

1 **Short-, medium- and long-term metabolic responses of adult ewes submitted to**
2 **nutritional and β -adrenergic challenges**

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11 **Abstract**

12 **Background:** In order to maintain homeostasis, ruminants submitted to alternating shortage
13 and refeeding situations manifest switches in metabolic pathways induced by undernutrition
14 and body reserves (**BR**) replenishment cycles. The objective of this experiment was to study
15 adaptive regulatory mechanisms present during subsequent feeding transition periods and the
16 inherent lipolytic activity of the adipose tissue in individuals with contrasted BR. Three diets
17 containing different levels of energy were offered to 36 mature, dry, non-pregnant Mérinos
18 d'Arles ewes in an experiment lasting 122 days. Ewes were selected with similar body weight
19 (**BW**), body condition score (**BCS**) and were allocated into three equivalent treatments
20 according to the plane of nutrition: normally fed (**Control**); underfed (**Under**) or overfed
21 (**Over**). The BW, BCS and individual energy metabolism were monitored. At the end of the
22 experiment, lipolytic activity of adipose tissue was studied through a β -adrenergic challenge
23 to the same ewes, with body conditions according to the offered diet (**Normal, Leans** and
24 **Fat**, respectively).

25 **Results:** Anabolic or catabolic responses to energy dietary manipulation were accompanied
26 by synchronised metabolic regulation, leading to contrasting metabolic and BR profiles.
27 Average BW and BCS were higher and lower in Over and Under ewes, respectively. The
28 higher and lower BR variations were observed for Under and Over ewes. Higher plasma non-
29 esterified fatty acids (**NEFA**) concentrations were accompanied by lower insulin, leptin and
30 glucose. Differences in leptin were consistent with the dietary energy load (Over > Control >
31 Under). After refeeding, a rebound in BW and BCS was observed for the three groups
32 whereas NEFA was drastically reduced in Under ewes. No differences among treatments were
33 detected in NEFA profiles at the end of the study but lipolytic activity responses to the β -
34 adrenergic challenge were different and coherent with the adipose tissue mass (Fat > Normal

35 > Lean) and, importantly, was also different between ewes from the same group or BR status,
36 thus evidencing diversity among individual adaptive capacities.

37 **Conclusions:** The ability of ewes to quickly overcome undernutrition situations by efficiently
38 using their BR was confirmed. There is potential for a simplified β -adrenergic challenge
39 protocol helping to identify differences in adaptive capacity among individuals.

40 **Keywords:** Sheep; Undernutrition; Metabolic profile; Energy balance; Body reserves;
41 Adipose tissue; Lipolytic activity; Adaptive capacity.

43 **Background**

44 Maintaining the consistency of the internal environment (homeostasis) and/or sustaining
45 productive functions (homeorhesis) are essential mechanisms of control in ruminants,
46 allowing them to adapt to physiological and environmental fluctuations [1].

47 How the animal partitions its nutrients when resources are limited, or imbalanced, is a
48 major way in which it is able to cope with such variations, and thus, determines its robustness.
49 In highly productive selected ruminants there is evidence that their reliance on body reserves
50 (**BR**) is increased and robustness is reduced [2]. The efficiency of BR mobilisation-accretion
51 processes, in order to overcome undernutrition events, is therefore recognised as an essential
52 trait in ruminants. These processes contribute to maintaining the resilience of the flock under
53 fluctuating circumstances, such as in tropical or Mediterranean regions, where seasonal forage
54 availability is highly contrasted.

55 In previous works characterizing the energy metabolism in a typical round productive
56 year of Romane [3] and Lacaune [4] meat and dairy ewes, respectively, the potential of
57 plasma non-esterified fatty acids (**NEFA**) for being used as predictor of the ruminant
58 nutritional status was confirmed. Furthermore, we know that adipose tissue (**AT**) lipolytic
59 potential can be estimated *in vitro* (by glycerol and NEFA responses from tissue explants into

60 the incubation medium) or *in vivo* by plasma glycerol or NEFA response to injection or
61 infusion of catecholamines or synthetic drugs (β -adrenergic agonists) [5]. Such lipolytic
62 potential could be seen as a sign of the ultimate necessity of the animal to compensate their
63 basic requirements by using their BR. When facing an undernutrition event, a quick BR
64 mobilization (illustrated by plasma NEFA) could be a symptom of the incapacity of the
65 animal to re-adjust its maintenance energy requirements (**MER**) which would lead to regulate
66 (reduce) its feed intake. Under the same conditions (i.e. species, breed, physiological state,
67 age, production system, feeding regimen...) less NEFA in the immediate response would
68 mean that the animal is less depending from its BR in the very short term.

69 For this study we hypothesised that offering restricted diets to adult Mérimos d'Arles
70 ewes would significantly increase their BR mobilization to meeting their MER. After
71 refeeding, the metabolic plasticity of the breed [6,7,8] would lead to recovery within a similar
72 period of time to that of feed restriction. We also hypothesised that those ewes with contrasted
73 body condition scores (**BCS**), resulting from receiving different dietary regimes, would
74 respond differently to an *in vivo* β -adrenergic challenge. That response will correspond to the
75 individual reactivity or adaptive capacity.

76 Thus, the objective of this study was to evaluate the impact of offering diets of differing
77 nutritional planes on the adaptive capacity of mature ewes at the short-, medium- and long-
78 term. Such adaptation will be characterized by studying trends in the individual BR
79 mobilisation-accretion and the associated metabolic profiles after dietary challenge. A second
80 objective was to evaluate the impact of different BCS on the individual lipolytic potential of
81 the adipose tissue of the ewes facing a β -adrenergic challenge. This would allow us to study
82 the potential of a simplified method for analysing the intraflock variability in individual
83 metabolic plasticity responses when facing nutritional alias.

84

85 **Methods**

86 **Location**

87 The experiment was conducted at the Montpellier SupAgro Domaine du Merle
88 experimental farm, located in Salon-de-Provence in the south-east of France (43°38'N,
89 5°00'E). All animals were cared for in accordance with the guidelines of the Institut National
90 de la Recherche Agronomique (INRA) animal ethics committee.

91 **Ewes, management, feeding and experimental design**

92 After weaning their litters in mid-January, 36 adult Mérinos d'Arles ewes between 6 and
93 10 years old and being lambed in October (average lambing on 10 October) were selected for
94 this study from the main research flock. The body weight (**BW**) and BCS was used to select
95 animals with similar body conditions. The initial BW and BCS were 44.4 ± 0.83 kg and $2.0 \pm$
96 0.05 , respectively.

97 A schematic representation of the experimental design is presented in Figure 1. The
98 experiment lasted 122 days, and was comprised of two consecutive periods. Firstly, the ewes
99 were allowed to acclimatise to the feeding regimen and the general environment of the
100 sheepfold for 22 days (under confinement). All ewes were managed as a single flock and fed
101 the same Control diet (composition included below) throughout this period. Following
102 acclimatisation was a measurement period of 100 days, beginning from the point at which the
103 experimental feeding regime began (day "zero"). Ewes were randomly assigned to one of
104 three covered pens, each with an area of approximately 30 m² and containing both concrete
105 and straw flooring in the same sheepfold. The 100 day measurement period was stratified into
106 two sub-periods of similar lengths (50 days each), including a dietary challenge period (from
107 day 0 to 49) followed by a refeeding period (from day 50 to 100; Figure 1). At the end of the
108 experiment (last day) a β -adrenergic challenge protocol was carried out (details included
109 below).

110 Ewes were pen-fed and feed was allocated according to treatment to the three groups in
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2 111 each of the covered pens (n = 12 ewes/ pen). The treatments included three contrasted diets of
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4 112 different nutrition planes depending on energy supply: i) underfed ewes offered 70% of
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7 113 theoretical MER (i.e. **Under** group), ii) ewes offered 100% of MER (i.e. **Control** group) and
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10 114 iii) ewes offered 160% of MER (i.e. overfed ewes, **Over** group).

11 115 At the start of the experiment, ewes were in a maintenance state. Considering the average
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14 116 BW (~45 kg BW; 17.4 kg BW^{.75}) the individual daily intake capacity was 1.3 fill units.
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17 117 According to the INRA tables [9], in order to meet their MER 0.033 fill unit/kg BW^{.75} for
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19 118 maintenance and meat production (UFV) and 2.3 g/ kg BW^{.75} of protein digestible in the
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22 119 small intestine (PDI) was required. Therefore, feeding regimes for each treatment were
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24 120 theoretically planned to achieve three different BCS of 1.75, 2.5 and 3.25 for Under, Control
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27 121 and Over ewes, respectively.

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29 122 The nutritive values of the ingredients included in the experimental diets are presented in
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32 123 Table 1. As the basal roughage, a wheat straw containing 3.5% of crude protein (CP), 1.34
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34 124 Mcal/kg dry matter (DM) of metabolisable energy (ME) and 2.4 UFV (INRA, 2010; [9]) was
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36 125 used. Dried and pelleted alfalfa (16% CP) was offered as the main protein source, whereas a
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39 126 dried and pelleted sugar beet pulp was supplied as the main energy source (2.7 Mcal/kg DM
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41 127 of ME and 1.0 fill unit for sheep). A mineral-vitamin premix, containing 90 and 126 g/kg DM
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43 128 of P and Ca, respectively, was supplied at the same dose (~10 g/ewe/day) for all treatments
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46 129 (Table 2), thus, ensuring the same amount of P and Ca (1 g/ewe/day).

