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Consequences of rearing feeding programme on the performance of rabbit females from first to second parturition

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Consequences of rearing feeding programme on the performance of rabbit females from first to second parturition

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Short title: Effects of rearing feeding programme in primiparous rabbit females

Abstract

To evaluated how rearing programmes could affected resources allocation and reproductive performance of primiparous rabbit females, a~~A~~ total of 118 rabbit females were used to evaluate the effects of five rearing feeding programmes on their performance from first to second parturition: CAL, fed *ad libitum* C diet (11.0 MJ digestible energy **(DE)**, ~~and~~ 114 g digestible protein **(DP)** and 358 g neutral detergent fibre **(NDF)**/kg DM) until first parturition; CR, fed *ad libitum* with C diet until 12 weeks of age and then C diet restricted (140 g/day) until first parturition; F,

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fed *ad libitum* with F diet (8.7 MJ DE, ~~and~~ 88 g DP and 476 NDF/kg DM) until first parturition; FC, fed with F diet *ad libitum* until 16 weeks of age, and C diet *ad libitum* until first parturition; FCF, fed with F diet *ad libitum* until 16 weeks of age, then C diet *ad libitum* until 20 weeks and then F diet *ad libitum* until first parturition. From first parturition, C diet was *ad libitum* offered to all the experimental groups until second parturition. CAL females presented ~~significantly~~ lower feed intake than females of F, FC and FCF groups in the first week of lactation (on av. –16.6%; P<0.05). During first lactation, the perirenal fat thickness change in CAL females was not ~~significantly~~ different from zero (+0.02 mm), while in the other four groups it increased (on av. +0.44 mm; P<0.05). Plasma of females fed with F diet during rearing (F, FC and FCF) had lower non-esterified fatty acids content than those exclusively fed with C diet (–0.088 and –0.072 mmol/L compared to CAL and CR, respectively; P<0.05). FCF litters had ~~significantly~~ higher weight than F litters at day 21 of lactation (+247 g; P<0.05), but FCF litter had significantly lower weight than FC litters at weaning (+170 g; P<0.05). CR females had the shortest average interval between the first and second parturition (49 days) and FCF females the longest (+ 9 days compared to CR; P<0.05). At second parturition, liveborn litters of F females were ~~significantly~~ larger and heavier than litters of FCF females (+2.22 kits and +138 g; P<0.05), probably due to the lower mortality at birth of F litters (–16.5 percentage points; P<0.05). In conclusion, rearing females on fibrous diets seems to increase the ability of primiparous rabbit females to obtain resources, especially at the onset of lactation.

Keywords: *Oryctolagus cuniculus, rearing programmes, fibrous diet, body condition, metabolic status, resources allocation.*

Implications

Obtaining well-developed rabbit females that produce a large number of healthy and marketable litters per mating over several parities is still one of the main priorities in rabbit production. This objective not only involves the use of suitable management programmes during reproduction, but also appropriate management of nutrition during pre- and post-pubertal growth to ensure adequate development of the future reproductive female. In this sense, the design of rearing programmes that consider the young rabbit female's nutritional requirements and priorities, while "training" their future ability to obtain and manage the available resources, is expected to help farmers achieve their reproductive objective.

Introduction

In a previous work (Martínez-Paredes *et al*., 2012), we were able to confirm that the *ad libitum* use of energetic reproduction diets during rearing had negative effects on young rabbit females until first parturition, such as higher risk of digestive troubles (Rommers *et al*., 2004) and gestational toxaemia (Viudes-de-Castro *et al*., 1991 and Rosell, 2000), smaller litter size at first parturition, probably due to a misuse of the available resources (both feed and body reserves), and inappropriate physiological development.

On the other hand, we verified that alternatives, such as restriction and some programmes based on high-fibre diets, allowed them to reach an adequate degree of maturity, without prejudice to the rabbit female or the first litter, when an adequate flushing was applied around first artificial insemination **(AI)**, as well as a greater uptake of resources during pregnancy (Pascual *et al*., 2002 and Manal *et al*., 2010). However, these improvements would have less impact if the benefits do not remain in the medium and long term, improving the further reproductive performance of rabbit females (feed intake, milk yield, litter size, survival...). Nonetheless, the number of works that have attempted to elucidate the effects of the restriction or use of fibrous diets on subsequent reproductive performance are few and present variable results. Rebollar *et al*. (2011) did not register improvements in feed intake during the first lactation when young rabbit females were restricted during rearing. Other works also failed to show improvements in the feed intake of primiparous lactating females when fibrous diets were used during rearing (Quevedo *et al*., 2005; Verdelhan *et al*., 2005; Rebollar *et al*., 2011). However, another of these works did report an improvement in feed intake capacity, which was addressed to recovery of reserves (Xiccato el al., 1999) or to milk yield promotion (Pascual *et al*., 2002). In the long term, some works (Nizza *et al*. 1997; Martínez-Paredes *et al*., 2018) have observed slight improvements in litter performance at birth or during lactation in females reared on a fibrous diet.

For a better understanding of the consequences that these rearing feeding programmes can have on the future reproductive capacity of our rabbit females, it is essential to assess the changes entailed by their implementation on the ability to obtain resources and their partition among the different vital functions of the

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females. To this end, the aim of the present work was to evaluate how five different feeding rearing programmes used in a previous work (Martínez-Paredes *et al.*, 2012) could have affected resources allocation and reproductive performance of rabbit females from first to second parturition.

Material and methods

Composition of experimental diets

Two experimental diets were formulated and pelleted. A control diet (**C**), similar to a commercial diet for reproductive rabbit does [11.0 MJ digestible energy **(DE)**, 114 g digestible protein **(DP)** and 358 g neutral detergent fibre **(NDF)**/kg ~~dry matter~~ (DM)], was formulated following the main nutritional recommendations of De Blas and Mateos (2010). In addition, a low-energy high-fibre diet (**F**) was also formulated (8.7 MJ DE, 88 g DP and 476 g NDF/kg DM). Details of ingredients and chemical composition of both diets can be seen in ~~Martínez-Paredes *et al.* (2012) and in supplementary~~ Table ~~S~~1. Methods for chemical analysis and *in vivo* determination of DE and DP of both diets can be consulted in Martínez-Paredes *et al*. (2012).

Animals and experimental procedure

In the present work, 118 rabbit females (line A of the Universitat Politècnica de València; **UPV**), which achieved the first parturition in a previous work (Martínez-Paredes *et al*., 2012), were controlled from first to second parturition. In this previous work, 190 young rabbit females were subjected to five different feeding programmes from 9 weeks of age to first parturition (Figure 1). In brief, C group

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was fed C diet *ad libitum* until first parturition; CR group was fed C diet *ad libitum* until 12 weeks of age and then 140 g/day until first parturition, with a 7-day *ad libitum* flushing period around the first AI; F group was fed F diet *ad libitum* until first parturition; FC group was fed F diet until 16 weeks of age and then C diet until first parturition, both *ad libitum*; and FCF group was fed F diet until 16 weeks of age, then C diet until 11 days of pregnancy, and finally F diet until first parturition, all of them *ad libitum*. Animals were housed in a traditional building under controlled environmental conditions, with light alternating in a cycle of 16 h light and 8 h dark. For more details of management and results with the different feeding programmes throughout the rearing period, see Martínez-Paredes *et al.* (2012).

