World
Rabbit
Science
Association

PROCEEDINGS OF THE 12th WORLD RABBIT CONGRESS

Nantes (France) - November 3-5, 2021
ISSN 2308-1910

Session
NUTRITION & FEEDING

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Full text of the communication
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How to cite this paper
Martínez-Paredes E., Nicodemus N., Pascual J.J., García J., 2021. Challenges in rabbit does feeding, including the young doe (invited paper). Proceedings 12th World Rabbit Congress - November 3-5 2021 - Nantes, France, Communication N-00, 24 pp. + presentation

CHALLENGES IN RABBIT DOES FEEDING, INCLUDING THE YOUNG DOE

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ABSTRACT

In this review is summarized the last knowledge on rabbit doe nutrition, to complement the current nutritional requirements and strategies for the young and adult rabbit does, considering the production, and health issues. The rabbit doe must reach an adequate maturity level (body condition) at first artificial insemination (AI) to face its productive life with minimal guarantees (around 7.0 mm of perirenal fat thickness, 2.8 ng/mL of plasma leptin concentration and around 18% and 15-20% of body protein and fat, respectively). This goal can be achieved by restricting feed intake from 12 weeks of age until first AI or feeding *ad libitum* with a fibrous diet (<10.5 MJ digestible energy/kg) from 60 d of age to first parturition. Once the doe is reproducing, the increase of the n-3 fatty acids (or reduction of the n-6/n-3 ratio), soluble fibre (under epizootic enteropathy) and the Arg/Lys and Gln/Lys ratios may help to improve the reproductive traits of rabbit does, although their optimal level of inclusion remain to be identified. It is recommended to limit an excessive negative energy balance before parturition, and the supplementation of glucose precursors to reduce the ketosis incidence could be useful. The formulation of different diets for the doe and the litter to fit better their requirements and assuring their health would be an option to consider when it would be applicable in the farm. The influence of the mother on the litter microbiota and immune status and its potential modulation through the diet open a new research area that will deserve more studies in the next future.

Key words: Body condition, Lactation, Nutritional requirements, Rabbit does, Rearing.

INTRODUCTION

Feed is the greatest cost in rabbit production. It accounts for almost 45% of the total costs of a rabbit farm in Spain (72.5% of the variable costs) and between 55 and 60% in France (Cartuche *et al.*, 2014; Coutelet *et al.*, 2015). Rabbit doe feeding only makes up around one third of the total feed cost (3.7 and 31.7% for the replacement and reproductive does, respectively; Cartuche *et al.*, 2014). In spite of the lower incidence in the total feed cost compared to fattening rabbits, the nutrition of the rabbit doe is highly relevant to the final farm profit. In fact, according to these authors, the traits with a higher economic weight were the feed conversion rate during fattening and the number of kits born alive, being the latter influenced by the genetic and management (Huneau-Salaün *et al.*, 2015), but also by the feeding of rabbit doe.

In this way, the feeding of the rabbit doe can directly affect the number of kits born alive, but also the milk yield and composition, and therefore the survival and growth of the kits during lactation (Pascual *et al.,* 2013). In fact, some studies indicated that the feeding and genetics of the rabbit can affect the immune status of the kits at weaning, and even the incidence of digestive disorders during the growth period (García-Quirós *et al.,* 2014). This indicates that the feeding of the rabbit does not only has a direct effect on the cost of feeding and productivity during the reproductive period, but also on the performance we will obtain during the fattening period.

As a consequence, research on rabbit does nutrition and management has focused on the reproductive period, because the nutritional requirements are more demanding (lactation, overlapping between cycles, resources recovery…). However, focusing our efforts only in this period is not enough to maximize the potential of our reproductive rabbit does. A large number of works carried out during the rearing period (Xiccato *et al.*, 1999; Pascual *et al.*, 2002; Rebollar *et al.*, 2011; Martínez-Paredes *et al.*, 2012, 2018 and 2019) have demonstrated the relevance of adequate management and feeding strategies in this period on the future performance of rabbit does.

The present work aimed to review the current knowledge available on rabbit doe nutrition, to establish nutritional requirements and strategies for the young and adults' rabbit does, which take into account the production, and health of rabbit does, and that allows us to progress to a rational rabbit production system.

NUTRITIONAL REQUIREMENTS AND STRATEGIES FOR REARING THE FUTURE RABBIT DOE

Rearing development and body status

Different works in different species suggest that reaching an adequate development at the beginning of the reproductive life could be key to both improve productivity in the short-medium term and persist for a longer time (Pascual et al., 2013; Martínez-Paredes et al., 2018). In rabbit does, many works consider the evolution of body weight, body condition and hormonal status as the relevant factors during the rearing period (Pascual et al., 2006). These traits are closely related to each other. But how do these traits affect and how should be their evolution to improve the potential of the reproductive rabbit does? The answer would be that status that allows females to face the cyclical effort with the highest productivity, by optimizing their fertility and life span, as well as the numebr and health of kits weaned.

Regarding how the growth of the young rabbit doe should be, we must consider the existence of different growth patterns according to the genetic type, the environment and/or the individual itself. Notwithstanding, regardless of these factors, gradual growth seems the best option for rabbit females during the rearing period. Some studies have observed that a controlled growth of young rabbit does, by quantitative or qualitative feeding restriction, allows a gradual development during the rearing period and better performance during the first reproductive cycles (Xiccato *et al.*, 1999; Pascual *et al.*, 2002). Recently, Martínez-Paredes *et al.* (2018) obtained worse results both in the first parturition (on av. –1.3 liveborn) and in the whole productive period (–6.1 liveborn and –4.8 weaned) for those young rabbit does that showed faster growth patterns (43 days in advance) during the rearing period.

On the other hand, the different works that evaluated the evolution of body resources and metabolic profile, help to reinforce the idea that a deviation from the adequate body condition pattern during the rearing can be harmful to the future performance of rabbit does (Savietto et al., 2016;Martínez-Paredes et al., 2018). In this sense, Friggens (2003) suggests that reserves mobilization could have negative consequences on reproduction when is far from its optimal level. The development of methods to determine *in vivo* body condition in rabbit does (Pascual *et al.*, 2004; Pereda *et al.*, 2009; Pereda, 2010) has been very useful to evaluate the evolution of body reserves of young rabbit does during the rearing and reproductive periods, reaching similar conclusions.

Martínez-Paredes *et al.* (2018) observed that rabbit does having a high perirenal fat thickness (PFT) at the first artificial insemination (AI) also had a lower reproductive performance at the first parturition and in whole productive period (–3.2 liveborn and –3.0 weaned for each mm of increment in the PFT value). This worse reproductive performance of thick young females may be related to an increased number of prenatal losses during gestation (Vicente *et al.*, 2012), and/or to an increased culling risk among females with excessive PFT (Theilgaard *et al.*, 2006; Martínez-Paredes *et al.*, 2018). About the risk of death or culling of females during the rearing period, high PFT at first AI followed by a high pre-partum mobilization of reserves, low pre-partum' digestible energy (DE) intake, as well as high non-esterified fatty acids and low glucose levels in the blood at first parturition, are all factors associated with pregnancy toxaemia (Martínez-Paredes *et al.*, 2012). In addition, the rabbit does that had a high PFT at the first AI, showed a PFT evolution pattern different to the rest of the females until the second parturition, not being able to recover the body condition that they had at the moment of the first parturition (Martínez-Paredes *et al.*, 2019). In contrast, the body composition at first AI (body protein, fat and energy measured by bioelectrical impedance) did not influence the fertility rate at the first parturition but did it at the second one (Taghouti *et al.*, 2011a,b). These authors reported that the increase of body protein and fat combined with a low body protein/energy ratio at first AI improved the fertility rate at the second parturition. They also found an increase in the percentage of kits born alive over the total born in the first parturition when the body protein and energy content increased at first AI. Similarly, in a rabbit line selected for growth rate, Naturil-Alfonso *et al.* (2016) observed better performance results in the first third cycles of the rabbit does with a higher PFT value at the first AI (+0.5 mm respect lean ones). These studies suggest that more mature nulliparous does (but no overfatted ones) with a body protein content near 18% might have a better performance, probably due to the less competition between growth and pregnancy may exist. The maturity level depends on the age, diet and the genetic type. The question is what the optimal range for body condition at first AI is to optimize performance? It is also important to consider that the variability of the body condition at the beginning of the productive life is a key point and may account for some of the observed differences among studies.

