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Feeding programs promoting daily feed intake stability in rabbit males reduce sperm abnormalities and improve fertility

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Abstract

Feeding programs promoting daily feed intake stability in rabbit males could be useful to ensure successful coverage of their nutritional requirements and for continued production of quality semen. To evaluate two feeding systems designed to reduce daily feed intake variability, 115 rabbit males at 1.2 years of age were randomly assigned to 3 different treatments for 294 days: CS, animals fed *ad libitum* with a control diet [127 g starch and 281g total soluble fiber (hemicellulose + soluble fiber) kg^{-1} dry matter (DM)]; SF, males fed *ad libitum* with diet enriched in soluble fiber [86 g starch and 330 g total soluble fiber kg^{-1} DM]; and R, animals fed with CS diet but daily restricted to maintenance requirements. Feed intake, body weight, body condition and variability of daily feed intake (DFI) were controlled every 42 days, and individual semen volume and sperm motility, concentration, acrosome status and abnormalities every 15 days. In 6 commercial farms, the number of females inseminated, pregnant and kindling, as well as the number of kits born alive, were registered for 15893 inseminations with pooled semen from each treatment. DFI was significantly lower for R males than for the other treatments (on av. −12±4 g/d; $P<0.001$). Daily weight gain of R males was close to zero and significantly lower than in the other groups (–1.42 g/d; $P<0.001$). Variability of DFI was significantly ($P<0.01$) lower for R males (7%) than for males of dietary treatments CS (13%), with SF males showing intermediate values (11%). Semen from R males presented lower sperm abnormalities (–5.9%; $P<0.05$) and higher percentages of normal and motile spermatozoa (–3.4% than SF males; $P<0.05$). Dietary treatments formulated to reduce DFI variability (SF and R) led to an improvement of kindling to pregnant and kindling to insemination ratio (+0.039 and +0.060±0.015, respectively; $P<0.05$) compared to CS treatment. In conclusion, a moderate restriction of rabbit males may be useful to fit their needs and to provide a constant daily supply of nutrients, with some

sperm morphological characteristics being improved, as well as the fertility of their pooled semen.

Keywords: Rabbit males, feed intake, restriction, soluble fiber, semen, fertility.

1. Introduction

The great development undergone by the practice of artificial insemination (AI) in modern rabbit farming over the last two decades encouraged the emergence of specific farms of rabbit males for semen production. From that moment on, the development of specific feeds for rabbit males began to make more sense, taking into account their nutritional needs and the purpose of their breeding, the production of high quality semen for dissemination [1,2].

However, there is not much scientific knowledge on adequate feeding programs for rabbit males, and even a lack of recommendations to cover their nutritional requirements in the most recent and accepted literature [3]. Although some of the studies have been addressed to determining the effect of dietary energy [4,5] and protein content [6] on the seminal production of these animals, most of the literature on this topic has focused on evaluating the effect of supplementation with micronutrients (n-3 fatty acids, vitamins, trace minerals…) on sperm membrane fluidity and integrity, or as antioxidants to prevent the high susceptibility to peroxidation of the highly unsaturated spermatozoa membrane (reviewed by Castellini et al. [2]).

On the other hand, an additional problem with this type of animals could be due to their feeding behavior. As animals under low production conditions (close to maintenance), rabbit males could show a lack of regular consumption. Pascual et al. [5] observed that adult males show periods of high consumption that can lead to overfattening, sometimes associated with an increase in abnormal spermatozoa and a high risk of fertility problems [7]), and other periods where the animals consume nothing or very little, with the consequent negative effects of occasional undernourishment on semen production and quality [8]. Therefore, the development of a well-adjusted feeding program promoting daily feed intake stability could be useful to cover the nutritional

requirements and for ongoing production of quality semen in rabbit males. The simplest method to ensure a constant intake is by daily feed restriction. Although excessive feed restriction in males is not recommended [9], adjustment of feeding to daily needs has been proposed as useful to reduce problems associated with fatness [8] and to ensure a regulate daily feed intake. An alternative to feed restriction could be the ad libitum provision of diets enriched with soluble fiber. High water-binding capacity of soluble fiber in some feedstuffs (such as pulps), which promotes digestive tract filling [10], could also be useful to regulate the voluntary feed intake of animals with overfeeding tendency.

Therefore, the present study evaluated two feeding systems designed to reduce daily feed intake variability, either by daily restriction to maintenance requirements or increasing the level of dietary soluble fiber, as well as their effect on semen characteristics and fertility in commercial farms.

