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Onset of feed intake of the suckling rabbit and evidence of dietary preferences according to pellet physical properties

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## Abstract

Weaning represents a critical period for rabbit, with a high susceptibility to gastrointestinal diseases. Stimulating early solid feed intake **could** contribute to preserving health in rabbit. Thus, the aims of this study were, first, to quantify early solid intake and, second, to determine young rabbit pellet preferences according to their physical properties. Double-choice tests of pellets processed with different dies were conducted in two trials. An original feeding system was used to specifically measure consumption of suckling rabbits in the nest independent of that of the does. Pellets were provided from 3 to **17** days in the nest box and intake was measured daily. From 15 to 35 days, kits received pellets in feeders and intake was measured every 4 days. In the first trial, four pellet diameters were tested in pairs against each other: (A) 2.0, (B) 3.0, (C) 4.0 and (D) 6.0 mm. In the second trial, pellets of identical diameter (2.5 or 4.0 mm) were manufactured with three die channel lengths: (E) 10, (F) 12, (G) 14 mm or (C) 18, (H) 20 and (I) 24 mm. For a given diameter, pellets were tested in pairs. This resulted in six combinations per trial (n = 10 litters per combination). Solid feed intake began at 8 days for 89 out of 118 litters. Over the first 17 days of life, a rabbit ingested a total of  $1.63 \pm 0.76$  g of **dry matter** in the nest in addition to milk. The solid feed intake at 2 weeks of age was correlated with litter weight at the beginning of the experiment ( $r = 0.50$  between 15 and 17 days;  $P < 0.05$ ). In the feeders, rabbits selected pellet A (61%, 67% and 86% of relative consumption when compared with B, C and D). In the second trial, pellet I was slightly disliked compared to C when provided in the nest ( $P < 0.05$ ), and compared to H in the feeders ( $P < 0.05$ ). It was concluded that suckling rabbits were able to ingest solid feed in the nest box and to discriminate pellets according to their presentation when a choice was given. Providing young rabbits with an easy-access feeding system and an optimal pellet presentation may help to stimulate early solid intake and thus pave the way for further studies on nutritional control of microbiota implantation.

*Keywords:* Rabbit; Weaning; Solid consumption; Pellet die; Feed preference

*Abbreviations:* ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; DM, dry matter; Lignin (sa), lignin determined by solubilization of cellulose with sulphuric acid; RC, relative consumption; SC, swelling capacity; SEM, standard error of the mean; WHC, water holding capacity

## **1. Introduction**

Despite consequent improvements in rabbit feed formulation and husbandry practices since the 1980s, a high incidence of digestive disorders around the time of weaning can still be observed. In this context, antibiotic use remains high on meat rabbit farms (Méheust et al., 2017). The cost of antimicrobials and the spread of antibiotic-resistant bacteria have increased the need for alternatives to antibiotic use.

Establishing a stable digestive microbiota at an early stage could enhance rabbit resilience to the stress of weaning (Fortun-Lamothe and Boullier, 2007; Combes et al., 2013). Indeed, relationships between enteric infection and disruption of the normal microbiota (known as dysbiosis) have been highlighted in different species such as rabbit (Sekirov and Finlay, 2009; Bäuerl et al., 2014). As in the case of human children and piglets (Konstantinov et al., 2004; Koenig et al., 2011), the rabbit kit digestive microbiota composition is strongly shaped by solid feed intake (Padilha et al., 1995, 1999). Previous studies demonstrated that ingestion by kits of doe feces left in the nest can speed up colonization of microbial cecum (Kovács et al., 2006; Combes et al., 2014). Solid intake via ingestion of fecal pellets or feed offered in the nest can start 2-3 days after birth (Gidenne et al., 2013; Combes et al., 2014; Kacsala et al., 2017). As opposed to fecal pellet ingestion, neonatal solid feed intake behavior has never been quantified.

Since newborn rabbits are able to ingest solid feed, and since this behavior can contribute to triggering the installation of microbiota, we hypothesize that providing palatable feed to the kits is an opportunity to strengthen rabbit gut health. However, in rabbit farming, kits only have access to pelleted feed when they start leaving the nest box at around 16-18 days (Gidenne and Fortun-Lamothe, 2002). Moreover, no data exists to validate the ability of the physical properties of the common industrial pellet to encourage early feed intake. Preliminary studies investigated the effect of pellet hardness on rabbit intake in rabbits from 2- to 5-weeks old, but this effect could have been combined with feed composition or pellet size effect (Gidenne et al., 2007; Travel et al., 2009). Maertens (1994) previously showed that pellet size had an effect on rabbit intake after weaning, with higher consumption when a large pellet diameter (4.8 mm) was offered, suggesting that the physical properties of feed are key factors to optimize solid feed intake.

The aims of this study were (i) to quantify kit feed intake from 8 to 35 d, and (ii) to determine the optimal pellet diameter and hardness necessary to stimulate the onset of feed intake. To reach these goals, an original feeding system in the nest was designed and nine pellets with different press die specifications (i.e., length and diameter) were offered in double-choice tests before weaning to evaluate kit feed preferences and intake.

## **2. Material and methods**

The study was conducted by the Groupe d'Experimentation Cunicole (GEC) network<sup>1</sup> and the experiments were carried out at the PECTOUL Experimental Unit (INRA, Castanet-

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<sup>1</sup> GEC is a French network of companies with expertise on rabbit production and nutrition. It includes CCPA, EVIALIS, INZO, MiXscience and TECHNA.

Tolosan, France). Animals were handled according to the recommendations for animal care in experimentation in agreement with the EU Directive 2010/63/EU (Anonymous, 2010) and the Journal Officiel de la République Française (Anonymous, 2013).

## 2.1. Animal management and housing

Female rabbits (hybrid breed PS19; Hypharm, France) were inseminated with semen from male commercial breeds for two successive reproductive cycles (first two lactations). The does and their litter ( $n = 60$  and  $63$  for the first and second trial, respectively) were raised together in a ventilated breeding unit in individual wire cages (width:  $61$  x length:  $69$  x height:  $61$  cm). Cages were equipped to allow the mother and its litter to be fed separately. In that respect, a wire mesh partition separated the doe from the area containing the kits' feeders. The doe's feeder in the cage was elevated so that the kits could not access to the doe's feed (Fig. 1). Two days after parturition (day 2), nest quality was assessed using a 3-point scale: 1 represented a nest of weak quality in which only wood shavings were found; 2 represented a nest of medium quality in which doe fur was present but insufficient to fully cover the litter; and 3 corresponded to a nest of good quality in which doe fur entirely covered the litter. After nest quality assessment, the litter size was standardized to ten kits per doe by cross-fostering or culling. The doe could access to the nest once a day for suckling (controlled suckling between 8:00 and 9:00 am for 10 min) until day 21. After day 21, the doe could freely access to the nest. Two water nipples were available: one in the doe area and one in the litter space. Feed was offered *ad libitum* to the youngs in the nest (width:  $25$  x length:  $38$  x height:  $20$  cm) from 3 to 17 days of age using two plastic cups (volume:  $30$  mL;  $\varnothing = 40$  mm; height:  $32$  mm; GOSSELIN®, Le Mans, France), placed opposite the doe's entrance (Fig. 2). The feed present in the nest was removed before suckling to prevent the doe from eating it, then weighed and finally reintroduced after suckling. Cups were carefully maintained in holders ( $6$ -cm high) and contained feed pellets that differed by their physical characteristics alone (same formulation).

