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Establishment of the biochemical and endocrine blood profiles in the *Majorera* and *Palmera* dairy goat breeds: the effect of feed restriction

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Feed restriction, and seasonal weight loss (SWL), are major setbacks for animal production in the tropics and the Mediterranean. They may be solved through the use of autochthonous breeds particularly well adapted to SWL. It is therefore of major importance to determine markers of tolerance to feed restriction of putative use in animal selection. Two indigenous breeds from the Canary Islands, *Palmera* and *Majorera*, are commonly used by dairy goat farmers and, interestingly, have different phenotype characteristics albeit with a common ancestry. Indeed, *Majorera* is well adapted to feed restriction whereas the *Palmera* is susceptible to feed restriction. In addition, regardless of their importance in dairy production, there are only a limited number of reports relating to these breeds and, to the best of our knowledge, there is no description of their blood metabolite standard values under control conditions or as affected by feed restriction. In this study we analysed the blood metabolite profiles in *Majorera* and *Palmera* goats aiming to establish the differential responses to feed restriction between the two breeds and to characterise their metabolite standard values under control conditions. We observed significant differences in creatinine, urea, non-esterified fatty acids (NEFAs), cholesterol, IGF-1 and T3 due to underfeeding. Furthermore, a PCA analysis, revealed that animals submitted to undernutrition could be distinguished from the control groups, with the formation of three separate clusters (*Palmera* individuals after 22 d of subnutrition (PE22); *Majorera* individuals after 22 d of subnutrition (ME22) and animals assigned to control conditions (MC0, MC22, PC0 and PC22)), highlighting different responses of the two breeds to undernutrition.

Keywords: Blood metabolites, seasonal weight loss, goat, *Majorera*, *Palmera*.

Animal production is increasing, particularly in developing countries (Morales de la Nuez et al. 2014; Hernández-Castellano et al. 2015a, b), and goats are considered to be particularly well adapted to a range of adverse conditions, particularly in relation to low feed availability during the dry season (Lérias et al. 2014). Indeed, it is estimated that approximately 130 million people across Western and Southern Africa alone own small ruminants, particularly goats, and that these are very important for their livelihood and food security (McDermott et al. 2010).

Both tropical and Mediterranean climates are defined by a rainy and a dry season that provide different feed availabilities, the rainy season being notable for its good quality

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pastures. On the contrary, during the dry season, the pasture quantity and quality is significantly reduced, causing feed restriction and consequently live weight decrease, commonly described as seasonal weight loss (SWL) (Lamy et al. 2012; Cardoso & Almeida, 2013). SWL is considered to be one of the major issues in animal production in tropical and subtropical climates, as the affected animals may lose up to 30% of their body live weight (BLW) (Cardoso & Almeida, 2013) as seen in Southern Africa (Almeida et al. 2006, 2007), West Africa (de Almeida & Cardoso, 2008a, b) and more recently in Western Australia (Almeida et al. 2013; Scanlon et al. 2013). Indeed, SWL can lead to significant economic losses to producers, enhancing the need to solve this problem, something the Food and Agriculture Organization of the United Nations has been addressing in recent years (Cardoso & Almeida, 2013). The use of supplementation is one proposed solution, however, it is very expensive and, consequently, difficult to implement in developing countries. The use of local breeds known to be tolerant to SWL is increasing, being much less expensive and easy to implement in such regions (Cardoso & Almeida, 2013).

The Canary Islands are a subtropical archipelago composed of seven islands with very different and contrasting micro-climates. Overall, the western region (*La Palma, El Hierro, La Gomera* and the northern part of the island of *Tenerife*) are humid, whereas the eastern islands (*Lanzarote, Fuerteventura, Gran Canaria* and the southern part of *Tenerife*) are extremely dry (Herrera et al. 2001), a consequence of not being in the path of the trade winds arising from the Atlantic and their exposure to the influence of the Sahara Desert. These two groups of islands have very different rain patterns that necessarily affect local ecosystems, agriculture, pasture abundance and animal production systems. As a consequence, the Canary Islands are home to an extraordinary variety of domestic animal resources, counting three goat local breeds, all descending from ancestors imported from Northern Africa and the Iberian Peninsula during the colonisation of the Islands. Different breeds developed as a result of particular adaptations of the animals to the different climates present on each of the islands. There are three different local goats breeds: the *Majorera*, the *Palmera* and two ecotypes of the *Tinerfeña* (Amills et al. 2004).

Currently, the *Majorera* goat, although distributed through all islands, is primarily found on *Fuerteventura* and *Gran Canaria* (Navarro-Ríos et al. 2011). In contrast, the *Palmera* breed is found primarily on *La Palma* , but also sporadically on other islands, particularly northern *Tenerife* (Escuder et al. 2006). Although *Palmera* and *Majorera* goats share a common ancestor, there are several differences between them. *Majorera* goats are adapted to dry climates and therefore have an acquired resistance to SWL (Fresno et al. 1994), whilst *Palmera* goats are adapted to rainy climates, and as such are susceptible to SWL (Navarro-Ríos et al. 2011). It is also noteworthy to mention that such adaptation is clear from a phenotypic point of view with *Majorera* goats showing strong similarities to breeds from the Sahel region (e.g. slender bodies, long legs and short hair) and accordingly have recently been successfully exported for milk production to the arid regions of Senegal in West Africa. In contrast, *Palmera* goats show strong similarities to goats from the northern Iberian Peninsula (bulkier body frame, spiral horns and long hair).