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49 130 Table 2 presents the dietary composition for each experimental treatment, including the
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51 131 amounts of each ingredient and the overall daily nutrient supply (per ewe), according to each
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53 132 nutritional plane used in the study. The Control diet was composed of 910 g of wheat straw,
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56 133 165 g of alfalfa and 170 g dried sugar beet pulp. For the Over group, the quantities of alfalfa
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58 134 and sugar beet pulp were increased (almost tripled) compared to Control, whereas the quantity
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135 of wheat straw was reduced by almost half. In contrast, the Under (feed-restricted) ewes were
136 offered only 1 kg of wheat straw daily. These experimental diets corresponded to the dietary
137 challenge period from day 0–49 following acclimatisation (Figure 1). During the second half
138 of the measurement period (refeeding), from day 50 to the end of the experiment (day 100),
139 an equivalent additional daily quantity (DM basis) of 100 g of alfalfa and 100 g of dried sugar
140 beet pulp per ewe were supplied to each of the three experimental groups. In this refeeding
141 period, the quantity of wheat straw supplied to the Under group was reduced to half of that
142 supplied during the 0 to 49 day measurement period (Table 2).

143 Ewes were group fed once daily at 0800 h, and diets were provided *ad libitum*, which was
144 weekly adjusted at 120% compared to the average intake for previous week. Feed refusals
145 were daily weighted and samples were weekly pooled for further analyses. Ewes in each
146 treatment group had free access to fresh drinking water.

147 **The β -adrenergic (isoproterenol) challenge**

148 The contrasting BCS attained among groups at the end of the experimental period allowed
149 to induce a β -adrenergic challenge to the thirty-six mature, dry, non-pregnant Mérimos d'Arles
150 meat ewes. The objective was to evaluate the lipidic potential of the AT of the 3 contrasted
151 BCS groups i.e. normal ewes issued from the Control diet (**Normal**, n= 12), underfed or lean
152 ewes (**Lean**, n= 12) and overfed, fatty ewes (**Fat**, n= 12). The previous day of the β -
153 adrenergic challenge the ewes were individually weighed and the BCS estimated.

154 All ewes (n = 36) were challenged early in the morning (~0800 h) of the same day (day
155 100). The challenge consisted on an intravenous injection (4 nmol/ kg BW) of isoproterenol
156 (ISO, IsuprelTM; Hospira France, 92360 Meudon-La-Fôret, France). IsuprelTM (0.2 mg
157 isoproterenol hydrochloride/mL sterile injection) is a potent nonselective β -adrenergic agonist
158 with very low affinity for α -adrenergic receptors. For individual monitoring of reactions,

159 blood samples (n= 10) were individually drawn from each ewe by jugular venipuncture at -15,
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2 160 -5, 0, 5, 10, 15, 20, 30, 45 and 60 min. relative to the β -adrenergic challenge time.
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4 161 **Measurements, blood sampling, hormones and metabolite assays**

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7 162 Measurements lasted a total of 122 days, starting with the acclimatisation period (22 days)
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9 163 and continuing throughout the 100 day measurement period (Figure 1). Ewes were
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11 164 individually and manually monitored for BW (n= 11) and BCS [10] at 28 and 11 days prior
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13 165 to the experimental period (-28 and -11, respectively), and at day 0, 6, 14, 21, 35, 49, 62, 77
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15 166 and 97 after the beginning of the dietary challenge. Similarly, plasma samples for the
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17 167 determination of metabolites and metabolic hormones associated with energy metabolism (n =
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19 168 18) were taken at 22, 15, 11 and 1 day prior to the experimental period (-22, -15, -11 and -1,
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21 169 respectively), and at day 0, 1, 3, 6, 8, 10, 14, 17, 21, 35, 49, 62, 77 and 97 following the
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23 170 beginning of the dietary challenge.
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29 171 The close monitoring of ewes (every two or three days) started the day before the dietary
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31 172 changes and lasted until 3 weeks after the beginning of the 100-day measurement period.
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33 173 Following this, approximately two sampling points per month were performed until the end of
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35 174 the experiment (see Figure 1 for details on the experimental design schedule).
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39 175 For monitoring the energy metabolism progression of each experimental group, individual
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41 176 concentrations of plasma metabolites, including non-esterified fatty acids (**NEFA**), beta-
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43 177 hydroxybutyrate (**β -OHB**) and glucose (**GLU**), and the metabolic hormones insulin (**INS**) and
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45 178 leptin (**LEPT**) were determined according to the protocols described by González-García et
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47 179 al. [3,4]. Blood samples were taken by jugular venipuncture before the first meal
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49 180 (approximately at 0800 h) on each sampling day. Two 9 mL samples were drawn from each
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51 181 ewe (1 tube with 18 IU of lithium heparin per 1 ml blood and 1 tube with 1.2–2 mg of
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53 182 potassium EDTA per 1 ml blood; Vacuette[®] Specimen Collection System, Greiner Bio-One
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55 183 GmbH, Austria). Samples were immediately placed on ice before centrifugation at $3600 \times g$
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184 for 20 minutes at 4°C. The plasma was collected and stored at -20°C in individual identified
185 aliquots (3 µL) for the metabolite and hormone analyses. Plasma NEFA was measured in
186 duplicate using the commercially available Wako NEFA-HR(2) R1 and R2 kit (manufactured
187 by Wako Chemicals GmbH, Neuss, Germany, and distributed by Laboratoires Sobioda SAS,
188 Montbonnot, Saint Martin, France); intra- and inter-assay variations averaged 4.9% and 3.5%,
189 respectively. Plasma GLU concentrations were measured in triplicate using a commercially
190 available glucose GOD-PAP kit (reference LP87809; manufactured and distributed by
191 Biolabo SAS, Maizy, France); intra- and inter-assay variations averaged 2.5% and 2.1%,
192 respectively. Plasma β-OHB were measured in duplicate using the enzymatic method
193 proposed by Williamson and Mellanby [11]; intra- and inter-assay variations averaged 8.8%
194 and 3.3%, respectively. Plasma INS was measured in duplicate using a commercially
195 available RIA kit (Insulin-CT; manufactured by MP Biomedicals–Solon, Ohio, USA and
196 distributed by Cisbio Bioassays, Codolet, France); intra- and inter-assay variations averaged
197 10.3% and 4%, respectively. Plasma LEPT was quantified using the double-antibody leptin
198 RIA procedures with some modifications as described by González-García et al. [3,4];
199 average intra- and inter-assay coefficients of variation were 5.4% and 4.8%, respectively.

200 For the β-adrenergic challenge, the 9 mL blood samples (1 tube with 1.2-2 mg of
201 potassium EDTA per 1 ml blood) drawn from each ewe at each sampling point of the kinetic
202 (n = 10) were placed immediately on ice before centrifugation at 3,600 × g for 20 min at 4°C.
203 Plasma was harvested and stored at -20°C until analyses in individual identified aliquots (3
204 µL). Concentrations of plasma NEFA were analysed in duplicate, similarly to the procedure
205 above described. Intra- and inter-assay variation for these samples averaged 4.74% and
206 6.98%, respectively.

207 **Calculation and statistical analyses**

208 Statistical analyses were performed using Statistical Analysis System package (SAS; v.
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2 209 9.1.3., 2002-2003 by SAS Institute Inc., Cary, NC, USA) [12]. Data were analysed using the
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4 210 PROC MIXED function with repeated measures. The least square means separation procedure
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7 211 using the PDIF option in SAS was used and the statistical model was as follows:

$$212 \quad Y_{ijk} = \mu + Plane_i + Ewe_{ij} + Time_k + (Plane \times Time)_{ij} + \varepsilon_{ijk}$$

11 213 where Y_{ijk} is the response at time k , for ewe j that consumed a diet at the nutritional plane i ,
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14 214 μ is the overall mean, $Plane_i$ is the fixed effect of the specific nutritional plane i ($i = 1-3$),
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16 215 Ewe_{ij} is the random effect of ewe j offered the nutritional plane i , $Time_k$ is the fixed effect of
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19 216 time k , $(Plane \times Time)_{ik}$ is the fixed interaction effect of the nutritional plane i for time k and
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22 217 ε_{ijk} is the random error at time k on ewe j offered the nutritional plane i .

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24 218 For the β -adrenergic challenge database, the NEFA response at each time after challenge
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26 219 was calculated as the change in concentration from basal (-15 min) value as described by
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29 220 Chilliard et al. [13]. The area under the concentration curve (AUC) was calculated by doing a
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32 221 definite integral between the two points or limits at time X , using the following formula:

$$33 \quad AUC_{1-2} = (B_1 + B_2) / 2 \times (A_2 - A_1)$$

34 222 where B is the y axis value (NEFA concentration) and A is the X axis value (time relative to
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36 223 challenge). The AUC was thus calculated for each ewe for the time intervals 0 to 5 min
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39 224 (AUC05), 5 to 10 min (AUC510), 10 to 15 min (AUC1015), 15 to 30 min (AUC1530), 30 to
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41 225 60 min (AUC3060) and, finally, from 0 to 60 min (AUC060).
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46 227 By using the data from the concentration-time plot, we calculated the NEFA elimination
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48 228 rate (turnover) constant of each ewe after the ISO challenge (i.e. rate at which NEFA was
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51 229 cleared from the body), assuming first order elimination. For this purpose we calculated K
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53 230 which is the slope of the regression line between time (hours). The measured concentration
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56 231 values of NEFA above the initial point ($t = 0$; time of injection of ISO) was firstly
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58 232 transformed to their natural logarithm. Extrapolation at zero time gives the theoretical
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233 maximal amplitude above initial point (**NEFAamp**). Since we did not have access to the
234 volume diffusion or volume of distribution (V) of NEFA, we used the individual BW of each
235 ewe to determine the clearance rate which was calculated as follows:

$$236 \quad CL = K \times BW$$

237 where CL is the NEFA clearance rate value from the body of each ewe, K is the slope of
238 the regression line and BW is the individual BW at the adrenergic challenge moment.