At first parturition, litters were standardised to nine kits and all groups were *ad libitum* fed on C diet until second parturition. Rabbit females were AI at 11 days after the first parturition and successive AIs were carried out every 21 days, as necessary. Ar-tificial insemination ~~I~~ was performed using polyspermic semen (line R of UPV), supplying gonadotropin-releasing hormone **(GnRH)** hormone by intramuscular injection. Pregnancy was tested by manual palpation at 11 days after AI. Litter was weaned at 28 days of age. At the 28th day of pregnancy, a nest equipped for the litter was provided.

The traits measured for all females were body weight and feed intake, weekly during the first lactation and at second parturition, as well as perirenal fat thickness **(PFT)** by ultrasound at first parturition, AI, weaning and second parturition. Daily milk production was measured using the weight(doe)-suckle-weight(doe) method. To prevent free nursing, nest boxes were closed between nursings from first

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parturition to 21 days of age. From this moment to weaning, litters were housed in a cage close to their mother to control milk production of the female and ~~litter~~ feed consumption of the litter. Two milk samples were collected on days 4 and 21 of the first lactation from 12 rabbit females per group, following the methodology described by Pascual *et al.* (1999). Litter size and weight were controlled at first parturition after standardisation and weekly until first weaning. Mortality was recorded daily. The interval from first to second parturition of rabbit females and the total and live size and weight of litters at second parturition were recorded. From the same 12 rabbit females per group, blood samples were collected at first parturition, AI, weaning and second parturition. On sampling day, feeders were closed at 0700 h and blood samples were taken from the central ear artery into ethylenediaminetetra-acetic acid ~~**(EDTA)**~~ -containing tubes from 1100 to 1300 h. Blood samples were centrifuged immediately after sampling (3 000 g, 4ºC and 10 min) and plasma was stored at -20ºC before being assayed for insulin, glucose, non-esterified fatty acids **(NEFA)**, leptin, cortisol and tri-iodothyroxine **(T3)** concentrations.

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Ultrasound measurements

The PFT of females was measured to evaluate body condition, as described by Pascual *et al.* (2000 and 2004). Images were obtained with an ultrasound unit (JustVision 200 'SSA-320A' real-time machine; Toshiba) equipped with image analyser software to determine ~~distances~~thickness measurements.

Hormone and metabolite assays

Plasma insulin concentrations were determined by the double antibody/ polyethylene glycol ~~PEG~~ technique using porcine insulin radioimmunoassay **(RIA)** kit (Linco Research Inc., St Charles, MO, USA). The antiserum was guinea pig anti-porcine insulin, while both labelled antigen and standards used purified recombinant human insulin. Glucose was analysed by the glucose oxidase method using the Glucose Infinity kit from Sigma (Sigma Diagnostic Inc., St. Louis, MO, USA). NEFA concentrations were analysed using enzymatic colorimetric assay from Wako (Wako Chemicals GmbH, Neuss, Germany) as previously reported (Brecchia *et al*., 2006). Leptin concentrations were determined by double antibody RIA using the multi-species leptin kit (Linco Research Inc.) as previously reported (Brecchia *et al*. 2006). Plasma cortisol was assayed by RIA, using the CORT kit (ICN Biomedicals Inc., Costa Mesa, CA, USA). CORT assay sensitivity was 0.15 ng/mL. Finally, total T3 was assayed by RIA according to the procedure provided by the manufacturer (Immunotech, Marseille, France). The assay sensitivity was 0.13 ng/mL, and the major analogues of T3 did not interfere with the assay. Dilution and recovery tests performed on insulin, leptin, T3 and corticosterone using five different samples of rabbit plasma showed linearity.

Milk chemical composition

Milk samples were analysed for total solids, ash, protein and energy. Total solids and ash contents of milk were obtained using the Association of Official Analytical Chemist ~~(AOAC)~~ (1999) methods ~~(1999)~~. Milk protein content was calculated by the Kjeldahl method according to FIL Standard: 20B~~8~~ (Federation Internationale de

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Lacterie, 1993). Adiabatic bomb calorimetry method was used to determine the energy content of lyophilised milk.

Statistical analysis

The model used to analyse performance, hormonal and metabolic data and milk composition of rabbit females from first to second parturition and litter weight throughout first lactation was a mixed model (PROC MIXED by SAS, Statistical Analysis System, 2002), in a repeated measure design that considered the variation between animals and covariation within them. Covariance structures were objectively compared using the Schwarz Bayesian criterion, as suggested by Littell *et al.* (1998). The model included the feeding programme (CAL, CR, F, FC and FCF), the overlapping between lactation and gestation (yes and no), the time (control levels for each trait) and their interaction as fixed effects. Random terms in the model included a permanent effect of each animal (p) and the error term (e), both assumed to have an average of zero, and variance σ^2_p and σ^2_e.

To analyse the ~~litter~~ solid feed intake of litter during last week of first lactation, interval between first weaning to second parturition and litter data at second parturition, a general linear model was used (PROC GLM of SAS, 2002) that included the feeding programme (CAL, CR F, FC and FCF) and the overlap between lactation and gestation (yes and no).

Different contrasts were computed to test the significance of the differences between treatments, CAL *vs.* CR, CAL *vs*. Fs and CR *vs*. Fs, Fs being 1/3[F+FC+FCF].

Results

No significant differences among rearing feeding programmes for the evolution of females' body weight were observed from first to second parturition (on av. 4 100±59 g). Figure 2 shows the evolution of the rabbit females' feed intake from first to second parturition depending on the rearing feeding programme received. CAL group females presented significantly lower feed intake than females from groups F, FC and FCF during the first week of lactation (on av. −38.8 g DM/d; P<0.05). In addition, FCF females showed significantly higher feed intake compared to the rest of the groups during this first week (+65.9, +42.3, +29.5 and +36.5 g DM/d compared to CAL, CR, F and FC, respectively; P<0.05). From this moment to second parturition, differences in daily feed intake among groups disappeared, with the exception of F group, which showed the lowest values at the second week of lactation (on av. −29.4 g DM/d; P<0.05). In the whole period, FCF females had a significantly higher feed intake than CAL females (+19.7±7.4 g DM/d; P=0.0088).

Figure 3 shows the PFT change in rabbit females throughout the first lactation and from first to second parturition. During first lactation, the PFT change in CAL group was not significantly different from zero (+0.02 mm PFT), while the other four groups increased PFT (on av. +0.44 mm; P<0.05). In fact, the PFT increase in CR during lactation was significantly higher in CAL females (+0.55 mm of PFT; P<0.05). From first to second parturition, CAL females showed a significantly different PFT change compared to FC females (–0.24 and +0.29 mm, respectively; P<0.05), while the other four groups kept PFT between parturitions.