From 15 to 35 d, the same kind of pellets **was** offered *ad libitum* outside the nest in the litter area using two feeders, one for each type of pellet tested. The kits' feeders were designed as presented by Fortun-Lamothe et al. (2000), with triangular-shaped openings to limit waste. To avoid confusion between feed placement and pellet preference, the location of cups or feeders containing each pellet type was reversed when feeding measurements were performed. The feeding scheme is summarized in Fig. 3. Milk production was measured as the difference of female weight before and after suckling at 3 or 4, 7, 10, 14, 17 and 21 **days**. Litter weight after suckling was recorded at 3 or 4, 10, 14, 21, 28 **days**.

At weaning (35 **days of age**), kits were assigned to cages of five rabbits/cage (width: 78 x length: 46 x height: 30 cm) according to the double-choice preference test conducted and litter of origin. Individual rabbit weight was measured at weaning, at 50 and 64 **days of age**. Following common feeding strategy in France, the animals were given restricted feed until 64 **days** in order to achieve an average daily gain of 40 g/day. Animals were then fed freely until slaughter (**day 72**). Rabbit mortality was recorded daily from 3 to 72 **days of age**.

## 2.2. Diet processing

Does were fed a commercial feed (without any antibiotics) adapted to the lactation stage (Table 1). The diet for suckling rabbit was formulated to meet the nutrient needs of both does and kits. The feed mash was manufactured by Euronutrition SAS (Saint-Symphorien, France). Nine kinds of pellets from different dies were then produced (Tecaliman, Nantes, France) from the same mash to ensure that pellets had identical chemical composition. The ingredients and chemical composition of the pelleted feeds are presented in Table 1. In the pelletizer, the meal was delivered to a conditioning chamber where the quantity and pressure of added steam were controlled to ensure that the mash was at 65°C before flowing into the mill die chamber. To process the pellets, the mash was forced through one of the nine dies with a specific channel length and diameter (Table 2). Die length-to-diameter ratio (also

referred to as compression rate) is known to influence hardness (Thomas and Van der Poel, 1996). Therefore, die size was the parameter chosen to obtain the desired pellets: pellets with four different diameters and similar hardness in Trial 1 (A, B, C, D), and pellets with three different hardnesses for two given diameters in Trial 2 (E, F, G and C, H, I).

### *2.3 Experimental design*

From 3 to 35 days, to assess solid feed preferences, each litter was offered two pellet types while they were still suckling the doe. A complete double-choice feeding protocol was performed in both trials. In the first trial, four pellets were tested in pairs, which resulted in six combinations (AB, AC, AD, BC, BD, and CD, where comparison treatment AB represented pellet A tested against pellet B, etc.). In this trial for diameter preference, we had run out of pellet A stock at 32 days, thus ending the preference trial. Hardness preference was assayed in Trial 2, comparing 2.5-mm-diameter pellets against each other and 4.0-mm-diameter pellets against each other. These comparisons of pellets with the same diameter but with different compression rates resulted in six comparison treatments (EF, EG, FG and CH, CI, HI). Pellet C was used in both trials. The litters were assigned to one double-choice treatment according to doe parity (only primiparous in Trial 1 and a parity rank of  $1.9 \pm 0.01$  in Trial 2) and to kit weight (average body weight at 2 days was  $66 \pm 1$  g per kit in Trial 1 and  $72 \pm 1$  g per kit in Trial 2). Allocation inside the farm was also equally divided between the treatments. Each treatment had ten or eleven litters of ten rabbits at the beginning of the experiment.

### *2.4 Evaluation of the feed intake*

Early feed intake was calculated daily based on the weight of feed offered and refused from 3 to 17 days of age in the first trial using a 0.001-g accuracy scale (OHAUS, Parsippany, NJ, USA). Water loss, measured as the difference of pellet weight between two days in the absence of animals, was measured the first days of the experiment for the four pellets A, B, C



and D. After correction for these intrinsic changes, it was found that feed intake was negligible the first week. For this reason, feed intake was measured daily from 8 to 17 days in the second trial. When waste or cup spillage occurred, we considered feed consumption in the two cups as missing data. In these cases, all of the nest material was renewed to ensure that no pellets were left inside the nest. Remaining feed in the feeders was weighed at 18, 21, 25, 28, 32 and 35 days of age. If one kit was found dead between these dates, the remaining feed in the cage was weighed to recalculate feed intake.

## 2.5 Physical characteristics of pellets

Pellet length and diameter were measured with a caliper with a resolution of 0.5 mm (ROCH, France). Diameters obtained were in accordance with the die used during the pellet processing (Table 3), with the biggest pellet (pellet D) 3-fold larger than the smallest one (pellet A). Pellet hardness was determined with the Kahl Pellet Hardness Tester (AMANDUS KAHL GmbH & Co. KG, Reinbek, Germany) on a minimum of ten pellets. Each pellet was inserted between two bars with an increasing static pressure to determine the force needed to crack it (Thomas and Van der Poel, 1996). Force values obtained in Newtons were then expressed as a pressure variable to take account of the length and diameter of each pellet tested as follows:

$$H = Hm / (\pi * l * r)$$

where  $H$  is the calculated hardness (MPa),  $Hm$  is the measured hardness (N),  $l$  is the length of the pellet (mm), and  $r$  is the pellet radius (mm)

Pellet durability is “the amount of fines returning from pellets after they were subjected to an abrading action” (Thomas and Van der Poel, 1996). For each measurement, 200 g of feed pellets were tumbled in a SABE drum (SABE Distribution, Chauche, France) at 500 rpm for 20 seconds. After tumbling, the fines (particles of less than 2 mm) were removed.

Durability was expressed as the percentage of remaining pellets (ISO 17831-1:2015). This procedure was repeated three times per pellet type.

The particle size distribution was determined after water and ultrasonic treatments according to the Lebas and Lamboley procedure (1999). Water-holding capacity (WHC) and feed-swelling capacity (SC) were measured for each type of pellet to check for confounding effects since these properties affect satiation (Tan et al., 2017). WHC was determined twice according to the Giger Reverdin (2000) method: 2 g of sample were poured into 10 mL distilled water and left to soak for 24 hours, so that water was in excess. After centrifugation, wet supernatant was removed and weighed to measure water absorption. WHC was calculated as the ratio of the supernatant weight to the sample dry matter weight. SC was obtained by dissolving 2 g of sample in 25 mL distilled water. After 1 hour at room temperature, the increased volume of the feedstuff due to hydration was measured to determine SC, as previously described by (Arroyo et al., 2012). WHC and SC were measured in triplicate.