Some of the blood metabolic parameters controlled during the rearing period can also help us to evaluate the body status and future performance of the rabbit does. One of them is the leptin, a metabolic indicator of body reserves that helps to regulate energy balance by modulating feed intake and fat storage in adipocytes. After reviewing the results of different studies that measured leptin during the rearing period (Brecchia *et al.*, 2006; Arias-Álvarez *et al.*, 2009; Rebollar *et al.*, 2011, and Martínez-Paredes *et al.*, 2012), it has been observed that to be successful at first mating, a minimum threshold of leptin must be reached (2.8 ng/mL), as a sign that the female is ready to start its reproductive life. Another good metabolic indicator, in this case of body reserves mobilization, is the non-esterified fatty acids level in the blood (NEFA). Rebollar *et al.* (2011) observed that a lower concentration of NEFA in the blood of rabbit does at first parturition was correlated with a better fertility at 11 d post-partum, and with a lower mortality of females and its litter at birth.

In brief, a proper management of physiological development of young rabbit does during the rearing period maximize both their productivity and lifespan. During the rearing period, the animal's growth pattern should be progressive (Figure 1), allowing the achievement of an adequate maturity degree at the beginning of its productive life, avoiding excessive fatness, to favor long-term reproductive performance and lifespan. In addition, *in vivo* tools to evaluate body condition could be useful to evaluate the management and feeding strategies to adjust growth patterns in the future.

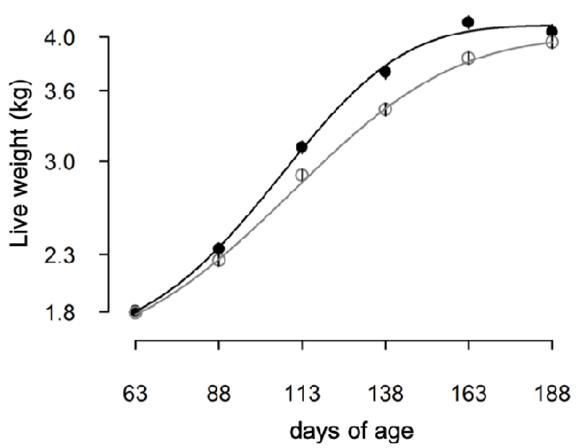

Figure 1: Evolution of live weight of young rabbit does with an uncontrolled feeding program (◆) or another programmed for a progressive development (○). Both programs allowed a similar live weight

at first parturition, but the uncontrolled one leads to an overweighing of females around first mating, which had negative consequences on further reproductive performance and lifespan. Adapted from Martínez-Paredes *et al.* (2018).

Rearing feeding programs: concentrate diets

To achieve the goals previously mentioned, the feeding and management programs during rearing must allow achieving adequate body condition and physiological development (both digestive and reproductive) to optimize future resource utilization. It will allow reproductive rabbit does to face the usual environmental and reproductive challenges with success. The definition of the type of feeding program, the period of application and age at first mating will be key to achieve these objectives.

Ad libitum programs. The use of *ad libitum* supply of reproductive or fattening diets at the onset of rearing (8-10 weeks old) was a feeding program widely used for many years in rabbit farms, addressed to achieve sexual maturity as soon as possible to reduce rearing costs. This is the opposite of the gradual development that we mentioned previously. In fact, the results obtained in *ad libitum* programs were increased risk of digestive problems in the first weeks of rearing period (Rommers *et al.,* 2004a; Martínez-Paredes *et al.* 2012), greater fatness and higher prenatal mortality (Viudes-de-Castro *et al.*, 1991), lower fertility at second parturition (Rebollar *et al.*, 2011), gestational toxaemia and lower feed intake at the start of lactation (Martínez-Paredes *et al.,* 2012), and reduced lifespan of rabbit does (Rosell, 2000; Martínez-Paredes *et al.,* 2018).

Restriction programs. Some authors (Partridge, 1986; Maertens, 1992) proposed the use of feed restriction of the reproductive or fattening diets as an alternative to the traditional *ad libitum* rearing programs. However, several studies indicated that some rearing programs based on feed restriction could delay growth and sexual maturity at first mating (Rommers *et al.*, 2004b), impair fertility (Szendrő *et al.*, 2006, Rebollar *et al.*, 2011) and prolificacy (Rommers *et al.,* 2001 and 2002, Naturil-Alfonso *et al.,* 2016). Such a strategy may also affect milk yield of lactating does (Menchetti *et al.*, 2015). These downsides could be the result of nonproper management of the restriction because there are many variables that can influence the program's success (the application period, restriction level, deficiency on some micronutrients, genetic type…). In fact, results from feed restriction trials have not provided conclusive results.

Suitable age to start and finish restriction. It seems appropriate not to start the restriction before 12 weeks of age in order to ensure the proper development of the main physiological structures of the female (Deltoro and Lopez, 1985). Pascual *et al.* (2002) started the restriction at 10 weeks of age (140 g/d) and the first insemination had to be delayed so that the females reached 3 kg of live weight. However, Martínez-Paredes *et al.* (2012 and 2019), with a similar restriction program that started at 9 weeks of age, reported a lower body weight of restricted females at the first insemination, but differences disappeared at parturition, having a better performance during the first two reproductive cycles. In other studies, it has been evaluated restriction or undernutrition after the first AI. Manal *et al.* (2010) restricted mature females (5 months old) during 15 or 20 days after first mating, showing an improvement of doe kindling performance and kit and litter traits from birth till weaning without adversely affecting progesterone level and embryonic mortality. However, Menchetti *et al.* (2015) observed that a severe restriction (90 g/d) of primiparous rabbit does at mid-late pregnancy (19-28 days of gestation) led to a negative balance and increased perinatal and pre-weaning mortality. The success of the use of feed quantitative restriction during the rearing period depends on the future rabbit female reaches an adequate physiological maturity at the first insemination to be able to cope first pregnancy with sufficient energy body reserves. In either case, the feed restriction should end at calving.

Restriction level. Eiben *et al.* (2001) compared young females fed at 100, 95 (full-fasting one day in a week), 82 (9 h of daily access to feed) and 76% (fixed daily maintenance provision) of *ad libitum* feeding, and only observed a delay in reaching the adequate body weight at first mating in the last two groups, without negative consequences on reproductive performance. Although there are not many studies with different genotypes, Matics *et al.* (2008) described a longer delay achieving the targeted weight (between 4.5 to 5 kg) when the restriction was applied in females with a larger format (around 6 kg of adult age). Similarly, in a rabbit line selected for growth rate, Naturil-Alfonso *et al*. (2016) observed worse reproductive performance results (–0.2 kindling rate, +0.18 and +0.29 gestational and foetal losses and –1.6 kits during the first three reproductive cycles) when rabbit does were restricted (130 g/d) a month before being mated. However, in other studies done with the same scheme and line, the differences on reproductive performance disappeared (Naturil-Alfonso *et al.,* 2017). In maternal crossbreed females, Martínez-Paredes *et al.* (2012) observed that a restriction of 140 g/d since 12 weeks of age, with a flushing 4 days before mating (18 weeks of age), led young rabbit does to show an energy intake below to the recommendations (Xiccato and Trocino, 2010), causing a delay of body development respect to those fed *ad libitum.* However, these restricted females with flushing reached the first AI with a desirable lower PFT level, without consequences on fertility and reproductive performance.

Long-term effects of restriction. There is practically no information on the long-term effects of restriction during the rearing period, but Martínez-Paredes (2008) did not find differences in lifespan nor in reproduction parameters (born alive and weaned per year) compared to other *ad libitum* feeding systems during nine reproductive cycles.

The results seem to indicate that the restriction does not produce negative effects if applied at the beginning of the reproductive life of the rabbit females if they assure sufficient physiological maturity at first AI. However, more studies in the long-term must be done to corroborate these results and understand the implications of this management system. With the results discussed above, we could conclude that moderate restriction during the rearing period can let the young rabbit does achieve reproductive success. However, the conditionings are many, which makes it difficult to define a clear management and restriction program during the rearing period.