239 Data of NEFA kinetics in the β -adrenergic challenge were analysed as repeated measures
240 ANOVA using the PROC MIXED function with least squares means separation procedure
241 using the PDIF option of SAS. The statistical model was as follows:

$$242 \quad Y_{ijk} = \mu + BCS_i + Ewe_{ij} + Time_k + (BCS \times Time)_{ij} + \varepsilon_{ijk}$$

243 where Y_{ijk} is the response at time k on ewe j with a BCS_i , μ is the overall mean, BCS_i is a
244 fixed effect of the BCS of the ewe at the moment of the challenge i ($i = 1-3$), Ewe_{ij} is a
245 random effect of ewe j with a BCS_i , $Time_k$ is a fixed effect of time relative to challenge k ,
246 $(BCS \times Time)_{ik}$ is a fixed interaction effect of the BCS of the ewe i with time relative to
247 challenge k , and ε_{ijk} is random error at time k on ewe j with a BCS i .

248 The BW, BCS, NEFA responses after challenge with regard to basal NEFA and AUC at
249 different periods were analysed by ANOVA of SAS considering the fixed effect of the BCS
250 group of the observation value. Results were considered significant if $P < 0.05$. Correlation
251 coefficients between basal plasma NEFA and plasma NEFA responses to the ISO challenge
252 and AUC at different ranges of time and from time 0 to 60 min. were determined by using the
253 PROC CORR of SAS.

254 **Results**

255 The final average individual daily feed balance is shown in Table 3. After calculating the
256 average feed refusal per treatment for each stage of the measurement period (dietary challenge
257 period from 0–49 days and refeeding period from 50–100 days), it was determined that the

258 ewes were 114, 68 and 190% of their MER for the Control, Under and Over groups,
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2 259 respectively. This was different to the 100, 70 and 160% MER theoretically planned,
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5 260 respectively (Table 2). However, the final objective of MER for each of the diets was
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7 261 attained.

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9 262 Overall changes in BW, BCS and plasma profiles are presented in Table 4. When all
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12 263 parameters were considered, significant effects were observed for the main sources of
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14 264 variation evaluated i.e. the feeding regimen, time after diet challenge and their first order
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17 265 interactions. A high level of significance was observed for the interaction of nutritional plane
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19 266 with time for all variables measured in this study. As expected, after beginning the feeding
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22 267 regimen (day “zero”) the average BW and BCS were higher and lower, respectively, in the
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24 268 ewes in the Over and Under groups (Table 4 and Figure 2). At the beginning of the refeeding
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27 269 period (day 50) a significant recovery for BW and BCS was observed.

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29 270 The differences and trends observed for BW and BCS were consistent with those obtained
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32 271 for NEFA profiles (Figure 2). The higher and lower average BR mobilisation, as illustrated by
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34 272 plasma NEFA concentration, were observed in the Under and Over group of ewes ($0.26 \pm$
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36 273 0.011 vs. 0.09 ± 0.017 mmol/L, respectively). However, the differences between the Over and
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39 274 Control groups were only evident until 1 week following the change of the feeding regimen
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41 275 (Figure 2). Once started the refeeding period, plasma NEFA was drastically reduced in the
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44 276 Under ewes. At the end of the study, no significant differences were detected between the
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46 277 groups of ewes, regardless of the feeding regimen (Figure 2).

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48 278 Differences in GLU were only observed when the Over ewes were compared to the other
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51 279 two experimental treatments. However, there were no differences observed when the Under
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53 280 and Control ewes were compared (Table 4 and Figure 3). Conversely, plasma INS
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56 281 concentrations were more consistent with the differences in BR mobilisation rates (i.e. NEFA
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58 282 profiles) shown above. At higher plasma NEFA concentrations a lower INS concentration was
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283 obtained. Therefore, plasma INS was higher in the Over ewes, followed by the Control ewes,
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2 284 and was the lowest for ewes in the Under group (Table 4 and Figures 2 and 3). A
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5 285 concomitant, parallel effect on INS and GLU was observed following refeeding. The peak in
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7 286 GLU concentration observed in the Over ewes was followed by the observation of a similar
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10 287 peak for the plasma INS profile at the same time point and in the same group of ewes. In
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12 288 general, either the plasma GLU or INS profiles were higher in the Over ewes throughout the
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14 289 experiment, but no differences were observed when the ewes offered the Control or Under
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17 290 diets were compared (Figure 3).

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19 291 No differences in β -OHB concentrations were found when the Control and Over ewes were
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22 292 compared (Table 4). A lower ($P < 0.003$) β -OHB profile was observed in the Under ewes
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24 293 compared to the average of Control and Over ewes combined (20.16 ± 0.684 vs. $23.47 \pm$
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26 294 0.876 mg/L, respectively). The lower β -OHB profile observed in the Under ewes was
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29 295 consistent throughout the experiment (Figure 4).

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31 296 Differences in plasma LEPT were also consistent with the feeding regimen (Over >
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34 297 Control > Under; Table 4). As expected, a higher LEPT profile was observed in the Over
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36 298 group throughout the experimental period, and these differences were significantly increased
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39 299 following refeeding (Table 4 and Figure 4).

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41 300 At the day of the β -adrenergic challenge (end of the experiment), significant differences (P
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44 301 < 0.0001) were verified when comparing average BW and BCS of the 3 experimental groups
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46 302 (Table 5; Figure 5). As a result of the previous 100 days feeding manipulation period, ewes
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49 303 belonging to the underfed (Lean) group (BW = 37.7 kg; BCS = 1.34) were more than 10 kg
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51 304 lighter than Normal (BCS = 1.79) and overfed (Fat) (BCS = 2.17) ewes (46.2 and 50.9 kg
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53 305 BW, respectively).

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56 306 Basal plasma NEFA (-15 min.) before the β -adrenergic challenge was higher ($P < 0.0002$)
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58 307 in Lean ewes compared to Normal and Fat groups (Table 5). In contrast, plasma NEFA
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308 response at 5 min. after ISO challenge was higher ($P < 0.0003$) in Fat ewes. After 10 min.
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2 309 plasma NEFA response was consistently higher in Lean ewes. Plasma NEFA maximal
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5 310 response (0.56 mmol/L) was higher ($P < 0.0001$) in Lean ewes and this occurred at 12 min.
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7 311 after the challenge (Table 5; Figure 5).

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9 312 The AUC were all higher ($P < 0.0001$) in Lean ewes. Thus, overall results showed that
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12 313 underfeeding increased basal plasma NEFA, plasma NEFA response at 10 and 20 min.,
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14 314 plasma NEFA maximal response after ISO challenge and all NEFA response areas ($P <$
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17 315 0.0001). The NEFA maximal response occurred later in underfed or Lean ewes when
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19 316 compared to Control and Over ewes.

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22 317 The plasma NEFA kinetics for the three experimental groups appears in Figure 5. The
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24 318 NEFA concentrations increased for 10 min. in all groups and were always higher ($P < 0.0001$)
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27 319 in Lean ewes during the 60 min. post-challenge with a peak of plasma NEFA concentration
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29 320 attaining 0.53 mmol/L. However, for all groups NEFA concentration decreased in a similar
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31 321 way and, after 60 min., it returned to values close to baseline.

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34 322 All correlations ($r = 0.54$ to 0.79) between basal NEFA and different parameters of NEFA
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36 323 response to ISO challenge were significant ($P < 0.0001$; Table 6) with the exception of the
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39 324 variable time between ISO challenge and maximal response ($r = 0.25$). The highest
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41 325 correlations between basal plasma NEFA and responses at different points after challenge
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44 326 were at 10, or 15 min ($r = 0.73$ and 0.69 , respectively). The highest correlation between
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46 327 plasma NEFA and AUC were at the area from 0 to 15 min ($r = 0.79$; 0.72 and 0.73 for 0 to 5,
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49 328 5 to 10 and 10 to 15 min, respectively).

