1	Effects of the feeding level in early gestation on body reserves and the productive
2	and reproductive performance of primiparous and multiparous sows
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9 ABSTRACT

10 Early gestation may be the best period for sows to recover body reserve losses from previous lactation. The aim of this study was to investigate the effect of different levels 11 12 of restricted feeding in early gestation on the body status, productive and reproductive 13 performance, and hormonal-metabolic status of primiparous and multiparous sows. A total of 130 sows were randomly assigned to one of three feeding levels: Treatment I, 14 which sows were fed at the level commonly used from day 3 to 28 of gestation (2.5 15 16 kg·d-1 of a diet with 2.18 Mcal NE·kg-1 and 13.72 g CP·kg-1), and Treatments II and III, where feed was increased by 25% and 50%, respectively. Sow body status, litter size 17 and weight, early mortalities, reproductive rates, weaning-to-estrus interval, and 18 19 hormones linked to metabolism were recorded. The highest weight gain, body condition score, and backfat thickness were found in sows fed Treatment III compared to those 20 21 fed the usual feeding level (Treatment I). No differences among treatment groups were found in litter size or litter weight, although a tendency for more live born piglets and 22 23 fewer stillbirths was found in sows fed Treatment III. In contrast, litters from sows fed 24 at higher feeding levels had a higher mortality at 72 h compared to those fed at the 25 lowest feeding level (I), which was partly linked to a higher percentage of piglets culled at birth and piglets weighing less than 800 g. There were no differences in conception 26 27 and farrowing rates, leptin, progesterone, insulin, or cortisol among treatment groups applied in early gestation. In conclusion, increasing the feeding level in sows during 28 early gestation to improve their short-term productive and reproductive performance 29 30 remains controversial. Further studies are needed to focus on how the restricted feeding level applied could affect the viability and proportion of low-weight piglets. 31

32 Keywords: feeding level, early gestation, sow, body reserves, performance, leptin

33 1. INTRODUCTION

Early gestation, which covers about the first 30 days, may be the best period for the sow to recover body reserve losses from previous lactation (Dourmad et al., 1996), and this is particularly relevant for gilts because they still have to grow to reach their mature body size. In addition, sows may be kept in individual crates in this period according to the European legislation (European Union, 2009/120/EC), which would allow specific feeding strategies to be adopted.

Knowledge of the nutrient requirements of modern sows and the feeding strategies to be applied to meet them under commercial conditions play an important role in achieving the best productive and reproductive performance. Furthermore, genetic selection for leanness has developed animals with lower fat reserves and limited feed intake. In fact, new hyper-prolific and leaner sows have higher culling rates than a few years ago, mainly due to reproductive failure (Sasaki and Koketsu, 2011).

This fact has been linked to both excessive losses of body weight and body reserves 46 during lactation, and to a sub-optimal level of body reserves at weaning in these genotypes 47 (Thaker and Bilkei, 2005; Ferreira et al., 2021). In this line, the restricted feeding 48 49 programs commonly used during gestation do not always allow for the recovery of body 50 reserves lost in the previous lactation. This is particularly problematic in primiparous 51 sows, as they have lower body reserves and a 20% lower feed intake during lactation 52 compared to multiparous sows (Eissen et al., 2000). In this sense, primiparous sows also show lower reproductive performance (a reduced farrowing rate, a lower litter size, or 53 both) resulting from relatively higher weight losses during the first lactation (Thaker and 54 55 Bilkei, 2005).

An increased feed intake during early gestation should help to ensure suitable levels of 56 body reserves at farrowing and at weaning. However, the effect of the feeding level after 57 insemination is controversial (Leal et al., 2019; Langendijk, 2021). Earlier studies with 58 gilts diets supplying 32.5MJ DE·d□ 1 (Jindal et al., 1997) or 29.4MJ ME·d□ 1 (Dyck 59 and Strain, 1983) in the first days after mating reduced the level of progesterone and 60 61 impaired the uterine environment, reducing embryo survival. For this reason, it has been traditionally recommended to keep the feeding level low (without energy content greater 62 63 than needed for body maintenance) in the first post-mating weeks. However, currently, 64 increasing the level of feeding in hyper-prolific sows could positively affect embryo development and survival. Indeed, Leal et al. (2019) in a meta-analysis study about this 65 topic, showed that restricted feed intake in early gestation is no longer relevant when 66 67 using modern sow lines, as dietary energy of up to 54 MJ ME·d 1 had no detrimental 68 effect on embryo survival. Otherwise, it is also well documented that increased levels of backfat at farrowing lead to a decrease in feed intake during lactation (Lavery et al., 69 70 2019). However, some studies suggest that higher body reserves at farrowing may play a 71 protective role in sow performance against the adverse effects of excessive body weight 72 loss during lactation, mainly in lean genotypes (Cerisuelo et al., 2008). In this sense, Schenkel et al. (2010) reported that a body weight loss higher than 10–12% decreases 73 74 reproductive performance in the subsequent productive cycle.

From a hormonal point of view, the feeding level stimulates the growth of luteal tissue and increases the synthesis of progesterone by the ovaries in the pre-implantation period (Athorn et al., 2013), which is supplied directly to the uterine horns. The establishment and maintenance of luteal tissue in early gestation are critical to endometrial function, development, and embryo survival. Before implantation, the effects on formation of luteal tissue and progesterone secretion would be independent of the luteinising hormone (LH)

and probably linked to metabolic signals via insulin and insulin-like growth factor 1 (IGF-81 82 1) (Langendijk et al., 2008). It is just during the first three days after ovulation when high feed intake can reduce systemic progesterone (while direct supply from the ovaries may 83 still be low), and thus can also reduce embryo survival according to Jindal et al. (1997). 84 After day 12 of gestation, the luteal function is dependent of LH, although variations in 85 energy intake may not lead to gestation failure, but may affect nutrient supply to the 86 embryos, as uterine capacity becomes limiting (Langendijk et al., 2016; Wang et al., 87 2017). Furthermore, to our knowledge, there is insufficient information about the effects 88 of a feeding strategy applied in early gestation on the serum levels of some non-89 90 reproductive hormones, such as leptin, related to body reserves and feed intake in sows. 91 Previous research has demonstrated that leptin is the principal regulator of energy balance during gestation, and the presence of leptin receptors in the placenta suggests effects on 92 93 essential processes to ensure an adequate nutrient supply to the fetus (Saleri et al., 2015).