Rearing feeding programs: high-fibrous diets

Another alternative could be the *ad libitum* use of high-fibrous or low-energy diets during rearing, formulated to fit the requirements of young rabbit does. Theoretically, their use could prevent over-fatting and promote digestive tract development to improve feed intake capacity before and after the first parturition, contributing thus to reduce possible energy imbalances that usually affects primiparous rabbit does. The results obtained of this feed strategy seem to depend on several factors such as the chemical composition of the diet, the management system of the females, the genotype and the environmental conditions of the farm.

Suitable age to introduce high-fibre diets. As the success of this strategy is partially given by the greater development of the digestive tract of the female (Fernández-Carmona *et al*., 1998), it would be advisable to begin the administration of fibrous diets before 12 weeks of age, when the development of digestive tract is almost completed (Deltoro and Lopez, 1985). For this reason, Pascual *et al*. (2013) proposed the application of fibrous diets as early as 60 days of life, because if these diets are included later the expected benefits are limited, regardless of the dietary amount of fibre [360-500 g neutral detergent fibre (NDF) per kg DM; Quevedo *et al*., 2005; Verdelhan *et al*., 2005; Pereda, 2010; Martínez-Paredes *et al*., 2012]. Respect to the effect on body condition, an early introduction of this type of diets allows to reach the recommended leptin levels to ensure the reproduction (Martínez-Paredes *et al*., 2012), and reduce the level of NEFA in blood during pregnancy or parturition (Rebollar *et al*., 2011). Also, no differences in body energy content (Xiccato *et al*., 1999), or similar o lower PFT values (Martínez-Paredes *et al*., 2012 and 2018, respectively) have been observed at first AI when their use was compared with the other feeding strategies during rearing. The high-fibre diets were usually offered until the first parturition without negative consequences on the performance.

Nutrient recommendations. Another important aspect of the high-fibrous diets is the level and nature of the fibre they contain. If the lignin levels are excessive (above 150 g of acid detergent lignin (ADL) per kg of DM), puberty is delayed and fertility is impaired respect to normal lignin values (50 g

ADL/kg DM; Arias-Alvarez *et al*., 2009). However, lower values (107 g of ADL per kg DM) promote a higher feed intake of young females, resulting in young females capable to take enough energy in to overcome gestation, lactation and growth, and at the same time being also capable to conceive again (Rebollar et al., 2011). These results are similar to those obtained with moderated ADL levels (59-75 g of ADL per kg DM) in multiparous does (Nicodemus *et al*., 1999a and 2007). On the other hand, the largest increases in rabbit doe intake during lactation (+11 to +18%) were observed with rearing diets that showed an NDF content of over 400 g/kg DM, although Rebollar *et al.* (2011) did not observe any change in lactating feed intake of females receiving a diet with 505 g NDF/kg DM during rearing. The reduced digestible energy content of high-fibrous diets does not seem to be a limiting factor, even when animals are subjected to fibrous diets containing 8 to 9 MJ DE/kg DM. In fact, when female is fed with such low levels, they could reach an adequate live weight between 18 to 19 weeks of age (Pascual *et al*., 2002), and improve the resources acquisition during the first lactation (Nizza *et al*., 1997; Pascual *et al*., 2002; Quevedo *et al*., 2006a,b; Rebollar *et al*., 2011; Martínez-Paredes *et al*., 2019). Even the combination of these fibrous diets with others of higher energy content can be an alternative to adjust to the development objectives discussed in the previous section. Martínez-Paredes *et al*. (2012 and 2019) used a fibrous diet (8.7 MJ DE/kg DM) throughout the whole rearing period but allowed the access of young females to a higher energy concentration diet (11.0 MJ DE/kg DM) around mating (from 16 to 20 weeks of age). When compared to other rearing feeding programs, this management allowed achieving the best performance results during the first reproductive cycle. Finally, Saidj *et al.* (2019) observed that, when increasing the crude protein (CP) content from 150 to 170 g/kg in diets with 10.9 MJ DE/kg, no significant differences were observed at the metabolic level (glucose, triglycerides, urea and total protein in blood) and in reproductive performance (feed intake, live weight and litter size at birth).

However, if we make a small metanalysis with the limited data available in the literature we can draw some conclusions about the possible effect of the chemical composition of rearing diets on primiparous rabbit does performance (Figure 2). It seems that an excess of ADF reduces the rate of conception at first mating, an excess of CP can increase the rate of stillbirths at first parturition and reducing DE can increase feed intake during the first lactation.

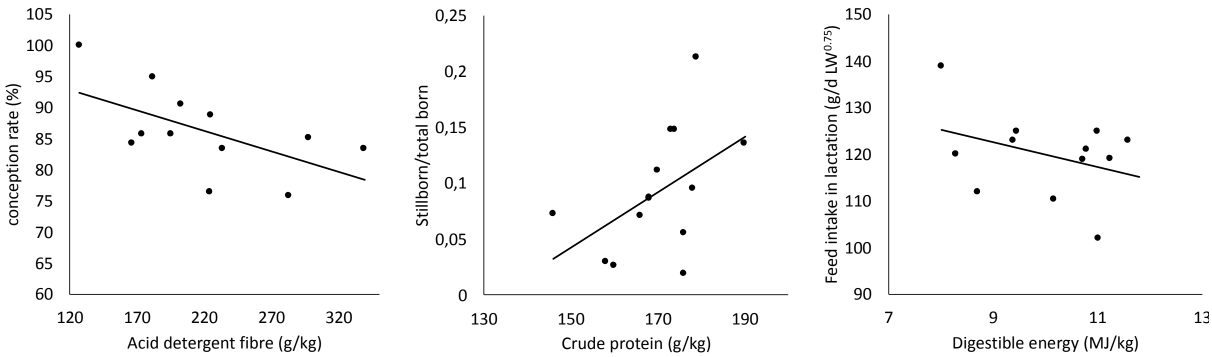

Figure 2: Meta-analysis on the effect of composition of the feed received during the rearing period on the conception rate in the first AI, stillborn ratio and feed intake during lactation of primiparous rabbit does. Built from literature (Xiccato *et al.*, 1995; Nizza *et al.*, 1997; Pascual *et al.*, 2002; Quevedo *et al.*, 2005; Arias-Alvarez *et al.*, 2009; Rebollar *et al.*, 2011; Martínez-Paredes *et al.*, 2012; Martínez-Paredes et al., 2018; Saidj *et al.*, 2019). LW: liveweight.

Long-term effects of high-fibre diets. Usually, reproductive and physiological differences observed between the different rearing feeding programs disappear from the second parturition. In this sense, Martínez-Paredes (2008) did not find long-term differences associated to the use of high-fibrous diets during the rearing period. However, some authors have described some positive long-term effects of the use of high-fibrous rearing diets: Nizza *et al*. (1997) obtained a higher litter size and heavier kits at weaning during the first four reproductive cycles; Pascual *et al*. (2002) observed increased number of weaned kits, lower interval between parturitions and longer lifespan of females for two years; and Martínez-Paredes *et al*. (2018) described a higher total number of born and weaned rabbits (+6 and +5, respectively) during 3 years, compared with young rabbit does fed with a reproduction diet. Although lifespan depends on a large number of factors, one of the most important particularities of rabbit females characterized by a higher lifespan is their lower dependence on fat mobilization to ensure reproduction (Savietto *et al.*, 2015), as body reserves are used as a safety factor. In fact, Theilgaard *et al*. (2006) observed that rabbit females with a low body condition (fatness group 1 vs. group 2), or showing higher mobilizations, had an increased relative risk of culling 1.00 vs. 0.45). Martínez-Paredes *et al.* (2018) observed that rabbit does fed with fibrous diet during rearing (436 g NDF/ kg DM) showed a +13% of life expectancy than other fed with reproduction diet in a population observed for three years. In contrast, Delgado *et al.* (2017a), for rabbit does reared with a fibrous diet fed *ad libitum* until one week before the first AI, observed that those with reproductive success along the first five AI (from 45 to 90%) had a greater fat mobilization (from -97 to +78 between the second AI and the first weaning than those who failed at least in one cycle. This successful group of rabbit does had a lower body fat content (15.7 *vs*. 17.2%) and liveweight at first AI compared with the other rabbit does. These results might indicate the different individual capacity to manage body reserves in critical periods, which might be linked to the individual genetic variability.