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51 329 From 20 min. after challenge, correlations were progressively lower in the sense of the
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53 330 declining tendency of the curve. Correlations with AUC from time 0 to 60 min. were very
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56 331 high for response at 10, 15, 20 min. ($r = 0.91$ to 0.99) and maximal response ($r = 0.80$).
57
58 332 Correlations between AUC from time 0 to 60 min. and AUC from time 10 to 15 min., or AUC
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333 from time 15 to 30 min. were very high ($r = 0.97$); correlations between AUC from time 0 to
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2 334 60 min. and AUC from time 30 to 60 min. (declining part of the curve) were also high ($r =$
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4 335 0.83; Table 6). Correlations between the maximal response and the response at 5, or 10 min.,
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7 336 and with the AUC from time 5 to 10 min. were high (ranging from 0.83 to 0.89; Table 6).
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10 337 Within BCS groups' correlations between AUC from time 0 to 60 min. and responses at
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12 338 10, 15 and 20 min were higher than those with basal plasma NEFA, or NEFA response at 5,
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14 339 30, 45 and 60 min. (Figure 6). Differences in the releasing NEFA turnover were observed
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16 340 between BCS groups and among individuals in the same group and with similar BW and BCS
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19 341 status (Figure 7).
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21 342 **Discussion**

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24 343 In the next future, sustainability of farming systems will rely on their ability to cope with
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26 344 a reduction of inputs usage (i.e. concentrate, irrigation, fertilizers...). In this context, a better
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29 345 understanding of the relationship between nutrients supply, nutritional status, their
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31 346 interactions with BR dynamics and the progression of the metabolic profile is essential for the
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34 347 development of a more comprehensive management approach of nutrition based on adaptive
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36 348 capacity of ruminants [14,15,16]. The objectives of this study were to evaluate and describe
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39 349 how dietary energy restriction and/or repletion influence changes in BW, BCS and metabolic
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41 350 status responses in Mérinos d'Arles ewes, considered to be a robust (rustic) and hardy sheep
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43 351 breed.
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46 352 We validated the previous estimation of energy requirements (INRA, 2007 [9]) of the dry
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48 353 ewe by a stabilized BCS and BW over the whole experimental period with the Control diet.
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51 354 Hence adaptive capacity of ewes fed Under and Over diets will be discussed by direct
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53 355 comparison with the ewes Control responses. We confirmed that offering restricted diets to
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56 356 ewes would induce significant increases in BR mobilisation in order to meet their energy
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58 357 requirements. We also verified that, after partial refeeding, the metabolic plasticity of ewes of
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358 this breed allowed the BW and BCS to start recover within a period of similar duration to that
359 of the feed restriction, whereas a high restriction of nutrient allowance would temporarily and
360 negatively affect the voluntary feed intake. The highest feed refusal rate was consistently
361 observed in the Under group, mainly after the first 3–4 weeks of feeding of the restricted diet
362 (data not shown). This is likely to be a consequence of a depressed ruminal environment
363 together with low roughage quality, which is known to negatively affect digestion,
364 metabolism and appetite in undernourished ruminants.

365 Differences in BW and BCS progression throughout the experiment were expected,
366 considering the dietary energy manipulation. The Under, Control and Over ewes reduced,
367 maintained and improved their BW and BCS, respectively. Ewes in the Under and Control
368 groups responded to the dietary manipulation by attempting to maintain their BW and BCS, as
369 observed with the reduced concentrations of GLU, INS and LEPT, compared to ewes that
370 consumed the Over diets (Table 4 and Figures 2, 3 and 4).

371 Throughout the experiment, the Under ewes (who consumed almost half of their MER)
372 presented lower BW and BCS, increased plasma NEFA concentration and lower INS and
373 LEPT concentrations when compared to adequately fed animals. The BR mobilisation status
374 was well illustrated by the consistently higher plasma NEFA concentrations observed in
375 Under ewes. This was expected as this group was exposed to a strong dietary energy
376 restriction, based on *ad libitum* wheat straw (containing 3.5 % CP and 1.3 Mcal/kg DM of
377 ME) during the first 50 days of the measurement period. After beginning the partial refeeding
378 period at day 50, the same Under ewes responded to the energy repletion with an immediate
379 short-term decrease in their NEFA concentrations, with more delayed recovery of BW and
380 BCS observed in this group (Figure 2), which is logical since it was only a partial refeeding.

381 The endocrine system, characterised by plasma INS and LEPT profiles in this study,
382 regulates metabolism by finely tuned peripheral information, which are ultimately aimed to

383 maintain homeostasis. These adaptive processes involve the interplay between several
1
2 384 hormones, which also include growth hormone (GH) and insulin-like growth factors (IGFs)
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5 385 [17, 18, 19, 20], which were not analysed in this study.

6
7 386 The typical characteristics of undernourished ruminants were observed in the Under
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9
10 387 ewes. The lack of glucose arriving to the rumen, in addition to a reduction in volatile fatty
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12 388 acids (VFA) production, induces gluconeogenesis accompanied by intense lipolysis,
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14 389 proteolysis and ketogenesis [1,17]. The reduction of gut metabolic activity is known to
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17 390 account for the decrease of energy requirement in underfed ruminants. Thus, the reduced
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19 391 oxidative or basal metabolism is characterised by a decrease in plasma GLU, INS, LEPT and
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22 392 prolactin concentrations, and an increase in other hormones such as GH, adrenalin, cortisol
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24 393 and glucagon (not measured in this study). This generally leads to shifts in metabolic
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27 394 pathways which aim to spare GLU (with the accompanied increase in NEFA) and proteins
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29 395 (with increased proteolysis and ketogenesis).

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31 396 Interestingly, the plasma level of β -OHB was lowest in the Under group. This may be due
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34 397 to limits in the supply of β -OHB precursors by this diet. Thus, even if higher BR mobilisation
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36 398 was present in Under ewes, the unexpected lower β -OHB plasma concentration is probably
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39 399 the consequence of the ingredients used in the experimental diets. The Under diet, which only
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41 400 offered wheat straw, did not contain the required precursors. However, with similar
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44 401 underfeeding situation [21] fat-tailed Barbarine ewes were able to produce and survive thanks
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46 402 to their significant ability to mobilise their BR. Plasma NEFA and β -OHB concentrations
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49 403 were initially almost doubled. The medium-term response of these ewes was very similar to
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51 404 what we observed: a steady decline of these metabolites, which was attributed to their ability
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54 405 to adjust their lipid metabolism in order to reduce the toxic effects of high concentrations of
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56 406 NEFA and β -OHB, and therefore, prolong survival. After partial refeeding Barbarine ewes
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58 407 were able to fully recover their initial BW, lipid and protein masses [21].
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408 Submitted to an opposite nutritional situation, the enhancement of the anabolic pathway
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2 409 response was clear in the Over ewes, with increased GLU, INS and LEPT concentrations and
3
4 410 decreased plasma NEFA (Figures 2, 3 and 4). However, differences toward dominant anabolic
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6 411 responses were not always evident when comparing the Control and Over ewes, despite the
7
8 412 clear differences in energy supply, which should have been sufficient to create greater
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10 413 differences than those observed. However, the BW and BCS progression were clearly
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12 414 different between the groups from 20 days after the introduction of the diet changes (Figure
13
14 415 2). Thus, the differences in responses between these two groups were not well described in
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16 416 this study from the analysis of the chosen metabolites and metabolic hormones. This led us to
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18 417 hypothesise that measuring other parameters, including GH, IGF-1 and polyunsaturated
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20 418 nitrogen (PUN), may provide an improved characterisation of the anabolic and catabolic
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22 419 responses. It is highly probable as reported by Delavaud et al. [22], that increasing amounts of
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24 420 stored body fat in the Over group, increased their MER thus reducing the energy balance gaps
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26 421 between Normal and Over ewes. Hence metabolic and endocrine profiles are blunted by this
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28 422 phenomenon. Such effects of body fatness on energy requirement were reported by Caldeira
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30 423 and Portugal [20] in underfed fat ewes. Ewes with low BCS had lower plasma GLU,
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32 424 triiodothyronine and thyroxine concentrations and serum INS, albumin, globulins and IGF-I,
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34 425 in addition to higher serum NEFA, urea and creatinine.
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43 426 When energy intake is high, INS concentrations are also high, which promotes growth
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45 427 and/or BR recovery [1, 19, 22]. Such a positive correlation between energy intake and INS
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47 428 concentrations has been reported and this response was confirmed when lowering energy
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49 429 supply: concentrations of INS decreased during energy restriction [18].
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53 430 **Adrenergic challenge**

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55 431 Our results regarding the individual responses to the β -adrenergic challenge are in
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57 432 agreement with Chilliard et al. [13]. These authors found that basal NEFA and NEFA
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433 response to an isoproterenol (ISO) challenge with a similar dose to that used in our experiment
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2 434 (4 nmol/kg BW) were higher in underfed than overfed cows. Consistent with our findings,
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4 435 high correlations between the response area or maximal NEFA response and NEFA response
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6 436 at 15 min. ($r = 0.95$ and 0.98 , respectively) were observed. A significant effect of BCS on the
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8 437 basal plasma level was also reported, thus concluding that NEFA response to ISO at 15 min.
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10 438 could provide an efficient method for *in vivo* studying the AT lipolytic potential. Our results,
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12 439 including trends of the response curves with regard to BCS groups, are very similar to those
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14 440 findings, except maximum value was obtained at 10 min. in our study. We also agree with the
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16 441 fact that maximal response occurred later when this response was higher which illustrates that
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18 442 lipolytic response to ISO take longer in underfed animals.
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24 443 The significant correlation ($r = 0.69$) between plasma NEFA and NEFA maximal
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26 444 response to ISO confirms results obtained in lactating ewes by Bocquier et al. [23] and
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28 445 suggests that the adrenergic component of the lipolytic cascade plays a significant role in the
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30 446 regulation of basal plasma NEFA. The NEFA response to ISO challenge in that well-fed
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32 447 lactating ewes depended on body lipid mass but not on energy balance. By the contrary, in
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34 448 underfed ewes the NEFA response depended on energy balance and not on the body lipid
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36 449 mass. Adrenergic challenge was also useful in explaining the differences in interindividual
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38 450 adaptive strategies to underfeeding in the ewe. In underfed ewes Bocquier et al. [23] observed
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40 451 that the relative variation in milk yield was negatively correlated to NEFA+10 ($r = -0.51$),
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42 452 which show that ability to support lactation was related to the ability to mobilise body lipids.
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48 453 **Potential contribution for a simplified method helping to identify individual adaptive** 49 50 51 454 **capacities or robustness (intraflock variability)** 52

53 455 There is evidence of the great potential of plasma NEFA as a powerful predictor of the
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55 456 nutritional status of the ruminant under determined circumstances. This parameter provides
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57 457 reliable information on the stage of the BR mobilization of the animal under exigent
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1 458 physiological status and/or when facing the consequences of being reared in fluctuating
2 459 environments [3, 4]. The NEFA is thus recommended as a good diagnostic tool for health or
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4 460 reproductive interpretations, and we think that it could also be considered as a pertinent
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7 461 variable to be included in models aiming to analyze metabolic plasticity of ruminants when
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9 462 facing variability in feed availability and quality in a given timespan (i.e. individual
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12 463 robustness).

