Clustering and genetic analysis of body reserves changes throughout productive cycles in meat sheep

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Introduction

To limit the use of feed inputs and the competition with agricultural surfaces used for human feeding, the livestock production sector needs to improve the efficiency and the robustness of farm animals. Ruminants should particularly increase the use of natural resources involving grazing in less controlled environments. In most of such systems, the ability of the animal to mobilize or to restore their body reserves (**BR**) is a key physiological mechanism to respond to qualitative and quantitative variations in the available feedstuffs bases and to ensure its productivity, health, survival and reproduction (Blanc *et al.*, 2006; Chilliard *et al.*, 1998). The availability of numerous and reliable measures to study such trait is not yet widespread. The body condition score (**BCS**) was defined as a subjective measure (5 classes) for fat quantity stored by the animals and assessed by palpations of the lumbar region (Murray, 1919; Jefferies, 1961). In sheep, heritabilities of BCS ranged from 0.10 to 0.30 (Borg *et al.*, 2005; Shackell *et al.*, 2011) according the breed and the time of measure but genetic determinism of BCS changes has not been yet investigated. The objectives of this study were i) to investigate temporal changes and profiles of BR dynamics throughout productive cycles, and ii) to analyze the genetic variability of BR dynamics on French Romane ewes reared in extensive conditions.

Material and methods

Data. Body condition score were recorded each year on 250 productive Romane ewes reared exclusively outdoors on about 280 ha of rangelands at the INRA Experimental Farm La Fage (Causse du Larzac, Roquefort-sur-Soulzon, France) since 13 years. A maximum of 2873 records per trait was registered over this period with 1146, 1068 and 414 ewes representing the productive cycles 1, 2 and 3 respectively (see details of La Fage management in González-García *et al.*, 2014). Eight BCS records were measured regularly according to a physiological stages schedule during each productive cycle of the female. The BCS evaluation was performed using an adapted grid (i.e. 1/10 scale) from the original one described by Russel *et al.* (1969). Differences between two individual measurements of BCS were computed to study mobilization and reconstitution of BR: 1) between early pregnancy and lambing (**BCS-Pa:L**), 2) between lambing and early suckling (**BCS-L:Sa**), 3) between early pregnancy and weaning (**BCS-Pa:W**), 4) between mating and early pregnancy (**BCS-M:Pa**), 5) between weaning and dry-off (**BCS-W:D**), 6) between weaning and mating (**BCS-W:M**).

Clustering of individual profiles. To investigate the variability in individual profiles of BCS, a Functional Principal Component Analysis (**FPCA**) was performed on smoothed BCS profiles, for each productive cycle, using the R package fdapace (Dai *et al.*, 2017). The eight measurements were considered regularly spaced out throughout the productive cycle, the time between two consecutive

measurements were not taken into account. Based on the principal component scores obtained, a cluster analysis was performed and the optimal number of clusters was researched between 2 and 7 clusters. The Akaike Information Criteria (**AIC**) and Bayesian Information Criterion (**BIC**) were used to determine it.

Genetic analyses on BCS differences. The variance components for BCS differences were estimated by restricted maximum likelihood methodology applied to the following animal mixed model using ASREML software, $\boldsymbol{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{a} + \mathbf{W}\boldsymbol{c} + \mathbf{e}$
where $\boldsymbol{Y}$ is the vector of BCS differences; $\boldsymbol{\beta}$ is the vector of fixed effects (age, parity of the ewe, litter size and year of measurement), $\boldsymbol{a}$ and $\boldsymbol{c}$ are vectors of random ewe additive genetic and permanent environmental effects with incidence matrices **X**, **Z**, and **W**, respectively, and **e** is the vector of residual effects.

Results and Discussion

Cluster analysis of BCS highlighted three clusters for each productive cycle (Figure 1) with more than 98% of variances explained by the two first principal components of FPCA. In productive cycle 1 and 2, two major clusters included 99 % and 85 % of the ewes respectively (clusters B1, B2, D1 and D2). In productive cycle 3, major cluster represented 76 % of the ewes while the two others represented 13 and 11 %. For each productive cycle, two clusters (i.e. the biggest clusters in cycle 1 and 2) showed paralleled profiles and similar profiles between cycles. These clusters differed in the level of BCS. Their profiles were characterized by a decrease in BCS during mid-pregnancy and the beginning of suckling, suggested that BR mobilization occurred during this period and by an increase in BCS from weaning to beginning of next pregnancy. During the first month of suckling, there was a loss of body condition despite the use of better paddocks. These losses in BCS were probably due to negative energy balance. The rest of the suckling period was characterized by stabilization in BCS which could be related to a decrease in the ewe's energy requirements for suckling. The BCS recovery started at weaning and lasted until the beginning of the next pregnancy. This increase in BR was linked to the low energy demands of the ewe and began in July (dry season). The BR accretion continued in autumn through grazing of new regrowth again available in native paddocks and lasted until the beginning of the next pregnancy, despite the winter, through feed supplementation started at mid-pregnancy (see overall feeding system schedule in González-García *et al.*, 2014). BCS changes were consistent with body weight variations recorded concomitantly (data not shown). The third cluster (i.e. the smallest clusters in cycle 1 and 2) (clusters B3, D3 respectively), was mainly characterized by higher levels of BCS than the two other clusters and a lower decrease in BCS during the mobilization period.