Females' milk yield during first lactation is shown in Table ~~1~~2. On average, FCF females produced more milk than CAL and F females (+10 and +13 g/d, respectively; P<0.05). Weekly, FC and FCF females yielded more milk than F females at the second week (+22 g/d; P<0.05) and FCF females to CR and F females at the third week (on av. +18 g/d; P<0.05). Milk composition at days 4 and 21 of first lactation is also presented in Table 2. Milk from CR females had more total solids (+4.6 and +2.7 g/100g at days 4 and 21, respectively; P<0.05) and lower ash contents (–0.22 g/100g at day 21; P<0.05) than the milk of the other four groups. At day 4 of lactation, F females produced less milk protein than FC and FCF (on av. –2.25 g/d; P<0.05) and less milk energy than FC (–0.21 MJ/d; P<0.05). However, at day 21 of lactation, milk of CR females had higher energy and protein content than FCF milk (+1.0 g/100g and +1.37 MJ/kg, respectively; P<0.05).

Average content of blood plasma parameters in the rabbit females from first to second parturition is shown in Table 3. Interaction between rearing feeding programme and time was not significant for any blood plasma trait. There were no significant differences in the insulin, leptin and cortisol content among the experimental groups (on av. 16.03 μUI insulin/mL, 2.95 ng leptin/mL and 4.6 μg cortisol/dL). Plasma of FC blood had higher glucose than CAL and FCF (+19.0 and +16.3 mg/dL, respectively; P<0.05). Plasma of females fed with F diet during rearing (F, FC and FCF) had lower NEFA content than those with C diet (–0.088 and –0.072 mmol/L compared to CAL and CR, respectively; P<0.05). Particularly, NEFA content was the lowest in F females and the highest in CAL females (P<0.05). Finally, plasma T3 content of the CAL, CR and FCF blood were significantly higher than for FC (on av. +0.43 mmol/L; P <0.05).

Table 4 shows the performance traits of litters during the first lactation. No significant differences were observed in litter mortality. After litter size standardisation at birth, no significant differences in litter weight at 1st, 7th and 14th day of lactation were observed. However, FCF litters had significantly higher weight than F litters at day 21 of lactation (+247 g; P<0.05). On the contrary, the FCF litter had significantly lower weight than FC litters at weaning (+170 g; P<0.05). No significant differences among groups were observed for litter feed intake during the last week of lactation.

Finally, the reproductive performance of rabbit females at second parturition according to rearing feeding programme is described in Table 5. CR females had the shortest interval between the first and second parturition (49 days), significantly different from that obtained for FCF females (–9 days; P<0.05). F females had a significantly higher number of kits born alive at second parturition compared to FCF females (+2.22 kits; P<0.05), probably due to the lower mortality at birth of F litters compared to FCF (–16.5 percentage points; P<0.05), but also compared to CR (–20.8 percentage points; P <0.05). Consequently, F litters had a significantly higher liveborn weight at second parturition than FCF litters (+138 g; P <0.05).

Discussion

The interest of specific rearing feeding programmes mainly lies in providing adequate resources to correctly cover the females' requirements (maintenance, growth and gestation), avoiding possible deficits or excesses (Pascual *et al*., 2013). A good rearing programme choice should promote an adequate physiological and reproductive development of the females, which should allow a good start to their reproductive life (Martínez-Paredes *et al*., 2012); but it should also improve the way they obtain and use the available resources, which could have positive effects on their reproductive capacity and lifespan (Martínez-Paredes *et al*., 2018). In our previous work (Martínez-Paredes *et al*., 2012), we described the effects of these same rearing programmes on the development of young rabbit females up to the first parturition. In that study, we observed that programmes based on feed restriction or fibrous diets reduced the risk of early death in females and led to achieving an adequate weight and fat mass at first AI, a reserve that was further used to ensure reproduction. On this basis, the present work was focused on how these rearing programmes could also have modified the way females acquire and use the resources available during their first reproductive cycle.

In order to better understand the effects observed from first to second parturition depending on the feeding programme applied during rearing, we decided to discuss each of the feeding programmes separately, to achieve a better view of the evolution of the rabbit females, with results from the previous work (Martínez-Paredes *et al*., 2012) as starting point.

In the previous work, CAL females were characterised by an overweight at the first AI and a smaller litter size at first parturition. As in previous works (Nizza *et al*.

1997; Pascual *et al*., 2002), we observed that females' *ad libitum* fed with a non-fibrous diet showed significantly lower feed intake during the first lactation, especially during the first weeks. Excessive overweight during the first gestation has been associated with a reduction in feed intake late in pregnancy, which seems to be maintained at least during the onset of the first lactation (Pascual *et al*., 2002 and the present work), as differences disappeared thereafter. As a consequence of their reduced ability to obtain resources, CAL females showed the lowest milk output and PFT recovery during first lactation. Blood metabolites confirmed this acquisition and use pattern, with CAL females showing both the lowest glucose and the highest NEFA and T3 concentrations in plasma, in agreement with previous works (Savietto *et al*., 2014; Arnau-Bonachera *et al*., 2018). Although the reduced resources acquisition in first lactation did not affect the reproductive performance of the CAL females at second parturition, the use of this rearing programme may lead primiparous females to suffer a higher negative balance in their body condition, with their possible associated risks in the long term (Pascual *et al*., 2013).

During rearing, CR females accomplished their performance goals, achieving an adequate energy feed intake and body reserves balance, without affecting fertility and litter size at first parturition. In the present work, restriction during the rearing period allowed CR females to show a good body balance during first lactation, which resulted in ~~an improvement in fertility and~~ a reduction in the interval between parturitions. Moreover, we reported no relevant differences in the ability to acquire resources or to use them to produce milk yield when compared to CAL females. Similarly, Bonnano *et al*. (2004) did not find differences in milk yield between females restricted and *ad libitum* fed during the rearing period. In fact, the plasma metabolites profile was similar to that of the CAL group, characterised by low glucose and high NEFA and T3 levels compared to Fs groups. As is well known, rich starch diets promote insulin sensitivity, and consequently glucose infusion rate (Daly *et al*., 1997). However, the shortest interval between parturitions had negative consequences on the body reserves recovery time, which could also explain the high levels of NEFAs and T3 in CR females. These levels denote a greater mobilisation of the acquired reserves, which may be behind the high mortality at birth observed among the litters of CR females at the second parturition.