As shown in sheep (Caldeira et al. 2007; Hyder et al. 2013) and in other goat breeds (Aboelmaaty et al. 2008; Hyder et al. 2013), blood metabolites are one of the most important indicators in the evaluation of the physiological state of animals, as well as determinants of the putative differential responses of these animals to specific conditions such as those posed by SWL. Besides, the lactation process has an autocrine regulation (Wilde et al. 1997) that can be affected by hormone changes during SWL. In addition blood metabolite analysis can be an expeditious tool to assess animal nutrition status and can be easily integrated into the farm's daily management. Their determination can be easily and rapidly conducted. In this work, we analysed the influence of feed restriction on the evolution of several blood metabolites (creatinine, urea, cholesterol, triglycerides, glucose, total proteins, creatine kinase, phosphorus, non-esterified fatty acids (NEFAs), sodium, chloride, hydroxybutyrate, haptoglobin) and hormones (cortisol, insulin, insulin-like growth factor 1 (IGF-1), triiodothyronine (T3) and leptin). To the best of our knowledge, this is the first time that these blood metabolites and hormones have been characterised in *Majorera* and *Palmera* goat breeds under feed restriction. Furthermore, this work, by conducting an extensive characterisation of the blood metabolites profiles in *Majorera* and *Palmera* breeds under control situations, was seen as serving as a reference for future works involving dairy goat breeds from the Tropics and the sub-Tropics. We will finally address the evolution of these metabolite and endocrinal profiles as a consequence of feed restriction by relating these findings to the differential adaptation of these two breeds to SWL.

Material and methods

Location, animals and nutritional treatments

As described in detail previously (Lérias et al. 2013), the study was conducted at the experimental farm of the Faculty of Veterinary Medicine of the ULPGC – Universidad de Las Palmas de Gran Canaria (Arucas, Gran Canaria, Spain) with 10 *Majorera* and 10 *Palmera* dairy goats (3 lactations with kidding in late February and a body condition score of 3) obtained from the experimental flock of the *Pico* Research Station (Valle Guerra, Tenerife, Spain) during May/June 2012. Animals were classified as clinically healthy at the onset and through all the experimental period (22 d) and were admitted to the trial at day 80 of the lactation, approximately at the peak of lactation. The goats were divided randomly into four sets, two for each breed: underfed and control groups. During the

experimental period animals were milked at a vacuum pressure of 42 kPa, a pulsation ratio of 90 pulses/min, and a pulsation ratio of 60/40, in accordance with Capote et al. (2006) and Torres et al. (2013). Body liveweights and daily milk production were 45·5 kg and 1·60 l for *Majorera* Control, 50·6 kg and 1·68 l for *Majorera* Underfed, 32·8 kg and 1·03 l for *Palmera* Control and 40·6 kg and 1·33 l for *Palmera* underfed, as previously reported (Lérias et al. 2013). During the whole trial, all animals had free access to drinking water.

With the view to reproducing the field conditions in regions prone to drought and SWL when the pasture is characterised by low protein and high fibre content the following nutritional restrictions were applied (Almeida et al. 2006). Animals from the underfeed groups were fed on standard wheat straw and a vitamin-mineral supplement (underfed diet, in order to achieve a 15–20% reduction of their initial BLW by the end of the experimental period). The wheat straw basic composition corresponded to a low level of crude protein (approximately 30 g/kg dry matter), high amounts of fiber (420 g/kg dry matter) and low energy content (5·5 MJ/kg dry matter) (McDonald et al. 1988). In contrast, control animals were fed on a balanced diet, sufficient to cover their maintenance and lactation needs by using standard supplements found on the Canary Islands. As per Lérias et al. (2013) and Martínez-de la Puente et al. (2011), goats from the control groups were fed above-maintenance needs with maize, soy 44 (crude protein 44%), dehydrated lucerne, dehydrated beetroot, lucerne hay and a vitamin-mineral supplement. The control diet provided 1·81 kg of dry matter, 1·46 UFL, 133 g of metabolisable protein, 12 g of Ca and 6 g of P in accordance with the guidelines issued by the *Institut National de la Recherche Agronomique* (INRA, 2007). Goats from the underfed groups were fed with straw, representing 52% of the total UFL provided to the control group (1·81 kg of dry matter, 0·76 UFL, 41·13 g of metabolisable protein, 1·33 g of Ca and 0·66 g of P). The experimental period lasted 22 d from the point when the animals in the underfed groups had reached a stable decrease in relative liveweight of 13–15%. At this date, liveweights and daily milk production were 48·2 kg and 1·99 l for *Majorera* Control and 44·1 kg with 0·22 l for *Majorera* Underfed, 33·9 kg and 1·15 l for *Palmera* Control and 35·4 kg and 0·17 l for *Palmera* Underfed, as previously reported by Lérias et al. (2013).

Blood collection and analysis

Blood samples (4–5 ml) were collected from the external jugular vein at day 0 and day 22 of the trial in the early hours of the morning and before the animals were milked and fed. Plasma was separated by centrifugation as described (Hernández-Castellano et al. 2014) and stored at −80 °C until use. Plasma metabolite concentrations were measured as previously described (Yang et al. 2011). Briefly, the following methods were used: glucose-dehydrogenase (GLDH) for urea; recommended IFCC reference method for creatine kinase; molybdate method for phosphorus; ion selective electrode method for sodium and chloride; Mercodia Ovine Insulin ELISA (Mercodia, Sweden) for insulin; multi-species leptin RIA kit (Millipore Corporation, USA) for leptin; Immulite Solid-phase, enzyme-labeled chemiluminescent immunoassay (Siemens, USA) for IGF-1; Immulite Solid-phase, competititve chemiluminescent immunoassay (Siemens, USA) for T3; cholesterol esterase/peroxidase enzymatic method (Beckman Coulter Reagent) for cholesterol; glycerol phosphate oxidade enzymatic method (Beckman Coulter Reagent) for triglycerides; hexokinase method (Beckman Coulter Reagent) for glucose; biuret method (Beckman Coulter Reagent) for total proteins; reagent NEFA C (Wako Chemicals GmbH, Germany) for NEFAs; reagent RANBUT D-3-hydroxybutyrate (Randox, UK) for hydroxybutyrate; reagent Haptoglobin Colorimentric Assay (Tridelta Phase, Ireland) for haptoglobin; salivary cortisol ELISA (DRG Instruments, Germany), for cortisol.