## *2.6. Chemical analysis of the feeds*

Chemical analysis was performed on the nine experimental pre-weaning pellets and on the commercial pellets provided to the does using the ISO methods and the procedures described by the European Group on Rabbit Nutrition (EGRAN, 2001). Dry matter (DM) was obtained after 24 h at 103°C (ISO 6496:1999) and ashes after 5 h at 550°C. Total nitrogen was determined with the DUMAS combustion method using an automatic analyzer (Vario El cube model; Elementar, Langenselbold, Germany) to approximate the crude protein content ( $N \times 6.25$ ) (ISO 16634-1:2008). Neutral detergent fiber (aNDFom) and acid detergent fiber (ADFom) were measured using the sequential method of Van Soest et al. (1991) with a heat-stable amylase pre-treatment (AFNOR, 1997). Results were expressed exclusive of residual ash. Lignin analyses were performed on ADFom residues by solubilization of cellulose with sulfuric acid (sa). Starch was determined with the enzymatic procedure described by

Kozłowski (1994): starch was solubilized in potassium hydroxide and then hydrolyzed with amyloglucosidase. Released glucose molecules were finally quantified with the hexokinase glucose 6-phosphate dehydrogenase system (D-Glucose®; Boehringer, Mannheim, Germany). Intense ether washing was performed using the Tecator Soxtec system HT (FOSS, Hilleroed, Denmark - acid hydrolysis pre-treatment) to determine crude fat content (ISO 6492:1999). Gross energy was established with adiabatic combustion (Model C5000 adiabatic calorimeter; IKA, Staufen, Germany) (ISO 9831:1998).

## 2.7. Calculation and statistical analysis

Before weaning, statistical models include the litter as the experimental unit, whereas after weaning, the individual animal is the experimental unit. Feed intake values in the nest (8-17 days) that were three standard deviations away from the mean were removed. These outliers represented less than 1.5% of the dataset. For feed consumption after 17 days of age, the number of kits was adjusted assuming that dead animals did not consume feed two days before their death.

Voluntary feed intake measurements were used to determine feed preferences: relative consumption (RC) was calculated for each pellet within a litter, which is the percentage contribution of a pellet to the total feed intake. For example, in the treatment AB of a given litter, the RC of pellet A was calculated as follows:

$$RC_A = \left[ \frac{\text{Amount of pellet A consumed}}{\text{Amount of pellet A consumed} + \text{Amount of pellet B consumed}} \right] * 100$$

This index was chosen to describe preferences because it is informative, intuitive and has practical appeal (Lockwood III, 1998). RC values could represent total aversion (0%), indifference (50%) or strong preference (100%; Seabolt et al., 2010). If RC was significantly higher than the neutral value of 50%, then a preference for the given pellet was observed inside the treatment. RC values of the first trial were calculated as of 13 days of age because

we encountered technical difficulties with the feeding system before this date (cups spilled by the rabbits). In the second trial, RC values could be calculated from 8 to 35 days.

Statistics were performed with R software (R Core Team, 2018). One sample t-test was performed to test the RC means compared to the neutral value of 50%. If the RC value did not fit a normal distribution, Arcsine transformation was used. Comparison treatment effect on feed intake, milk intake and growth performance was controlled to ensure that preference responses could be compared between treatments by using one-way analysis of variance. In the second trial, the intake of 2.5-mm pellets was also compared with the intake of 4-mm pellets with ANOVA procedure. A linear mixed model was fitted to investigate inter-litter variability of the feed consumption measurements in the second trial from 8 to 21 days since no milk data were available beyond this date. Daily milk data between 8 and 21 days were obtained with extrapolation of the farm measurements by modeling the lactation curve with a quadratic model (Casado et al., 2006). Time, comparison treatment and nest quality scores were set as fixed categorical factors, and litter weight at equalization and milk consumption were set as quantitative cofactors; the litter was considered to be a random effect. A linear mixed procedure was also applied on post-weaning data with comparison treatment before weaning and rabbit sex as fixed effects, litter and fattening cage as random effects. Mortality data was analyzed using adjusted chi-square according to the Donner and Banting procedure (1989), so that litter cluster was accounted for (Reed, 2004; Princée, 2016). Differences were considered to be significant when  $P \leq 0.05$ . If P-value was less than 0.10 but greater than 0.05, then differences among means were considered to suggest a tendency.

### 3. Results

#### 3.1. Pellet physical characteristics

Measurements of pellet physical characteristics are given in Table 3. In Trial 1, by ranking in increasing order of hardness, we obtained pellets D, A, B and C (pellet C was 36%

harder than pellet D). In Trial 2, for the 2.5-mm-diameter pellet group, pellet G had a greater hardness than pellets E and F (+ 0.3 MPa), whereas for the 4-mm diameter, pellet C was softer than pellets H and I (- 0.2 MPa). These differences in hardness were related to the die length-to-diameter ratio used: 5.6 for G (highest compression rate of its group) and 4.5 for C (lowest compression rate of its group). Pellet D showed a notably low durability ( $85.7 \pm 1.1\%$ ) compared to the other pellets ( $94.3 \pm 1.9\%$  on average).

### 3.2. Feed and milk intake

Considering the whole pellet intake, regardless of the comparison treatments in Trials 1 and 2, we observed that solid feed ingestion in the nest started at 8 days of age, with a mean of  $0.03 \pm 0.03$  g of DM consumed per kit (Fig. 4). One fourth of the litters did not start solid feed intake at 8 days, while one day later, only 9% had not started. At 12 days of age, all the litters were consuming the pellets. Pellet intake progressively increased with age and reached  $0.54 \pm 0.19$  g of DM per rabbit at 17 days. Between 15 and 17 days, the feed was accessible in both the nest and the feeders. The young rabbits preferred to consume feed in the nest rather than in the feeders during this period ( $+0.20$  g of DM/rabbit from 15 to 17 days in the nest compared to the feeder origin;  $P = 0.003$ ). Over the first 17 days of life, a rabbit consumed a total of  $1.63 \pm 0.76$  g of DM in the nest and a total of  $2.50 \pm 0.44$  g of DM from the pellets in both the nest and the feeders. In relation to fresh matter, the pellet intake from 8 to 17 days corresponded to 1.3% of the total milk intake over this period. The feed-to-milk intake ratio increased from 0.1% of the milk intake at the beginning of the experiment (day 8), to 2% of the milk intake at 17 days of age. Interestingly, post-natal feed intake was highly variable between litters (CV = 89% for the total feed intake at 12 days, for example). A statistical model was therefore built to determine which factors had an effect on the daily feed intake using data obtained in the second trial until 21 days of age. Daily milk intake and nest quality score did not affect the solid feed intake (NS). Instead, the body weight at 3 days of

age (average weight at 3 days:  $109 \pm 9$  g/rabbit) correlated positively with the litter's post-natal pellet consumption from 12 to 17 days ( $P=0.003$ ), and the correlation coefficients ranged from 0.3 to 0.5 (Fig. 5). After 21 days of age, the amount of feed consumed quickly increased (Fig. 6) to reach  $65 \pm 14$  g of DM per kit on average at weaning. Concomitantly, the milk yield curve reached a plateau at around 21 days ( $292 \pm 75$  g of milk/doe).