In summary, increasing the level of feeding in early gestation may offer advantages over later stages. Furthermore, the level of feeding in hyper-prolific sows during early gestation still needs to be refined in order to clarify some conflicting results found in the scientific literature on feeding management of sows. The aim of this study was to evaluate the effect of the feeding level from day 3 to 28 of gestation on body status and litter traits of primiparous and multiparous sows, as well as the effects on their hormonal-metabolic status.

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2. MATERIAL AND METHODS

102 The experimental procedures and handling of animals, following the European 103 regulations (European Union, 2010/63/EU Directive) for the protection of animals used 104 for scientific purposes, were approved by the Ethics Committee of Animal 105 Experimentation of the University of Murcia and the Administrative Authorities (A-106 13170805).

107 2.1 Location and Animals

108 The trial was carried out in a commercial farm located in the southeast of Spain (Murcia, Spain). A total of 130 sows (Landrace x Large-white; Topigs Norsvin TN70), 61 109 primiparous (second gestation sows) and 69 multiparous (from third to eighth parity), 110 were used for this experiment. When estrus was detected, sows were blocked by parity, 111 body weight, and body condition status (by decreasing order of preference) and assigned 112 113 randomly to one of three gestation feeding programs (described below). Sows were then 114 inseminated with semen from Duroc boars, after following the standard procedure for assessing sperm motility, which included total sperm motility (percentage showing any 115 form of motility), progressive sperm motility (percentage showing rapid, linear 116 movement) and sperm velocity (on a scale of 0 to 4, from immotile to rapidly motile). 117 The estrus detection and insemination protocol are described below. 118

2.2 Experimental Design and Treatments

The feeding program during gestation was divided into two periods: early gestation (from 120 day 3 to day 28) and the rest of the gestation period until a few days before farrowing 121 122 (from day 29 to day 110). All sows were fed the same commercial gestation diet, which 123 was formulated according to the standard recommendations of the Spanish Foundation for the Development of Animal Nutrition (FEDNA, 2013) and manufactured in a dry 124 mash form. The ingredients and chemical composition of the gestation diet are 125 126 summarized in Table 1. All sows were fed once a day (at 07:00 h). Three feeding levels were assessed in the early gestation period. In Treatment I, sows were fed at the level 127 commonly used on this farm (2.5 kg·d \square 1 of a diet with 2.18 Mcal NE·kg \square 1 and 13.72 128

g CP·kg□ 1), whereas feed was increased by 25% and 50% in Treatments II and III, 129 respectively (3.125 and 3.750 kg·d \square 1, respectively). From day 29 onwards, all sows 130 131 were fed the same amount (2.5 kg·d \square 1 until day 110), regardless of the feeding level applied in early gestation. Sows were kept in the gestation facilities during the weaning-132 to-estrus interval and the first 28 days, including gestation diagnosis; which was 133 134 performed at 25 days after first insemination by transabdominal ultrasonography 135 (Echoscan T-300 S, Barcelona, Spain). They were housed in individual crates (0.60 m \times 2.00 m). Non-pregnant sows were removed from the study when they returned to estrus 136 137 (n = 9). Water was offered ad libitum by a drinking nipple, while feed was restricted in 138 an individual feeder according to the experimental design (as described above). Feed refusals in early gestation were monitored to check that all sows ate the total amount of 139 feed offered. Room temperature was kept at approximately 20 °C, and ventilation was 140 thermostatically controlled. On day 29 of gestation, sows were moved to outdoor pens (5 141 142 $m \times 8$ m) and kept loose in mixed-treatment groups of 15 sows each, in which they were also fed in a restricted way but with the same amount of feed, as noted previously. The 143 144 feeder was a long common cement trough with access to water by means of nipple 145 drinkers. Throughout lactation and the first post-weaning days, the feeding regime was also common for all sows. When sows were moved to individual crates (0.60 m \times 2.20 146 147 m) about one week before farrowing (day 110 of gestation), they were fed 2.0 kg d = 1 of 148 a commercial lactation diet (Table 1). After farrowing, sows were fed three times a day: 149 at 7:00, at 12:00, and at 18:00 h. The initial amount of feed was adjusted for each lactating 150 sow by gradually increasing the daily amount supplied by 0.5 kg when no refusal was observed for 2 consecutive days, until the maximal feed intake was reached (about day 151 152 10 of lactation). From weaning (at 25 days of lactation) to the next heat period, sows were fed 4.0 kg·d \square 1 of the gestation diet (Table 1). 153

154 **2.3 Measurements**

155 Body status traits of sows were monitored during gestation and lactation. Body weight (BW), body condition score (BCS), backfat thickness (BF), and loin depth (LD) were 156 recorded individually at days 0 (d0), 28 (d28), 60 (d60), 90 (d90), and 110 (d110) of 157 158 gestation and at weaning (dW). The changes in body condition during gestation (d110d0) and from day 0 until weaning (dW-d0), known as the global balance, were also 159 calculated. The BCS was always evaluated by the same person on an ordinal scale from 160 161 1 (very thin) to 5 (very fat) according to Bonde et al. (2004); BF and LD were measured by ultrasound scan equipment with a linear probe (SF1, Wireless Backfat and Loin Depth 162 163 Scanner, Sonivet, Beijing, China) at the P2 position (last rib, 65 mm from the center line of the back). The measurements were repeated three times, and their average was used 164 for further calculations, following previous protocols (Maes et al., 2004). The productive 165 performance per sow (litter size and weight) was recorded at farrowing. Live born piglets, 166 167 stillborn, and mummies were recorded and weighed within 24 h after birth. Piglets of litters born from 9:00 to 18:00 h were individually weighed the following day in the 168 169 morning (at 7:30 h), while those born during the night were weighed at 15:00 h. The 170 mortality rate at birth (percentage of stillborn in relation to total born) and within-litter birth weight coefficient of variation were calculated. The number of dead piglets during 171 172 the first three days was also recorded to calculate the mortality rate at 72 h as a percentage 173 of live piglets at birth. In addition, piglets culled at birth, as they are unlikely to survive and unable to reach market weight at the same rate as normal-weight littermates (low 174 175 vitality, crushed but still alive, malformations, cannibalism, etc.), and piglets born 176 weighing less than 0.8 kg were recorded to calculate their proportions within each litter. Reproductive parameters, such as the conception rate and the farrowing rate, were 177 178 calculated within treatment. The conception rate was calculated as the proportion of sows

that did not return to estrus relative to the total number of sows inseminated, while the 179 180 farrowing rate was calculated as the proportion of inseminated females that farrowed. The 181 weaning-to-estrus interval (WEI) of sows in the next reproductive cycle, defined as the interval from weaning to the first standing heat reflex, was also determined. Sows were 182 183 checked for estrus twice a day (at 08:00 and at 16:00 h) using the back-pressure test in the presence of a mature teaser boar. They were inseminated twice by a post-cervical 184 185 artificial insemination method, 16–24 h after onset of standing heat and again 24 h later; 186 considering the first insemination as day 0 of gestation.