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14 464 In previous works carried out by our team and aiming to characterize the energy
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16 465 metabolism of ewes in a typical round productive year, such a NEFA potential for illustrating
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19 466 the dynamic of individual BR status was confirmed in Romane [3] and Lacaune [4] meat and
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22 467 dairy ewes, respectively.

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24 468 In the present study we evaluated the *in vivo* method with a β -adrenergic challenge. We
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26 469 confirmed our hypothesis that ewes with different, contrasted BCS would respond differently
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29 470 to a β -adrenergic challenge and that this response could be predicted at a given point (10
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32 471 min.) of the plasma NEFA kinetic after the challenge, in function of the relationships between
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34 472 the different parameters responses at different times.

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36 473 Chilliard et al. [13] using the same method, looked for a simplified procedure for
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39 474 predicting the lipolytic response curve with a smaller number of samples after ISO challenge.
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41 475 Consistent with our results (Figure 5, 6), they obtained a very good prediction of AUC from
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44 476 time 0 to 60 min either by the partial AUC from time 0 to 20 min ($r = 0.95$) or by the sole
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46 477 response at 15 ($r = 0.95$) or 20 min ($r = 0.97$). Measuring plasma NEFA in blood samples
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49 478 taken just before and at 15 (or 20) min after an ISO injection was thus considered as an
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51 479 efficient and simple way of predicting the maximal NEFA response of an individual and an
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54 480 AUC equivalent to one hour of sampling. Extra blood samplings at 5, 10, and 20 (or 15) min
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56 481 after ISO challenge only slightly increased the prediction of these parameters.

482 The adaptive capacity of a ruminant to an adrenergic challenge alias (i.e. pronounced
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2 483 energy shortage) is expressed by subsequent individual physiological responses at the short-,
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5 484 medium- and long-term timespans. Differences in the amplitude (gap between maximum
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7 485 NEFA response and basal NEFA), turnover (exponential slope when reducing plasma NEFA
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10 486 after maximal response) and length of those specific and combined processes are expected to
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12 487 be consistent with adaptive capacities' differences between individuals reared under similar
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14 488 conditions.

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17 489 A stronger lipolytic potential could be seen as a sign of the ultimate necessity of the
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19 490 animal to compensate their basic requirements by mainly using their BR. Indeed, when facing
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22 491 an undernutrition event (i.e. challenge), a higher and quicker BR mobilization peak
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24 492 (illustrated by plasma NEFA) could be a symptom of the incapacity of the animal to readjust
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27 493 its MER at the short-term. This would be in close relationship with their more or less efficient
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29 494 capacity of regulating (reducing) its feed intake and thus the individual MER, which would
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32 495 mean a higher dependency of their BR *per se* to cover energy requirements. Thus, under
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34 496 uniform conditions (i.e. same species, breed, physiological state, age, production system,
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36 497 feeding regimen...) less NEFA at a given point after the challenge would mean that the
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39 498 animal is less depending from its BR. That individual, with more pronounced NEFA
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41 499 amplitude and quicker NEFA turnover would be, a priori, a better adapted animal when
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44 500 compared to its cohort. Such differences at the intragroup level were observed in our
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46 501 experiment (Figure 7). This could enable us to the potential effective use of this relative easy
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49 502 and quick method, for contributing to give useful information for identifying existing
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51 503 intraflock variability in individual robustness in practice at a given field situation.

53 504 **Conclusions**

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56 505 The findings confirmed the ability of these mature, dry, non-pregnant Mérinos d'Arles
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58 506 ewes to quickly overcome undernutrition situations by efficiently using their body reserves.
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507 The anabolic or catabolic responses to energy dietary manipulations were accompanied by
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2 508 synchronised metabolic regulation, resulting in differences in their metabolic and BCS
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5 509 profiles.

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7 510 Because of the fact that lipolytic activity of adipose tissue differed among ewes with
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10 511 similar body condition status in the same group, our results also indicate the potential of using
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12 512 a simplified β -adrenergic challenge protocol for identifying, at the intraflock level, individual
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14 513 differences in adaptive capacity to undernutrition.

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20
21
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23
24 517 animal care and data collection.

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Table 1 Nutritive value of ingredients included in the experimental diets

Ingredient	¹ DM, %	Organic constituents, g/ kg DM					Energy, Mcal/ kg DM			Minerals, g/ kg DM			Net energy, /kg DM		Protein value, g/ kg DM		Fill value
		² OM	³ CP	⁴ CF	⁵ GE	⁶ ME	Ash	P	Ca	⁷ UFL	⁸ UFV	⁹ PDIA	¹⁰ PDIN	¹¹ PDIE	¹² SFU		
Wheat straw	88	920	35	420	4.34	1.34	80	1	2	0.42	0.31	11	22	44	2.41		
Alfalfa (pelleted)	91	885	160	310	4.40	1.84	115	-	-	0.67	0.56	50	101	87	0.80		
Dried sugar beet pulp	89	912	98	206	4.01	2.73	88	1	13	1.01	0.99	40	63	106	1.36		
Mineral- vitamin premix	90	-	-	-	-	-	-	90	126	-	-	-	-	-	-		

¹DM= Dry matter content; ²OM= Organic matter content; ³CP= Crude protein content; ⁴CF= Crude fibre content; ⁵GE= Gross energy; ⁶ME= metabolizable energy; ⁷UFL= net energy for lactation; ⁸UFV= net energy for maintenance and meat production; ⁹PDIA= dietary protein undegraded in the rumen which is digestible in the small intestine; ¹⁰PDIN= PDIA + PDIMN (microbial protein that could be synthesized from the rumen degraded dietary N when energy is not limiting); ¹¹PDIE= PDIA + PDIME (microbial protein that could be synthesized from the energy available in the rumen when degraded N is not limiting); ¹²SFU= Fill unit for sheep

Table 2 Diet composition and daily nutrient supply according to treatment during the dietary challenge period (day 0 to 49). Values between brackets correspond to the added or reduced quantities applied during the refeeding period (day 49 till 100)

Treatment	Ingredient ^a	Distributed (as-fed), kg	Nutrient supply per ewe (DM basis)							
			DM, kg	¹ UFV	² PDIN, g	³ PDIE, g	Fill units	⁴ CP, g	P, g	Ca, g
Control (100% ⁶ MER)	Wheat straw	0.91 (=)	0.80 (=)	0.25 (=)	18 (=)	35 (=)	1.28 (=)	28 (=)	1 (=)	2 (=)
	Alfalfa	0.17 (+11)	0.15 (+0.10)	0.08 (+0.06)	15 (+10)	13 (+9)	0.12 (+0.8)	24 (+16)	1 (+0.5)	1 (+0.5)
	Dried sugar beet pulp	0.17 (+11)	0.15 (+0.10)	0.15 (+0.10)	9 (+7)	16 (+11)	0.12 (+0.8)	15 (+10)	0 (+0.3)	2
Underfed (70% MER)	Wheat straw	1.00 (-0.50)	0.88 (-0.38)	0.27 (-0.11)	19 (-8)	39 (-17)	1.41 (-0.61)	31 (-13)	1 (=)	2
	Alfalfa	0 (+10)	0 (+0.10)	0 (+0.06)	0 (+10)	0 (+9)	0 (+0.08)	0 (+16)	0 (=)	0 (=)
	Dried sugar beet pulp	0 (+11)	0 (+0.10)	0 (+0.10)	0 (+6)	0 (+11)	0 (+0.08)	0 (+10)	0 (=)	0 (+1)
Overfed (160% MER)	Wheat straw	0.57 (+11)	0.50 (=)	0.16 (=)	11 (=)	22 (=)	0.80 (=)	18 (=)	1 (=)	1 (=)
	Alfalfa	0.44 (+10)	0.40 (+0.10)	0.22 (+0.06)	40 (+11)	35 (+9)	0.32 (+0.08)	64 (+16)	1 (+0.5)	1 (+0.5)
	Dried sugar beet pulp	0.45 (+11)	0.40 (+0.10)	0.40 (+0.10)	25 (+7)	42 (+11)	0.32 (+0.08)	39 (+10)	0 (+0.5)	5 (+1.5)