The total feed intake in the feeders was similar between comparison treatments (Table 4) except at the beginning of the first trial (15 – 17 days). However, the precision of the mean estimates for this period was low as indicated by the standard error of the mean (SEM). We compared intake of 2.5-mm pellets vs. 4-mm pellets in Trial 2, a situation where no choice between diameters was given (EF, EG and FH treatments compared to CH, CI and HI treatments). Between 21 and 27 days, the 2.5-mm pellet intake tended to be higher than the 4-mm pellet intake ( $P = 0.068$ ), but no effect was observed from 15 to 35 days ( $P = 0.645$ ).

### 3.3 Pellet preferences

RC value of the 6-mm-diameter pellet (D) was greater than 50% when offered with the 2-mm-diameter pellet (A) in the nest ( $P=0.002$ ). No other preference was expressed in the nest within treatments of the first trial (Fig. 7). In contrast with what was observed in the nest, clear-cut aversion was found for pellet D compared to A, B (3.2 mm) and C (4 mm) in the feeders, with RC values below 50% of total feed intake (RC of 14% in the double-choice group AD, 28% in BD and 27% in CD;  $P<0.001$ ; Fig.7). Relative consumption of A was greater than 50% when it was offered against B (61%;  $P=0.021$ ) and D (86%;  $P<0.001$ ), and tended to be greater than 50% compared to C (67%;  $P=0.081$ ).

In the second trial, RC values within the double-choice groups EF, EG and FG did not significantly differ from 50%, meaning that no preference was expressed throughout the whole trial (Fig. 8). In the nest, slight avoidance was noticed for I compared to C (RC of 40%;  $P=0.014$ , when compared to the 50% neutral value) but this response was no longer

significant in the feeders ( $P=0.117$ ). When H was present with I in the feeders, the latter was avoided since its relative consumption was less than 50% (39%;  $P=0.020$ ).

### *3.4 Health and growth performances according to treatments*

Throughout both trials, all animals remained healthy: the mortality was low (4.5% from 3 to 35 **days** and 1.8% from 36 to 72 **days**) and was not affected by the pellets offered before weaning (NS). Milk intake was similar between groups as well as feed intake from 18 **days of age** to weaning.

Growth performances are reported in Tables 5 and 6. Rabbit weights before weaning were not affected by the comparison treatments (NS). Double-choice treatment had an effect on the daily weight gain between 21 and 35 **days of age** in the first trial ( $P = 0.039$ ), but the Tukey follow-up test performed on the six group means was not significant for any pair of means. The gender did not affect growth after weaning, thus the sex effect was removed from the final model. Rabbit growth performances during the fattening period did not differ between comparison treatments.

## **4. Discussion**

We hypothesized that stimulating early solid feed intake would contribute to preserving the health of young rabbits through a positive action on cecal microbiota implantation. We thus aimed to, first, quantitatively describe early feed intake and, second, to determine young rabbit pellet preferences according to their physical properties.

### *4.1 Characterization of feed intake patterns*

To our knowledge, this study is the first to provide comprehensive quantitative data on the solid intake of suckling rabbits. A nest feeding system was especially developed to measure post-natal rabbit feed intake. Previous studies dealing with the feeding capacity of young rabbits provided gradual amounts of pellets in the nest. They visually observed solid

intake (Kacsala et al., 2017) or measured the consumption by counting the number of feed pellets left (Gidenne et al., 2013). By providing quantitative feed intake records, this study provided new insights into the occurrence, magnitude and variability of this behavior.

Suckling rabbits were able to consume some feed pellets as of 8 **days of age**. Before this age, the diet of young rabbits depends on milk supply alone. The absence of solid intake during the first week might be explained by a lack of capability since mastication is functional as of 13 **days** and kits are still learning to masticate until 4 weeks of age (Langenbach et al., 2001). Furthermore, while the olfactory system is fully developed at birth, the onset of visual functions is estimated to be at around 7-8 **days of age** (Gottlieb, 1971). These ontogenetic parameters should be considered when developing feed adapted to the young rabbit, for example, by adding sensory functional ingredients in the feed or by offering feed with an optimal texture for the kit's masticatory system. In this study, pellet size and hardness were investigated since we assumed that rabbits would prefer soft and small pellets because they are easier to chew.

In the present study, we were able to determine the evolution of solid feed intake in kits. As previously described (Gidenne et al., 2010), the amount of solid feed ingested outside the nest remained low until 21 **days**, before a sharp increase. The early onset of solid feed intake allowed by a nest feeding system did not modify this general pattern. In the nest, the between-litter variability of the solid feed intake was high, especially in the early period. Our results showed that the maternal ability of the doe, assessed together with milk intake and nest quality, did not affect the early feeding in the nest or in the feeders. Bonachera et al. (2017) found, on the contrary, that the higher the milk intake was during early lactation (0-17 **days**), the higher the feed intake (17-28 **days**) was, which suggests that high milk intake could promote solid intake. However, these authors did not study the correlation between the feed intake and the corresponding milk intake at an early stage (before 17 **days of age**). At this age,



we hypothesized that other factors could explain dietary intake, including litter weight at standardization, which was positively correlated with the early feed intake of the litter. At the within-litter level, a similar conclusion was reached by Pajor et al. (1991) who studied the variation of solid feed intake of suckling piglets. They demonstrated that piglets of high birth weight ate more pre-weaning feed than their littermates of lower birth weight. This could be explained by greater needs and a more developed digestive system in heavier animals (de Passillé et al., 1989). However, these findings were not consistent with the research of Bruininx et al. (2004) who did not report a relationship between the piglets' birth weight and individual early feed consumption. Better knowledge of the animal related factors that affect solid feed intake variability is of importance in order to increase both the number of rabbits that exhibit early feed intake and the levels of ingestion.