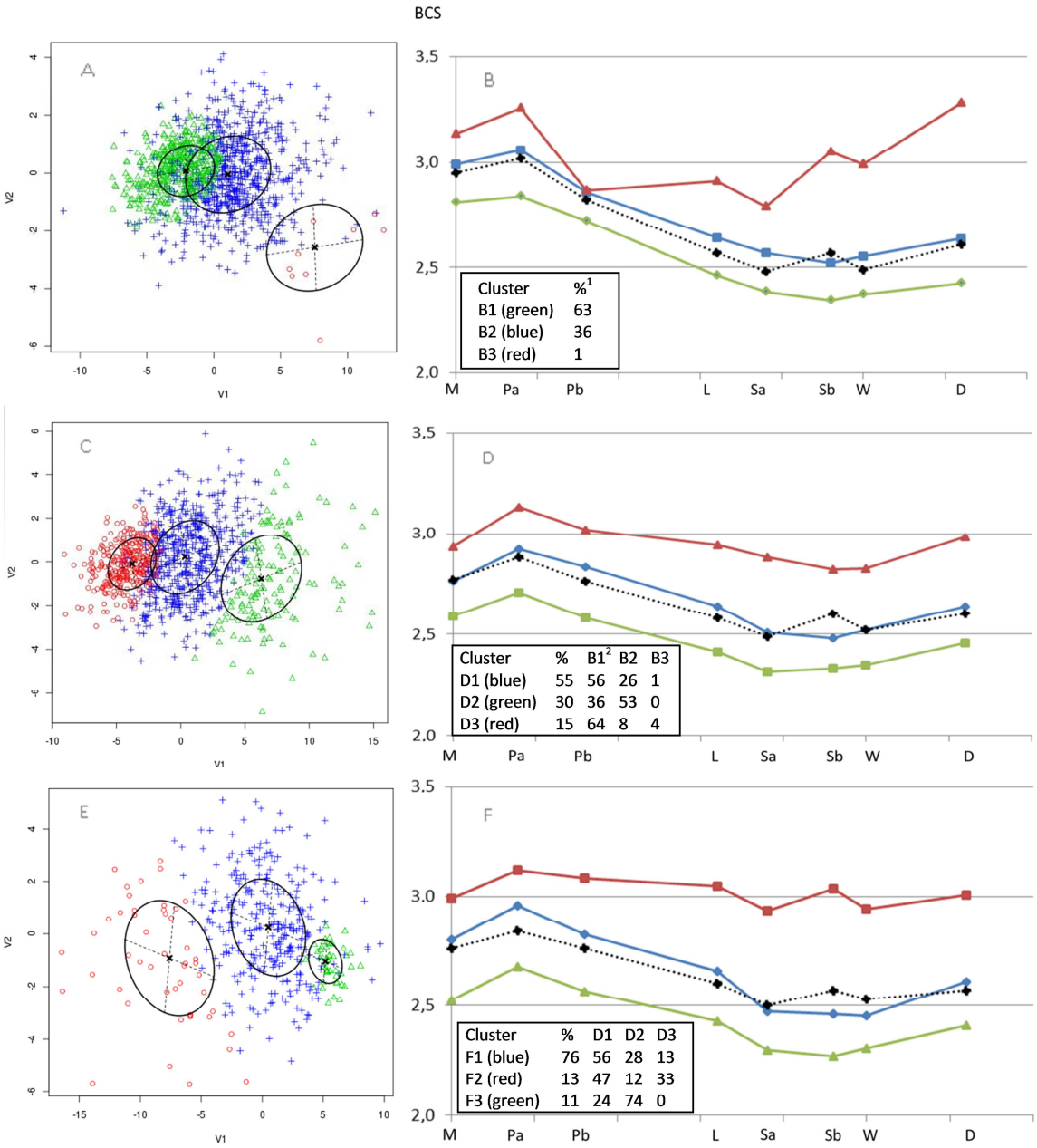

*Figure 1. Representation of clusters on the two first principal components from FPCA (**A, C, E**); cluster profiles **(B, D, F)** for Body Condition Score and mean BCS curve in black dot for each productive cycle (**A, B** for cycle 1; **C, D** for cycle 2 and **E, F** for cycle 3).[1]Proportion of animals in each cluster is given in percentage. [2]Composition of each cluster at productive cycle n is given by indicating proportion of animals from clusters found at previous cycle n-1.*

The cluster origin of ewes at previous cycle was not associated with repartition of ewes in clusters at following cycle. Two main biological factors influenced repartition of ewes between clusters: litter size and age of the ewes (Additional Table 1). In cycle 1, younger ewes were in cluster B1 and B2. In the two others productive cycle, the age was not discriminating the clusters. It appeared that ewes with smallest average litter size were mostly included in clusters showing highest BCS levels (clusters B3, D3 and F2). The cluster profile showing the lowest BCS in cycles 2 and 3 included ewes with the highest litter size (clusters D2 and F3). Interestingly, cluster analysis in ewes having similar litter size (i.e. 2 lambs born and suckled) still showed three clusters as those described here

suggesting that other sources of variation than known biological factors influenced BR variations (data not shown).