¹UFV= net energy for maintenance and meat production; ²PDIN= PDIA + PDIMN (microbial protein that could be synthesized from the rumen degraded dietary N when energy is not limiting); ³PDIE= PDIA + PDIME (microbial protein that could be synthesized from the energy available in the rumen when degraded N is not limiting); ⁴CP= Crude protein; ⁶MER= maintenance energy requirements. ^aThe mineral-vitamin premix was supplied at the same rate for all treatments i.e. 10 g/ewe/d thus providing the same amount of P and Ca (1g/ewe/d and 1 g/ewe/d, respectively)

Table 3 Final individual daily feeding balances, after calculating average feed refusal per treatment

Treatment	¹ UFV					² PDIN				
	Requirements	Actual daily intake			Balance, % of ³ MER	Requirements	Actual daily intake			Balance, % of ⁴ MPR
		0-49 d	50-100 d	Average			0-49 d	50-100 d	Average	
Control	0.592	0.63	0.72	0.675	114	41	47	60	53	129
Underfed	0.592	0.41	0.39	0.403	68	41	22	30	26	62
Overfed	0.592	1.09	1.16	1.123	190	41	102	107	104	252

¹UFV= net energy for maintenance and meat production; ²PDIN= PDIA + PDIMN (microbial protein that could be synthesized from the rumen degraded dietary N when energy is not limiting); ³MER= maintenance energy requirements; ⁴MPR= maintenance protein requirements

Table 4 Average body weight (BW), body condition score (BCS) and plasma profiles of mature, dry, non-pregnant *Mérinos d'Arles* ewes (n = 36) receiving 70% (Underfed; n = 12), 100% (Control; n = 12) or 160% (Overfed; n = 12) of their maintenance energy requirements during the dry-off period

Item	Nutritional plane or treatment			Effects, <i>P</i> value		
	Control	Underfed	Overfed	Regimen	Time	Regimen × Time
BW, kg	44.27 (± 0.285)	41.59 (± 0.285)	47.48 (± 0.446)	<.0001	<.0001	<.0001
BCS, 1-5	1.92 (± 0.017)	1.78 (± 0.017)	2.00 (± 0.027)	<.0001	<.0001	<.0001
NEFA, mmol/L	0.12 (± 0.011)	0.26 (± 0.011)	0.09 (± 0.017)	<.0001	0.0002	<.0001
β -OHB, mg/L	23.36 (± 0.684)	20.16 (± 0.684)	23.58 (± 1.068)	0.003	<.0001	<.0001
Glucose, g/L	0.55 (± 0.005)	0.55 (± 0.005)	0.59 (± 0.008)	0.025	<.0001	0.0061
Insulin, μ IU/mL	12.98 (± 0.520)	11.58 (± 0.520)	14.96 (± 0.813)	0.004	0.0033	0.0001
Leptin, ng/mL	4.56 (± 0.058)	4.52 (± 0.058)	5.09 (± 0.091)	0.003	<.0001	<.0001

Table 5 Average body weight (BW), body condition score (BCS), basal plasma NEFA (at -15 min.) and plasma NEFA responses to a β -adrenergic challenge with isoproterenol injection in mature, dry, non-pregnant *Mérinos d'Arles* ewes (n = 36) with different body condition scores

Item	BCS group						Effect, P <
	Normal		Lean		Fat		
	LSmeans	SEM	LSmeans	SEM	LSmeans	SEM	
BW, kg	46.22	0.786	37.69	0.786	50.92	0.923	<.0001
BCS, 1-5	1.79	0.049	1.34	0.049	2.17	0.058	<.0001
Basal NEFA, mmol/L	0.06	0.02	0.11	0.02	0.05	0.02	0.0002
Response at 5 min., mmol/L	0.31	0.04	0.33	0.04	0.37	0.04	0.0003
Response at 10 min., mmol/L	0.23	0.04	0.42	0.04	0.22	0.04	<.0001
Response at 20 min., mmol/L	0.08	0.03	0.30	0.03	0.05	0.03	<.0001
Maximal response, mmol/L	0.38	0.04	0.56	0.06	0.42	0.03	<.0001
Time [†] , min.	5.83	0.54	12.00	1.93	5.00	0.00	<.0001
AUC [∅] , mmol.min/L							
from 0 to 5 min.	1.1	0.14	1.4	0.23	1.2	0.09	<.0001
from 5 to 10 min.	1.6	0.22	2.4	0.33	1.7	0.14	<.0001
from 10 to 15 min.	1.2	0.20	2.6	0.31	1.1	0.12	<.0001
from 15 to 30 min.	1.9	0.38	5.6	0.62	1.4	0.23	<.0001
from 30 to 60 min.	2.8	0.58	5.5	0.65	2.0	0.23	<.0001
from 0 to 60 min.	5.4	0.87	13.3	1.53	5.5	0.59	<.0001

[†]Time between isoproterenol challenge and maximal response; [∅]Area under the concentration curve and above baseline

Table 6 Correlation coefficients* between basal plasma NEFA and plasma NEFA responses to isoproterenol challenge in fat, lean or normal body condition score meat ewes (n = 36)

	Response at 5 min	Response at 10 min	Response at 15 min	Response at 20 min	Maximal response	Time [†]	AUC [∅] 0-60	AUC 0-5	AUC 5-10	AUC 10-15	AUC 15-30	AUC 30-60
Basal NEFA	0.61	0.73	0.69	0.66	0.54	0.25	0.69	0.79	0.72	0.73	0.65	0.67
Response at 5 min		0.77	0.63	0.51	0.84	-0.08	0.67	0.96	0.92	0.72	0.52	0.44
Response at 10 min			0.91	0.85	0.83	0.30	0.91	0.83	0.96	0.98	0.84	0.72
Response at 15 min				0.97	0.77	0.43	0.99	0.70	0.84	0.98	0.98	0.82
Response at 20 min					0.72	0.47	0.97	0.61	0.75	0.93	1.00	0.89
Maximal response						-0.07	0.80	0.84	0.89	0.82	0.73	0.69
Time							0.40	0.01	0.15	0.37	0.47	0.44
AUC 0-60								0.74	0.86	0.97	0.97	0.83
AUC 0-5									0.94	0.79	0.61	0.59
AUC 5-10										0.92	0.75	0.64
AUC 10-15											0.93	0.79
AUC 15-30												0.89

*All significant at $P < 0.0001$ except for the variable Time

[†]Time between isoproterenol challenge and maximal response; [∅]Area under the concentration curve and above baseline

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4 **Figure captions:**
5

6 **Figure 1.** Schematic representation of the experimental design. The distribution of experimental
7 ewes (n = 36) submitted to three contrasted nutritional planes and the body weight (n = 11), blood (n =
8 18) sampling points and a final β -adrenergic challenge are illustrated. After 3 weeks of adaptation,
9 energy diet content was changed in the two extreme groups (overfed and underfed). The measurement
10 period (100 days) consisted in two periods i.e. dietary challenge (from 0 to 49 days) and refeeding
11 (from 50 to 100 days) period. Sampling was structured in a close (3 weeks) and a more extended
12 (biweekly) individual monitoring periods.
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23 **Figure 2.** Body weight (BW), body condition score (BCS) and non-esterified fatty acids (NEFA)
24 blood plasma profiles of mature, dry, non-pregnant *Mérinos d'Arles* ewes (n = 36) offered 60%
25 (Underfed; n = 12), 100% (Control; n = 12) or 170% (Overfed; n = 12) of maintenance energy
26 requirements. Diet challenge started at day 0, after an overall 3 week adaptation. Arrow represents
27 commencement of refeeding period. Error bars represent SEM.
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35 **Figure 3.** Energy metabolism (**glucose** and **insulin** blood plasma profiles) of mature, dry, non-
36 pregnant *Mérinos d'Arles* ewes (n = 36) offered different nutritional planes during the dry-off period
37 i.e. 60% (Underfed; n = 12), 100% (Control; n = 12) or 170% (Overfed; n = 12) of maintenance energy
38 requirements. Diet challenge started at day 0, after an overall 3 week adaptation period. Arrow
39 represents commencement of refeeding period. Error bars represent SEM.
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48 **Figure 4.** Energy metabolism (β -hydroxybutyrate - **β -OHB-** and **leptin**) blood plasma profile) of
49 mature, dry, non-pregnant *Mérinos d'Arles* ewes (n = 36) offered different nutritional planes during the
50 dry-off period i.e. 60% (Underfed; n = 12), 100% (Control; n = 12) or 170% (Overfed; n = 12) of
51 maintenance energy requirements. Diet challenge started at day 0, after an overall 3 week adaptation
52 period. Arrow represents commencement of refeeding period. Error bars represent SEM.
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4 **Figure 5.** Effect of an induced β -adrenergic challenge injection with isoproterenol (ISO, 4 nmol/kg
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6 BW) on plasma non-esterified fatty acids (NEFA) kinetics of mature, dry, non-pregnant *Mérinos*
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8 *d'Arles* ewes (n = 36) with contrasted body condition scores i.e. high (FAT, +BCS; n = 12), average
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10 (NORMAL, BCS; n = 12) or low (LEAN, -BCS; n = 12). Error bars represent SEM.
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14 **Figure 6.** Correlations between plasma area under the concentration curves (AUC) from time 0 to
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16 60 min. and basal plasma NEFA, or plasma NEFA responses at different times after isoproterenol
17
18 challenge in normal (-o-), lean (-▲) or fat (-•-) *Mérinos d'Arles* meat ewes.
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21 **Figure 7.** Individual distribution of non-esterified fatty acids (NEFA) release turnover in *Mérinos*
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23 *d'Arles* meat ewes with contrasted body weights (Fat, Normal or Lean) resulting from different dietary
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25 challenges (overfed, normally fed or underfed, respectively). Notice that, at similar body weights, ewes
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27 belonging to the same group showed contrasted adaptive capacity as illustrated by their short-term
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29 responses after a β -adrenergic challenge.
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Figure 1