#### *4.2 Pellet processing and effects on suckling rabbits' preferences*

This study attempted to analyze separately diameter and hardness effects by using pellets with different diameters and constant compression rate, and vice versa. However, hardness differences were found between pellets of different diameters in Trial 1, whereas the pellet compression rates were similar. Inversely, for pellets in Trial 2 with the same diameter, different compression rates led to similar hardness. These unexpected results might be explained by the heterogeneity of the internal pellet structure inherently caused by the diverse raw materials that make it up. Thomas and Van der Poel (1996) confirmed the large variation of fragmentation-type measurements for a given hardness device. For this reason, hardness measurements should be carefully interpreted. As expected, the pellet processing of this experiment did not alter pellet chemical composition and hydration properties, which suggests that post-ingestive effects were similar between feeds. Thus, dietary preferences were considered to be a good indicator of feed palatability, which is defined as the "physical and chemical properties of the diet that are associated with promoting or suppressing feeding

behavior during the pre-absorptive or immediate post-absorptive period” (McArthur et al., 1993). In the first trial, results suggested a shift of preferences between the nest and the feeder, with a slight preference for the pellet with the greatest diameter over the lowest (6.0 mm over 2.0 mm) in the first days of the experiment, whereas the rabbits rejected 6.0-mm-diameter pellets a few days before weaning. The initial preference expressed for the 6.0-mm pellets may be explained by an overestimation of their intake due to their low durability index. Indeed, pellets of low durability produce a greater amount of fines, which are not consumed by the rabbits (Maertens, 2010) but are counted as consumed with food weighing method. Pellet durability could not be controlled with our experimental design during the pelletizing since die size has an intrinsic effect on durability (Oduntan and Koya, 2016). In the feeders, we were able to rank the pellets by order of preference: first: 2.0-mm-diameter pellets; second: 3.0- and 4.0-mm-diameter pellets; and third: 6.0-mm-diameter pellets. Regarding pellet physical properties, pellets with diameters ranging between 2.0 and 4.0 mm had similar hardness, durability and hydration properties, so we could therefore conclude that the higher palatability of 2.0-mm-diameter pellets was due to their small diameter. Pellet presentation can modify feed sensory characteristics such as appearance and feed texture sensation. Ease of prehension of small pellets may partly explain their attractiveness for the litters since rabbits are known to eat pellets one by one (Maertens, 1994). Regarding the largest pellets, they were found to have a low durability index, which could negatively affect their palatability since rabbits do not like fines as previously discussed. Therefore, it remains unclear to what degree the large diameter of these pellets is responsible for their low palatability. In the second trial, rabbits slightly avoided eating the 4.0-mm-diameter pellet processed with the highest compression rate when facing another kind of 4.0-mm-diameter pellet. However, this avoidance was only visible in one of the two comparison combinations in the nest and in the feeders. Limited statistical power due to the sample size used in the

present study (n = 10 litters) may have reduced the chance to detect moderate magnitude preferences for pellets with a lower compression rate both in the nest and the feeders. Physical measurements performed on the pellet with the highest compression rate did not highlight any specific characteristics when compared to other 4.0-mm-diameter pellets. Consequently, the effects of compression rate on pellet palatability still need to be investigated.

Overall, in the present study, no clear preference pattern in the nest was determined. The small quantity of feed consumed in the nest, which requires high measurement accuracy, may explain why we did not detect preferences. This phenomenon can also be explained by the low activity of the kits during the two first weeks of life: their exploration of the nest is limited at that time and feed consumption probably occurred randomly. In contrast, in the feeders, suckling rabbits were able to differentiate pellets according to their diameter.

#### *4.3 Stimulation of the early feed intake and implications*

Currently, rabbit pellets are manufactured with a diameter of 3.0 or 4.0 mm (Acedo-Rico et al., 2010). High palatability of 2.0-mm-diameter pellets in the present study suggests that a preferential use of small-diameter pellets could be advocated to stimulate early consumption. However, some studies have pointed out that when domestic caged rabbits expressed a preference for a specific feed rather than another, the daily feed intake could be similar between the two types of feed once they were offered alone (Gidenne et al., 2010). Nevertheless, these studies were performed on fattening rabbits, and suckling rabbits may present specific feed intake regulations. In the first double-choice trial, total feed intakes of treatments providing the smallest pellet did not differ from the feed intake of other treatments, except between 15 and 17 days (however those results might be meaningless due to low precision). It could suggest that the higher palatability of the small pellets is not sufficient to increase feed intake. On the contrary, in the second trial in this study, there was a trend towards a higher feed intake in the feeders for the treatments with 2.5-mm pellets when

compared to treatments with 4.0-mm pellets. Experiments where one kind of pellet is tested per litter (no-choice tests) are now required to ensure that the feed intake increases with small pellets and to evaluate further consequences on rabbits growth.

In the current study, the onset of solid feed was advanced by eight days compared to conventional rabbitries where the solid feed intake behavior starts at around 16 days of age (Gidenne and Fortun-Lamothe, 2002). The substrates precociously reaching the gut from the solid feed are likely to stimulate the microbiota implantation process. Indeed, it was shown that intake of fecal pellets left behind by the doe in the first 10 days after delivery plays a role in cecal microbiota colonization (Kovács et al., 2006; Combes et al., 2014), even if the ingestion is small (a mean of ten fecal pellets per litter with free milking conditions). The feed is also a key factor for microbiota activity, as highlighted by Kovács et al. (2006) through an early weaning experiment: when the rabbit solid feed intake was stimulated as of 21 days of age by preventing milk supply, the cecal microbiota fermentation activity significantly increased. Seeding the microbiota of neonatal gut with healthy commensal bacteria is essential to optimize rabbit health since it is known that microbiota strongly interacts with mammal health (Mage et al., 2006; Combes et al., 2018). The effect of early feeding on the rabbit health needs to be further investigated since it could not be studied with our experimental design (all the litters had access to pellets as of 3 days of age).

## 5. Conclusion

Intake of supplemental solid feed provided in the nest was observed as of 8 days of age. The high variability of feed consumed during the early suckling period was partially explained by the litter weight at the beginning of the experiment: heavier litters were associated with higher solid feed intake at two weeks of age. Outside the nest, suckling rabbits were able to discriminate pellets according to their diameter with the following preferences: 2 mm > (3.2 and 4 mm) > 6 mm diameter pellets. The effect of compression rate on the kits' feeding

behavior needs to be further investigated. In terms of implications, this research shows that it is possible to promote post-natal feed intake through a nest feeding system and that pellet palatability can be improved before weaning with an optimal presentation. The acceleration and the increase in the solid intake before weaning could contribute to the acquisition and maturation of the kit microbiota and, hence, contribute to improved health.

Declarations of interest: none

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**Fig. 1.** Housing used to control separately the doe's feed intake and that of its litter, with a limited access to the nest box for the doe. On the top is mapped the view of the cage seen from the top. Below is represented the view of the same cage seen from left side.

**Fig. 2.** View of the nest box when rabbits were 4-days old. The two cups on the right contained two types of feed pellets differing in either diameter (Trial 1) or compression rate (Trial 2).

**Fig. 3.** Experimental design used to characterize suckling rabbit intake and pellet preferences.

**Fig. 4.** Dynamic of feed consumption in 8 to 17-day-old rabbits receiving pellets in the nest as of 3 days of age (Trials 1 and 2). From 15 to 17 days, feed was also available in feeders outside the nest. The intake is the sum of the consumptions of the two types of pellets tested. Results are presented as the mean and standard deviation. The different letters stand for significant differences ( $P < 0.05$ ).