187 2.4 Blood Samples and Hormone Analysis

188 Blood samples, from ten sows per treatment, were taken from the cranial vena cava in sterile tubes without additives (Vacuette®, Greiner Bio-One, Kremsmünster, Austria), at 189 190 the day of first artificial insemination (d0) and at days 28, 60, and 110 of gestation. Sows within each treatment were chosen according to their BW as being close to the average 191 192 weight of the treatment at day 0; the same sows were subsequently bled over time. The serum was separated by centrifugation (1600 \times g for 10 min), and it was then frozen at \Box 193 194 80 °C until further analysis of leptin, progesterone, insulin, and cortisol. The serum 195 samples were submitted to the Interdisciplinary Laboratory of Clinical Analysis of the 196 University of Murcia (Interlab-UMU, Spain) and were analyzed in duplicate with an 197 automated chemiluminescent immunoassay (Immulite System, Siemens Health 198 Diagnostics, Deerfield, IL, USA). Leptin concentration was analyzed at days 0, 28, 60 199 and 110 using RIA (Multi-species Leptin Assay Kit, Linco Research, St. Louis, MO, 200 USA), previously validated for porcine plasma (Govoni et al., 2005). Paired differences 201 in leptin relative to the initial concentration (d0) were calculated taking into account the 202 sow effect ([Leptin]d28- [Leptin]d0, [Leptin]d60-[Leptin]d0, and [Leptin]d110-[Leptin]d0). The other hormones were determined at day 28. Progesterone concentrations 203

were analyzed using the Immulite System (Siemens Health Diagnostics, Deerfield, IL,
USA). Insulin was determined by an Enzyme- Linked Immuno Sorbent Assay (ELISA)
Kit (Mercodia, Porcine Insulin ELISA ref. 10–1200-01, Winston Salem NC, USA).
Cortisol was analyzed with an automated chemiluminescent immunoassay (Immulite
System, Siemens Health Diagnostics, Deerfield, IL, USA). All methods showed an interand intra-assay coefficient of variation lower than 15%.

210 2.5 Statistical analysis

Data were analyzed using SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL, 211 212 USA). Body measurements of sows at different days throughout the study, and their changes in gestation and lactation (d110-d0 and dW-d0), were analyzed through an 213 ANOVA model using the General Linear Model procedure. For the productive 214 215 performance of sows at farrowing, an ANOVA model was also used. Both models 216 included treatment (feeding level in early gestation) and parity order (primiparous vs. multiparous) as main factors as well as their first-order interaction. The value of each 217 body variable measured at day 0 and the total born were used as a covariate for body 218 219 status measurements and the productive performance of sows (litter data), respectively. 220 Piglet mortality rates, the proportions of piglets culled at birth and of piglets of low birth weight, and the reproductive rates of sows were coded as binary variables within litter 221 222 (sow) and treatment, respectively, and then analyzed by logistic regression. Therefore, a 223 binomial distribution (either the number of events occurring in a set of trials or a binary 224 response) with a logit model was fitted to evaluate them, where treatment, parity order, 225 and their first-order interaction were used as dependent variables. Furthermore, 226 correlations by Spearman rank correlation analysis were examined for ordinal categorical 227 variables, such as the number of piglets dead at 72 h, the number of piglets culled at birth, and the number of low-weight piglets within litters. For hormone analyses, paired 228

differences in leptin within sow relative to the initial concentration (d0) were calculated 229 230 to ignore the intra-individual variability and analyzed with a model including treatment 231 and parity order as main factors as well as their first-order interaction. The same model 232 was used for the rest of the hormones (progesterone, insulin, and cortisol), which were measured only at day 28. The sow (or litter) was considered the experimental unit. Results 233 are presented as least square means or as percentages (reproductive rates and piglet 234 235 mortalities). A pairwise comparison of means was performed by using the least significance difference (LSD) test. The significant value was set at $p \le 0.05$, while 0.05 236 was considered as a tendency.237

3. RESULTS

239 **3.1** The Body Status and Body Changes of Sows

At the beginning of the study (day 0), there were no differences in sow BW and BCS 240 241 among dietary treatments (p > 0.05) (Fig. 1A). However, differences in both traits were 242 found at day 28 and throughout gestation ($p \le 0.05$). Sows fed the highest level of feed 243 (III) showed a higher BW at 28 and 60 d of gestation than those fed the lower feeding levels (I and II). On days 90 and 110 of gestation and at weaning, sows fed Treatment III 244 had also a higher BW than those fed Treatment I, although without differences compared 245 246 to sows fed Treatment II. Sows fed Treatment III had a higher BCS than sows fed 247 Treatments II and I at days 28, 60, and 90. There were also differences in BCS between Treatments I and II at day 28. However, no differences between Treatments I and II were 248 249 found from day 60 onwards. At 110 d of gestation and at weaning, there were also differences in BCS between Treatments I and III, whereas Treatment II showed 250 251 intermediate scores. The BF and LD results of the treatments were not different at day 0 252 (p > 0.05) (Fig. 1B). On day 28, sows fed Treatments III and II had a higher BF thickness