Therefore, theoretically, rearing feeding programs allowing the gradual provision of resources, proportional development of young breeding animals and proper mobilization at reproductive challenge events should provide breeding animals with a lower risk of culling throughout their reproductive life.

Rearing feeding programs: polyunsaturated fatty acids

In recent years, other complementary and interesting nutritional proposals have been tested to improve young rabbit female's reproductive performance, such as the use of diets enriched with polyunsaturated fatty acids (PUFA) during the rearing period. Rebollar *et al.* (2014) observed that kits born of rabbit does fed with a diet enriched with n-3 PUFA (+3.2 g/kg) weighed more and were longer, as well as they have a lower number of stillborn (–0.8) in the second parturition. This could be related to the ability of n-3 PUFA supplements from fish products to reduce 2-series prostaglandin secretion by the endometrium, preventing early embryonic death. Due to these results, the same research team used the same n-3 PUFA supplement but in a dose fourfold higher (+14.3 g/kg), obtaining a greater physiometric parameters, an improve on fertility rate (+13%) in the second parturition and a higher PUFA profile on milk respect a control diet not enriched (Rodriguez *et al.*, 2018). Other studies (Mattioli *et al.*, 2019) have observed similar improvement on fertility rate in the first cycle and from second to fourth cycle (+16 and +13%, respectively) as a consequence of dietary PUFA supplementation from different sources (extruded flaxseed or fish oil) during rearing and reproductive period.

NUTRITIONAL REQUIREMENTS AND FEEDING STRATEGIES FOR RABBIT DOES

The current nutritional requirements standards for rabbit does were developed in the last century, as well as clarified the differentiated role of energy sources (fat and starch) in these diets (Xiccato, 1996; Pascual *et al.*, 2003; Xiccato and Trocino, 2010; De Blas and Mateos, 2010). Since then there has not been any update, in spite of the increase of rabbit doe productivity and possibly of its nutrient requirements. Nowadays rabbit does have even a higher prolificacy –that poses management difficulties for farmers-, show an important productive difference between primiparous and multiparous ones, and pregnant or not, and they remain a relatively high replacement rate (Rosell and de la Fuente, 2009), suggesting potential unbalances in the productive system. Regarding the feeding strategy, there is a lack of studies evaluating nutritional requirements in rabbit does in the long term, as well as along the different physiologic states. In this section we revise whether new recommendations can be derived based on the results obtained with highly productive rabbits does in this century.

Arginine and glutamine supplementation

The interest in these amino acids derives from their potential influence on placental, embryonic and fetal growth during pregnancy. It is well known the economic impact of litter size in rabbit farms (Cartuche *et al.*, 2014), which is affected mainly by ovulation and prenatal survival rates, being the latter around 0.35 both in rabbit does and sows (Blasco *et al.*, 1993). In rabbit does, when the number of fetuses increases the vascular supply to each implantation site is reduced (Duncan, 1969). This limited blood supply may produce smaller fetuses and a higher fetal mortality rate, reducing litter weight and size at birth (Argente *et al.*, 2003). In this context, Arginine (Arg) is used as a precursor for the synthesis of nitric oxide, polyamines and other compounds (Wu and Morris, 1998), playing nitric oxide an important role in vasodilatation, regulating the uterine blood flow promoting the transference of nutrients to the fetus (Moncada and Higgs, 1995; Bird *et al.*, 2003). Regarding glutamine (Gln), free Gln is abundant in plasma, milk and fetal fluids (Wu, 2009), representing the uterine uptake of Gln in pregnant gilts the highest compared to other amino acids (Wu *et al.*, 1999). Nevertheless, the Gln uptake by porcine mammary glands is not adequate for milk protein synthesis (Li *et al.*, 2009).

In standard diets for rabbit does the Arg/Lys and Gln/Lys ratio is around 1.45 and 3.51, respectively (Nicodemus *et al.*, 1999b). When low Arg and Gln diets for rabbit does were supplemented with 4 g Arg/kg (1.23 *vs*. 1.66 total Arg/Lys ratio, or 1.33 *vs*. 1.85 apparent ileal digestible Arg/Lys ratio) or 4 g Gln/kg (3.30 *vs*. 3.80 total Gln/Lys ratio, or 3.75 *vs*. 4.29 apparent ileal digestible Gln/Lys ratio) tended to increase the number of kits born per litter (+0.8), and the litter size once made the adoptions (+0.6) with no impairment of the rabbit doe body condition along the first three cycles (Delgado *et al.*, 2017b, 2019a). This effect was reflected at weaning in a trend to increase the litter size (+0.7), and consequently the litter weight. The possible positive effect of Arg might be associated with an increase of the uterine blood flow. In fact, in rabbit does the probability of mortality of fetuses is three times higher if they receive a single vein than if they receive two or more veins (Damico *et al.*, 2013). Otherwise, the effect of Gln might be related to a better development of oocytes, an effect already observed *in vitro* in hamsters and rabbits (Gwatkin and Haidri, 1973; Bae and Foote, 1975), and embryos (in hamsters and pigs; Carney and Bavister, 1987; Petters *et al.*, 1990), which might be linked to the use of Gln as an energy source. Curiously, there was no additive effect of the simultaneous supplementation of Arg and Gln. These results suggest that the physiological period to supplement these amino acids might be different if a synergistic effect is wanted and possibly with a higher dose than the one used in this study (total Arg/Lys ratio of 2.64 in sows, Mateo *et al.*, 2007, 2008). The different way of action of Arg and Gln to promote prolificacy might recommend to supplement Gln few days before insemination until at least embryo implantation (7-8 d after insemination), when fertilization and implantation occurs (Harper, 1961; Denker, 1977), whereas Arg supplementation could be made few days before implantation until the end of pregnancy. At this moment, it is not possible to supplement these amino acids in rabbit diets (due to their price/availability) but their use might be reduced by using ingredients rich in Arg and Gln although it should be evaluated whether they exert similar effects.

Enrichment in n-3 fatty acids

The requirements of essential fatty acids n-3 and n-6 and the optimal n-6/n-3 ratio are unknown in rabbits, and there are still no clear recommendations probably due to the variability of the results obtained. The n-6/n-3 ratio in diets with no added fat range between 4 and 15, whereas it is usually below 4 in diets enriched in n-3 fatty acids. The reduction of the n-6/n-3 ratio (from 7.3 to 2.2, and 34 g fat/kg DM) using fish oil (7.5 g/kg) reduced the number of stillborn in the second parturition and increased the weight and size of newborn kits (Rebollar *et al.*, 2014; Rodríguez *et al.*, 2017). Using similar diets, reducing the n-6/n-3 ratio (from 8.7 to 1.0) with a higher dose of fish oil (30 g/kg), Rodríguez *et al.* (2018) found again a greater size of newborn kits, better embryo quality and higher fertility in the second insemination, although also a trend to reduce litter size at weaning (-0.6) was observed. According to these authors, these results might be associated with the hormonal changes, due to the increase of oestradiol and leptin during lactation, and progesterone around the implantation phase of pregnancy. It might be related to the influence of these PUFA on both prostaglandin and steroid metabolism (Wathes et al., 2007). The reduction of the n-6/n-3 ratio (from 13.4 to 3.5) using linseed oil rendered different results, increasing the number of kits per litter once homogenized (+0.6) with no other effect on rabbit doe productivity and body condition along the first four cycles (Delgado *et al.*, 2018a), but a reduction in the replacement rate of rabbit does when high n-6/n-3 ratio and high soluble fibre levels were combined (Figure 3).