**Fig. 5.** Relationship between starter feed intake and litter weight (at 3 days of age) in Trial 2. The total feed intake from 12 to 14 days is represented on the left, and the total feed intake from 15 to 17 days is represented on the right. Dot size is proportional to milk consumption.  $r$  stands for the Pearson Correlation,  $P$  for the significance level of the correlation, and  $N$  for the sample size.

**Fig. 6.** Feed and milk consumption of suckling rabbits receiving solid pelleted feed as of 3 days of age (Trials 1 and 2). For each litter, the intake is the sum of the consumptions of the two pellets tested. Results are presented as the mean and standard deviation.

**Fig. 7.** Relative pellet consumption in the nest (A) and in the feeders (B) for the first trial. The animals had the choice between two pellets that differed in diameter (A: 2 mm; B: 3.2 mm; C: 4 mm; and D: 6 mm). Results are presented as the mean and standard deviation. The symbols represent significantly different preference values ( $\dagger$ :  $P < 0.1$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ).

**Fig. 8.** Relative pellet consumption in the nest (A) and in the feeders (B) for the second trial. The young rabbits had the choice between two pellets of the same diameter (E, F, G: 2.5 mm, and C, H, I: 4 mm) that differed by compression rate. Results are presented as the mean and standard deviation. The symbols represent preference values significantly different by 50% (\*:  $P < 0.05$ ).

654 **Table 1** Ingredients and chemical composition of the experimental meals.  
655

	Kits <sup>a</sup>	S.E. <sup>a</sup>	Does
Ingredients (g/kg as-fed basis)			
Wheat	50		54
Barley	148		120
Wheat bran	150		300
Rapeseed whole seed	20		
Rapeseed meal	82		100
Rapeseed oil	5		2
Sunflower whole seed			73
Sunflower meal	240		120
Sunflower husk			77
Alfalfa meal	112		
Sugar beet pulp	110		76
Sugar beet molasses			50
Apple pomace	27		
Cane molasses	30		
Sodium chloride			6
Dicalcium phosphate	1		
Calcium carbonate	6		
L-Lysine, 20%	5		3
Methionine, 15%			2
Threonine, 20%			12
Mineral and vitamin premix <sup>b</sup>	10		5
Coccidiostat <sup>c</sup>	5		
Chemical composition (g/kg as-fed basis)			
Dry matter	909	7.4	890
Ash	81	5.6	80
Crude protein	176	2.3	172
aNDFom <sup>d</sup>	337	5.9	316
ADFom <sup>e</sup>	189	3.3	176
Lignin (sa) <sup>f</sup>	72	1.2	53
Starch	52	3.8	86
Fat content	33	0.6	27
Gross energy (MJ/kg)	17.0	0.3	16.4

656 <sup>a</sup> Means and standard errors for chemical composition of the nine pre-weaning pellets.

657 <sup>b</sup> Premix provided per kg of complete feed: vitamin A, 10,000 IU; vitamin D3, 1000 IU; vitamin E, 50.  
658 IU; Cu, 10 mg; Fe, 100 mg; Zn, 50 mg; Mn, 5 mg; I, 0.2 mg; Co, 0.1 mg; Se, 0.2 mg.

659 <sup>c</sup> Containing 0.5% diclazuril.

660 <sup>d</sup> Neutral detergent fiber expressed exclusive of residual ash.

661 <sup>e</sup> Acid detergent fiber expressed exclusive of residual ash.

662 <sup>f</sup> Lignin obtained with sulfuric acid method.

663

664 **Table 2** Pellet press dies specifications used to process young rabbit feeds.  
665

Pellet	Trial	Die specification		
		Channel diameter (mm)	Channel length (mm)	Length-to-diameter ratio
A	1	2	10	5
B	1	3.2	16	5
C	1 and 2	4	18	4.5
D	1	6	24	4
E	2	2.5	10	4
F	2	2.5	12	4.8
G	2	2.5	14	5.6
C	1 and 2	4	18	4.5
H	2	4	20	5
I	2	4	24	6

666

667 **Table 3** Physical characteristics of the pellets processed with nine different dies.

	Trial 1					Trial 2				Trial 2			
	A	B	C	D	SEM	E	F	G	SEM	C	H	I	SEM
Die length-to-die width ratio	5	5	4.5	4	NC	4	4.8	5.6	NC	4.5	5	6	NC
Diameter (mm)	2.0	3.0	4.0	6.0	0	2.5	2.5	2.5	0	4.0	4.0	4.0	0
Length (mm)	8.9	8.6	8.8	9.4	0.5	8.2	8.8	8.5	0.2	8.8	8.2	8.5	0.2
Hardness (MPa)	1.19	1.36	1.45	1.06	0.15	1.54	1.52	1.76	0.09	1.45	1.69	1.66	0.11
Durability (%)	95.4	94.8	92.2	85.7	2.3	93.3	95.14	95.4	1.8	92.2	93.8	93.9	1.4
WHC <sup>a</sup> (g of H <sub>2</sub> O/g of DM)	4.70	5.02	4.95	5.07	0.14	4.96	4.94	5.04	0.27	4.95	5.07	5.28	0.10
SC <sup>b</sup> (ml of H <sub>2</sub> O/g of DM)	4.98	4.86	4.39	4.16	0.42	4.29	4.24	4.41	0.25	4.39	4.21	4.64	0.36
Particle size distribution (%)													
> 1 mm	45.7	47.5	43.4	40.5	2.8	46.4	45.4	46.9	2.3	43.4	44.8	48.0	3.8
0.5-1 mm	8.9	13.4	10.8	10.3	1.6	10.2	10.7	10.7	1.2	10.8	11.3	10.9	2.1
0.3-0.5 mm	6.9	6.6	5.6	7.2	1.4	8.1	5.9	6.1	1.7	5.6	4.2	5.8	2.3
< 0.3 mm	38.5	32.5	40.3	42.0	2.3	35.3	37.9	36.4	1.5	40.3	39.8	35.3	2.6

681

682 <sup>a</sup>WHC: Water holding capacity.

683 <sup>b</sup>SC: Swelling capacity.

**Table 4** Effects of the physical properties of the pellets on the feed intake in the feeders from 15 to 35 days (weaning). Values are means of the amount of solid feed ingested per rabbit per day. Means denoted by a different letter indicate significant differences between treatments.