2. Material and methods

2.1. Animals and housing

A total of 115 adult rabbit males aged 1.18±0.22 years and weighing 5.40±1.00 kg were used in this trial. Rabbit males belonged to three different genetic types: Hyplus PS40 paternal line (Grimaud Frères, n=62), Caldes paternal line (IRTA, n=24) and Prat maternal line (IRTA, n=29), housed in the same room at the INCO Artificial Insemination Centre (Valderrobres, Teruel, Spain). Previously, males were trained for 3 weeks and selected for semen production when 20 weeks old (selection was performed according to their libido and adaptation to an artificial vagina), and housed in individual wire cages designed for rabbit males in AI (60 ×50×50 cm), all equipped with slats, feeders and cup drinkers. From selection to culling, the males were subject to the same semen collection management, two ejaculates a week.

Housing and husbandry conditions followed the current recommendations on principles of ethical care and protection of animals used for experimental purposes in the European Union [11]. Animals were housed with light altering on a cycle of 16 light hours and 8 dark hours, and the room was equipped with environmental control equipment such as hot air heaters or cooling systems (trying to maintain the temperature at 20ºC in summer and 18ºC in winter). Figure 1 shows the status of daily maximum and minimum temperatures in the experimental room throughout the trial.

2.2. Dietary treatments

All the diets were formulated following the recommendations given by Pascual [1] and Pascual et al. [5] for rabbit males in AI. Ingredients and chemical composition of the experimental pelleted diets used in this trial are summarized in Table 1. Diet CS, similar to commercial feeds for reproductive rabbits, was formulated promoting the inclusion of cereal starch [127 g starch and 281 g total soluble fiber (hemicellulose + soluble fiber)

96 kg^{-1} dry matter (DM)], while the SF diet promoted the inclusion of soluble fiber
97 ingredients such as beet and apple pulps [86 g starch and 330 g total soluble fiber kg^{-1}
98 DM]. Using these diets, 3 different treatments were evaluated: the common program for
99 adult rabbit males (CS), where 41 animals were fed *ad libitum* with diet CS; and two
100 programs addressed to promote daily feed stability: SF, where 37 males were fed *ad*
101 *libitum* with diet SF; and R, where 37 animals were fed with CS diet daily restricted to
102 maintenance requirements (calculated at the beginning of the trial as 400 kJ day^{-1} kg^{-1}
103 body weight $(BW)^{0.75}$ [12]).
104 Chemical analyses of diets were performed following the AOAC methods [13]: 934.01
105 for DM, 942.05 for ash, 976.06 for crude protein (CP), and 920.39 with previous acid
106 hydrolysis of samples for ether extract (EE). Starch content was determined according
107 to Batey [14], by 2-step enzymatic procedure with solubilization and hydrolysis to
108 maltodextrins with thermostable α-amylase, followed by complete hydrolysis with
109 amyloglucosidase (both enzymes from Sigma-Aldrich, Steinheim, Germany), and the
110 resulting glucose was measured by the hexokinase/glucose-6 phosphate
111 dehydrogenase/NADP system (R-Biopharm, Darmstadt, Germany). Neutral detergent
112 fiber (NDF), ADF and acid detergent lignin (ADL) fractions were analyzed sequentially
113 ([15] by AOAC procedure 973.187 [13] and [16], respectively) with a thermo-stable α-
114 amylase pre-treatment and expressed exclusive of residual ash, using a nylon filter bag
115 system (Ankom, Macedon, NY, USA). Soluble fiber content was calculated by
116 difference as: organic matter – (CP + EE + soluble sugars + starch + NDF) [17].
117 Hemicellulose content was also calculated by difference (NDF – ADF).
118 *2.3. Experimental procedure*
119 The experiment was carried out from November 2013 to August 2014 (294 days). At
120 the beginning of the trial, dietary treatments were randomly assigned to rabbit males

with a similar age (1.1 to 1.3 years). Feed intake, BW and perirenal fat thickness (PFT) was controlled at 42 day intervals (a total of 8 individual controls, always Friday), as in the adult buck spermatogenesis lasts approximately 38-41 d [18]. PFT was determined following the recommendations of Pascual et al. [19] with an ultrasound equipment JustVision 200 'SSA-320A' real-time machine (Toshiba; Medical Systems Co., Ltd, Tokyo, Japan). During the control week, feed intake of each animal was daily controlled at 11:00 (from Monday to Friday) to determine the variability of daily feed intake (DFI) as the intra-animal coefficient of variation of DFI in 4 consecutive days.