In our previous paper, F diet allowed young females to increase their intake capacity already during the rearing period, without any noticeable negative consequence on the reproductive outcomes at first parturition. As a consequence of these effects, most works (Nizza *et al*. 1997; Xiccato *et al*., 1999; Pascual *et al*., 2002) have observed an increase in feed intake during first lactation when females were fed with high-fibre diets, compared to commercial diets given *ad libitum*, during the rearing. In the present work, F females only showed higher feed intake during the first week of lactation compared to CAL females, but quite similar to CR females during the first lactation. In any case, receiving a poor diet (rich in fibre and low in starch) throughout rearing may have induced physiological changes in how females may address the acquired resources to the different life functions. Friggens *et al*. (2011) proposed that the nutritional environment may slightly affect gene expression and thus genetically driven partition of nutrients to the different life functions. Therefore, although the F and CR females showed similar resources acquisition and body condition during first lactation, the metabolism of the F females seems to be less dependent on the body reserves to ensure reproduction (lower NEFA levels to CAL and CR groups). In fact, the discrete lower feed intake observed at the second week of lactation in F females, and their possible tendency to safeguard reserves, had as consequences both low milk delivery and low effectiveness in the insemination at that week. Perhaps the females' safeguarding of reserves could also be behind the larger litter size and lower mortality at second parturition of F litters. In fact, Martínez-Paredes *et al*. (2018) described long-term reduced numbers of stillborn and offspring that died during lactation in females fed with a F diet during rearing.

In our previous work, F females that were changed to C diet at two weeks before first AI (FC) showed higher energy intake from that moment onwards and, as a consequence, higher body reserves than F females at the first AI, but similar performance at the first parturition. This feeding programme allowed FC females to show similar feeding and body reserves patterns during the first lactation to that obtained with the F programme, as well as to undergo a similar homeorhetic change to safeguard their body reserves. However, earlier introduction of C diet could have led to additional changes in the females' metabolism and improved adaption to the reproductive feed. This fact can be shown by the promotion of milk metabolism (higher plasma glucose level, milk energy and protein delivery and litter performance) compared to maintenance (reduced T3 level) from similar available resources, especially at the onset of lactation. This preferential use of the energy intake for milk may explain why the litter performance observed at the second parturition for F females was not achieved by the FC females.

Finally, in our previous work, F females fed with a flushing with C diet around first AI (16 to 20 weeks of age; FCF) had the best performance litter traits at first parturition. As a consequence of the larger litter size at birth and/or the adequate feeding management during rearing period, FCF females did achieve one of the main goals proposed for these programmes, an increase in the ingestion capacity during the first lactation (Pascual *et al*., 2013). FCF females showed the highest feed intake observed during the first lactation, even compared to F females during the first two weeks. Although PFT evolution and plasma energy metabolites were not much different from that observed for the other F groups, the higher feed intake observed in FCF was directly addressed to a clear increase in milk yield and litter growth until the third week of lactation. However, diverting the acquired energy mainly to lactation came with some costs, such as a longer interval between parturitions and the lowest number of kits born alive at the second parturition. In this sense, some previous works have also observed that the use of F diets during rearing has been associated with an increased feed intake and milk yield during lactation of both primiparous and multiparous females (Nizza *et al*.,1997), but no negative effects on litter performance at birth have been described in the long term (Nizza *et al*.,1997; Pascual *et al*., 2002; Martínez-Paredes *et al*., 2018).

Conclusions

The results of the present work have confirmed that the possible overweight at the end of the rearing period ~~of~~when young rabbit females *ad libitum* fed with reproductive commercial diets ~~during the rearing period~~ seems to have negative consequences~~, not only at first parturition but also~~ until the second parturition~~in the middle term~~. This *ad libitum* programme decreases primiparous females' ability to obtain resources and leads them to suffer possible negative body balances~~, which could be associated with the long-term risks frequently described for these rearing programmes~~. ~~With this programme, t~~The restriction of these reproductive diets during rearing to avoid the cited overweight, although it did not increase the ability of primiparous females to obtain resources, led females to a better energy balance. As an alternative, three different rearing programmes based on the use of a high-fibre low-energy diet have been proposed. ~~In a previous work, Martínez-Paredes *et al*. (2012) demonstrated the interest of these fibrous programmes for improving health, body condition and performance of nulliparous rabbit females. In this work, w~~We have confirmed the usefulness of these fibrous programmes to increase the ability of primiparous females to obtain resources, especially at the onset of their first lactation and when a previous flushing was applied around first insemination. In addition, the use of these low-energy rearing diets seems to provoke homeorhetic and metabolic changes in females' resources use, which enables females to be less dependent on their body reserves for reproduction. In this way, the additional feeding intake was mainly addressed to milk yield, and although the greater lactational effort could affect next litter size at birth, other works have confirmed that fibrous rearing programmes do not seem to have effects on reproduction in the long term.

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Declaration of interest

Author declares no conflict of interest of any sort.

Ethics statement

All experimental procedures were approved by the Animal Welfare Ethics Committee of the ~~Universitat Politècnica de València (~~UPV~~)~~, which follows Spanish Royal Decree 1201/2005 on the protection and use of animals for scientific purposes and carried out following the advice for applied nutrition research in rabbits according to the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005).

Software and data repository source

Data is property of the ~~Universitat Politècnica de València~~UPV and may be available from the authors upon request.

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Table 1 *Ingredients and chemical composition of experimental diets for rabbit females*.

	Diet C	Diet F
Ingredient (g/kg)		
Barley	312	78
Alfalfa hay	450	570
Sunflower meal	94	51
Soybean meal	85	-
Sugar beet pulp	-	152
Cereal straw	-	100
Soybean oil	30	10
HCl L-lysine, 780	2	3.9
DL-methionine, 990	-	0.85
L-threonine, 980	-	1.45
L-tryptophan, 980	1	1.5
L-Arginine, 990	-	4
Dicalcium phosphate	17	1.8
Monosodium phosphate	-	16.5
Salt	5	5
Vitamin-mineral mixture[1]	4	4
Chemical composition (g/kg DM)		
Dry Matter (DM, g/kg)	899	900
Ash	90	103
Starch	205	63
Ether Extract	52	29
Crude Protein	179	146
Neutral Detergent Fibre	358	476
Acid Detergent Fibre	277	394
Acid Detergent Lignin	59	88
Gross Energy (MJ/kg DM)	18.24	18.67
Digestible Energy (DE; MJ/kg DM)[2]	11.03	8.72
Digestible Protein (DP; g/kg DM)[2]	114	88
DP/DE (g/MJ)	10.3	10.1

[1] Per Kg of feed: Vitamin A: 8 375 IU; Vitamin D3: 750 IU; Vitamin E: 20 mg; Vitamin K3: 1 mg; Vitamin B1: 1 mg; Vitamin B2: 2 mg; Vitamin B6: 1 mg; Nicotinic acid: 20 mg; Choline chloride: 250 mg; Mg: 290 mg; Mn: 20 mg; Zn: 60 mg; I: 1.25 mg; Fe: 26 mg; Cu: 10 mg; Co: 0.7; Butyl hydroxylanysole+ethoxiquin: 4 mg.

[2] *In vivo* determination of DE and DP was performed in Martínez-Paredes *et al*. (2012).