All the assays were performed with an Olympus AU400 analyser following the manufacturer's recommendations for metabolites and with Immunite 1000 (Siemens, Germany) for hormone measurements, using standard commercial practices.

Statistical analysis

Data collected (*Majorera* and *Palmera* blood metabolites for day 0 and 22) for each feed group, breed and trial day was analysed for normality with the Shapiro-Wilk test and then further analysed by ANOVA repeated measures. When significant P values ($P < 0·05$) were observed, mean comparisons were performed with a Tukey post hoc test. Means were considered significantly different when $P < 0·05$. Statistical analysis was performed using the STATISTICA 8·0 software (Tulsa, OK, U.S.A.).

To identify the structure of the interdependences of the main parameters assessed in the animals of the study, joint principal components analysis (PCA) was performed on the following variables: NEFAs, creatinine, creatine kinase, chloride, haptoglobin, sodium, cholesterol, urea, total proteins, hydroxybutyrate, IGF-1, insulin, glucose, T3, phosphorus, leptin, cortisol and triglycerides. The standardised variables were subjected to PCA allowing the extraction of the rotated orthogonal components, as well as, their relative scores. Only principal components (PC) with an eigenvalue higher than 1 were considered for discussion.

Animal welfare disclaimer

Spanish and European Union guidelines and legislation on care, use and handling of experimental farm animals were followed. Author AM Almeida holds a FELASA grade C certificate enabling the design and conduction of animal experimentation in the European Union.

Results and discussion

Considering the importance of SWL and the use of indigenous breeds with an acquired tolerance to SWL (Lamy et al. 2012;

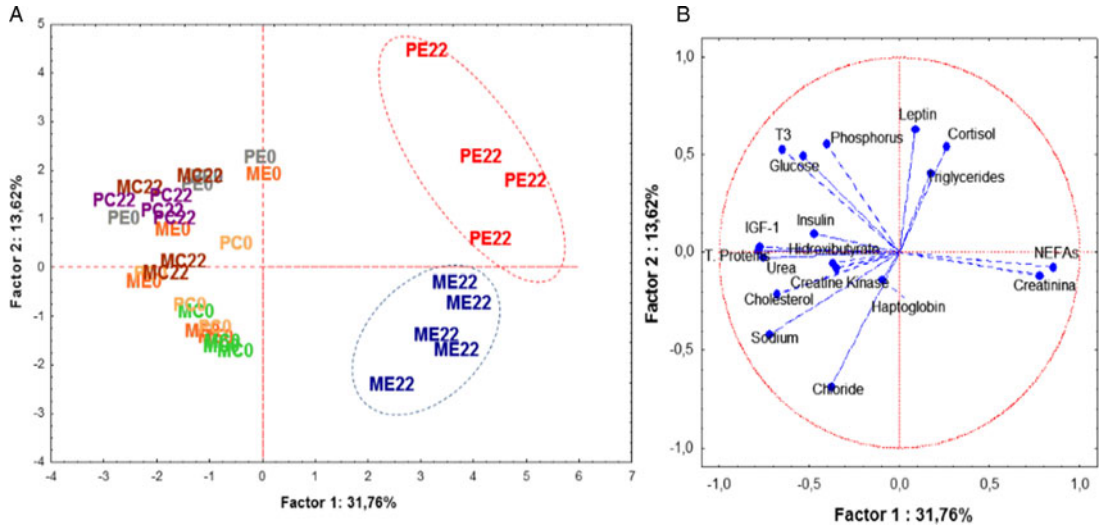

Fig. 1. PCA scatterplot of the *Majorera* and *Palmera* animals (control and underfed) at 0 and 22 d (A) and position of the variables projected in the plane as determined by the first two principal axes (B) (45·38% of the % explained variance). MC0 – *Majorera* Control, day 0; MC22 – *Majorera* control at day 22, ME0 – *Majorera* Underfed at day 0, ME22 – *Majorera* Underfed at day 22; PC0 – *Palmera* Control, day 0; PC22 – *Palmera* control at day 22, PE0 – *Palmera* Underfed at day 0 and PE22 – *Palmera* Underfed at day 22.

Cardoso & Almeida, 2013) in animal production, it is clear that the determination of the metabolic profiles in *Majorera* and *Palmera* goats are of extreme importance. In fact, and to the best of our knowledge, there has been no blood metabolic profiling of these two breeds, further enhancing the importance of these results. In addition, it is crucial to understand the effect of feed restriction in the metabolic and endocrine parameters of indigenous goats with different tolerances to SWL so that such information could be used in selection programs together, for instance, with genomic, transcriptomic or proteomic data in a systems biology perspective (Almeida et al. 2015). Accordingly, in this work we describe for the first time the blood metabolite and endocrinal profiles of in the *Majorera* and *Palmera* goat breeds from the Canary Islands (Spain) and at the same time we study how these profiles change in the two breeds as a consequence of feed restriction.