From the ejaculates collected (twice a week), one ejaculate every 42 days was evaluated during the experimental period. The volume was recorded. Ejaculates were initially diluted 1:5 with a gel-supplemented extender for solid storage of rabbit semen provided by IMV technologies (CUNIGEL, L'Aigle Cedex, France), and re-diluted after evaluation to ensure 30 10^6 spermatozoa per mL. To determine the effect of dietary treatment on fertility and prolificacy ability of semen obtained, pooled semen from each dietary treatment was used to inseminate a total of 15893 rabbit females. Rabbit females belonged to 6 commercial farms from this same geographical area (having from 250 to 1000 females), which were inseminated from January to July 2014. The number of females inseminated, pregnant (tested by palpation at 12 days after AI) and kindling, as well as the number of kits born alive (NBA), were registered for each pool.

2.4. Semen evaluation

Ejaculates comprising urine and calcium carbonate deposits were discarded. Gel plugs, when present, were removed before the volume of the ejaculates was determined using a graduated tube.

Sperm motility and morphological evaluations were performed as described previously [20]. Sperm motility parameters and dosage calculation were assessed using a

computer-assisted sperm analysis system (ISAS; Proiser R + D, Paterna, Spain). Briefly, 10 µL aliquot samples from ejaculates were diluted 1:20 with Tris–citrate–glucose extender and each sample was placed in a SPERMTRACK chamber (Proiser R + D, Paterna, Spain). ISAS software calculated a subpopulation of good quality spermatozoa, as assessed by motility, morphology and concentration at the same time. The percentage of good quality spermatozoa (%) was calculated as the ratio: [(Motile and morphologically normal spermatozoa) /(Total spermatozoa)] × 100.

For the manual morphological analyses, an aliquot from each sample (20 µl) was fixed with 180 µl of a solution of glutaraldehyde 2% in Dulbecco's phosphate-buffered saline. A minimum of 100 spermatozoa were evaluated at a magnification of 400X by phase positive contrast microscopy. Spermatozoa abnormalities (AS) and apical ridge status of normal spermatozoa [intact (IA) or reacted (RA)] were assessed. Spermatozoa with morphologic defects in head, tail or neck-midpiece were classified as abnormal (AS). The percentage of abnormal spermatozoa was calculated as the ratio: [AS/(IA+RA+AS)] x 100. The percentage of sperm with normal apical ridge was calculated as the ratio: [IA/(IA + RA)] x 100 [21]. Sperm concentration (10^6 spermatozoa per mL) was determined using a Thoma-Zeiss counting cell chamber (Marienfield, Germany).

2.5. Statistical analysis

Data from performance and semen evaluation were analyzed using a repeated measures model. Time effect was initially the control (every 42 days, 0 to 7), but considering the change in room temperature variability from April, a new variable was defined as period (cool or hot for before and after April, respectively), which was also used as time effect in place of the control. A mixed model (SAS Institute Inc., Cary, NC, USA) was used,

171 according to a repeated measures design that takes into account the variation between
172 animals and covariation within them. Covariance structures were objectively compared
173 using the most severe criteria (Schwarz Bayesian criterion [22]). The model included
174 the dietary treatment (CS, SF, R), the genetic type (Hyplus, Caldes, Prat), as well as the
175 time (control or period) and their interactions as fixed effects. Random terms in the
176 model included a permanent effect of each animal (p) and the error term (e), both
177 assumed to have an average of zero, and variance σ^2_p and σ^2_e. To evaluate the possible
178 effect of DFI variability on semen characteristics, intra-animal coefficient of variation
179 of DFI in 4 consecutive days was included as a covariate.
180 Data from the use of pooled semen in the commercial farms were analyzed using a
181 general linear model (SAS Institute), including the commercial farm, the period (cold,
182 hot), the dietary treatment (CS, SF, R), the genetic type (Hyplus, Caldes, Prat) and their
183 interaction as fixed effects. As the number of females inseminated was different
184 depending on the farm, the following weight variables were used: females inseminated
185 (for pregnant/inseminated or NBA/insemination ratios), females pregnant (for
186 kindling/pregnant ratio) and parturitions (for NBA/parturition ratio).
187

188 **3. Results**

189 Environmental conditions of the room were better controlled from November to April
190 than from April to September (Figure 1). Standard deviation (SD) of maximum and
191 minimum temperatures between controls until April (1.01 and 0.65ºC, respectively) was
192 almost half of those registered thereafter (1.96 and 1.14ºC, respectively).