The benefits of the n-6/n-3 ratio reduction do not seem derived from the n-3 source. In fact, the partial substitution of soybean oil with extruded linseed or fish oil (dietary n-6/n-3 ratio: 1.7, 1.1, 1.1, and different fat contents: 3.0, 5.6 and 4.0 respectively) both improved fertility (on av. +13% percentage units; without oestrus synchronization), the number of kits born alive (on av. +0.6) and weaned (on av. +0.3; without homogenization), milk production and perirenal fat at insemination in rabbit does along the first four cycles (Mattioli *et al.*, 2019). They also reported a reduction of the n-6/n-3 ratio in the ovaries due to the enrichment with n-3 long-chain PUFA. The influence of n-3 fatty acids on the hormonal status, as was commented before, might be behind these positive effects. Nevertheless, using very similar diets, Menchetti *et al.* (2018) found no improvement of reproductive traits considering two cycles in multiparous rabbit does. Similarly, the enrichment of the diet with n-3 fatty acids from extruded linseed (n-6/n-3 ratio: 4.8 *vs*. 1.0) did not modify fertility or prolificacy (Maertens *et al.*, 2005). There are authors reporting even negative results when marine algae PUFA was supplemented in the diet, as the impairment of kit weight at birth and at weaning in nulliparous does (1 cycle; Mordenti *et al.*, 2010). A consistent effect among different studies is that the modification of the dietary n-6/n-3 ratio led to parallel changes in the milk fatty acid profile (Pascual *et al.*, 1999; Maertens *et al.*, 2005; Delgado *et al.*, 2018a; Rodríguez *et al.*, 2018; Mattioli *et al.*, 2019). Nevertheless, most authors did not find any influence of the n-6/n-3 ratio before weaning on litter performance and survival after weaning (Rodríguez *et al.*, 2018; Delgado *et al.*, 2019b; Mattioli *et al.*, 2019). However, Maertens *et al.* (2005) reported a reduction of fattening mortality when n-6/n-3 ratio decreased from 4.3 to 1.0 during the growing period. The lack of agreement among studies suggest that other factors may be influencing the response to n-3 fatty acids enrichment.

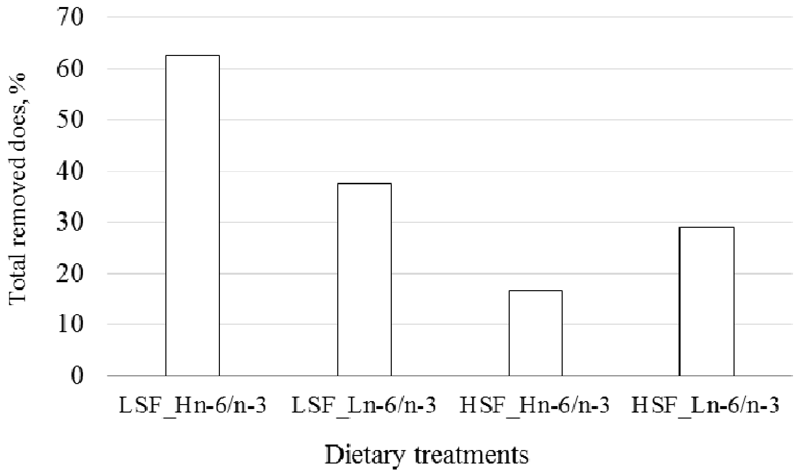

Figure 3: Effect of level of dietary soluble fibre and n-6/n-3 fatty acids profile on the proportion of total removed does along four cycles (HSF and LSF: high and low soluble fibre; Ln-6/n-3 and Hn-6/n-3: high and low n-6/n-3 fatty acid ratio. $P_{\text{Soluble fibre}} = 0.005$; $P_{\text{Soluble fibre} \times \text{n-6/n-3 ratio}} = 0.056$) (Delgado *et al.*, 2018a)

These heterogeneous results (both for rabbit does and kits) may be accounted for the different content of single PUFA (that could compete for the desaturase/elongase enzymes), the potential oxidation of these fatty acids that depends on the dietary antioxidant capacity, the different interaction with the intestinal microbiota or differences in the experimental procedure (estrous synchronization, the period of supplementation or age of does). Anyway, in most studies, no negative effects on reproductive traits of rabbit does were reported for the dietary n-3 enrichment.

Particle size

The range of dietary level of insoluble fibre for rabbit does is known, but the relevance of particle size had not been evaluated. It is important because of its influence on the mean retention time of the digesta (Gidenne, 1993) and thus possibly on voluntary feed intake, although the difficulty to quantify the fibrous large particles (> 0.3 mm) may have limited the available information. The quantification of large particles has to be made using wet sieving, to 'dissolve' the pellet. The main problem is the swelling capacity of particles rich in starch and soluble fibre (like grain cereals and sugar beet pulp), that produce very bulky particles being quantified as large particles when they do not stimulate the rate of passage. It can be partially corrected determining the insoluble fibre content (NDF) in the residue collected in each sieve. The inclusion of a very low proportion of large particles (<20% in diets with around 360 g NDF/kg DM) obtained by substituting mainly alfalfa with paprika meal impaired feed and DE intake, as well as reproductive traits (average of two cycles with multiparous does; Nicodemus *et al.*, 2006). Nevertheless, it is difficult to attain that low levels of large particles unless increasing the content of some unusual byproducts. Dietary particle size may be also modified by using different grinding sizes for fibrous sources. In this way, the inclusion of alfalfa and straw coarsely ground (9 *vs*. 1 mm) in standard or low fibrous diets (containing 35 to 20% particles > 0.3 mm, 23 to 14% NDF > 0.3 mm, and 10 to 1% NDF>1.25 mm, on DM basis) reduced the proportion of culled rabbit does during the experiment (3 *vs*. 22%. Nicodemus *et al.*, 2010). These authors did not find any other benefit, but an increase of feed and DE intake in the weaning-parturition period and the fertility when coarsely ground fibres were included in low fibrous diets (270 NDF/kg DM). The opposite occurred in rabbit does fed diets with 340 NDF/kg DM where the inclusion of coarse ingredients impaired feed and DE intake (in the same period) and fertility. These results are in agreement with those of Fortun-Lamothe (1998), who reported that an energy restriction of multiparous rabbit does in late pregnancy had a detrimental effect on receptivity and might suggest that the pre-partum DE intake might be more relevant to reproduction than pre-mating DE intake (Quevedo et al., 2016a,b), probably due to the great mobilization of body reserves at the end of pregnancy (Fortun-Lamothe, 2006; Savietto *et al.*, 2016). Finally, when highly fibrous diets are used, might be of interest to reduce the particle size to improve the digestibility. In this way, the reduction of grinding size of alfalfa and barley (from 4.5 to 1.5 mm, and from 16 to 11 % NDF>1.25 mm) in a high fibrous diet (420 NDF/kg DM) impaired the feed intake in the first lactation but increased it in the second one, with no influence on reproductive traits and body composition of rabbit does along the first three parturitions (Romero *et al.*, 2011). The wide differences observed in the proportion of NDF>1.25 mm obtained by Nicodemus *et al.* (2010) and Romero *et al.* (2011) derive from the different level of fibre but also from the different type of fibre used. The latter authors used a significant amount of sugar beet pulp, which increases the amount of large fibrous particles (due to its high-water holding capacity) although this fibre source does not seem to stimulate the rate of passage compared to hay (Gidenne *et al.*, 1987). This is a shortcoming of this methodology that difficult the establishment of a more robust value of minimal large fibre requirements.

Accordingly, there is no advantage in reducing grinding size in high fibrous diets, but it may be worth increasing it in very low fibrous ones, always preserving the pellet quality. Further evaluation of diets with coarse particle size might be also of interest to confirm its potential effect on limiting feed intake in rearing does fed high fibrous diets, and its positive effect on the culling rate of does in production.