In this section we will address first the Principal Component Analysis (Fig. 1) for all the metabolites and hormones and in the four experimental groups. Later, we will address one by one all the metabolites and hormones studied. The standard values for the plasma concentrations of each individual metabolite in the *Majorera* and *Palmera* breeds are presented finally in Figs 2–4 (control groups at day 0). As the establishment of the standard values for each breed is essentially descriptive, they will not be compared with results from other researchers in the literature.

Principal Component Analysis (PCA)

For the metabolite profiling of the animals of the study, three factors were extracted from the PCA, accounting for 55·2% of the variance. Factor 1 accounted for 31·8% of the variance and was highly correlated with NEFAs (0·85) followed then total proteins (−0·78), IGF1 (−0·77), creatinine (0·77), urea (−0·75). Factor 2 accounted for 13·6% and was potentially correlated with chloride (0·69) and leptin (0·63). Factor 3 accounted for 9·9% was correlated with haptoglobin (0·73).

The data were plotted according to PC1 and PC2, as shown in Fig. 1, from which three clusters were evident corresponding to: (1) individual *Palmera* animals after 22 d of undernutrition (PE22); (2), individual *Majorera* animals after 22 d of undernutrition (ME22) and (3) animals assigned to control groups (MC0, MC22, PC0 and PC22) as well as the data referring to the animals allocated to the experimental group at the beginning of the trial (PE0, ME0). Of these the most relevant observation is that it is clearly possible to separate animals submitted to undernutrition (right side of the plot, Fig. 1A) from those kept under normal feeding, irrespective of the breed. In fact, underfed animals presented higher concentrations of NEFAs (PE22 1·25 ± 0·31 and ME22 1·03 ± 0·23 mmol/l), tryglicerides only for PE (PE22 15·33 ± 0·82 and ME22 12·40 ± 2·15 mg/dl) and creatinine (PE0 0·79 ± 0·08 and ME22 11·46 ± 6·22 mg/dl), but not for leptin (PE22 4·00 ± 0·99 and ME22 3·05 ± 0·55 ng/ml HE) and cortisol (PE22 42·66 ± 12·34 and ME22 11·46 ± 6·22 ng/ml) (see subsequent sections), as plotted in Fig. 1B. In addition, NEFAs, total protein, IGF-1 and creatinine could be considered as the best parameters to be used to discriminate control from underfed animals, irrespective to the breed studied. Finally, a clear separation between breeds is noticed in the plot presented in Fig. 1A, showing that the reply of the two breeds to weight loss is indeed different, albeit a lower variation was accounted for this axis. As *Palmera* Underfed group has higher concentrations of cortisol (PE0 43·24 ± 47·18 and PE22 42·66 ± 12·34 ng/ml) and triglycerides (PE0 12·33 ± 1·47 and PE22 15·33 ± 0·82 mg/dl) (see subsequent sections), such metabolites