than those fed Treatment I ($p \le 0.05$). These differences were found between Treatments 253 254 I and III ($p \le 0.05$) at days 60, 90, and 110 and at weaning, while the BF of sows fed Treatment II was not different from the above-mentioned treatments (p > 0.05). For LD 255 at day 28 of gestation, sows fed Treatment I showed a lower average than those fed 256 Treatments II and III ($p \le 0.05$). On day 60, these differences were found in sows fed 257 258 Treatments I and III, but there were no differences between Treatments I and II. On day 259 90, Treatment I resulted in a lower LD than Treatment III. However, no differences in LD were found between treatment groups at day 110 and at weaning. Similar results to 260 those described above were partly reflected in body changes (Table 2). From day 0 to day 261 262 110 of gestation, sows improved their body status. The highest BW gain, BCS, and BF thickness were found in sows fed Treatment III compared to those fed the usual restricted 263 264 feeding level (I), whereas sows fed Treatment II showed intermediate averages. As for 265 changes in LD during gestation, there was a trend (p = 0.087) in which sows fed Treatment III showed the greatest LD. When results were examined by parity, BW and 266 267 LD changes in primiparous sows were higher than in multiparous sows ($p \le 0.05$). In addition, no interaction effects between feeding level and parity were found for any 268 variable mentioned above (p > 0.05). Regarding changes during the global period 269 270 (including lactation losses), differences among treatment groups were found ($p \le 0.05$); sows fed Treatment III showed the best balance in BW, BCS, and BF. Furthermore, there 271 were differences in BW and BF thickness according to parity order ($p \le 0.05$). While 272 273 primiparous sows showed higher weight gain compared to multiparous sows, they also had higher losses in BF. There were no significant interactions for body changes during 274 275 the global period between feeding level and parity.

276 **3.2 The Productive Performance of Sows: Litter Traits**

No differences among dietary treatments were found in the total born at farrowing (p > p)277 278 0.05) (Table 3), nor when piglets at birth segregated into live born, stillborn, and 279 mummies (p > 0.05). However, a tendency for more live born piglets and fewer stillbirths was found in sows fed Treatment III (p = 0.069 and p = 0.071, respectively). In addition, 280 the number of live born piglets was higher among primiparous sows than among 281 multiparous sows ($p \le 0.05$), while a reverse trend was found in mummies (p = 0.070). 282 283 There were no differences in any variable of litter weight analyzed (p > 0.05), either between treatment groups or between parity order. Moreover, there were also no 284 interaction effects between feeding level and parity order on litter size or litter weight (p 285 286 > 0.05). No differences in mortality rate at birth were found among dietary treatments (Table 3), while litters from sows fed at higher feeding levels (II and III) had a higher 287 mortality at 72 h compared to those fed at the lowest feeding level (I) ($p \le 0.05$). An 288 289 unfavorable effect was also found in litters fed Treatments II and III on the percentage of piglets culled at birth and those weighing less than 800 g ($p \le 0.05$). The mortality rate at 290 291 72 h included the rate of piglets culled at birth, so the number of piglets dead at 72 h was 292 positively correlated with both the number of piglets culled at birth and the number of low-weight piglets (p < 0.001) (Spearman rank correlations of 0.759 ± 0.052 and $0.535 \pm$ 293 294 0.077, respectively). In addition, piglets culled at birth were correlated with low-weight piglets (rs = 0.669 ± 0.068 , p < 0.001). When results were examined by parity order, 295 primiparous sows tended to have a higher piglet mortality at 72 h than multiparous sows 296 297 (p = 0.086), and there was a significant interaction effect between feeding level and parity order on the mortality rate at 72 h, the piglet culling rate, and the rate of low weights at 298 299 birth. Differences between primiparous and multiparous sows on the mortality at 72 h were observed in Treatment I. However, on the piglet culling rate at birth, these 300 differences in parity order were found in sows of Treatment I and III. On the low weight 301

piglet rate, differences between primiparous and multiparous were only found in sows of
Treatment II (Supplementary Fig. 1). Despite the mortality results described above, no
differences in litter size at 72 h were found for any of the factors analyzed (Table 3).

305 **3.3 Reproductive Performance and Serum Hormonal Concentrations**

306 There were no differences (p > 0.05) among the three different levels of restricted feeding applied in early gestation in terms of reproductive rates, either the conception rate or the 307 308 farrowing rate (p > 0.05) (Table 4). Neither an effect of parity order nor a first-order 309 interaction with the feeding level was found in regard to reproductive rates (p > 0.05). 310 Similarly, no factor affected the WEI (p > 0.05), whose mode and median was 4 days in 311 all treatments. Preliminary analyses of the serum leptin showed no differences among treatment groups at d0 (p > 0.05; data not shown). Paired differences in leptin (within-312 313 sow from the initial concentration at day 0) increased significantly as gestation progressed ([Leptin]d60-[Leptin]d0), returning to baseline values at d110 ([Leptin]d110-[Leptin]d0) 314 (Fig. 2). In any case, no effect was found on leptin concentration among treatment groups 315 (p > 0.05). Furthermore, no effect of parity order or first-order interaction with feeding 316 level on paired differences in leptin was found (p > 0.05). Regarding the rest of the 317 318 hormones (progesterone, insulin, and cortisol), measured at d28, none of the factors 319 examined in this study was associated with their serum concentrations (Table 5), except 320 for the first-order interaction for progesterone concentration (p = 0.011). The highest 321 serum concentrations were found in multiparous sows fed at the intermediate feeding 322 level (II), compared to primiparous sows in Treatment II, or in multiparous sows in 323 Treatment I, while the other sows showed intermediate concentrations (Supplementary 324 Fig. 2).

