour had little effect on morphological measurements (Table 1). Live weight of the adult ewes averaged 44.26 (±6.74) kg with no significant differences between white and black sheep. The average TC was 104.05 (±.58) cm, HW 65.72 (±3.7) cm and BL from chest to tail 72.93 (±4.25) cm. The average live weight and HW were higher than described by Barrio, Falceto and Doménech (1991) (live weight 36.6 kg, HW 59.8 cm). Chamarita sheep may have a lower body weight after food shortages in extensive systems, which was not the case in our experimental herd where sheep were fed twice daily with concentrate and had water and straw ad libitum. However, BL was shorter than in Barrio, Falceto and Doménech (1991) (90.5 cm), but the latter authors did not find differences between body measurements of white and black sheep. Although body weight was not significantly higher in white ewes, there was a tendency for black sheep to be lighter.

The small size of Chamarita ewes compared with more productive breeds (e.g. Rasa aragonesa: 45–50 kg; Manchega: weighing over 45 kg; Merino: 50–70 kg; and Assaf: 60–70 kg) is consistent with the morphology and weights of most breeds considered rustic and well adapted to a harsh environment (Catalogue Livestock Breeds in Spain). Such is the case of Ojalada (between 35 and 45 kg of body weight), Mallorquina (30–40 kg of body weight) or Gallega (25–35 kg in the mountain eco- type). This adaptation is due to food shortages, thereby reducing their energy needs (Álvarez and Arruga, 2007), allowing them to make use the resources at their disposal. In that regard, black sheep that tended to be smaller than white sheep may be more adapted to a harsh environment than white sheep (Álvarez and Arruga, 2007).

Productive traits

The average litter size of the whole flock was 1.29 (±0.36) lambs born per ewe lambing with no significant differences between coat colours (Table 2). Litter size is slightly higher than in Doménech et al. (1992), who found a tendency for single births (1.1 lambs per ewe). However, the latter authors suggested that an increased food supply could increase prolificacy to 1.5 lambs per ewe and delivery. These data confirm the potential for improvement of the Chamarita sheep when environmental conditions are optimal. The lambs weighed 3.63 (±0.59) kg at birth, and those born to white mothers were significantly ($P \leq 0.05$) heavier than these born (+9 percent) black ones. Balda, Chavarri and Doménech (1981) reported a birth weight of 2.6 kg in pure lines and 3.6 kg in industrial crossings. In our study, the herd was pure and the crosses were with Chamarito males, so probably the increased birth weight in lambs may be due to higher feeding rate in our experimental group. The fact that lambs from white females weighed more at birth

than those from black females is probably due to larger body size of their mothers is associated with higher consumption of concentrate. This hypothesis partially corroborates the data on blood glucose levels and social interactions of the herd, which suggest that white sheep were dominant (see below). Similarly, lambs from white ewes had a higher ADG during the milking period (+14.7 percent, $P \leq 0.05$), compared with the ADG of the whole herd 215.56 (±48.56) g. The ADG data support the hypothesis that white females could eat more and therefore produce more milk for their lambs. However, the average weaning weight of the lambs (11.95 ± 2.12 kg) was not significantly different among different coat coloured mothers. That implies that a homogeneous product can be obtained at weaning regardless of coat colour.