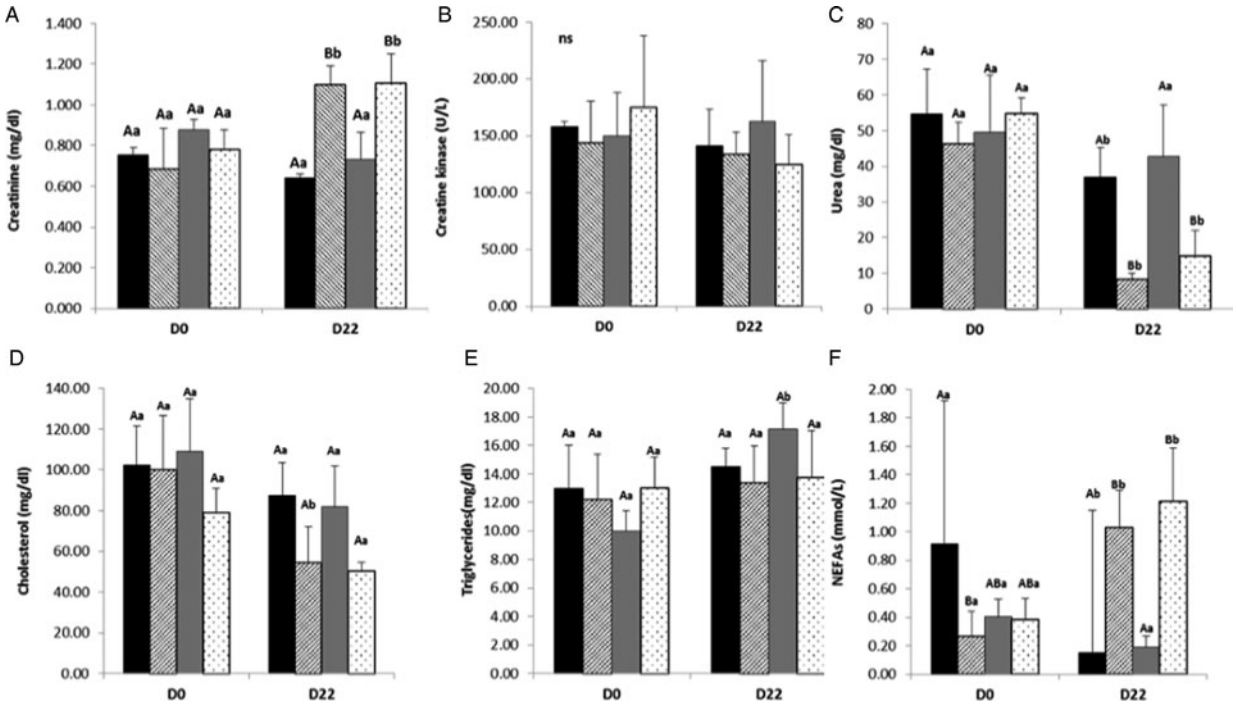

Fig. 2. Mean values and standard deviations for *Majorera* and *Palmera* control and underfed groups at days 0 and 22 of the trial for the metabolites Creatinine (A), Creatine kinase (B), Urea (C), Cholesterol (D), Triglycerides (E) and NEFA (F). ABC Columns with different uppercase letters superscripts indicate differences ($P < 0{\cdot}05$) within groups of the same trial day. abc Columns with different lowercase letters superscripts indicate to differences ($P < 0{\cdot}05$) between the same groups at days 0 and 22 of the trial. Black bars – *Majorera* Control; Striped Bars – *Majorera* Underfed; Grey Bars – *Palmera* Controls; Dotted Bars – *Palmera* Underfed.

could be proposed as associated to the responses of this breed to undernutrition.

Creatinine and Creatine Kinase

Creatine and creatine phosphate have key roles in energy metabolism, as a rapid source of high-energy phosphate via creatine kinase enzyme (CK) (creatine phosphate + ADP – creatine + ATP). Creatinine is a breakdown product of creatine and creatine phosphate, which is then excreted in the urine (Brosnan & Brosnan, 2010). This metabolite can be related to kidney function and in general to the body mass (Brosnan & Brosnan, 2010).

The results relative to the creatinine values in underfed and control groups from both breeds are described in Fig. 2A. Concerning *Majorera* and *Palmera* creatinine evolution in underfed groups, there was a significant increase ($P < 0{\cdot}05$) at day 22. There was however no differences between the two underfeed groups at day 22. Nevertheless, there was a clearly difference as a result of feed restriction, demonstrated in both statistical analysis.

There were no significant differences between the control groups ($P > 0{\cdot}05$) in the ANOVA repeated measures analysis, whereas the differences between control and underfed groups indicate that undernutrition led to an increase in creatinine values. Indeed, creatinine increase is likely linked to the negative energy balance that underfed animals are subjected to, being characterised not just weight loss, but also the mobilisation of adipose tissue and muscle protein (Carbone et al. 2012) So, the increase of creatinine was mainly due to the higher mobilisation of muscle protein, which increased the creatine levels, that were further broken down to creatinine. Nevertheless, there is no evidence of the influence of evolution on the breed, since both underfed groups behaved similarly during the trial. These findings are consistent with those of Jia et al. (1995) who studied the variation of creatinine concentrations in goats fed with different levels of crude protein (8 and 16%). It should also be emphasised that creatinine is a metabolite with a high variation due to breed, sex, diet, among other factors (Liu & McMeniman, 2006).

The results for creatine kinase are presented in Fig. 2B. No differences ($P > 0{\cdot}05$) between all groups at day 0 and day 22 were found. This is consistent with what would be expected as this enzyme is released to the plasma in case of muscular damage, which was not the case of the animals used in the study.

Urea

Urea is an important source of nitrogen, through its direct intake in the diet or its reentrance in the rumen across the ruminal epithelium (Huntington & Archibeque, 1999). Figure 2C shows the urea concentration in underfed and

control groups from both *Majorera* and *Palmera* breeds at days 0 and 22, a significant reduction at day 22 was observed in the underfed groups and the *Majorera* Control group. The differences in underfed groups reveal that only feed restriction had an effect in urea values, since both breeds had a similar trend for this variable. Similar results were obtained by Celi et al. (2008) using Red Syrian goats under feed restriction (80% of energy requirements). These authors observed higher urea values in the restricted group when compared to the control group (140% of energy requirements). Therefore, the reduction of urea concentration was probably due not only to the reduction of nitrogen intake, but also to the fact that these animals were in lactation and under negative energy balance. Nevertheless, and as milk yield was highly reduced along the trial (Lérias et al. 2013), these changes in urea blood concentrations are primarily due to the negative energy balance. Furthermore, and as previously suggested (Caldeira et al. 2007), an increment of the ammonia production resulting from the catabolism of mobilised endogenous N compounds could also be a likely explanation for our results. Finally, there are additional reports regarding the lack of a relationship between serum urea concentration and feed restriction, such as those described by Dashtizadeh et al. (2008) or Caldeira et al. (2007). These results indicate that urea concentrations are on one hand heavily dependent on the level and type of feed restriction imposed and on the other hand on breed and individuals. In fact, it has been demonstrated that this parameter has a strong variability among animals of the same breed (Kasumu, 2011).

Cholesterol and triglycerides

Cholesterol is essential for all mammals, including ruminants, being sourced mainly as a result of its ingestion or endogenous production (Bauchart, 1993). Triglycerides are one of the most important sources of energy in mammals being composed by a glycerol esterified with three fatty acids, which can be broken down into glycerol and NEFAs (Alila-Johansson, 2008).

The results for cholesterol in underfed and control groups' are represented in Fig. 2E. Here we observed a significant reduction ($P < 0{\cdot}005$) in the underfed groups only for the *Majorera* at day 22 (54·44% of the initial values) with no significant changes being observed for the *Palmera*. Nevertheless, though no significant differences were observed in the *Palmera*, there appears to be a trend towards a decrease in the levels of cholesterol in this breed as well (see Fig. 3F). There are a number of potential reasons for these differences. While they are most likely due to a reduced amount of fat ingestion, the fact that *Majorera* animals are heavier and produce more milk than *Palmera* animals may be one factor but may also be a consequence of a putative more efficient metabolism regarding cholesterol storage in the *Palmera* breed. Finally, the observed reduction in cholesterol concentration may have also been due to a decreased lipogenesis rather than an increased lipolysis. In support of our results Aboelmaaty et al. (2008) obtained similar results for Egyptian native goats, that was attributed to a reduction of fat ingestion and consequently of its absorption, and a higher metabolism rate.