325 **3. DISCUSSION**

326 4.1 The Body Status and Body Changes of Sows

327 Sows require intensive nutritional management due to a genetic for prolificacy and a leaner body condition. A sub-optimal level of body reserves at weaning in hyper-prolific 328 329 sows has been related to a lower productive and reproductive performance (Dourmad et 330 al., 1996; Thaker and Bilkei, 2005; Lavery et al., 2019). Increasing feeding levels during gestation should preserve higher levels of body reserves at farrowing and at weaning. 331 This study showed that a greater feeding level from 3 to 28 d of gestation in primiparous 332 333 and multiparous sows increased body reserves throughout gestation. The weight gain of sows fed at the highest level was an increase of about 20% (+10.4 kg) compared to sows 334 335 in the control group, which was reflected in the higher BCS (+0.48 points) and BF thickness (+1.43 mm). Furthermore, these improvements were also confirmed from a 336 global point of view when accounting for lactation losses, where sows at the highest level 337 maintained the differences compared to those in the control group (+9.49 kg, +0.49 338 339 points, and + 1.53 mm for BW, BCS, and BF, respectively). This is consistent with previous findings about increased levels of restricted feeding in early gestation 340 341 (Virolainen et al., 2004; Hoving et al., 2011; Mallmann et al., 2020), where this feeding 342 strategy improved BW gain and body reserves throughout gestation and at farrowing. Likewise, the extent of these differences in BW (+8.7 kg) and BF (+1.5 mm) compared 343 344 to the standard feeding level was similar to that found by Hoving et al. (2011) in first and second parity sows with a higher feed intake (+30%, 3.25 kg·d \square 1) from day 3 to day 32 345 346 after insemination. More recently, Seoane et al. (2020), by increasing the feeding level 347 during the first month of gestation (from 2.5 to 3.5 kg·d \square 1 of a diet with 2.11 Mcal 348 NE·kg \Box 1 and 13.85 g CP·kg \Box 1), showed an increase in sow BW from previous weaning to farrowing, but this effect was not found between two successive weanings. In this line, 349 Ren et al. (2017b), who changed feeding levels in three 7 d periods throughout gestation 350

(by modifying the amount of maintenance energy intake from a common corn-soybean 351 352 meal-based diet with 3.30 Mcal ME·kg \Box 1 and 15.70 g CP·kg \Box 1), showed that both BW gain and BF change (from day 27 to day 109) increased linearly with increasing feeding 353 levels; however, during lactation, BW gain and BF loss decreased and increased linearly, 354 respectively, due to the reduction of feed intake. Thus, a negative relationship was 355 356 described by linear regression between BF at farrowing and feed intake during lactation, mainly in the first week (Dourmad, 1991). Although it is well known that an excessive 357 increase in BF levels at farrowing leads to a decreased feed intake during lactation 358 (Mullan and Williams, 1989; Sinclair et al., 2001), this negative relationship could be not 359 360 linear; and there is no agreement in the literature on the threshold level above which feed 361 intake during lactation is reduced. In addition, this threshold may have changed in the 362 current genotypes, as suggested by Cerisuelo et al. (2008). Current findings showed that BF losses during lactation ranged from 2 to 2.5 mm and BF never fell below 14 mm at 363 364 weaning, regardless of the dietary treatment applied. In this line, data from several studies 365 showed that BF levels below 16 mm at weaning may compromise the next reproductive cycle (Schenkel et al., 2010), although this threshold value could also be dependent on 366 the sow genotype. On the other hand, global balance results also demonstrate that sows 367 368 can gain BW and lose BF simultaneously, indicating the limited practical usefulness of BW changes to predict changes in BF. In addition, our study showed that weight gain and 369 370 fat loss was more pronounced in primiparous sows compared to multiparous sows. These 371 results are in agreement with Cerisuelo et al. (2009), who suggested that BW gain in pregnant gilts was mainly in the form of protein and less as fat. It is also worth 372 highlighting the fact that the degree of improvement in body reserves throughout 373 374 gestation was close to that of global changes, suggesting that the mobilization or loss of reserves during lactation was similar among treatment groups. Massive tissue 375

mobilization could be expected from farrowing to weaning in sows with higher milk
production (Strathe et al., 2017), which would suggest no differences between treatment
groups; nevertheless, milk production was not measured in this study.

379 4.2 The Productive Performance of Sows: Litter Traits

Our results showed that the level of feeding after insemination had no influence on the 380 productive performance of sows, assessed by litter size and litter weight. The average of 381 the total born was 15.5, with no differences between treatment groups. In contrast, studies 382 conducted to assess the impact of feeding during early gestation reported an associated 383 384 increase in litter size. In this line, Hoving et al. (2011) found an increased litter size by 2 385 total piglets born in the plus-feed group (+30%) in comparison with those in the control group (3.25 kg·d \square 1 vs. 2.5 kg·d \square 1, respectively). However, they reported that birth 386 weight of piglets was similar in both groups, suggesting improved embryonic and/or 387 388 placental development. Sørensen and Thorup (2003) also found significant effects on 389 litter size (+0.5 piglets) when the daily energy supply to sows (49.9 vs. 31.2 MJ ME·d \square 390 1) was higher in the first 28 d after insemination. Nevertheless, our data are in agreement 391 with several studies that showed no effect of feeding levels during gestation on productive 392 performance (Dwyer et al., 1994; Ren et al., 2017a, 2018; Mallmann et al., 2019). 393 Furthermore, a higher level of feeding in hyper-prolific Large White gilts during early 394 gestation (50 vs. 25 MJ ME·d 1), 7 d after insemination, did not affect embryonic survival, size, or variability (Quesnel et al., 2010). There was also no effect of feeding 395 396 level on any of the litter weight variables tested. This lack of effect of increasing feeding levels during early gestation is in line with findings on the average weight of piglets at 397 birth (Hoving et al., 2011; Mallmann et al., 2019). Nissen et al. (2003) also found no 398 399 effect on average piglet birth weight when sows were fed ad libitum at different stages of gestation (25-50 and 25-70 days of gestation) compared to a control group (restricted 400

diet), suggesting no beneficial effect on fetal growth. However, several authors have 401 402 shown that increasing feeding levels during short periods throughout gestation or in late gestation increased piglet birth weight linearly or quadratically (Cromwell et al., 1989; 403 404 Ren et al., 2017b, 2018). Consequently, heavier piglets are generally expected to be associated with higher levels of feeding during late gestation, when fetal growth occurs. 405 406 Thus, the time during which higher levels of feeding were applied in our study could have 407 been determinant of a failure to influence fetal growth, since no differences in the average birth weight of piglets at birth were found. Differences in litter size and litter weight 408 409 among studies could be related to either the sow genotype or the feeding program, i.e., 410 the amount of feed (energy and/or nutrients supplied), the length of time of extra feed, 411 and the gestation period in which a higher feeding level is applied. On the other hand, an 412 increase above the standard restricted feeding level was associated with higher piglet 413 mortality at 72 h, although no differences in litter size or litter weight were found among treatment groups. When disaggregating the reasons recorded for early piglet mortality, 414 415 this finding was partly linked to a higher percentage of piglets culled at birth, which, in 416 turn, was also related to the proportion of piglets weighing less than 800 g. This 417 relationship between early mortality and the proportion of small piglets has been 418 previously reported in the literature (Quiniou et al., 2002), where a lower percentage of survival until weaning was observed in piglets weighing less than 1 kg (with more 419 stillbirths and more piglets dead within 24 h). Quiniou et al. (2002) also described an 420 421 increased proportion of smaller piglets in larger litters and a simultaneous decrease in average piglet birth weight and litter homogeneity, which had negative effects on piglet 422 423 viability. Thus, differences in early mortality and low-weight piglets of our study may also be in line with the trend found for the higher number of live born piglets from sows 424 fed the highest feeding level (+0.5 piglets), as well as the greater numerical variation in 425