193 Dietary treatment had no effect on the average BW and PFT of rabbit males (Table 2),
194 but daily weight gain (DGW) of R males was close to zero and significantly lower than
195 in the rest of the groups (−1.42 g/d; $P<0.001$). In fact, a significant interaction ($P<0.001$)
196 between the dietary treatment and the control time was observed for BW (Figure 2a).
197 BW was similar for the different dietary treatments at the start of the trial (on av.
198 5210±100 g). However, whereas R males maintained their BW until the end of the trial
199 (5270±102 g), the males from the other 2 treatments showed a constant gain in BW (on
200 av. until 5555±102 g). DFI was significantly lower for R males than for the rest of
201 treatments (on av. −12±4 g/d; $P<0.001$). These differences were mainly due to the lower
202 DFI of the R males during the cool period (−26±6 g/d at control 2; $P<0.001$), DFI not
203 being too different during the hot period (153 and 157±7 g/d for R and rest of the
204 treatments, respectively; Figure 2b). As expected, variability of DFI was significantly
205 ($P<0.01$) lower for R males (7%) than for males under dietary treatments CS (13%),
206 with the SF males presenting intermediate values (11%). These differences were mainly
207 due to the lower variability of DFI observed for R males during the hot period (5%)
208 compared to the rest of the dietary treatments (on av. 14%), there being no great
209 differences between dietary treatments for the cool period (Figure 2c).

210 In general, dietary treatment had no relevant effects on average semen characteristics of
211 males (Table 3). However, semen from R males presented lower sperm abnormalities
212 (contrast R–[(CS+SF)/2] = –5.9%; $P<0.05$) and higher percentages of normal and

213 motile spermatozoa (–3.4% than SF males; P<0.05). When the pooled semen of males
214 was used in the commercial farms, dietary treatment had no effect on the female's
215 pregnant to inseminated ratio (on av. 0.84%). However, dietary treatments designed to
216 reduce DFI variability (SF and R) led to an improvement of kindling to pregnant and
217 kindling to insemination ratio (+0.039 and +0.060±0.015, respectively; P<0.05)
218 compared to CS treatment. Differences in kindling to inseminated ratio were mainly due
219 to the higher values registered by females AI with the R pooled semen compared to the
220 rest of the treatments during the hot period (0.886 vs. 0.771, respectively; P<0.01;
221 Figure 3a). Although no significant effects of diets were observed for the NBA, during
222 the hot period females AI with pooled semen of SF males had a lower NBA per
223 parturition (–0.823 kits; P<0.05; Figure 3b) and those with R semen had higher NBA
224 per insemination (+1.480 kits; P<0.05; Figure 3c) compared to the other groups.
225

4. Discussion

The main goal of this study was to develop a feeding system which promotes the homogeneity of daily feed intake in rabbit males, as a possible strategy to also improve their semen production or quality. As expected, daily restriction of feed to maintenance requirements led to a constant intake of males throughout the trial, as well as to a significant reduction of daily feed intake variability. However, this improvement on feed intake homogeneity of restricted males took place only during the hot period.

During the cold period, males fed ad libitum showed greater consumption, which led to an increase of their BW in comparison to those restricted. This higher intake could be partially justified by the lower temperature [–2ºC; +28 kJ/d of heat production (+2 g of feed/d) [23]]; in fact, restricted males showed a slight decrease in their BW, but mainly by the fattening of males during this period [+3 g BW/d; +71 kJ digestible energy/d (+13 g/feed/d) [12]]. Previous works describing the growth curve of males selected for growth rate have proposed an achievement of mature weight at about 40 weeks of age [24,25], although a residual growth (1-2 g/d) has been described from this moment, but more related to fattening than to growing. Therefore, the needs of one year old males under winter conditions led to a less variable daily feed intake, showing no differences in this respect compared to those restricted.