No differences among groups due either to feed restriction or breed was detected in the triglycerides (Fig. 2E), with the exception of *Palmera* Control (higher values at day 22). Interestingly, and in contrast to our findings, Aboelmaaty et al. (2008) and Celi et al. (2008), observed a decrease in triglyceride concentration in situations of feed restriction. It could be hypothesised that an initial decrease in triglyceride concentration would be followed by an increase of the triglyceride values, via NEFA pathway inhibition with consequent re-esterification occurring. Working with Serra da Estrela ewes, Caldeira et al. (2007) has proposed such a theory that, in our case, may be corroborated by the fact that hydroxybutyrate, (see below) which is a product of NEFAs oxidation, was reduced and stabilised along with the feed restriction.

Non-esterified fatty acids (NEFAs) and hydroxybutyrate

Hydroxybutyrate as well as acetone and acetoacetate, is considered a ketone body. Ketones are produced in the liver from the metabolism of NEFAs and volatile fatty acids in negative energy balance conditions (Bauchart, 1993).

We observed a significant increase ($P < 0{\cdot}001$) in NEFA among the underfed groups of *Majorera* and *Palmera* (Fig. 2F). There was a significant reduction ($P < 0{\cdot}05$) in the *Majorera* control group at day 22, however, the standard deviation for this group was high. On the basis of this, feed restriction appears to lead to the increase of the concentration of NEFAs, but breed did not seem to have a significant effect. Several authors have reported similar results in goats (Dunshea et al. 1988; Ríos et al. 2006; Tsiplakou et al. 2012) and sheep reared under feed restriction (Caldeira et al. 2007). In our results, the increase in NEFAs was likely related to an increment of fatty acid mobilisation, due to the animals' energy negative balance (Lérias et al. 2013). Additionally, Dunshea et al. (1988) reported an increment in plasma of the NEFA:glycerol ratio in animals under feed restriction, suggesting a decrease in lipogenesis rather than increased lipolysis. The decrease in the control group may be due to lower degree of nutritional stress in a more advanced period of lactation, since the animals were in the 100th day of lactation. No differences were observed between day 0 and 22 in hydroxybutyrate for any of the studied breeds (Fig. 3A), with the exception of a significant difference between *Majorera* groups at both dates. As previously described, the inhibition of NEFA pathways and its re-esterification could explain these results.

Glucose and insulin

Insulin effects are evident in several tissues including muscles, adipose tissues, liver and mammary glands and are important in the regulation of glucose homoeostasis (Sasaki, 2002). No differences between groups due to either feed restriction or