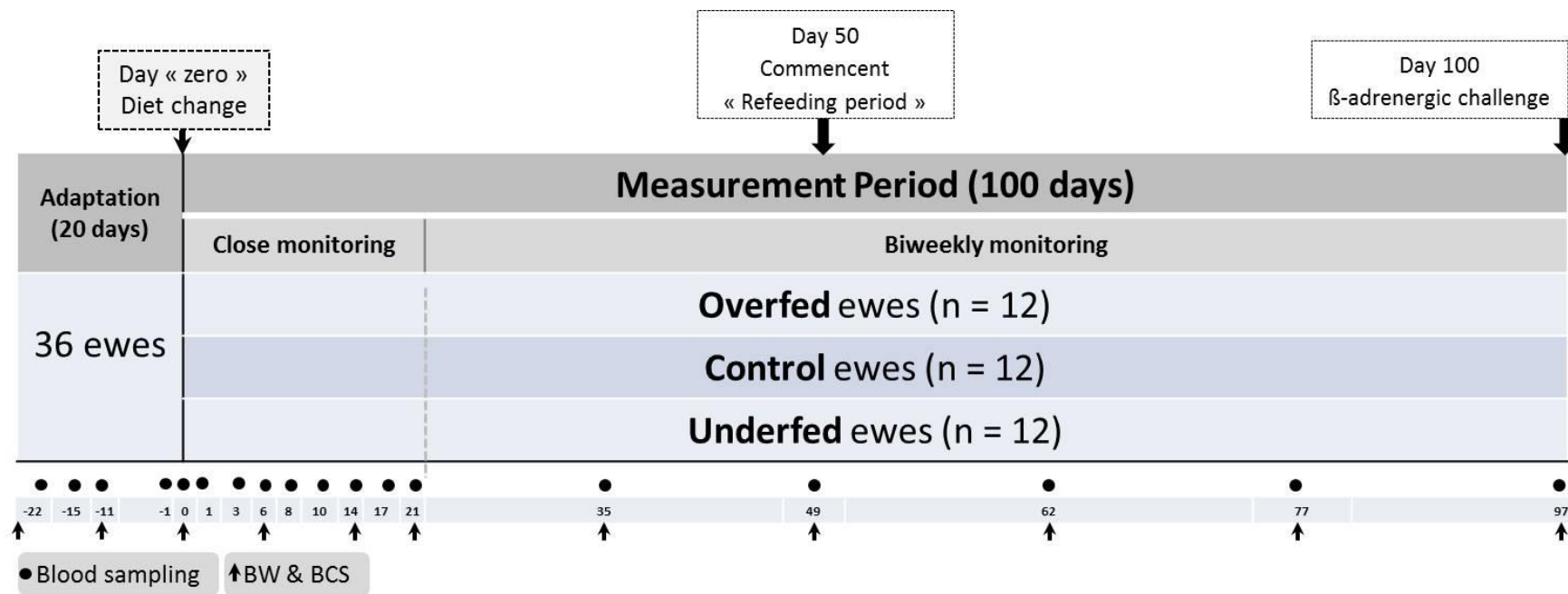


Figure 2

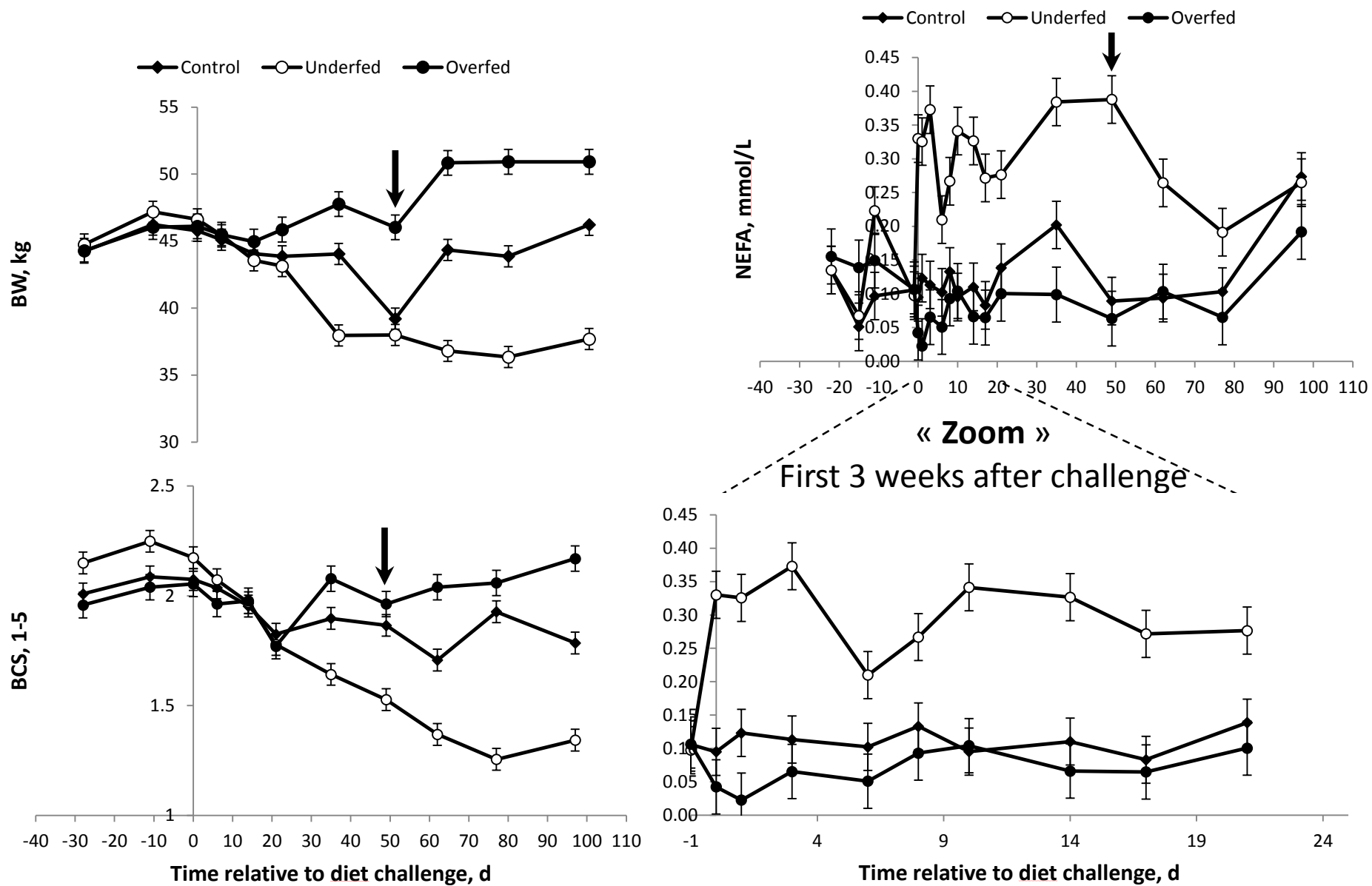
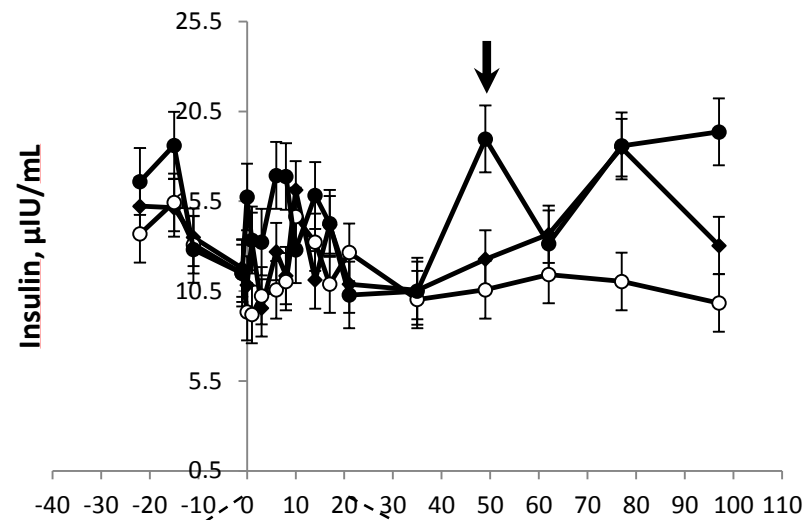
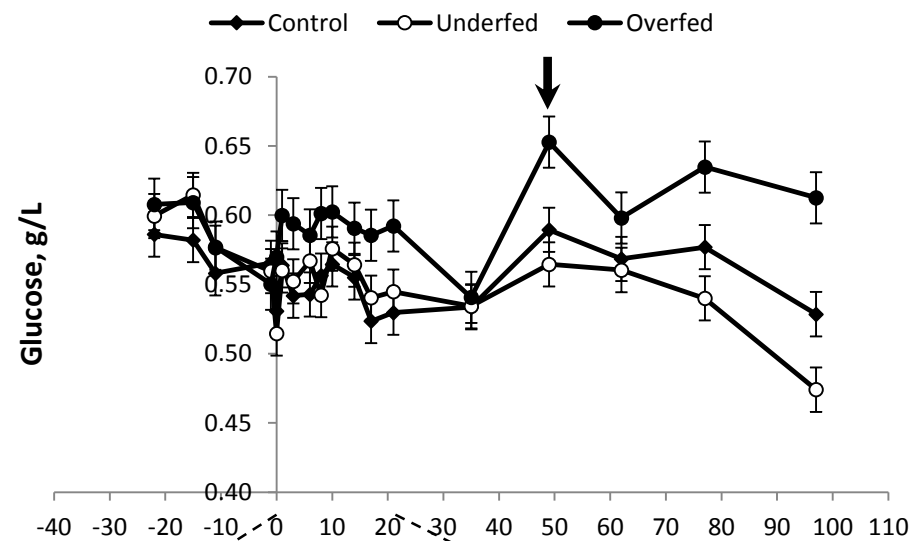