However, as summer drew near, requirements were reduced (lower heat production and fattening), with rabbit males fed ad libitum showing significant reduction of their feed intake (especially those fed with diet C; –16%) and fattening, while restricted rabbit males maintained their feed intake. Reduced requirements combined with higher variability in the room temperature (Figure 1) might be behind the increased variability on daily feed intake of males feed ad libitum, especially in those receiving a diet with a higher level of starch, which could lead to greater satiety. Le Magnen and Devos [26]

proposed that the size of the meal was not determined by the metabolic deficit incurred prior to the meal in rats. It seems that satiety ratio is more important than the hunger ratio, the energy intake from the meal determining the length of the subsequent interval. Therefore, occasional overfeeding with a higher satiety diet could increase the length between meals and daily feed stability.

The beet pulp enriched diet was proposed to promote the daily digestive tract filling of rabbit males, due to its higher level of soluble fiber with a high water-binding capacity [12,27]. Although no differences were found during the cold period, rabbit males ad libitum fed with a diet enriched in soluble fiber showed a lower reduction of their feed intake, as well as a lower variability on daily feed intake, than C males during the hot period. It can be hypothesized that physical satiety signals linked to higher filling and reduced glucose provision might promote a more constant feeding behavior throughout the days and consequently even higher feed intake. However, a 4 percent increase of dietary high digestible fiber at the expense of starch did not lead to the low variability on daily feed intake reached with the feeding restriction.

Regarding the possible effect of feed restriction on semen production and quality, it seems clear that earlier application of restriction or severe restriction negatively affected sperm production. Limiting access to feed in young animals has led to reduced semen volume in boars [28] or to delayed production in rabbits [29]. In adults, dietary energy provision clearly under the maintenance requirements has been related with reduction in blood testosterone or the number of sperm cells produced in boars [30,31] and rabbit males [9,32]. However, restriction adjusted to requirements, as in the present work, has not been associated with negative effects on sperm production. Sulabo et al. [33] observed a similar seminal volume and sperm concentration for boars fed ad libitum or restricted to maintain body condition. Furthermore, rabbit males have not reduced their

276 sperm production when subjected to moderate restriction [32] or feeding during the
277 night only [34]. On the other hand, the effect of feed restriction on common semen
278 quality traits (motility, percentage of normal cells…) seems to be small. The slight
279 improvement in semen quality observed in the present study could be related to a more
280 constant provision of nutrients or to an improvement in feeding behavior of moderately
281 restricted rabbit males.
282 The economic relevance of the contribution of a rabbit male to fertility has increased
283 with AI development [18]. In commercial farms, AI is performed with pooled semen at
284 high sperm dosage to overcome the possible negative effects on fertility of semen with
285 suboptimal characteristics [35], and consequently the relationship between the
286 characteristics of the individual ejaculates and fertility traits has not been clearly
287 established. Some quality traits have been proposed to have a relevant phenotypic
288 correlations with rabbit male performance, such as motility, percentage of abnormal
289 spermatozoa, pH of the ejaculate or the presence of cytoplasmatic droplets
290 [20,36,37,38], but there is a lack of studies or the precision of the genetic correlations
291 obtained are poor [37]. In the present study, pooled semen from males restricted to
292 maintenance, and characterized by sperm with lower abnormalities and higher normality
293 and motility, resulted in improved fertility traits on commercial farms. Differences from
294 the other dietary treatments especially occurred during the hot season, where variability
295 of daily intake of these animals was significantly reduced. Lavara et al. [20], using
296 semen from males selected for growth rate, proposed the percentage total motile and
297 abnormal spermatozoa as the semen morphological traits most correlated with the
298 kindling rate (+0.31 and –0.32, respectively).
299 In any case, it seems clear that rabbit male contributions to fertility and litter size after
300 AI were low, but higher in magnitude than those observed after natural mating [39]. In

addition, the possible male effect would be diluted as parturition draws near, as fetal survival is mostly determined by the female. This fact could explain the impact of male feeding system on kindling traits and its disappearance at parturition.

5. Conclusions

From these results, it could be concluded that in rabbit male lines, with a well-known tendency to overfeeding and overfattening, a moderate restriction may be useful to meet their needs and to provide a constant daily supply of nutrients. This moderate restriction improved some sperm morphological characteristics, as well as the fertility of their pooled semen, especially during the seasons where this program allowed higher daily feed intake stability. Finally, the ad libitum administration of a diet with higher digestible fiber content led to intermediate results compared to those obtained with the feeding restriction. Further studies on the adequate level of restriction and dietary digestible fiber content depending on the environmental conditions and genetic type are needed.

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Table 1. Ingredients (g/kg) and chemical composition (g/kg DM) of the experimental diets.