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Table 1 *Average milk yield (g/d) of rabbit females at first parturition according to rearing feeding programme.*

	Rearing feeding programme[1]							Contrasts[2]		
	CAL	CR	F	FC	FCF	SEM	P-value	CAL–CR	CAL–Fs	CR–Fs
No. of litters	18	23	25	26	26					
Milk yield:	172^{a}	174^{ab}	169^{a}	176^{ab}	182^{b}	5	0.0018	-2.4 ± 4.4	-4.3 ± 3.5	-1.9 ± 3.5
1st week	119	121	122	127	131	6	0.1512	-3 ± 9	-8 ± 7	-5 ± 7
2nd week	185^{ab}	183^{ab}	170^{a}	192^{b}	192^{b}	6	0.0092	2 ± 9	1 ± 7	-1 ± 7
3rd week	208^{ab}	204^{a}	206^{a}	212^{ab}	223^{b}	6	0.0221	4 ± 9	-6 ± 7	-10 ± 7
4th week	175	187	178	175	181	6	0.1443	-13 ± 9	-4 ± 7	9 ± 7

[1] Rearing feeding programme: CAL group received the C diet *ad libitum* until first parturition; CR group received the C diet *ad libitum* until 12 weeks and then, 140 g/day until first parturition; F group received the F diet *ad libitum* until first parturition; FC and FCF group received F diet *ad libitum* until 16 weeks and then, FC group received the C diet *ad libitum* until first parturition and FCF group the C diet *ad libitum* until 20 weeks and then the F diet *ad libitum* until first parturition.

[2] Fs: 1/3[F+FC+FCF]; mean±standard error.

SEM: Pooled standard error of the means.

^{a,b} Means within a row not sharing any superscript are significantly different at P<0.05.

Table 2 *Average milk yield and composition of rabbit females at ~~4th and 21st day of lactation~~first lactation according to rearing feeding programme.*

		Rearing feeding programme[1]							Contrasts[2]		
~~Day of lactation~~		CAL	CR	F	FC	FCF	SEM	P-value	CAL–CR	CAL–Fs	CR–Fs
No. of females		18	23	25	26	26					
Milk yield:		172^{a}	174^{ab}	169^{a}	176^{ab}	182^{b}	5	0.0018	-2.4 ± 4.4	-4.3 ± 3.5	-1.9 ± 3.5
1st week		119	121	122	127	131	6	0.1512	-3 ± 9	-8 ± 7	-5 ± 7
2nd week		185^{ab}	183^{ab}	170^{a}	192^{b}	192^{b}	6	0.0092	2 ± 9	1 ± 7	-1 ± 7
3rd week		208^{ab}	204^{a}	206^{a}	212^{ab}	223^{b}	6	0.0221	4 ± 9	-6 ± 7	-10 ± 7
4th week		175	187	178	175	181	6	0.1443	-13 ± 9	-4 ± 7	9 ± 7
Day of lactation											
Day 4	No. of females	12	12	11	11	12					
	Total solids (g/100g)	31.9^{a}	36.4^{b}	32.7^{ab}	31.6^{a}	31.0^{a}	1.5	0.0185	-4.5 ±2.1*	0.2 ±1.6	4.6 ±1.9*
	Ash (g/100g)	1.65^{a}	1.73^{ab}	1.71^{ab}	1.68^{a}	1.85^{b}	0.07	0.0186	-0.08 ±0.11	-0.10 ±0.07	-0.02 ±0.10
	Protein (g/100g)	10.7	10.7	10.9	10.6	11.1	0.3	0.1816	0.0 ±0.4	-0.2 ±0.3	-0.2 ±0.4
	Protein (g/d)	13.2^{ab}	12.9^{ab}	12.1^{a}	14.3^{b}	14.4^{b}	0.8	0.0383	0.4 ±1.1	-0.4 ±0.8	-0.7 ±1.0
	Energy (MJ/kg)	8.92	8.93	9.33	9.01	9.02	0.45	0.4664	0.02 ±0.76	-0.21 ±0.45	-0.19 ±0.70
	Energy (MJ/d)	1.09^{ab}	1.12^{ab}	1.00^{a}	1.21^{b}	1.16^{ab}	0.07	0.0171	-0.03 ±0.11	-0.04 ±0.06	-0.01 ±0.10
Day 21	No. of females	12	12	11	11	13					
	Total solids (g/100g)	28.3^{a}	32.1^{b}	30.4^{ab}	30.1^{ab}	28.7^{a}	0.09	0.0056	-3.7 ±1.3*	-1.4 ±0.9	2.3 ±1.1*
	Ash (g/100g)	2.12^{b}	1.86^{a}	2.07^{b}	2.04^{b}	2.07^{b}	0.05	0.0013	0.26 ±0.08*	0.06 ±0.06	-0.20 ±0.07*
	Protein (g/100g)	10.6^{ab}	11.1^{b}	10.8^{ab}	10.4^{ab}	10.1^{a}	0.3	0.0435	-0.4 ±0.5	0.2 ±0.4	0.6 ±0.4
	Protein (g/d)	21.8	19.9	21.5	21.3	20.7	0.9	0.1798	1.9 ±1.4	0.7 ±1.1	-1.3 ±1.2
	Energy (MJ/kg)	8.52^{ab}	9.47^{b}	8.77^{ab}	8.71^{ab}	8.10^{a}	0.36	0.0141	-0.95 ±0.54	-0.01 ±0.39	0.94 ±0.47*
	Energy (MJ/d)	1.75	1.71	1.74	1.77	1.66	0.09	0.3112	0.05 ±0.12	0.03 ±0.09	-0.01 ±0.11

[1] Rearing feeding programme: CAL group received the C diet *ad libitum* until first parturition; CR group received the C diet *ad libitum* until 12 weeks and then, 140 g/day until first parturition; F group received the F diet *ad libitum* until first parturition; FC and FCF group received F diet *ad libitum*

until 16 weeks and then, FC group received the C diet *ad libitum* until first parturition and FCF group the C diet *ad libitum* until 20 weeks and then the F diet *ad libitum* until first parturition.

~~As defined in Table 1.~~

[2] Fs: 1/3[F+FC+FCF]; mean±standard error. * Contrast significant at P<0.05.

SEM: Pooled standard error of the means.

[a,b] Means within a row not sharing any superscript are significantly different at P<0.05.

Table 3 *Average blood plasma insulin, glucose, non-esterified fatty acids (NEFA), leptin, cortisol and tri-iodothyroxine (T3) concentrations in rabbit females from first to second parturition according to rearing feeding programme.*

	Rearing feeding programme[1]							Contrasts[2]		
	CAL	CR	F	FC	FCF	SEM	P-value	CAL−CR	CAL−Fs	CR−Fs
No. of females	12	12	12	12	12					
Insulin (μUI/mL)	15.67	18.29	14.82	15.92	15.46	2.67	0.3616	−2.62 ± 3.78	0.27 ± 3.02	2.89 ± 3.14
Glucose (mg/dL)	90.8[a]	93.9[ab]	95.0[ab]	109.8[b]	93.5[a]	5.5	0.0191	−3.1 ± 7.8	−8.6 ± 6.3	−5.5 ± 6.5
NEFA (mmol/L)	0.653[c]	0.637[bc]	0.515[a]	0.590[b]	0.590[b]	0.024	0.0001	0.015 ± 0.034	0.088 ± 0.027*	0.072 ± 0.028*
Leptin (ng/mL)	3.05	3.24	2.78	2.87	2.79	0.25	0.2007	−0.19 ± 0.36	0.24 ± 0.28	0.43 ± 0.30
Cortisol (μg/dL)	4.31	4.61	4.59	4.47	4.82	0.32	0.2510	−0.30 ± 0.45	−0.31 ± 0.36	−0.01 ± 0.37
T3 (mmol/L)	2.81[b]	2.81[b]	2.56[ab]	2.40[a]	2.87[b]	0.11	0.0061	0.00 ± 0.16	0.20 ± 0.13	0.20 ± 0.13

[1] As defined in Table ~~1~~2.