Figure 3



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First 3 weeks after challenge

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First 3 weeks after challenge

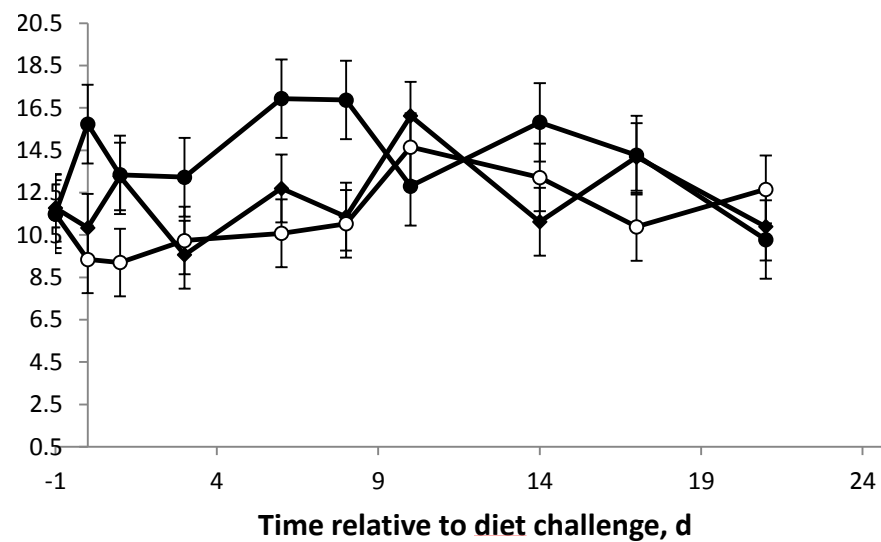
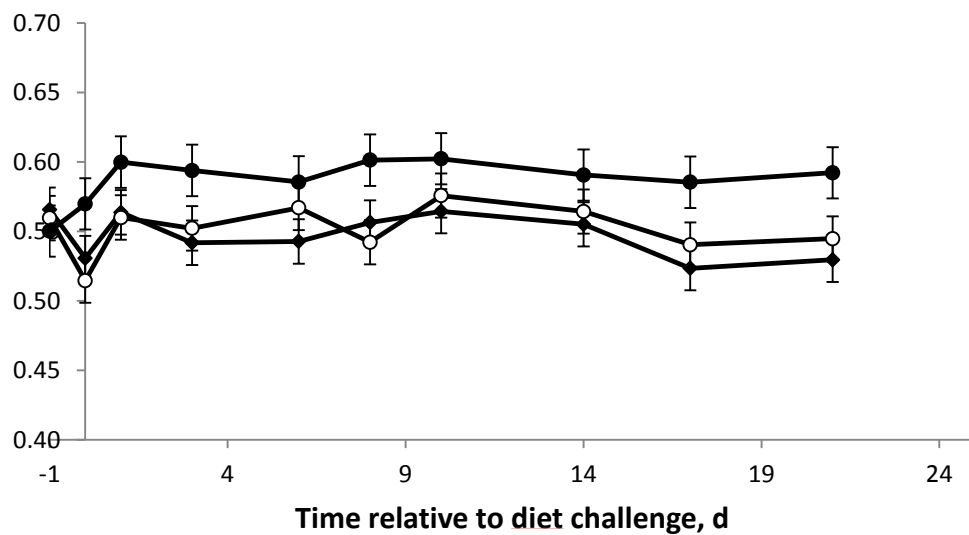
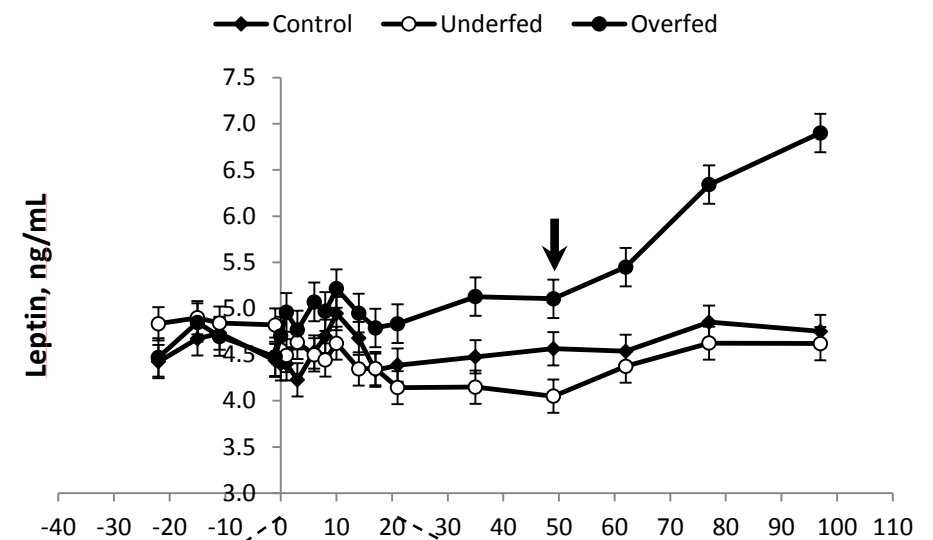
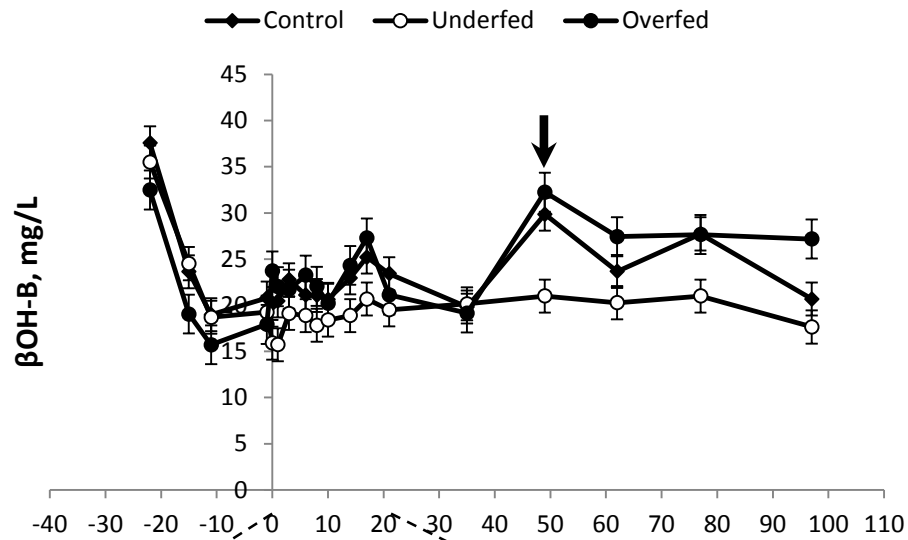
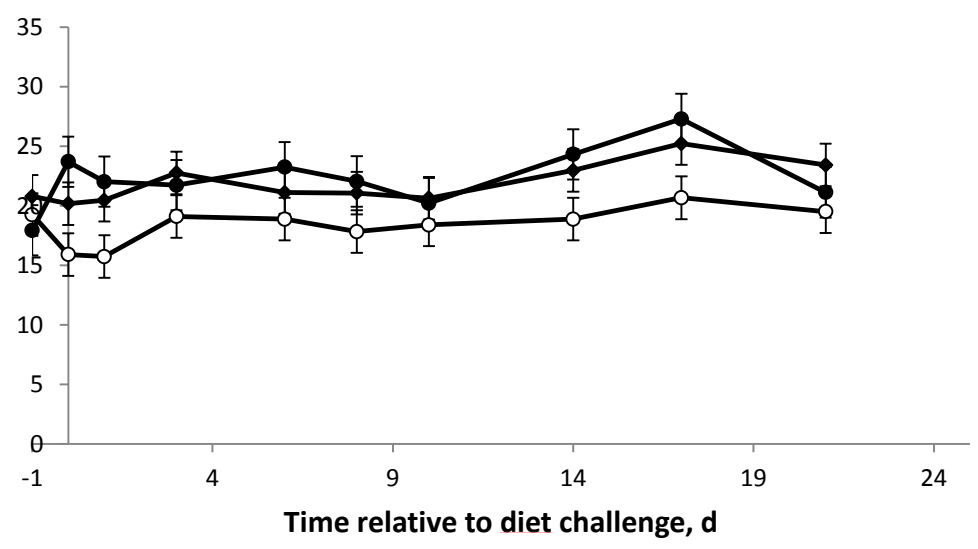


Figure 4



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First 3 weeks after challenge



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First 3 weeks after challenge