	Diets[1]	
Ingredients	CS	SF
Barley grain	100	18
Lucerne hay	276	38
Beet pulp	50	181
Apple pulp	0	80
Sunflower meal (30% CP)	220	320
Wheat bran	250	250
Palm kernel meal	60	60
Palm oil	7.9	3.6
Molasses	20	20
L-Lysine	2.1	1.9
DL-Methionine	0.3	0
Calcium carbonate	6.7	16.5
Sodium chloride	1.0	5.0
Robenidine	1	1
Levofeed	1	1
Vitamin/trace element premix[2]	4	4
Chemical composition		
Dry matter (DM, g/kg)	890	894
Ash	95	87
Ether extract	37	34
Crude protein (CP)	185	186
Neutral detergent fiber	388	399
Acid detergent fiber	228	223
Acid detergent lignin	64	61
Crude fiber	202	201
Soluble fiber	121	154
Starch	127	86

[1] CS, enriched in cereal starch; SF, enriched in soluble fiber;
[2] Supplied 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; Magnesium: 290 mg; Manganese: 20 mg; Zinc: 60 mg; Iodine: 1.25 mg; Iron: 26 mg; Copper: 10 mg; Cobalt: 0.7 mg; Butyl hydroxylanysole and ethoxiquin mixture: 4 mg.

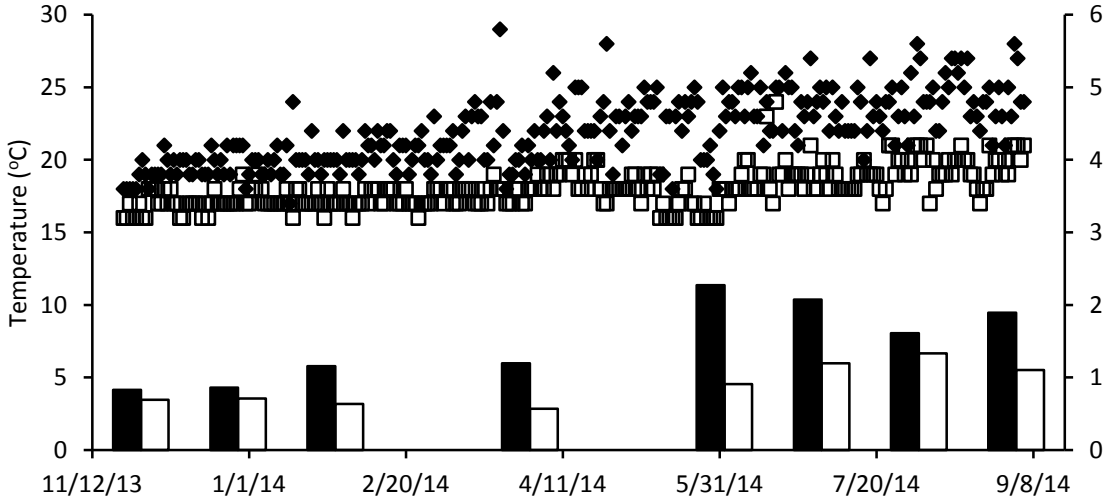

Figure 1. Evolution of maximum (◆) and minimum (□) temperatures in the experimental farm throughout the trial, as well as the standard deviation of maximum (black bars) and minimum (white bars) temperatures during the previous 45 days.

Table 2. Effect of dietary treatment on rabbit male performance.

	Dietary treatment[1]				
	CS	SF	R	SEM	P-value
No. of rabbit males	41	37	37		
Body weight (kg)[2]	5460	5445	5227	97	0.3165
Daily weight gain (g/d)[2]	1.472[b]	1.364[b]	0.084[a]	0.310	0.0010
Perirenal fat thickness (mm)	8.38	8.57	8.34	0.15	0.5370
Daily feed intake (DFI; g/d)[2]	160.7[b]	168.4[b]	152.6[a]	3.6	0.0034
Variability of DFI[3,4]	0.132[b]	0.112[ab]	0.069[a]	0.017	0.0068

[1] Dietary treatment: CS, enriched in cereal starch; SF, enriched in soluble fiber; R, CS restricted to maintenance requirements.
[2] Interaction dietary treatment and control (P<0.001).
[3] Interaction dietary treatment and period (P=0.004).
[4] Variability of DFI: Intra-animal coefficient of variation of DFI in 4 consecutive days.

Table 3. Effect of dietary treatment on rabbit individual male semen characteristics and further utilization of pooled semen in commercial farms.