Soluble fibre

This fibre fraction was not taken into account for a long time. In the last 15 years it was observed its utility in growing rabbits because of its positive influence on the health status of young rabbits after weaning in a context of epizootic rabbit enteropathy, probably through its effects on the intestinal mucosa and microbiota (Gómez-Conde *et al.*, 2007; Trocino *et al.*, 2013). In rabbit does the increase of soluble fibre (80 *vs*. 130 g/kg DM and 330 g NDF/kg DM) reduced the replacement rate on rabbit does along the first four cycles, especially when combined with n-6 than with n-3 fatty acids (Figure 3; Delgado *et al.*, 2018a). The positive effect of soluble fibre on the replacement rate might be related to the changes exerted in the intestinal microbiota (Delgado *et al.*, 2015). Soluble fibre might also

explain the reduction of mortality around parturition, which would be related to the slower starch digestion *in vitro* in high soluble fibre diets that might extend the glucose availability along time (Farías-Kovac *et al*., unpublished), or to the longer fermentation time of sugar beet pulp observed *in vitro* (Abad-Guamán *et al*., 2018), which might both contribute to limit the ketosis incidence. In fact, in nulliparous pregnant rabbit does, a higher soluble fibre level has been associated with lower both prepartum mortality and number of stillborn, females showing lower NEFA and higher glucose concentration in blood at parturition (Martínez-Paredes *et al.*, 2012). No other positive effects of soluble fibre have been recorded, and DE and DP intake during lactation, milk production and litter weight at weaning tended to reduce with the high soluble fibre level. Similarly, the increase of soluble fibre (106 to 126 g/kg DM, and 320 g NDF/kg DM) in late lactation (21-35 d) obtained by the partial substitution of alfalfa and wheat straw for apple pulp also reduced feed intake and kit weight at weaning (Álvarez *et al.*, 2007). These negative effects of soluble fibre were more clearly observed in high fibrous diets (460 g NDF/kg on average) with a high soluble fibre content (140 *vs*. 180 g/kg) only offered to rabbit does in late lactation (Martínez-Vallespín *et al.*, 2011).
Further research is required to confirm the interest of a minimal soluble fibre level in diets for rabbit does.

Flushing
An adequate energy supply before insemination seems to be important to obtain good fertility rates in most species and also in rabbits. In fact, rabbit doe receptivity impaired when they were restricted from weaning to parturition, while the stimulation of energy intake from parturition to mating seemed to improve the conception rate (Fortun-Lamothe, 1998). Nowadays it is not still clarified which of these two periods is more important if not both, and what can we do to limit the negative energy balance before parturition, which might be associated with a higher ketosis incidence. The selection of the main energy source to increase the DE intake might depend on the period, as starch might be preferable before parturition and fat after parturition. One option is to feed rabbit does with a more energetic diet (using fat) than the recommended one (11.5 *vs*. 10.6 g DP/kJ DE). However, in the long term (6 cycles) in spite of the increase DE intake during pregnancy and lactation observed (and accordingly of milk production), a reduction of prolificacy (-0.7), and higher litter mortality after weaning (+4 percentage units) were found (Quevedo *et al.*, 2006a,b). In contrast, when rabbit does were fed a diet to stimulate milk production (302 g NDF, 161 g starch and 49 g fat/kg as fed, with 11.6 g DP/kJ ED) the first 25 d of lactation combined with a standard diet (343 g NDF, 161 g starch and 24 g fat/kg, with 11.3 g DP/kJ ED) from 25 to 42 d (next parturition) no impairment (to rabbit does and their litters), but also no benefit (including fertility), was reported compared to offering to rabbit does the standard diet as sole diet (Read et al., 2016). It would be interesting to evaluate whether a specific diet in the weaning-parturition period might be of interest.

Glucose precursors for rabbit does. The last days of pregnancy feed intake of rabbit does is sharply reduced (Oger *et al.*, 1978), although the fetuses still demand a high amount of nutrients, which derives in an important mobilization of the body reserves (Savietto *et al.*, 2016), and an increase of the plasma non-esterified fatty acid and β-hydroxybutyrate (Minuti *et al.,* 2015). In this period, the increase of the number of fetuses reduced the plasmatic glucose concentration and changed its aminoacid profile (Minuti et al., 2020). Possibly, a glucose deficit may lead to ketosis and to higher mortality around parturition, especially if the dietary starch is low, similar to that reported in cows (Nielsen and Ingvartsen, 2004), or impair fertility. This situation might be counterbalanced by an increase of the dietary energy increasing the starch or other glucose-precursors in this period. However, the optimal starch level was 200 g/kg DM (with 355 g NDF and 35 g fat/kg DM), and both higher or lower starch levels (combined with a reduction or increase of NDF+fat, respectively), impaired the productive traits of rabbit does (De Blas *et al.*, 1995). Using a different approach, Nicodemus *et al.* (2005) compared three dietary strategies with a similar DP/ED ratio (12.4 g/MJ) along the first three cycles. A control diet high in starch (191 g/kg DM) and 39 g fat/kg DM, a diet with less starch and more fat (106 and 58 g/kg DM, respectively), and a third treatment in which the latter diet was supplied the first 21 d of lactation, and from this moment to parturition rabbit does were fed a diet with 163 g starch, 38 g fat and 25 g propylene glycol/kg DM. In this work, the combination of these two diets compared with the enriched fat group reduced mortality of rabbit does (17.4 *vs*. 0%) and improved fertility (81.3 *vs*. 88.2%), and also reduced kit mortality at birth respect to the high starch group (with no effect on feed intake). Similarly, the use of a diet containing a supplement of glucose precursors combined with minerals-vitamins around parturition (from -7 to +7 d) compared to the same diet but with no supplement improved their body fat content, fertility (84.7 *vs*. 75.0%) and litter size at birth, with no effect on rabbit doe mortality (Alfonso *et al*., 2014). These effects might be associated with a reduction of the ketosis incidence. The glucose supply of propylene glycol has also the potential to increase plasma insulin that might inhibit the fat mobilization like in cows (Nielsen and Ingvartsen, 2004). Propylene glycol can be also provided in drinking water in order to increase the energy supply. In this way, its supplementation (2% in water) for 4 d before insemination improved fertility in 10 percentage units (64 *vs*. 53%; Luzi *et al.*, 2001). On the opposite, the supplementation of propylene glycol (2.5% in water) from mid-pregnancy to the end of lactation or only during lactation showed no benefits for the doe or the litter, and even its supplementation in pregnancy-lactation impaired the body fat reduction at the beginning of lactation, and increased litter mortality after weaning (García-García *et al.*, 2010; Sakr *et al.*, 2011; Arias-Álvarez *et al.*, 2013). According to these results, more information about the dose, the form and the period of supplementation of propylene glycol is required to optimize its use.

Feeding and body condition

The evolution of the body condition, and especially of energy balance, of rabbit does has been extensively studied and reviewed due to the interest in limiting the negative energy balance in specific moments of the productive cycle (Parigi-Bini and Xiccato, 1993; Fortun-Lamothe, 2006; Pascual *et al.*, 2006; Pascual *et al.*, 2013). These traits are mainly influenced by non-nutritional factors as the genetic type, physiological state, reproductive rhythm, reproductive success, litter size and weaning age. Nutrition also play a role in the variations of the body condition and energy balance, being the most relevant factor the level and source of energy (starch, animal fat or vegetal oil) as reviewed by Pascual *et al.* (2003) or reported by Fortun-Lamothe *et al*. (2005). In addition, the effect of the energy source on the use of body reserves depends on the genetic type and priorites of the rabbit female (Arnau-Bonachera et al., 2018a). In lines selected for growth rate, females are characterized by a high dependence on their body reserves to cope with the high demand of the current lactation, obtaining better results when using diets that promote milk production (animal fat). However, in lines selected for hyper-prolificity, the foundation criteria have promoted a pattern based on the body reserves accretion during lactation to cope with future reproduction, obtaining better results when using diets that promote body condition (starch).

Besides nutrition, other factors like particle size, arginine/glutamine supplementation, level of soluble fibre and n-6/n-3 fatty acid ratio failed to exert any influence in the body condition of rabbit does along at least three cycles (Romero *et al.*, 2011; Delgado *et al.*, 2017b, 2018a). It might suggest that rabbit does have an important adaptability to the diet, as long as the diet do not deviate a lot from the nutritional standards (De Blas and Mateos, 2010; Xiccato and Trocino, 2010). In addition, we must bear in mind that reproductive rabbit does naturally show positive and negative balances in their body condition, genetically driven by their homeorhetic system, both throughout the reproductive cycle and life. We should not fight against these natural trajectories, but we should find a feeding program that avoids an excessive deviation of the body condition from this normal trajectory, which could put the animal at risk. These deviations occur frequently (a complicated parturition, seasonal environmental variations, presence of some pathogen ...) and the diet must facilitate the female's homeostatic ability to resume her trajectory (Pascual *et al.,* 2013).