Social behaviour

Of the 163.88 (±107.03) aggressive interactions observed (Table 1), most were performed by white sheep (+71.5 per- cent, $P \leq 0$, 05). That suggests a higher dominance status for white sheep (Orgeur, Mimouni and Signoret, 1990; Barroso, Alados and Boza, 2000), tied with the fact that they were heavier and larger (Miranda-de Lama et al., 2011). Dominance is a mechanism that regulates social behaviour and priority access to available resources, since high dominance animals have priority access to resources in intensive production conditions (Barroso, Alados and Boza, 2000). That coincides with the higher glucose levels in white sheep. The levels of aggressive interactions observed within a stable herd are consistent with the idea of Doménech et al. (1992) that Chamarita behaviour is more like goat behav- iour, with a lively temperament and instinct for survival, linked to foraging behaviour and browsing. Affiliative inter- actions totalled 18.74 (±21.55) for the whole herd, with no significant differences regarding coat colour. Affiliations are a very important part of the social cohesion of a group (Miranda-de la Lama and Mattiello, 2010), but under conditions of social competition for limited resources, animals tend to do without them (Miranda-de la Lama et al., 2011). It is likely that an adaptation to very scarce resources has led to Chamarita to favour aggressive interactions in detriment of affiliative ones in the context of the social group.

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References

- Álvarez, L. & Arruga, M.V. 2007. Caracterización de la oveja Chamarita mediante marcadores microsatélites. Estructura genética, genotipado del gen Scrapie y creación de un banco de ADN. Beca de estudios Arnedanos.
- Apple, J.K., Dikeman, M.E., Minton, J.E., McMurphy, R.M., Fedde, M.R., Leith, D.E. & Unruh, J.A. 1995. Effects of restraint and iso- lation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen metabolism, and incidence of dark-cutting longissimus muscle of sheep. J. Anim. Sci. 7: 2295–2307.
- Balda, I., Chavarri, J.B. & Doménech, J.M. 1981. La oveja Chamarita I Jornadas Técnicas de ganado ovino en La Rioja-Logroño.
- Barnett, J.L. & Hemsworth, P.H. 1990. The validity of physiological and behavioural measures of animal welfare. Appl. Anim. Behav. Sci. 25: 177–187.
- Barrio, A.R., Falceto, M.D. & Doménech, J.M. 1991. La raza Chamarita: Estudio etnológico y morfométrico. XVI Jornadas Científicas de la S.E.O.C.
- Barroso, F.G., Alados, C.L. & Boza, J. 2000. Social hierarchy in the domestic goat: effect on food habits and production. Appl. Anim. Behav. Sci. 69: 35–53.
- Chacón, G., García-Belenguer, S., Illera, J.C. & Palacio, J. 2004. Validation of an EIA [enzyme immunoassay] technique for the determination of salivary cortisol in cattle. Span. J. Agric. Res. 2: 45–51.

- Doménech, J.M., Barrio, A.R., Falceto, M.D. & González Jiménez, J.A. 1992. La oveja Chamarita. Serie Estudios no 25, Gobierno de La Rioja. Logroño, La Rioja, España.
- European Commission. 1986. Scientific procedure and breeding of ani- mals for use in scientific procedure establishments. Directive 86/609/ ECC 1986.
- Hargreaves, A.L. & Hutson, G.D. 1990. The effect of gentling on heart rate, flight distance and aversion of sheep to a handling procedure. Appl. Anim. Behav. Sci. 26: 243–252.
- Lawrence, A.B. & Rushen, J. 1993. Stereotypic animal behaviour: fun- damental and application to welfare. UK, CAB International.
- Miranda-de la Lama, G.C. & Mattiello, S. 2010. The importance of social behaviour for goat welfare in livestock farming. Small Rumin. Res. 90: 1–10.
- Miranda-de la Lama, G.C., Sepúlveda, W.S., Montaldo, H.H., María,
- G.A. & Galindo, F. 2011. Social strategies associated with identity profiles in dairy goats. Appl. Anim. Behav. Sci. 134: 48–55.
- Orgeur, P., Mimouni, P. & Signoret, J.P. 1990. The influence of rearing conditions on the social relationships of young male goats, Capra hir- cus. Appl. Anim. Behav. Sci. 27: 105–113.
- Statistical Analysis System Institute. 1998. SAS/STATS user's guide
- (Release 6.03). Cary, NC, SAS Institute.
- Warren, J. 2011. Chamarito Lamb Promoted among Restaurant Owners and Butchers. http://www.foodsfromspain.com.