[2] Fs: 1/3[F+FC+FCF]; mean±standard error. * Contrast significant at P<0.05.

SEM: Pooled standard error of the means.

[a,b,c] Means within a row not sharing any superscript are significantly different at P<0.05.

Table 4 *Average weight, mortality and solid feed intake of rabbit litters in the first lactation according to rearing feeding programme.*

	Rearing feeding programme[1]							Contrasts[2]		
	CAL	CR	F	FC	FCF	SEM	P-value	CAL−CR	CAL−Fs	CR−Fs
No. of Litters	18	23	25	26	26					
Litter Weight (g) at:										
1st day of life[3]	531	534	538	536	512	51	0.7153	-4 ± 77	2 ± 64	5 ± 59
7th day of life	1 132	1 144	1 173	1 180	1 218	74	0.4182	-12 ± 107	-58 ± 88	-46 ± 85
14th day of life	1 924	1 963	1 871	1 967	2 034	74	0.1181	-39 ± 107	-33 ± 89	5 ± 86
21st day of life	2 657^{ab}	2 686^{ab}	2 553^{a}	2 748^{ab}	2 800^{b}	75	0.0191	-29 ± 107	-44 ± 89	-15 ± 86
28th day of life (weaning)	4 466^{ab}	4 456^{ab}	4 441^{ab}	4 489^{b}	4 319^{a}	52	0.0203	9 ± 78	49 ± 66	40 ± 60
Mortality (%)	5.1	7.3	4.5	4.2	5.9		0.6267[4]			
Feed intake from 21st to 28th days of life (g/day)	69.0	69.8	81.1	71.0	81.1	5.2	0.0718	-0.9 ± 7.7	-9.3 ± 6	-8.4 ± 6.1

[1] As defined in Table 21.

[2] Fs: 1/3[F+FC+FCF]; mean±standard error.

SEM: Pooled standard error of the means.

[3] Litter size standardised at nine pups.

[4] Probability of Chi-Square.

^{a,b} Means within a row not sharing any superscript are significantly different at $P<0.05$.

Table 5 *Average reproductive performance of rabbit females at second parturition according to rearing feeding programme.*

	Rearing feeding programme[1]							Contrasts[2]		
	CAL	CR	F	FC	FCF	SEM	P-value	CAL–CR	CAL–Fs	CR–Fs
No. of females	18	23	25	26	26					
Interval 1st to 2nd parturition (days)	52.53^{ab}	49.22^{a}	57.52^{ab}	51.52^{ab}	58.04^{b}	3.22	0.0429	3.31 ± 4.72	-3.17 ± 4.01	-6.48 ± 3.57
Litter size at birth:										
Total born	10.63	10.75	10.35	9.39	9.52	0.62	0.1334	-0.13 ± 0.97	0.87 ± 0.78	1.00 ± 0.75
Born alive	7.58^{ab}	7.44^{ab}	9.30^{b}	7.69^{ab}	7.08^{a}	0.82	0.0389	0.15 ± 1.28	-0.44 ± 1.02	-0.58 ± 0.99
Mortality at birth (%)[3]	26.75^{ab}	31.91^{b}	11.07^{a}	16.25^{ab}	27.52^{b}	6.12	0.0328	-5.17 ± 9.58	8.03 ± 7.66	13.20 ± 7.40
Litter weight at birth (g):										
Total born	566	577	555	539	536	31	0.1762	-11 ± 47	27 ± 38	39 ± 36
Born alive	419^{ab}	408^{ab}	515^{b}	448^{ab}	377^{a}	43	0.0155	11 ± 67	-28 ± 54	-39 ± 52
Individual weight at birth (g):										
Total born	56.87	54.94	54.34	60.39	56.34	2.78	0.0803	1.94 ± 4.31	-0.30 ± 3.46	-2.23 ± 3.34
Born alive	57.59	55.16	55.92	61.31	57.66	2.97	0.1314	2.42 ± 4.81	-0.91 ± 3.59	-3.33 ± 3.88

[1] As defined in Table 2.~~1~~

[2] Fs= 1/3(F+FC+FCF); mean±standard error.

SEM: Pooled standard error of the means.

[3] Interaction feeding programme x overlapping degree was significant at P<0.01.

[a,b] Means within a row not sharing any superscript are significantly different at P<0.05.

Figure captions

Figure 1 Diagram of the different ~~rabbit females'~~ feeding programmes carried out by the rabbit females from rearing to the second parturition for the 5 ~~-~~experimental groups~~-~~.~~:~~ ~~(~~CAL group received the C diet ad libitum until first parturition, CR group received the C diet ad libitum until 12 weeks and then, 140 g/day until first parturition, F group received the F diet ad libitum until first parturition, FC and FCF group received F diet ad libitum until 16 weeks and then, FC group received the C diet ad libitum until first parturition and FCF group the C diet ad libitum until 20 weeks and then the F diet ad libitum until first parturition. ~~(~~* flushing 4 days before artificial insemination.~~; C: C diet ad libitum; CR; C diet restricted at 140g per day; F: F diet ad libitum;~~ AI1: effective 1^{st} artificial insemination; AI2: effective 2^{nd} artificial insemination; wk: weeks of age~~CAL, CR, F, FC and FCF)~~.

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Figure 2 Daily feed intake of rabbit females from first to second parturition according to the rearing feeding programme (abbreviations as in Figure 1). All the animals, independently of the rearing programme, were fed with the same feed (diet C) from first to second parturition. Bars not sharing any superscript are significantly different at P<0.05.