Concerning genetic parameters, direct heritabilities for BCS variations ranged between 0.06 ± 0.01 and 0.15± 0.02 (Table 1). The highest heritabilities were found for BCS-W:D and BCS-Pa:W. Lower heritabilites were found for the others BCS variations (BCS-M:Pa, BS-L:Sa, BCS-Pa:L and BCS-W:M). These results suggested that the biological capacities determining the nature of BR accretion and mobilization processes are heritable and could be selected. The heritabilities were similar to those found for punctual BCS ranging between 0.10 and 0.30 (Borg *et al.*, 2005; Shackell *et al.*, 2011). High negative genetic correlations were found between BCS-Pa:W and BCS-W:D or BCS-W:M, suggesting that mechanisms implied either in BR mobilization and/or accretion processes were genetically related. Moderate negative genetic correlations were found between BCS-M:Pa and BCS-Pa:W, between BCS-Pa:L and BCS-W:D, BCS-W:M and between BCS-L:Sa and BCS-W:D, BCS-W:M and confirmed the negative relationship between BR mobilization and accretion processes. Moderate positive genetic correlations were found between BCS-Pa:W and BCS-Pa:L, BCS-L:Sa indicating that the large BR mobilization period from pregnancy to weaning was correlated to shorter mobilization periods. High positive genetic correlation was found between BCS-W:D and BCS-W:M, suggesting that the anabolic BR process was predominant during this period. Phenotypic correlations followed the same variations than genetic correlations but were lower. Surprisingly, we did not find any genetic correlation for BCS variations between the dry-off period and early pregnancy whereas increases in BCS indicated that BR accretion continued at early pregnancy.

Table 1. Heritability, genetic and phenotypic correlations (± standard errors) for BCS variations.

Variables	BCS-M:Pa	BCS-Pa:W	BCS-Pa:L	BCS-L:Sa	BCS-W:D	BCS-W:M
BCS-M:Pa	**0.06 (0.01)**	-0.44 (0.14)	NS	NS	NS	NS
BCS-Pa:W	-0.41(0.02)	**0.14 (0.02)**	0.48 (0.09)	0.49 (0.12)	-0.71 (0.08)	-0.73(0.14)
BCS-Pa:L	-0.42 (0.01)	0.59 (0.01)	**0.09 (0.02)**	NS	-0.46 (0.12)	-0.48 (0.21)
BCS-L:Sa	NS	0.17 (0.02)	-0.31 (0.02)	**0.07 (0.02)**	-0.45 (0.14)	-0.52 (0.18)
BCS-W:D	NS	-0.49 (0.02)	-0.09 (0.02)	-0.08 (0.02)	**0.15 (0.02)**	0.75 (0.10)
BCS-W:M	NS	-0.46 (0.02)	-0.14 (0.03)	-0.07 (0.03)	0.59 (0.02)	**0.11 (0.04)**

Heritabilities on the diagonal in bold; phenotypic correlations below the diagonal; genetic correlations above the diagonal; NS, non significant.

Conclusion

Major clusters found for BCS variations were mainly characterized by BR accretion during dry-off and early pregnancy and BR mobilization during late pregnancy and suckling. These clusters were influenced by litter size and age effects. This study is the first demonstration of a genetic determinism for BR variations in the females of a meat sheep breed throughout productive cycles. Moderate heritabilities were found for both BR accretion and mobilization processes and they were highly genetically correlated. These results showed that a genetic selection is possible using BR accretion/mobilization as a trait for improving individual robustness in sheep.

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Additionnal Table 1. Biological characteristics of ewes included in each cluster. For each biological factor (litter size and age) and productive cycle, data represents percentage of distribution from the population present on each cluster.

			Litter size					Age				
			1	2	3	4	Avg.	1	2	3	4	Avg.
BCS	Cycle 1	B1	30	45	17	8	2.03	48	52	0	0	1.52
		B2	19	47	19	15	2.31	53	47	0	0	1.47
		B3	34	44	11	11	2.00	0	100	0	0	2.00
	Cycle 2	D1	17	19	34	30	2.76	0	56	44	0	2.44
		D2	6	17	44	33	3.03	0	64	36	0	2.36
		D3	44	22	23	11	2.02	0	45	55	0	2.55
	Cycle 3	F1	11	12	29	48	3.15	0	0	53	47	3.47
		F2	39	31	18	12	2.04	0	0	59	41	3.41
		F3	2	9	30	59	3.46	0	0	61	39	3.39