Feed for the doe or for the litter

Considering the difficulties to provide different diets to the rabbit doe and the litter in the current productive systems, and assuming their different nutritional requirements (Xiccato *et al.*, 2006), although roughly defined, especially to minimize digestive troubles in the litter after weaning, several attempts tried to adapt the diet of the rabbit doe to the litter. In this context, the substitution from 18 d of lactation to weaning of the rabbit doe diet, increasing the NDF and fat level (from 276 to 305 and from 30 to 55 g/kg as fed, respectively) and decreasing the starch content (from 190 to 95 g/kg) allowed both to maintain the body condition and fertility of rabbit does and reduce litter mortality after weaning (Fortun-Lamothe *et al.*, 2005). When the substitution was done increasing the NDF (up to 329 g NDF/kg) but reducing both the starch and fat content (95 and 32 g/kg, respectively), litter mortality after weaning was also reduced, but impaired body condition and fertility of rabbit does. These results suggest the importance to meet the energy requirements of rabbit does, but also the minimal fibre requirements of suckling rabbits at the onset of feed intake. Using a similar design, Álvarez *et al.* (2007) increased the dietary soluble fibre (from 106 to 126 g/kg DM, with 322 g NDF/kg DM) from 21 to 35 d of lactation, but it reduced kit weight at weaning, with minor effects on rabbit does, and the influence on litter health status was not observed due to the very low mortality rate. Martínez-Vallespín *et al.* (2011) followed a similar strategy and substituted at 17-d of lactation a standard breeding diet for another one in which starch was partially substituted with insoluble (ADF) and soluble fibre, and with a low dietary protein content (and that was also offered to the litter after weaning). The latter diet reduced the litter mortality after weaning, but impaired feed and DE intake, milk production, and body condition of rabbit does and litter weight at weaning. These effects observed in rabbit does might be related to the caecal filling effect produced by the combination of a high dietary NDF and sugar beet pulp level (Carabaño *et al.*, 1997) that might limit both feed and DE intake (García *et al.*, 2002). Using a similar approach, Gerencsér *et al.* (2011) changed the rabbit doe (and litter) from a breeding (136 g crude fibre, 46 g fat and 185 g starch/kg as fed) to a growing diet (172 g crude fibre, 31 g fat, 151 g starch/kg) at late lactation (21-35 d), although no positive effect was obtained neither in the litter (medicated after weaning) or the rabbit does along five reproductive cycles. However, this type of strategy reduced litter mortality after weaning with no negative effects on rabbit does along three reproductive cycles (Read *et al.*, 2016). These authors fed rabbit does from kindling to 25 d and from 35 (weaning) to 42 d a standard diet (343 g NDF and 161 g starch/kg as fed, and 11.6 g DP/kJ ED), while from 25 to 35 d offered a fattening diet (415 g NDF and 70 g starch/kg, and 9.7 g DP/kJ ED) compared with a standard reproduction diet along all the cycle or the combination of a reproduction and a milk enhancer diets. These results enhance the interest to provide different diets to the mother and the litter, mainly to optimize litter health after weaning avoiding any impairment of rabbit doe traits.

New ingredients for rabbit does

Fibrous sources, legumes, glycerol. Non-usual ingredients, mainly industry by-products, are included in rabbit diets with caution due to the scarce information regarding its effect on feed intake and nutritive value. However, there are few studies testing 'new' ingredients in rabbit does. Nicodemus *et al.* (2007) reported that soybean hulls and defatted grape seed meal can be included up to a 220 and 50 g/kg level in substitution of alfalfa, straw and sunflower hulls with no impairment of performances. A higher level of soybean hull reduced feed intake in spite of the high lignin level of the diet. Regarding the protein sources, the replacement of soybean and sunflower meals by rapeseed meal (100 g/kg) and white lupin seeds (140 g/kg; Volek *et al.*, 2018), by white lupin seeds (250 g/kg; Volek *et al.*, 2014) or by dehulled white lupin seeds (180 g/kg; Uhrilova and Volek, 2019) did not modify the productive traits of rabbit does, but enriched their milk in n-3 fatty acids. Similarly, the inclusion up to 50 g/kg of glycerol for starch had no influence on the productive traits of rabbit does (Iñigo *et al.*, 2011).

Probiotics. The interest to study the effects of probiotics in rabbit does is scarce considering the few number of publications. The use of *Bacillus cereus* var. toyoi in rabbit does (2×10^8 spores/kg diet) reduced the parturition interval and increased feed efficiency and numerical productivity (Nicodemus *et al.*, 2004). The same probiotic and dose produced different results, reducing kit mortality the first 18 d of lactation (in the second lactation), increasing the feed intake of rabbit does in the second part of lactation and the litter growth rate during lactation (Pinheiro *et al.*, 2007). These results were similar to those obtained with Bacillus CIP 5832 (Maertens *et al.*, 1994). Another probiotic with positive results is *Saccharomyces cerevisiae*. In rabbit does, it improved fertility and reduced mortality of does and suckling rabbits (Belhasen *et al.*, 2016). Nevertheless, the precise mechanisms behind these effects of probiotics are not clearly understood.

NEW OPORTUNITIES IN NUTRITION FOR RABBIT DOES

Effect of diet on the rabbit doe microbiota and its transmission to the litter

The main inheritances left by the rabbit doe to it is litter are its genetic and microbiota, being the latter the only that can be influenced by the diet. The microbiota might play a relevant role in the rabbit doe longevity, but also in the future litter health and performance. The transfer of intestinal microbiota from the mother to the litter is showed when the similarity of caecal microbiota was studied at 26 d of lactation in rabbit does (fed no antibiotic) and their litters with different cross-fostering policies (Abecia *et al.*, 2007). These authors reported that the caecal microbial profile of kits from the same litter clustered together, independently if they shared or not their biological mother. The biological mother seemed to have an influence on the intestinal microbiota but weaker than the effect of the mother who finally suckled them. But how the mother transfers its microbiota to the litter? Apparently, there are two periods to do that, during the short passage of the kits through the birth canal, and along lactation through the bacteria present in the milk, in the faeces, that present in the skin around the nipples and in the hair used as nest material. If we assume the absence of bacteria in the placenta of mammals, that is not completely discarded (Jiménez *et al.*, 2008; Willyard, 2018), the first maternal bacteria to be in contact with the kits would be that present in the birth canal. This microbiota comes from the digestive tract and would reach the reproductive tract (and the mammary gland) by translocation as observed in mice and cows (Donnet-Hughes *et al.*, 2010; de Andrés *et al.*, 2018; Klein-Jöbstl *et al.*, 2019), although there is no data in rabbits.

The second contact with the maternal microbiota would be with that present in the nest material (hair combined with the 'foreign' microbiota in wood shavings or straw), that around the nipples (no data for both), in the milk like in other mammals (Martín *et al.*, 2004), and that present in hard faeces excreted in the nest (Kovacs *et al.*, 2006). The intake of fecal pellets was observed even two days after birth, although it is more frequent from 7 d after birth onwards (Combes *et al.*, 2014; Nicodemus *et al.*, 2015). The increase of faecal pellets intake (supplementing the nest with additional pellets) accelerated the microbiota implantation in kits caecum and improved their health status after weaning (Combes et al, 2014), although this is not always observed (Nicodemus *et al.*, 2015). It might depend on the faecal pellet composition (microbial and chemical), which in turns depends on the diet, and anyway, it seems that no benefit is obtained from removing the maternal faeces from the nest. In this way, the separation of rabbit does from the litter, only joined for nursing (2 times/d, and all mother faeces were removed), decreased the number of caecal bacteria, the villus height/crypt depth ratio and the development of the appendix in the kits (Zhang *et al.*, 2018). The supply of selected faeces in the nest might be of interest to modify the microbiota profile of the litter when trying to avoid antibiotic-resistant genes (Archard et al., 2019)