Trial 1.								
	Double comparison groups						SEM	P-value
	AB	AC	AD	BC	BD	CD		
Feed intake (g of DM/rabbit/day)								
15-17 days	0.3 <sup>ab</sup>	0.4 <sup>ab</sup>	0.5 <sup>b</sup>	0.2 <sup>a</sup>	0.3 <sup>ab</sup>	0.2 <sup>ab</sup>	0.26	0.025
18-24 days	5	5	6	4	4	5	1.29	0.257
25-31 days	32	31	23	27	28	27	6.30	0.199
32-35 days	NC	NC	NC	56	59	57	NC	NC
Trial 2.								
	Double comparison groups						SEM	P-value
	EF	EG	FG	CH	CI	HI		
Feed intake (g of DM/rabbit/day)								
15-17 days	0.9	0.7	0.8	0.9	1.0	0.7	0.30	0.349
18-24 days	6	6	5	5	4	5	1.63	0.387
25-31 days	41	39	37	40	36	36	5.32	0.523
32-35 days	62	59	61	61	63	52	10.7	0.563

NC: not calculable because we ran out of pellet A as of 32 days.



**Table 5** Effects of physical properties of the pellets on growth performance before weaning.

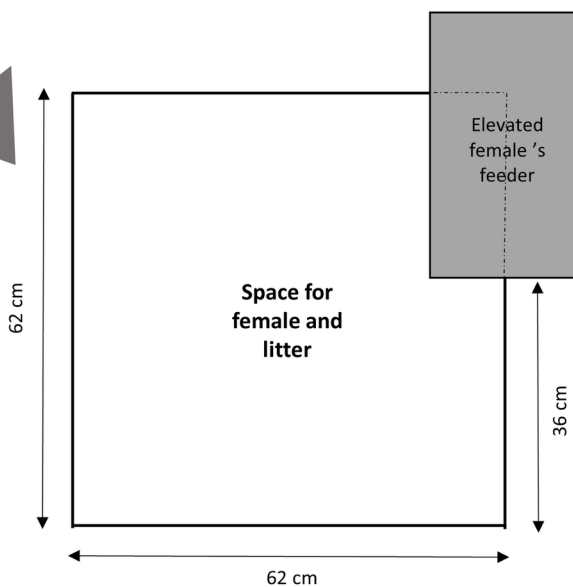
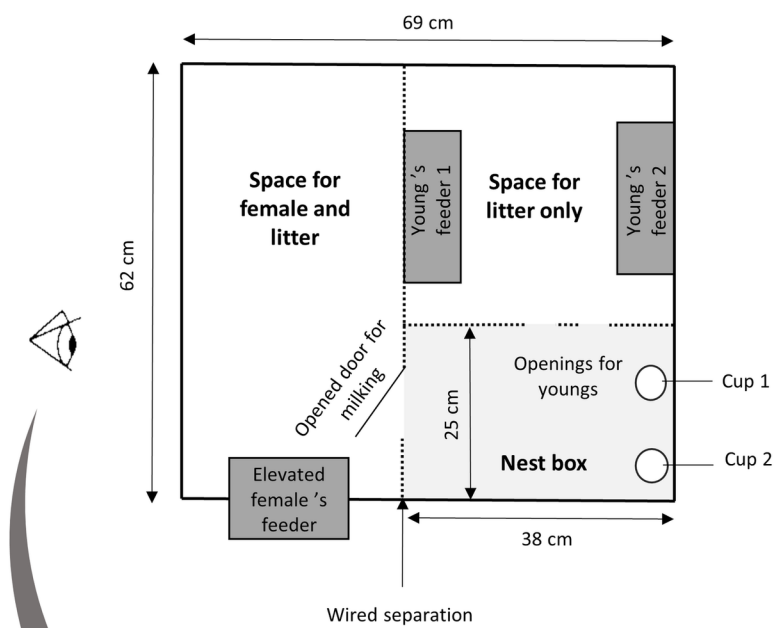
Trial 1.								
	Double comparison groups						SEM	P-value
	AB	AC	AD	BC	BD	CD		
Number of litters	10	8	10	10	10	10		
Live weight (g/rabbit)								
3 days	86	86	84	86	85	84	7	0.976
10 days	171	176	168	173	177	174	16	0.941
14 days	222	229	216	222	234	224	20	0.689
21 days	302	317	303	311	326	309	28	0.687
28 days	532	558	525	509	530	520	42	0.511
Average daily gain (g/d)								
3 – 21 days	12.0	12.8	12.2	12.5	13.4	12.5	1.4	0.627
21 – 35 days	38.6	38.4	33.8	33.6	35.6	35.9	3.3	0.039
Feed conversion ratio								
21 – 28 days	0.46	0.50	0.54	0.47	0.49	0.52	0.05	0.081
28 – 35 days	NC	NC	NC	1.04	1.04	0.97	NC	NC
Trial 2.								
	Double comparison groups						SEM	P-value
	EF	EG	FG	CH	CI	HI		
Number of litters	10	11	11	10	11	10		
Live weight (g/rabbit)								
4 days	110	112	108	109	105	107	7	0.713
14 days	280	279	269	282	273	272	22	0.880
21 days	381	385	378	387	378	372	31	0.960
28 days	646	643	628	639	606	601	44	0.353
Average daily gain (g/d)								
4 – 21 days	15.9	16.1	15.9	16.4	16.0	15.5	1.5	0.967
21 – 35 days	43.2	40.1	40.6	40.1	40.8	37.0	3.5	0.140
Feed conversion ratio								
21 – 28 days	0.46	0.47	0.46	0.45	0.42	0.48	0.06	0.660
28 – 35 days	1.14	1.20	1.13	1.22	1.09	1.15	0.10	0.226

NC: not calculable because we ran out of pellet A as of 32 days.

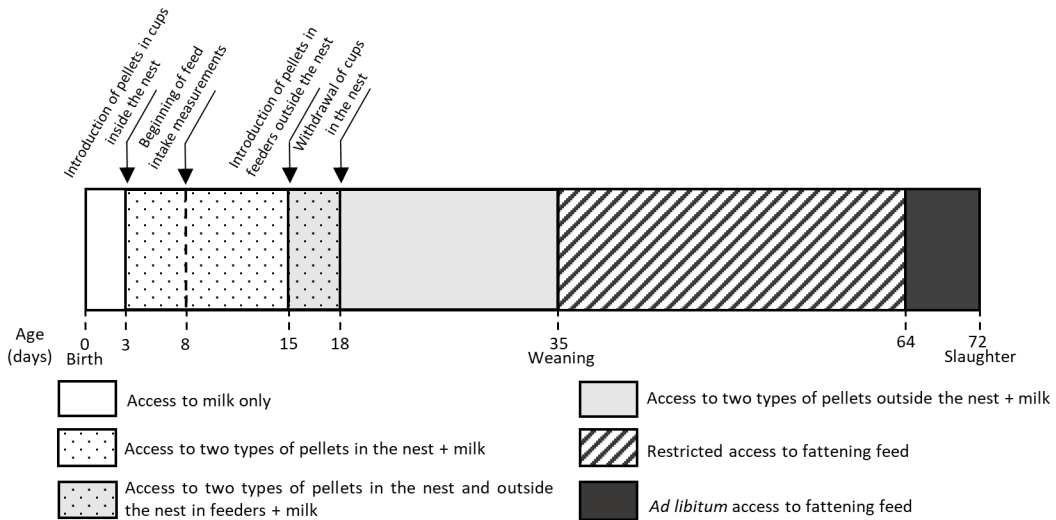
697 **Table 6** Effects of physical properties of the pellets on rabbit growth performance after weaning.  
698