within-litter weight of sows fed Treatments II and III compared to the control group
(+1.8%). In fact, within-litter birth weight variation is an economically important trait due
to its positive correlation with piglet losses from birth to weaning (Campos et al., 2012),
which was also previously described in hyper-prolific Large White sows by Wolf et al.
(2008). Furthermore, Moreira et al. (2020) recently reported, in a review and metaanalysis study, an increase in the coefficients of variation in piglet birth weight of around
4% for sows with high prolificacy compared to sows with low prolificacy.

433 **4.3 Reproductive Performance and Serum Hormonal Concentrations**

434 Our findings showed no differences among the three different levels of restricted 435 feeding applied in early gestation in terms of reproductive rates (p > 0.05); nevertheless, the farrowing rate was lower, albeit non-significantly, in sows fed Treatment III in 436 437 comparison with other groups (close to \Box 8%). It is worth noting that Hoving et al. (2011) also found that the increased feeding level (+30%) numerically reduced the 438 farrowing rate (\Box 13%). In contrast, Virolainen et al. (2004) showed that a high feeding 439 440 level during early gestation improved the percentage of pregnant gilts at day 34 post-441 insemination (54 vs. 27 MJ·d 1 for those 34 days using a commercial diet with 13 MJ 442 DE·kg \Box 1 and 14.5 g CP·kg \Box 1). However, this study was conducted with a reduced 443 number of gilts (three groups of eight females) of lower weight (7-8 months old and 127 kg of BW), and no data on body status were reported. These results emphasize the 444 445 importance of managing body reserves throughout gestation and at farrowing and their influence on fertility rates. In fact, further studies should examine how the farrowing 446 447 rate can be affected by increased feeding levels as a function of body reserves in new 448 leaner genotypes. Few studies on gestational feeding levels have provided information on the WEI. For instance, Whittemore et al. (1988) and Ren et al. (2017a, 2017b) found 449 450 no differences between sows fed different feeding levels, while Dourmad (1991)

reported that the WEI was extended in sows fed higher levels during gestation. 451 452 Furthermore, Whittemore et al. (1988) described a positive association by regression 453 analysis between readiness to rebreed (days from weaning to conception) and body fat at weaning, as well as with the BW of the sow at weaning. In this line, the different 454 455 results could be explained by the extent of BW and BF gains during gestation, body 456 reserves at farrowing and weaning, and the degree of body reserve mobilization. The 457 current findings show that there were no differences in the effects of dietary treatment groups on blood metabolites measured at day 28: progesterone, insulin, and cortisol. 458 When serum concentrations of different reproductive hormones were reported in studies 459 460 on feeding levels in early gestation, they mainly referred to progesterone and LH 461 (Virolainen et al., 2004). It is well known that the feeding level has a positive effect on the formation of luteal tissue and progesterone secretion in the pre-implantation period, 462 463 which is essential for endometrial remodeling (Langendijk and Peltoniemi, 2013). 464 Before implantation, these effects are independent of LH and probably triggered by 465 metabolic signals, such as insulin and IGF-1, but the potential of nutritional strategies in these pathways remains to be investigated (Langendijk, 2021). On the other hand, sow 466 467 stress (or elevated cortisol) before or during the pre-implantation phase is associated 468 with reproduction disruption (Du et al., 2020). Thus, there is evidence that stress may interfere with the release of LH at the pituitary level (Dobson and Smith, 2000). No 469 470 effect was found on leptin throughout gestation, regardless of dietary treatment, sow 471 parity order, or their interaction. To our knowledge, there are no data on the effect of the feeding level in early feed intake. In fact, leptin is recognized as a major regulator of fat 472 473 mass and energy balance during gestation (Grattan et al., 2007). In gestation, the placenta is the main source of leptin, which is an important factor linking metabolic 474 status to reproduction (Schneider, 2004). Prunier et al. (2001), who assessed two 475

feeding levels from day 23 of gestation (high (H) vs. medium (M)) in primiparous sows, 476 477 reported that leptin on days 4, 11, 18, and 25 of lactation was higher in H than in M 478 sows. Regarding leptin concentrations, Saleri et al. (2015), monitoring this hormone in Large White×Landrace multiparous sows, found that plasma levels increased from day 479 72 of gestation and remained high until farrowing, reaching the highest concentrations 480 at day 107 and at farrowing. These authors suggested that hyperleptinemia with 481 482 gestational progression in restrictively fed pregnant sows confirms the presence of 483 leptin resistance, which contributes mainly to allowing increased nutrient availability for the fetus. This increasing evolution of leptin concentrations throughout gestation are 484 485 in line with those described by Superchi et al. (2019) in groups of sows with high and 486 low BF, although no differences were found between them. However, the evolution described above differed from our results, as a higher concentration was found at mid-487 488 gestation (d60), and a lower concentration was found at days 0 (insemination) and 110 (pre-partum). These lower leptin levels in the peripartum period, also described by 489 490 Papadopoulos et al. (2009), could be related to the negative energy balance of sows due to the increased energy requirements in the last 12 days of gestation (up to 60% 491 492 according to Feyera and Theil (2017)). Therefore, the mechanisms by which leptin 493 concentrations are modified in pregnant sows need to be investigated.

494 **5. CONCLUSION**

495 No clear productive or reproductive benefits of increasing the amount of feed in early 496 gestation, other than an improvement in the body reserves of sows throughout gestation 497 and at weaning, were found. Therefore, increasing the level of restricted feeding during 498 early gestation to improve the short-term productive and reproductive performance of 499 sows remains controversial. Further research is needed to establish which feeding 500 strategy and what level of restricted feeding best fits the increasing demands of modern

- 501 genotypes and to assess how a restricted feeding level could affect the viability or
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506 ETHICAL STATEMENT

- 507 This study was carried out according to the protocol approved by the Animals
- 508 Experimentation Ethics Committee of the University of Murcia and the Authorities of the
- Region of Murcia (4 August 2017, No. A-13170805), and following the European Union
- 510 guidelines for the care and use of research animals (Directive 2010/63/EU of the EU
- 511 Parliament and of the Council of 22 September 2010 on the protection of animals used
- 512 for scientific purposes).