	Dietary treatment[1]				
	CS	SF	R	SEM	P-value
Semen quality traits					
No. samples	328	271	272		
Volume (mL)	0.96	0.92	0.88	0.04	0.4666
Concentration (sperm$\times 10^6$/mL)	521	542	485	39	0.4681
Abnormal spermatozoa (%)[2]	33.4	34.5	28.1	2.3	0.0931
Normal apical ridge (%)	96.5	96.6	97.1	0.4	0.7193
Total sperm motility (%)	82.9	83.1	85.9	1.2	0.2435
Good quality spermatozoa[3] (%)	52.2	49.9	54.2	1.7	0.3690
Semen utilization					
No. of females inseminated	2106	6441	1877		
Pregnant/ inseminated	0.817	0.849	0.851	0.017	0.3387
Kindling/pregnant[2]	0.762[a]	0.802[b]	0.800[b]	0.017	0.0462
Kindling/inseminated[2,4]	0.750[a]	0.792[ab]	0.828[b]	0.020	0.0489
No. of born alive/parturition[4]	9.59	9.34	9.59	0.21	0.6493
No. of born alive/inseminated[4]	7.23	7.48	7.93	0.28	0.6443

[1] Dietary treatment: CS, enriched in cereal starch; SF, enriched in soluble fiber; R, CS restricted to maintenance requirements.
[2] Contrast [(SF+R)/2−CS] significant at $P<0.05$.
[3] Determined automatically by ISAS program
[4] Interaction dietary treatment and period ($P<0.05$).