Figure 3 Perirenal fat thickness changes of rabbit females during whole lactation and from first to second parturition according to the rearing feeding programme (abbreviations as in Figure 1). Bars not sharing any superscript are significantly different at P<0.05.

animal minor technical revision checklist

Last updated January 2018

Manuscript number: 18-21058R1
Title in Editorial Manager: Consequences of rearing feeding programme on the performance of rabbit females from first to second parturition
Corresponding author: Juan José Pascual

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Discoverability issues

Tick when done	Requirements
⊗	Abstract ○ The abstract follows the same general structure as the text: 1) the context and 2) the scientific rationale of the study (= what is the scientific problem?), 3) the objectives, 4) materials and methods, 5) main results, 6) conclusion. In your abstract, the context and rationale are missing. Please revise. An initial sentence has been included to introduce the context and rational of the study. ○ The whole abstract is merged into a single paragraph. The abstract is presented in a single paragraph. ○ There is no need to define abbreviations in the abstract if they are not subsequently used in the abstract itself. All the abbreviations defined in the abstras were subsequently used in the abstract itself.
⊗	General Please also consider the editor's comments. Following editor's recommendations, we have included the NDF content of the diets in the abstract. We agree that a fibre/DE range could be interesting in commercial situations, but we believe that a study not designed to provide this rate must not include this type of recommendations in the conclusion. We believe that it could be proposed in a review paper, perhaps in the near future. We have also removed the "word" significantly when P-values were given in the abstract. Thank you so much to improve our manuscript.

Formatting issues

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	▪ European Union 2003. Protection of animals used for experimental purposes. Directive 86/609/EEC of 24th November 1986, amended 16th September 2003. Done ▪ Federation Internationale de Lacterie. 1993. Determination de la teneur en azote. FIL standard: 20B, 1993. Secrétariat General Federation Internationale de Lacterie, Bruxelles. Done Please check and revise accordingly. Note that for journal references (a) all journal names must be given in full (not in abbreviated form) and (b) all issue numbers must be removed; for publisher information, it must be listed as Publisher/Organisation's name, City, State (2-letter abbreviation) for US places, Country; for website references, the accessed date must be given in DD Month YYYY format. Revised and cheked. Please refer to the reference checklist appended below for specific formatting instructions.
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Figure 1