513 **RESEARCH DATA**

514 Data sharing is not applicable to this article.

515 CONFLICTS OF INTEREST

516 The authors declare no conflict of interest.

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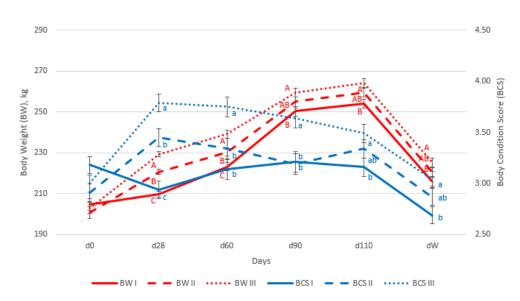
	Die	ets
Item	Gestation	Lactation
Ingredients, %		
Barley	45.00	18.77
Wheat flour, 30% starch	18.00	12.00
Sunflower seed 28	12.00	2.95
Sunflower seed 35	-	0.88
Wheat	6.69	32.00
Corn	6.00	10.00
Sugar beet	5.00	2.50
Colza 34	2.11	-
Soybean meal 47% CP	2.00	14.57
Calcium carbonate	1.35	1.43
Calcium phosphate	0.40	0.71
Fat 3/5	0.40	2.25
Salt	0.35	0.20
Vitamin-mineral premix 0.3 FIT EC ¹	0.30	0.30
L-lysine	0.24	0.41
Sodium bicarbonate	0.11	0.66
L-threonine	0.05	0.18
DL-Methionine	-	0.10
L-Valine	-	0.09
Composition calculated, % ²		
Net Energy, Mcal· kg ⁻¹	2.18	2.35
Crude Protein (CP)	13.72	16.80
Lys	0.72	1.06
Ca	0.80	0.85
Р	0.61	0.59

¹Nutrients supplied per kilogram: Vitamin A, 4,000,000 UI; Vitamin D₃, 666,666 mg; Vitamin E, 13,333 mg, Vitamin B₁₂, 6.67 mg, Vitamin B₆, 666.00 mg; Vitamin K₃, 333.00 mg; Vitamin B₁, 333.00 mg; Vitamin B₂, 1.33 mg; Folic acid 666.00 mg; Nicotinic acid 6.66 g; Pantothenate, 3.00 mg; Biotin, 83.00 mg; Choline Chloride , 41.67 g; Anhydrous betaine 19.20 g; Iron (Iron sulphate monohydrate), 33.33 g; Manganese (manganese oxide), 10.67 g; Selenium E₂ (Sodium selenite), 100.00 mg; Zinc (Zinc oxide), 30.00 g; Copper (copper sulphate), 3.33 g; Iodine (potassium iodate), 333.00 mg. ² According to FEDNA (2010).

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Table 1. Ingredients and composition of diets (as-fed basis).

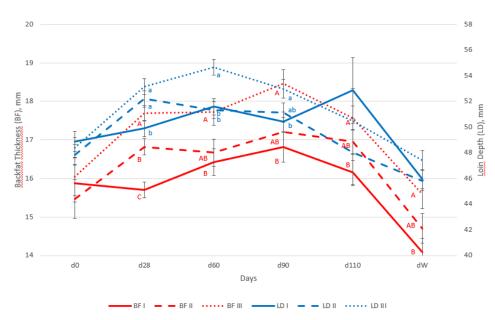
- Figure 1. Effect of the feeding level in early gestation on body weight (BW, red color),
- body condition score (BCS, blue color) (Figure 1A), backfat thickness (BF, red color),
- and loin depth (LD, blue color) (Figure 1B) of sows at Days 0, 28, 60, 90, and 110 and at
- 719 weaning (dW).



720 Figure 1A



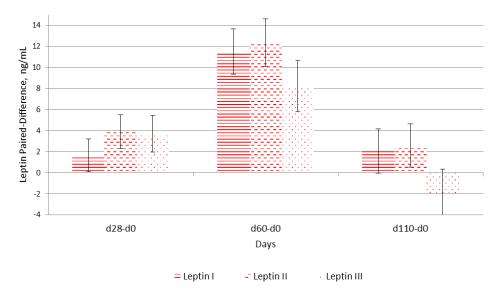




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Treatments I, II, and III: The feeding level from Day 3 to Day 28 of gestation was increased by 25 and 50% in Treatments II and III (dashed and dotted lines), respectively, in comparison to Treatment I (solid lines). Different capital and lowercase letters for left and right y-axis means, respectively, indicate significant differences at $p \le 0.05$ between treatment groups within a day.

Figure 2. Effect of the feeding level in early gestation on paired differences in leptin
concentration in relation to the initial concentration at Day 0 ([Leptin]_{d28}-[Leptin]_{d0},
[Leptin]_{d60}-[Leptin]_{d0}, and [Leptin]_{d110}-[Leptin]_{d0}).



732 Treatments I, II, and III: The feeding level from Day 3 to Day 28 of gestation was increased by 25 and 50%

- in Treatments II and III (bars with dashed and dotted lines), respectively, in comparison with Treatment I
- 734 (bar with solid lines). A non-significant effect of the feeding level was observed (p > 0.05).