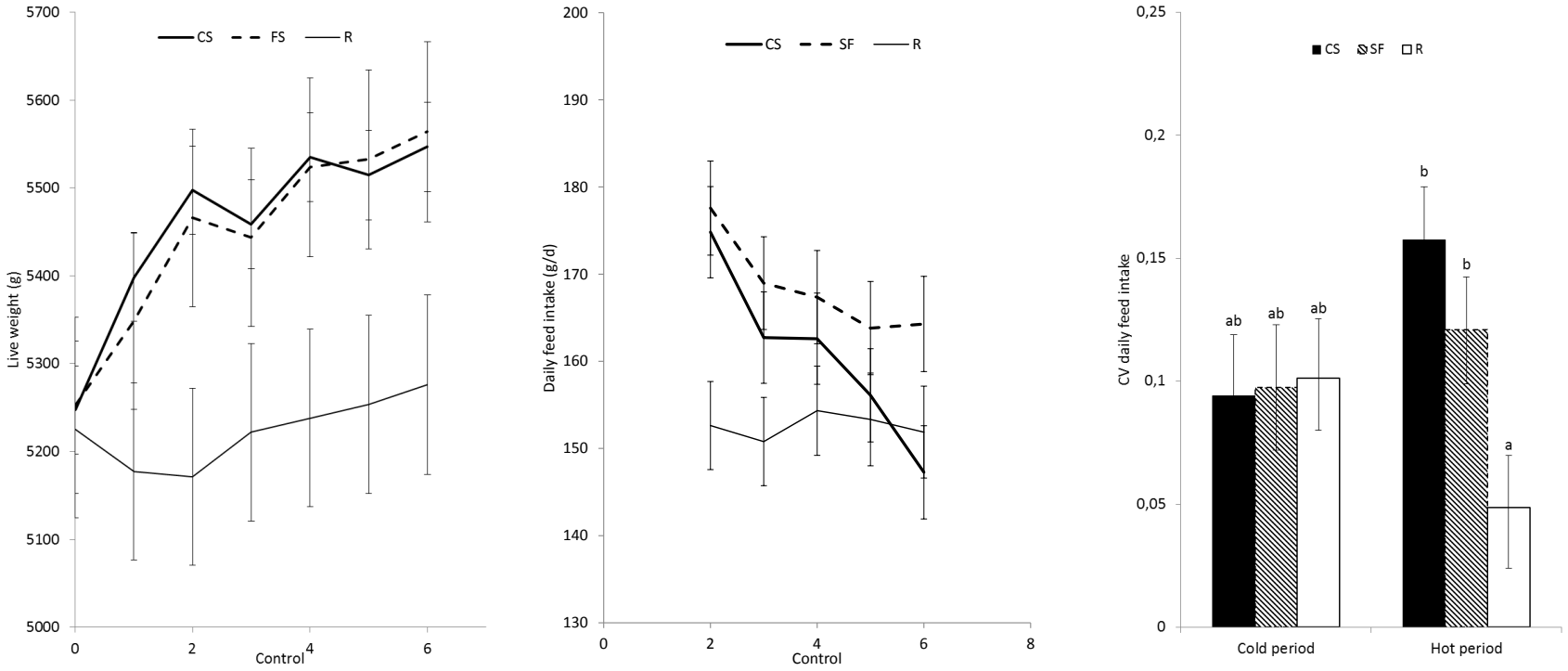

Figure 2. Effect of dietary treatment (CS, enriched in cereal starch; SF, enriched in soluble fiber; R, CS restricted to maintenance requirements) in the evolution of live weight and daily feed intake of rabbit males throughout the experiment (9 months) and intra-animal coefficient of variation of daily feed intake at cold and hot periods.

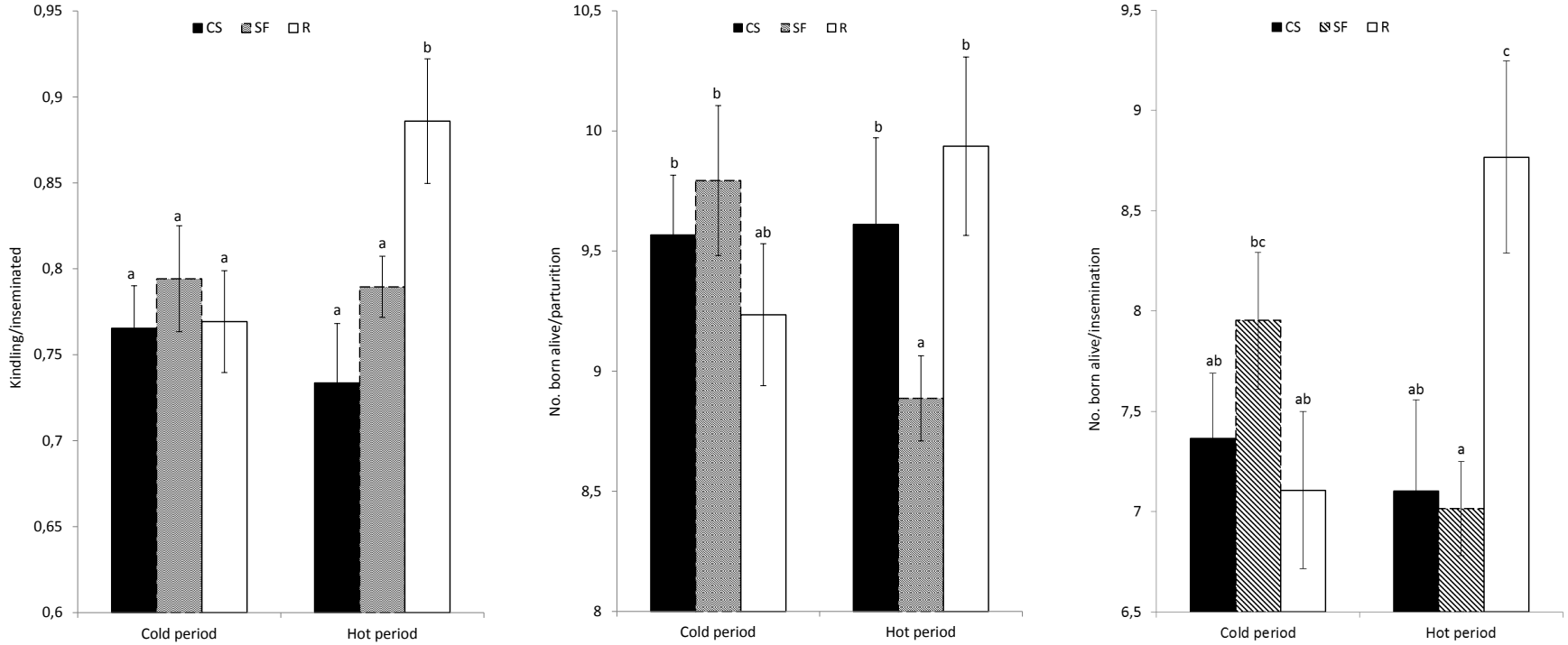

Figure 3. Effect of dietary treatment (CS, enriched in cereal starch; SF, enriched in soluble fiber; R, CS restricted to maintenance requirements) in the kindling/insemination ratio and number of born alive per parturition or insemination at cold and hot periods.