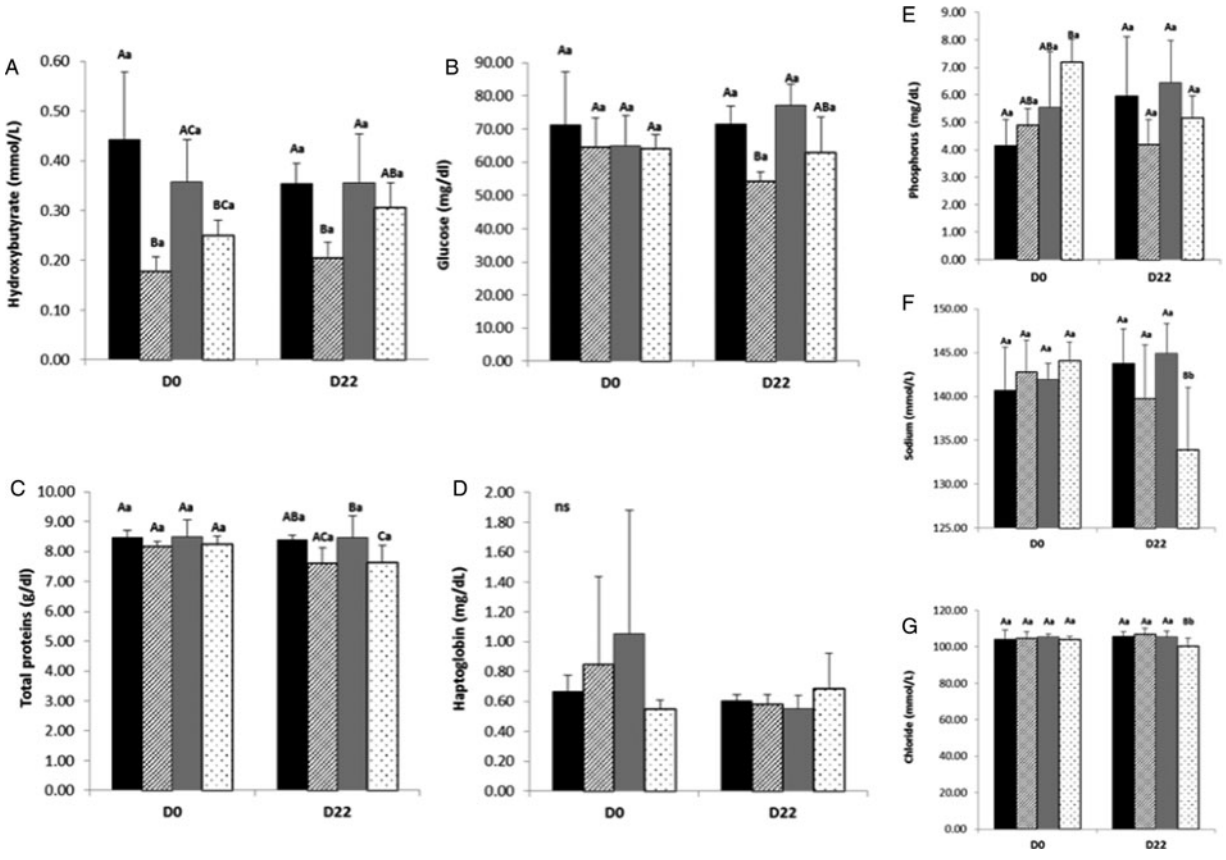

Fig. 3. Mean values and standard deviations for *Majorera* and *Palmera* control and underfed groups at days 0 and 22 of the trial for the metabolites Hydroxybutyrate (A), Glucose (B), Total Protein (C), Haptoglobin (D), Phosphorus (E), Sodium (F) and Choloride (G). ABC Columns with different uppercase letters superscripts indicate differences ($P < 0{\cdot}05$) within groups of the same trial day. abc Columns with different lowercase letters superscripts indicate to differences ($P < 0{\cdot}05$) between the same groups at days 0 and 22 of the trial. Black bars – *Majorera* Control; Striped Bars – *Majorera* Underfed; Grey Bars – *Palmera* Controls; Dotted Bars – *Palmera* Underfed.

different breeds were observed in these two metabolites. Despite the fact that several authors have reported a reduction in insulin values due to feed restriction (Caldeira et al. 2007; Ollier et al. 2007; Celi et al. 2008), it is important to emphasise that some have found inconsistent daily patterns for several metabolites and hormones, particularly insulin (Alila-Johansson et al. 2004). In our study the standard deviation of this variable was high for all studied groups, reflecting a high variability of the insulin concentration among animals (see Fig. 4B). Glucose concentration (Fig. 3B), was found similar to what has been observed by Aboelmaaty et al. (2008) and Celi et al. (2008). Nevertheless, it has to be emphasised that glucose, similarly to insulin may show considerable changes between the same breed.

Total proteins and haptoglobin

Haptoglobin is an α2-glycoprotein that binds to free haemoglobin (Javid, 1978; Sadrzadeh & Bozorgmehr, 2004). This acute phase protein is one of the most used indicatives for stress conditions (Hernández-Castellano et al. 2015c) such as feed restriction. Furthermore, total protein concentration can be used as an indicator of liver disease, nutritional status, kidney disease, among others (Dhinaa & Palanisamy, 2010).

No significant differences were found in the total proteins and haptoglobin concentration due to either feed restriction or to breed effect (see Fig. 3C, D), with the exception of *Palmera* groups at day 22 for total proteins (higher value for the control group). Even though some authors have reported a reduction of total proteins due to feed restriction (e.g. Caldeira et al. 2007; Aboelmaaty et al. 2008), Celi et al. (2008) reported a reduction of total proteins in their trial but it bore no relation to feed restriction. Indeed, protein levels can vary considerably with the diet (Hatfield et al. 1998) and also between breeds, which lead to a high variability between results.

Cortisol and leptin

Cortisol is a glucocorticoid hormone involved in stress response (Alila-johansson, 2008). Leptin is involved in body weight regulation and related to food intake and energy balance (Ahima et al. 1997; Alila-Johansson, 2008). Cortisol hormone levels are strongly related to the feeding