	Fee	eding level ¹ (A	A)	Parity	$v^{2}(B)$	SEM ³		<i>P</i> -value		
	Ι	II	III	Р	М		А	В	AxB	
Sample size, sows	37	36	39	50	62					
Gestation changes										
BW, kg	50.25 ^b	56.60^{ab}	60.65 ^a	62.25	49.42	2.293	0.007	0.000	0.894	
BCS	0.00^{b}	0.38 ^{ab}	0.48^{a}	0.31	0.26	0.109	0.006	0.718	0.373	
BF, mm	0.54^{b}	1.41^{ab}	1.97ª	0.99	1.62	0.329	0.009	0.101	0.884	
LD, mm	0.04	0.18	2.14	1.65	-0.08	0.748	0.087	0.048	0.248	
Global balance ⁴	37	36	36	52	57					
BW, kg	12.67 ^b	18.35 ^{ab}	22.16^{a}	20.92	14.53	2.293	0.015	0.018	0.351	
BCS	-0.46 ^b	-0.10 ^a	0.03 ^a	-0.22	-0.13	0.100	0.002	0.448	0.413	
BF, mm	-1.61 ^b	-0.92 ^{ab}	-0.08 ^a	-1.58	-0.16	0.368	0.015	0.001	0.917	
LD, mm	-2.52	-2.28	-0.92	-1.76	-2.05	0.808	0.324	0.762	0.293	

Table 2. Effect of the feeding level in early gestation and parity (primiparous vs. multiparous) on body weight (BW), body condition score (BCS), backfat thickness (BF), and loin depth (LD) changes in gestation (d110-d0) and the global balance of sows.

¹ Treatments I, II, and III: The feeding level from Day 3 to Day 28 of gestation was increased by 25 and 50% in Treatments II and III, respectively, in comparison with Treatment I (2.5 kg·d⁻ ¹). ² Parity: P (primiparous) and M (multiparous) sows.

³ SEM: standard error of the mean.

⁴ Changes from Day 0 until weaning (calculated as dW-d0).

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	Fe	eeding Leve	$el^{1}(A)$	Parit	$\mathbf{x}\mathbf{y}^{2}$ (B)	SEM ³		<i>P</i> -value		
	Ι	II	III	Р	М		А	В	A*B	
Sample size, litters	38	37	39	52	62					
Litter size										
Total born, n	15.21	15.39	16.00	15.77	15.30	0.527	0.532	0.442	0.585	
Live born ⁴ , n	14.57	14.59	15.05	14.94	14.54	0.164	0.069	0.036	0.716	
Stillborn ⁴ , n	0.67	0.84	0.40	0.52	0.76	0.135	0.071	0.124	0.570	
Mummies ⁴ , n	0.28	0.08	0.07	0.06	0.22	0.076	0.103	0.070	0.543	
Piglets alive at 72 h ⁴ , n	13.99	13.37	13.60	13.79	13.52	0.133	0.158	0.312	0.456	
Litter weight										
Total born weight ⁴ , kg	22.92	22.46	22.14	22.62	22.39	0.466	0.497	0.663	0.351	
Average live born weight ⁴ , kg	1.53	1.50	1.47	1.50	1.50	0.031	0.402	0.949	0.308	
Within-litter variation ⁴ , %	19.92	21.70	21.77	21.27	21.00	0.009	0.275	0.800	0.277	
Mortality and low birth weight										
rates ⁵										
Mortality rate at birth, %	4.14	5.23	2.73	3.29	4.62	0.485	0.170	0.117	0.412	
Mortality rate at 72 h, %	2.96 ^a	8.26 ^b	9.93 ^b	7.59	5.20	0.693	0.000	0.086	0.045	
Piglet culling rate at birth ⁶ , %	2.12 ^a	5.04 ^b	5.46 ^b	4.82	3.14	0.535	0.029	0.120	0.010	
Low birth weight rate ⁷ , %	2.85 ^a	4.99 ^b	6.76 ^b	4.49	4.70	0.547	0.013	0.845	0.050	

Table 3. Effect of the feeding level in early gestation and parity (primiparous vs. multiparous) on sow productive performance, i.e., the litter size and litter weight of sows, and on mortality and low birth weight rates within litters.

¹ Treatments I, II, and III: The feeding level from Day 3 to Day 28 of gestation was increased by 25 and 50% in Treatments II and III, respectively, in comparison with Treatment I (2.5 kg·d⁻¹).

² Parity: P (primiparous) and M (multiparous) sows.

³ SEM: standard error of the mean.

⁴ Adjusted mean: Total born was used as a covariate for these variables in the statistical model.

⁵ Data analyzed by logistic regression.

⁶ Piglets culled at birth, as they are unlikely to survive and unable to reach market weight at the same rate as normal-weight littermates.

⁷ Piglets with low weight at birth (alive born weighing less than 0.8 kg)

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	Fe	Feeding Level ¹ (A)			\mathbf{y}^{2} (B)	SEM ³	<i>P</i> -value		
	Ι	II	III	Р	М	-	А	В	A*B
Sample size, sows	42	41	47	61	69				
Conception rate ⁴ , %	93.24	95.12	92.39	90.97	95.62	2.235	0.878	0.306	0.731
Farrowing rate ⁴ , %	90.48	91.41	82.75	85.70	91.15	2.964	0.414	0.360	0.722
WEI, days	4.00	4.09	4.34	4.29	4.00	0.184	0.397	0.180	0.397

Table 4. Effect of the feeding level in early gestation and parity (primiparous vs. multiparous) on sow reproductive performance: fertility rates and weaning-to-estrus interval (WEI).

¹ Treatments I, II, and III: The feeding level from Day 3 to Day 28 of gestation was increased by 25 and 50% in Treatments II and III, respectively, in comparison with Treatment I (2.5 kg/d).

² Parity: P (primiparous) and M (multiparous) sows.

³ SEM: standard error of the mean.

⁴ Data analyzed by logistic regression.

Table 5. Effect of the feeding level and parity (primiparous vs. multiparous) on some hormonal parameters: the progesterone, insulin, and cortisol of sows.

	Feeding level ¹			Par	rity ²	SEM ³	<i>P</i> -value		
	Ι	II	III	Р	М	-	А	В	A*B
Sample size, sows	10	10	10	15	15				
Hormonal parameters									
Progesterone, ng⋅mL ⁻¹	15.40	18.97	17.32	16.57	17.89	1.206	0.133	0.358	0.011
Insulin, µUI·mL ⁻¹	48.39	57.91	43.48	54.47	45.39	4.729	0.460	0.346	0.222
Cortisol, µg·mL ⁻¹	2.67	2.86	2.94	2.73	2.94	0.279	0.919	0.712	0.475

¹ Treatments I, II, and III: The feeding level from Day 3 to Day 28 of gestation was increased by 25 and 50% in Treatments II and III, respectively, in comparison with Treatment I (2.5 kg/d). ² Parity: P (primiparous) and M (multiparous) sows. ³ SEM: standard error of the mean.

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