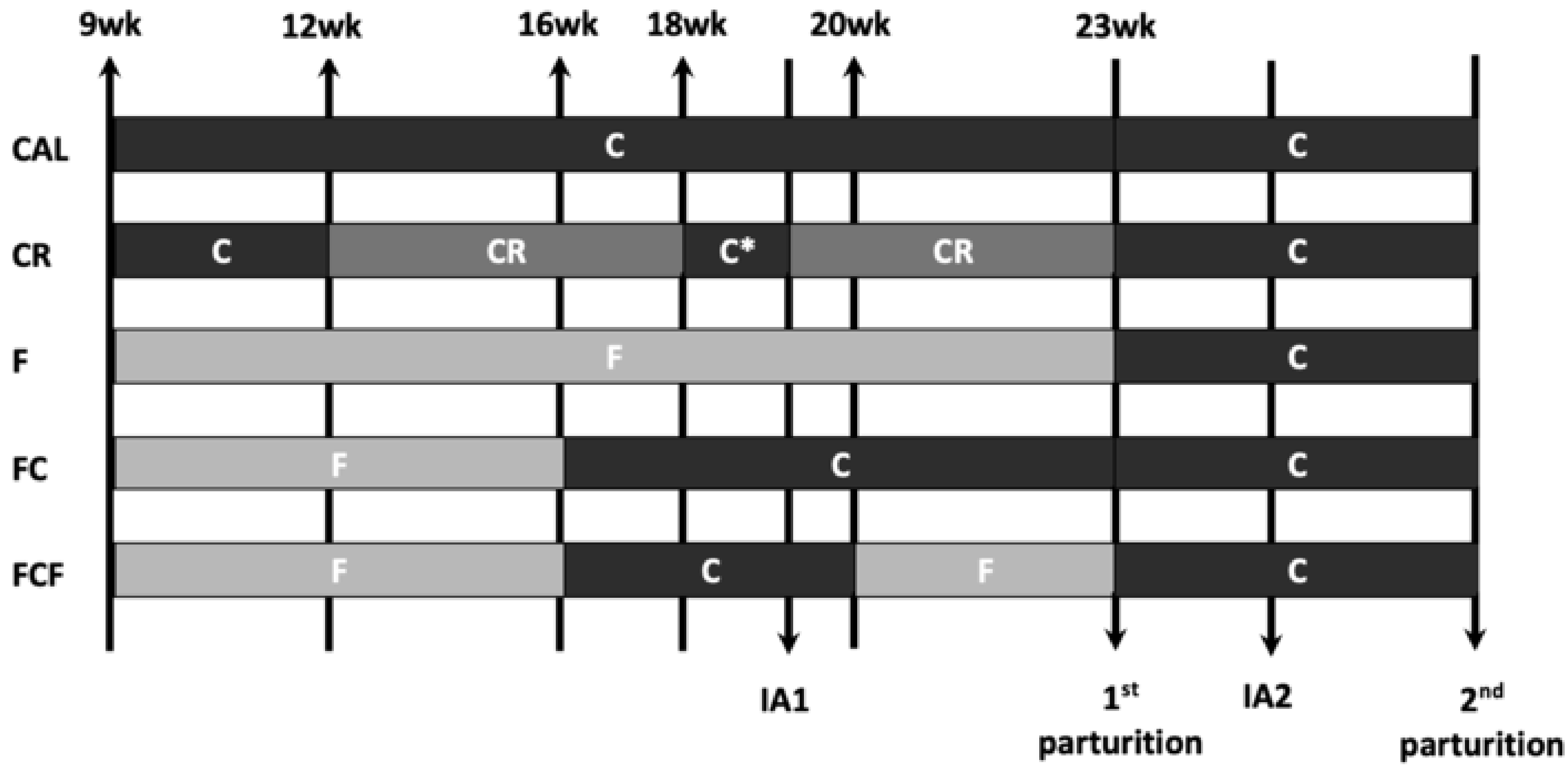
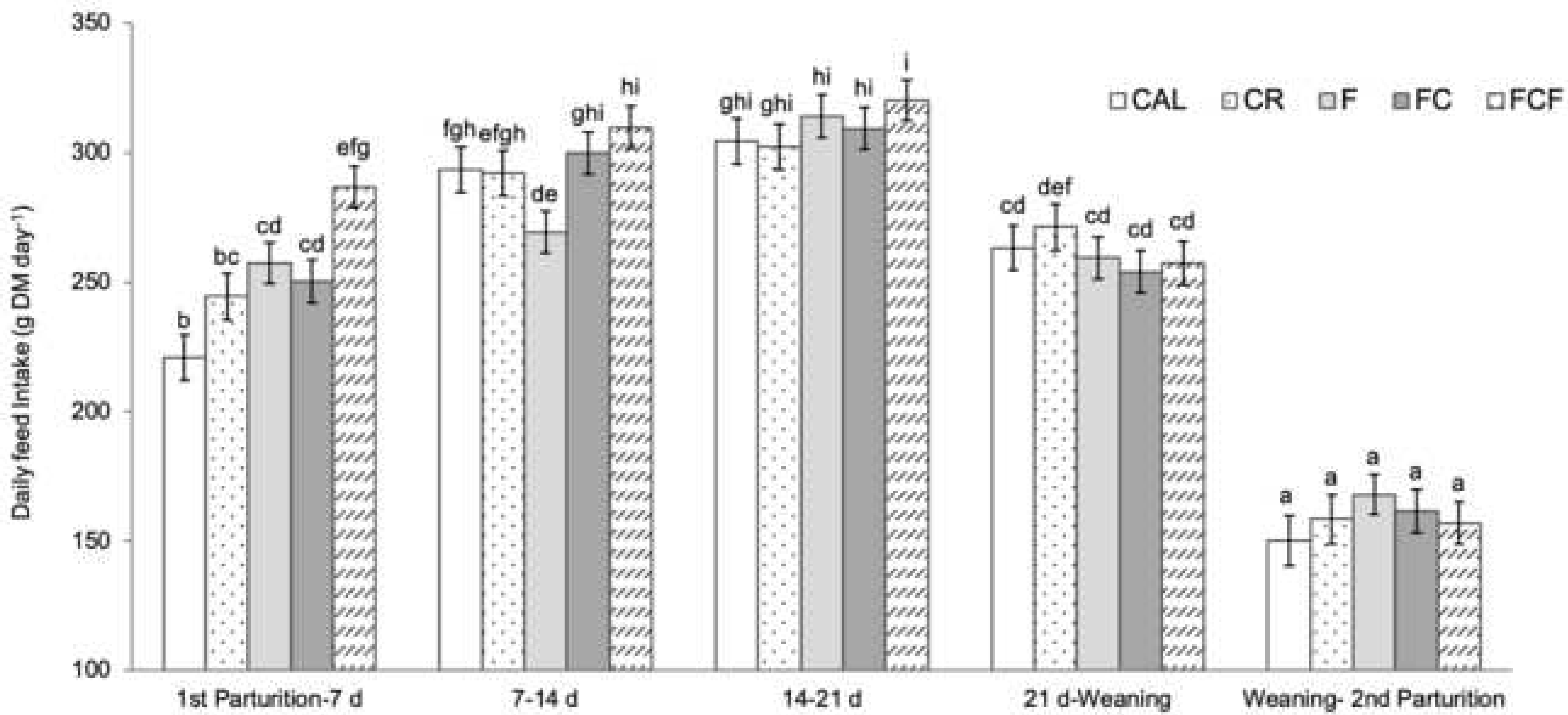
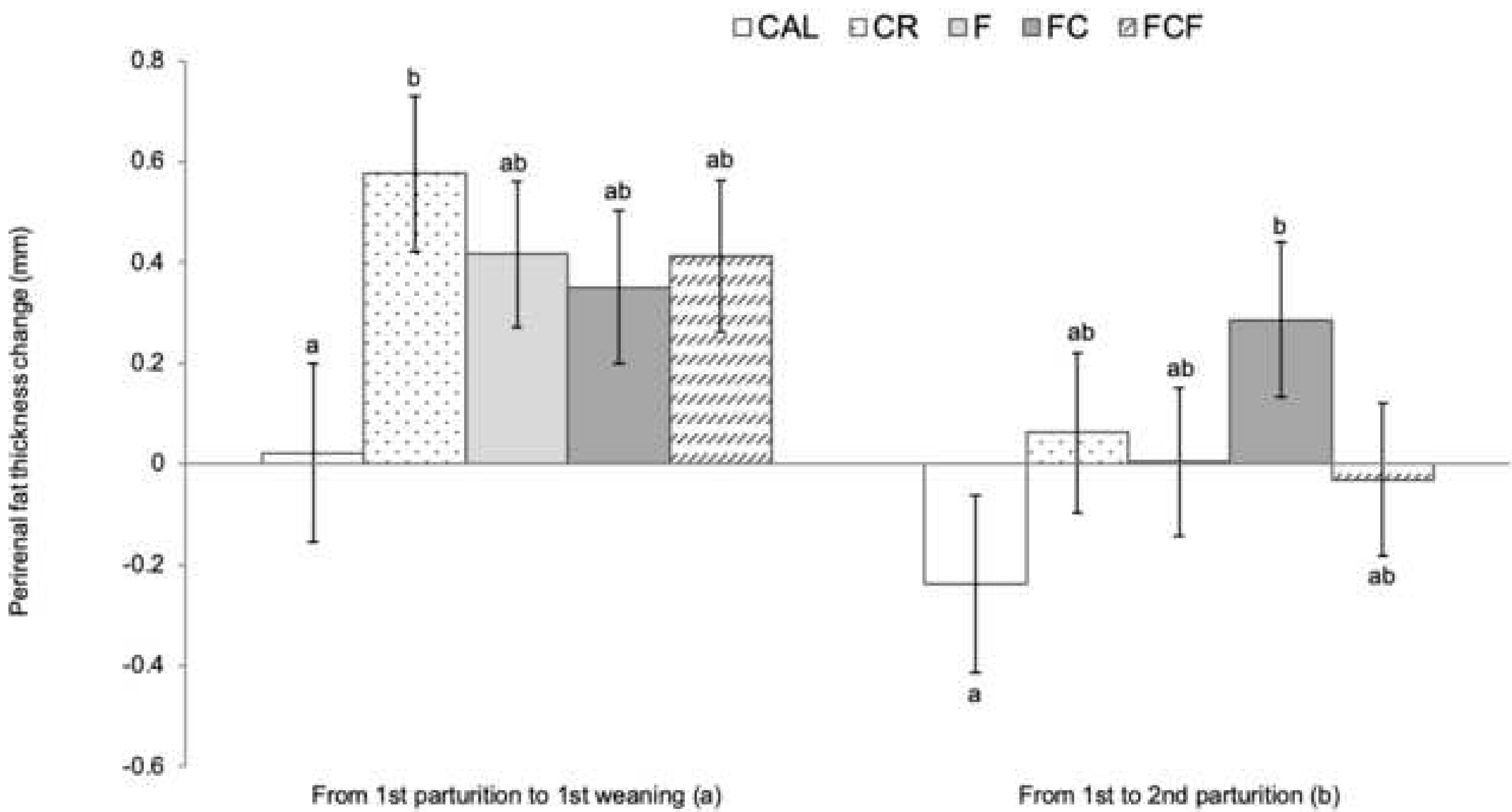

9wk
12wk
16wk
18wk
20wk
23wk
CAL
C
C
CR
C
CR
C*
CR
C
F
F
C
FC
F
C
C
FCF
F
C
F
C
IA1
1st parturition
IA2
2nd parturition

Figure 2



350
300
250
200
150
100
Daily feed intake (g DM day⁻¹)
CAL
CR
F
FC
FCF
b
bc
cd
cd
efg
fgh
efgh
de
ghi
hi
ghi
ghi
hi
hi
i
cd
def
cd
cd
cd
a
a
a
a
a
1st Parturition-7 d
7-14 d
14-21 d
21 d-Weaning
Weaning- 2nd Parturition

Figure 3


CAL
CR
F
FC
FCF
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Perirenal fat thickness change (mm)
a
b
ab
ab
ab
a
ab
ab
b
ab
From 1st parturition to 1st weaning (a)
From 1st to 2nd parturition (b)