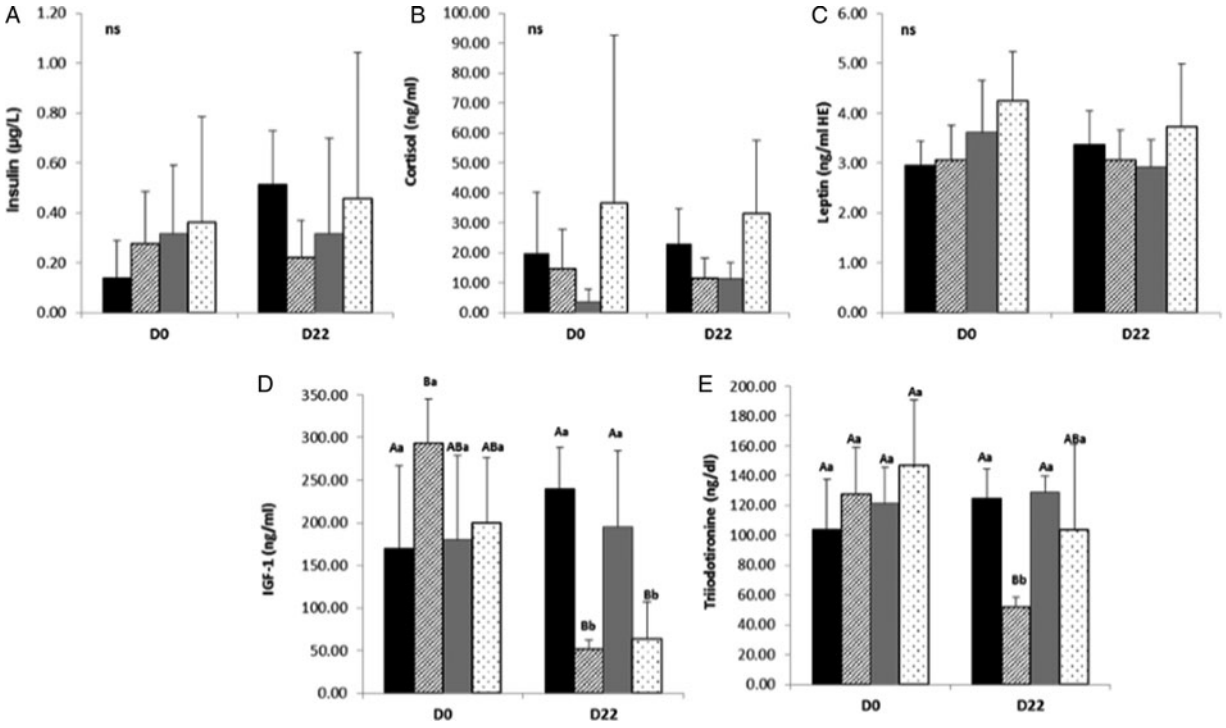

Fig. 4. Mean values and standard deviations for *Majorera* and *Palmera* control and underfed groups at days 0 and 22 of the trial for the hormones Insulin (A), Cortisol (B), Leptin (C), IGF-1 (D) and Triiodotironine (E). ABC Columns with different uppercase letters superscripts indicate differences ($P < 0{\cdot}05$) within groups of the same trial day. abc Columns with different lowercase letters superscripts indicate to differences ($P < 0{\cdot}05$) between the same groups at days 0 and 22 of the trial. Black bars – *Majorera* Control; Striped Bars – *Majorera* Underfed; Grey Bars – *Palmera* Controls; Dotted Bars – *Palmera* Underfed.

time in goats (Eriksson & Teräväinen, 1989; Alila-Johansson, 2008), so this may explain the high standard deviation within groups and the lack of statistical differences (Fig. 4B). It may be expected that underfed groups would have a higher level of cortisol due to the feed restriction stress situation and a decreased concentration of leptin (Fig. 4C), caused by the reduction of body fat and food ingestion. In agreement with our findings, Celi et al. (2008) reported that leptin alterations over time in underfed animals was not due to feed restriction and Tsiplakou et al. (2012) reported no significant differences between over and underfeed groups. In fact, leptin and cortisol have a high variability due to several factors, with special emphasis to stress conditions, and regular management operations such as milking leading in turn to a strong result heterogeneity as the one observed in our study.

IGF-1 and T3

Insulin-like growth factor 1 (IGF-1) stimulates tissue differentiation and cellular proliferation and is also involved in lipogenesis and gluconeogenesis (D'Ercole et al. 1984; Clemmons & Van Wyk, 1985; Zulu et al. 2002; Guerra-García et al. 2009). The thyroid hormones have an effect on the metabolic rate increment, protein synthesis and lipid metabolism stimulation, among other effects (Capen & Martin, 1989). Our results showed a tendency towards lower values for both hormones in the underfed groups, albeit with statistical differences due to feed restriction only being recorded in the *Majorera* groups (Fig 4D, F) respectively for IGF-1 and T3). Some groups (Aboelmaaty et al. 2008) found similar results for IGF-1 to those described in the present study, which are expected considering the role of this hormone on the metabolism regulation. The concentration of the T3 hormone would be expected to reduce, to allow the animals to decrease their energy requirements, as has been reported by other authors (Caldeira et al. 2007; Celi et al. 2008), and in our results.

Ions: Chloride, sodium and phosphorus

Minerals are important elements in goat nutrition, since their defficiency may lead to reproductive complications and production losses (Bueno & Vitti, 1999). Sodium and chloride are two of the most important ions for pH regulation and osmotic pressure (Underwood & Suttle, 1999). Our results (Fig. 3E–G), found no differences in any of the studied minerals between groups (comparing days 0 and 22) and between day 22 values, with the exception of sodium and chloride (lower values for day 22 of *Palmera* underfed group compared to day 0). These results may not be surprising given that the main consequence of the feed restriction imposed

was essentially related to energy and protein requirements, with minimal changes regarding mineral nutrition.

Conclusions and future perspectives

The results showed in this study reveal important aspects regarding goat physiology under feed restriction and provide the first in-depth characterisation of blood metabolite and hormonal profiles in the two studied breeds, *Majorera* and *Palmera*. These breeds are extremely important to the goat dairy production sector in the Canary Islands and are also gaining importance in the Spanish mainland, as well as other countries in arid tropical regions such as Senegal and Mauritania, where dairy goat production has been implemented and where the small ruminant dairy industry is expanding considerably. These results are also particularly interesting given the limited number of such assays in dairy goats. Herein, we determined that several of the studied metabolites and hormones showed significant differences due to feed restriction (creatinine, urea, NEFAs, cholesterol, IGF-1 and T3), due either to the lower amount of protein and fat ingestion (urea and cholesterol reduction), higher metabolisation of muscular proteins and fatty acids (creatinine and NEFAs increment) or an overall metabolism aiming at energy conservation (IGF-1 and T3 reduction). These results are supported also by a Principal Component Analysis that showed the presence of three clusters: one for each underfed group at day 22 and a third one that included animals in control conditions in both days 0 and 22 and the experimental groups prior nutritional stress i.e. day 0. The existence of these clusters is likely explained by: NEFAs, total proteins, IGF-1 and creatinine, which are probably the best parameters to differentiate control from underfed animals. In addition, *Palmera* animals appear to have a tendency towards higher cortisol and triglycerides levels, which could be of use as a putative biomarker of the specific response of this breed to feed restriction.

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