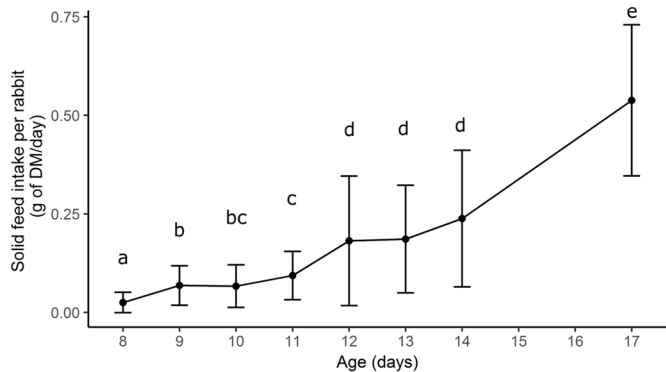
Trial 1.								
	AB	Double comparison groups					SEM	P-value
		AC	AD	BC	BD	CD		
Number of rabbits at weaning	98	75	92	93	85	89		
Live weight (g/rabbit)								
35 days	842	859	777	782	824	812	58	0.118
50 days	1225	1259	1195	1222	1227	1196	46	0.487
64 days	1808	1907	1822	1824	1855	1798	67	0.517
Average daily gain (g/d)								
35–50 days	25	27	28	29	27	26	3	0.259
50–64 days	41	46	44	43	45	43	3	0.370
Trial 2.								
	EG	Double comparison groups					SEM	P-value
		FG	EF	CH	CI	HI		
Number of rabbits at weaning	102	102	74	91	89	81		
Live weight (g/rabbit)								
35 days	986	947	934	889	948	959	62	0.228
50 days	1506	1510	1552	1511	1512	1477	43	0.591
64 days	2132	2138	2218	2152	2130	2133	61	0.518
Average daily gain (g/d)								
35–50 days	37	37	38	35	38	39.	2	0.784
50–64 days	44	45	47	45	44	47	3	0.344

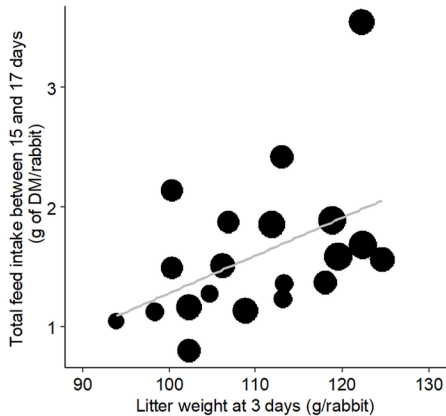
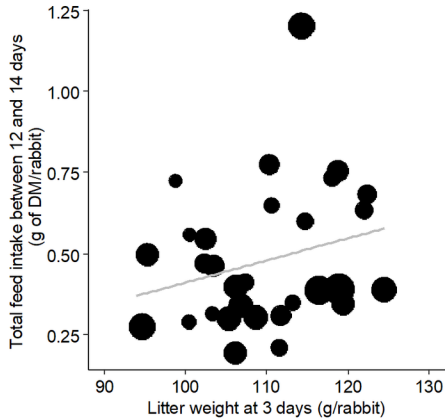
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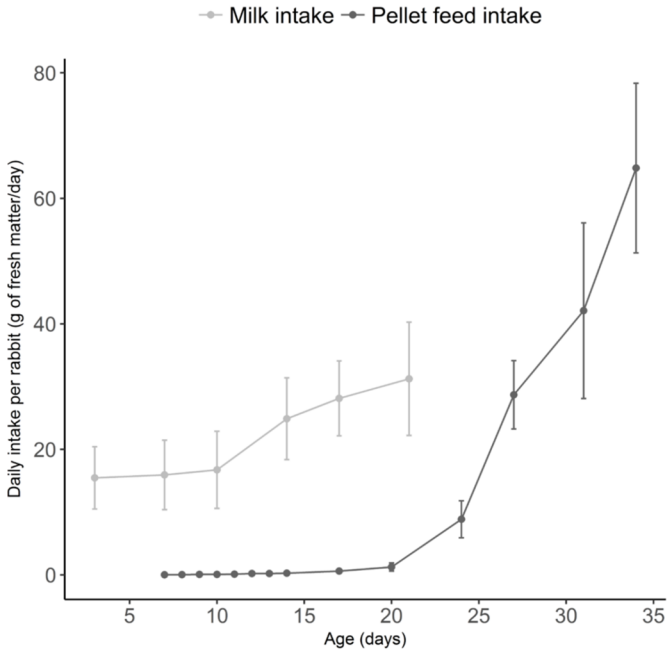






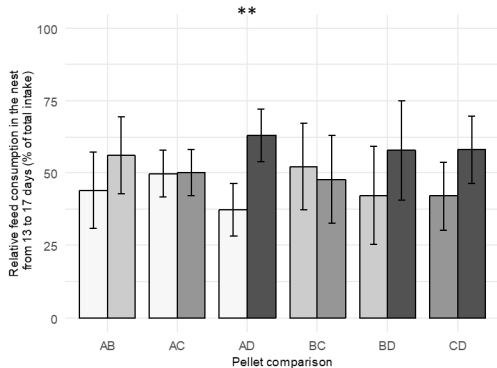




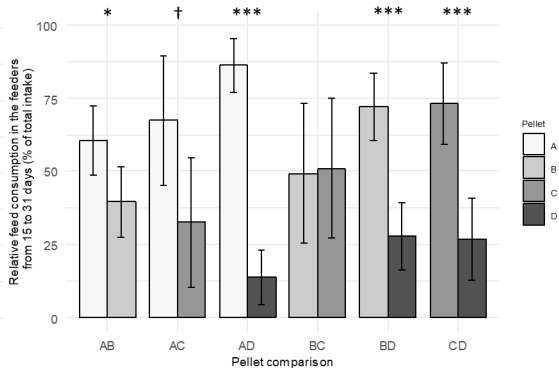




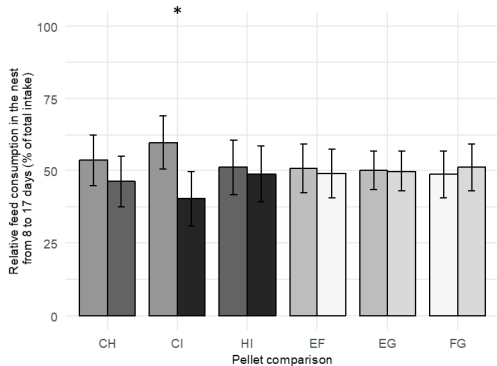
A) in the nest



B) in the feeders



A) in the nest



B) in the feeders