Another source of bacteria is maternal milk. Milk intake begins just after birth, and now is well known its endogenous microbial content in mammals confirmed both by DNA detection as well by bacterial isolation (Fernández *et al.*, 2013). The sequencing of 16s DNA in milk of 6-d lactating rabbit does revealed the main operational transfer units (OTUs) (Table 1; Delgado *et al.*, 2015 and unpublished), and some of the most abundant are also present in milk of sows and humans (Fernández *et al.*, 2013; Chen *et al.*, 2018). It seems that at least some bacteria could translocate during pregnancy from the maternal digestive tract to the mammary gland and milk (de Andrés *et al.*, 2018). However, the key question is what role play these bacteria and whether its composition can be manipulated through the diet. Previous studies described in kits in the first week of age that once the small intestine is colonized exists bacterial translocation to the mesenteric lymph nodes (Urao *et al.*, 1995, 1996; Delgado *et al.*, 2019c). In this way, Delgado *et al.* (2015 and unpublished) studied the microbiota in the milk and faeces of rabbit does (6-d lactation) and mesenteric lymph nodes of 6-d old kits, when rabbit does were fed a combination of two dietary levels of soluble fibre (8 *vs*. 13% on DM basis) and two n-6/n-3 fatty acids ratios (13.5 *vs*. 3.5). One of the main surprises was the identification of several species of Lactobacillus in the milk and mesenteric lymph nodes (Figure 4), as it was considered absent in the rabbit. They also reported that most of the bacteria detected in the mesenteric lymph nodes were also present in the maternal milk and/or in the faeces. The microbiota of mesenteric lymph nodes clustered according the n-6/n-3 fatty acid ratio, that also affected the milk fatty acid composition (Delgado *et al.*, 2015, 2018a), and was closer to the milk than to the faecal microbiota, that instead clustered according to the soluble fibre level (although it also affected some important OTUs in the mesenteric lymph nodes; Figure 4). Consequently, the bacteria found in the mesenteric lymph nodes of kits probably come from the milk, the faeces or from both (Table 1; Figure 4). Curiously, only the level of soluble fibre exerted a positive influence on the rabbit health status after weaning (in an epizootic rabbit enteropathy context) when fed the same diets than before weaning (Delgado *et al.*, 2018b). However, when these weaned rabbits were fed a common standard diet, no effect on health status was observed (Delgado *et al.*, 2019b), indicating that these changes before weaning are not enough to improve rabbit health after weaning.

These results indicate that this microbiota can be at least partially modified by the diet, although still pose the question about what role plays this initial microbial colonization. At present, it is still unknown the role of the microbiota found in the mesenteric lymph nodes on the colonization process or in the immune system development, and the potential influence of its manipulation on the future litter health. It is not clear whether this initial microbiota might be behind the influence of the litter on the rabbit health, and the relevance of the maternal influence on the kit intestinal microbiota compared with that of the post-weaning diet.

The influence of the diet on milk microbiota suggests that it might modify the microbiota of the mammary gland, which is a relevant issue considering the increasing mastitis incidence and *Staphylococcus aureus* strains resistant to antibiotics (Moreno-Grua *et al.*, 2018). In fact, in humans, the supplementation for the last 9 weeks of pregnancy of *Lactobacillus salivarius PS2* reduced the mastitis incidence (Fernández *et al.*, 2016). What bacteria may limit the mastitis development, when and how should it be supplied and whether the type of diet can contribute to promoting udder health are new challenges to be explored in rabbit does in the next future.

Effect of diet on the rabbit doe immune system

In this same context, taking into account that the health of the rabbit doe and its litter is one of the most determining issues of the current farms' profitability, it is also important to evaluate the possible effect of the feeding system on the immune status of the rabbits and their litters. The immune system of animals, and among them rabbits, develops with age. Jeklova *et al.* (2009) observed that the count of the different populations of leukocytes in the blood of rabbits increases with age up to six weeks of life. However, after that age, the counts are already quite similar to those of an adult rabbit. However, Guerrero *et al.* (2011) and Penades *et al.* (2018) observed that the count of the different populations of lymphocytes in the blood of breeding rabbits changes significantly throughout the reproductive cycle, and that these counts could be influenced by the availability of resources and/or the reproductive effort of these animals. In fact, they observed that the counts of major blood lymphocyte populations were generally lower in those rabbit does with weaning at 42 compared to 28 days postpartum, probably due to their greater lactational effort.

The immune status of rabbit does and their evolution throughout their reproductive life can be essential to help improve their health and lifespan. In this sense, Ferrian *et al.* (2012) observed that the evolution of the lymphocyte populations of rabbit does belonging to a genetic line characterized by greater robustness and survival was significantly different from a line selected by reproductive criteria, especially when they were subjected to a challenge with heat stress. But could the rabbit does' diet affect the immune status of the females and the development of the immune status of their litter? We know that a diet rich in fat favors the production of milk while another rich in starch more favors the recovery of body reserves, regardless of the genetic type used (Arnau-Bonachera, *et al.,* 2018b). This could affect the productive effort and recovery capacity of the rabbits, and therefore their immune status. Penades *et al.* (2018) studied the evolution of the immune status of rabbit does of three different genetic types fed with two different isoenergetic diets, differing in their main energy source (starch or fat). In general, the type of diet given during reproductive life did not affect the leukocyte population counts. However, females from a specialized line had lower total lymphocytes (xx) and those from a robust line had higher granulocytes counts (+x%) with the starch-enriched diet (xx vs yy g/kg). The authors concluded that diet could affect the immune system of rabbit does in function of the way of managing their body resources, and it could have consequences in the health of the females and its litter, since there is a correlation between the immune status of the mother and its litter at weaning (Guerrero *et al.*, 2011). In fact, with the animals and diets of this same trial, García-Quirós *et al.* (2014) observed that the animal fat-enriched diet led to higher milk yield in females, resulting in greater development of kits during the lactation period. Moreover, these young rabbits reached weaning with higher live weight and increased B lymphocyte counts. Bienertova-Vasku *et al.* (2012) described that the B-cell activating factor (BAFF) expression was tightly related to adipose tissue, and its plasma level was also correlated with the energy derived from the diet. Therefore, the higher milk energy output of mothers fed the fat-enriched diet could be responsible for the greater B lymphocytes counts in weaned rabbits.

CONCLUSIONS

The rabbit doe requires a minimal maturity (body condition) at first AI to face its productive life with minimal guarantees (around 7.0 mm perirenal fat thickness, 2.8 ng/mL of plasma leptin concentration and around 18% and 15-20% of body protein and fat, respectively). This goal can be achieved by restricting feed intake from 12 weeks of age until first AI or feeding *ad libitum* with a fibrous diet (<10.5 MJ DE/kg) from 60 d of age to first parturition. Once the doe is lactating the increase of the n-3 fatty acids (or reduction of the n-6/n-3 ratio), the soluble fibre (under epizootic enteropathy) and the Arg/Lys and Gln/lys ratios may help to improve the reproductive traits of rabbit does, although their optimal level of inclusion remain to be identified. In this period, it is important to limit the negative energy balance before parturition, and the supplementation of glucose precursors reduce the ketosis incidence. The formulation of different diets for the doe and the litter to fit better their requirements and assuring their health would be an option to consider when it would be applicable in the farm. The influence of the mother on the litter microbiota and immune status and its potential modulation through the diet will deserve more studies in the next future.

Table 1: The first twenty operational transfer units (OTUs) more abundant in in mesenteric lymph nodes (MLN) of 6-d old kits and their relevance order in milk and faeces of rabbit does at the same time. Values represent the order for each type of sample (values in grey indicate OTUs within the 20 most abundant in milk or faeces that are also abundant in MLN) (Delgado *et al.*, unpublished)

Operational transfer units	**Milk**	**MLN**	**Faeces**
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae	1	1	65
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	126	2	9
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Stenotrophomonas; s__maltophilia	6	3	92
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__ovatus	143	4	28
k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__	2	5	84
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Odoribacteraceae]; g__Odoribacter; s__	230	6	34
k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Staphylococcaceae; g__Staphylococcus; s__	3	7	85
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	30	8	6
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Tepidimonas; s__	5	9	93
k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__	7	10	103
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Rikenellaceae; g__; s__	181	11	12
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__distasonis	232	12	19
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Odoribacteraceae]; g__Butyricimonas; s__	227	13	68
k__Bacteria; p__[Thermi]; c__Deinococci; o__Deinococcales; f__Deinococcaceae; g__Deinococcus; s__geothermalis	82	14	120
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	100	15	8
k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__[Weeksellaceae]; g__; s__	4	16	104
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides	169	17	37
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	13	18	4
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__fragilis	209	19	66
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	26	20	1

k: kingdom, p: phylum, c: class, o: order, f: family, g: genus, s: specie. MLN: mesenteric lymph nodes of 6 d-old kits. Milk and faeces of does in 6-d of lactation

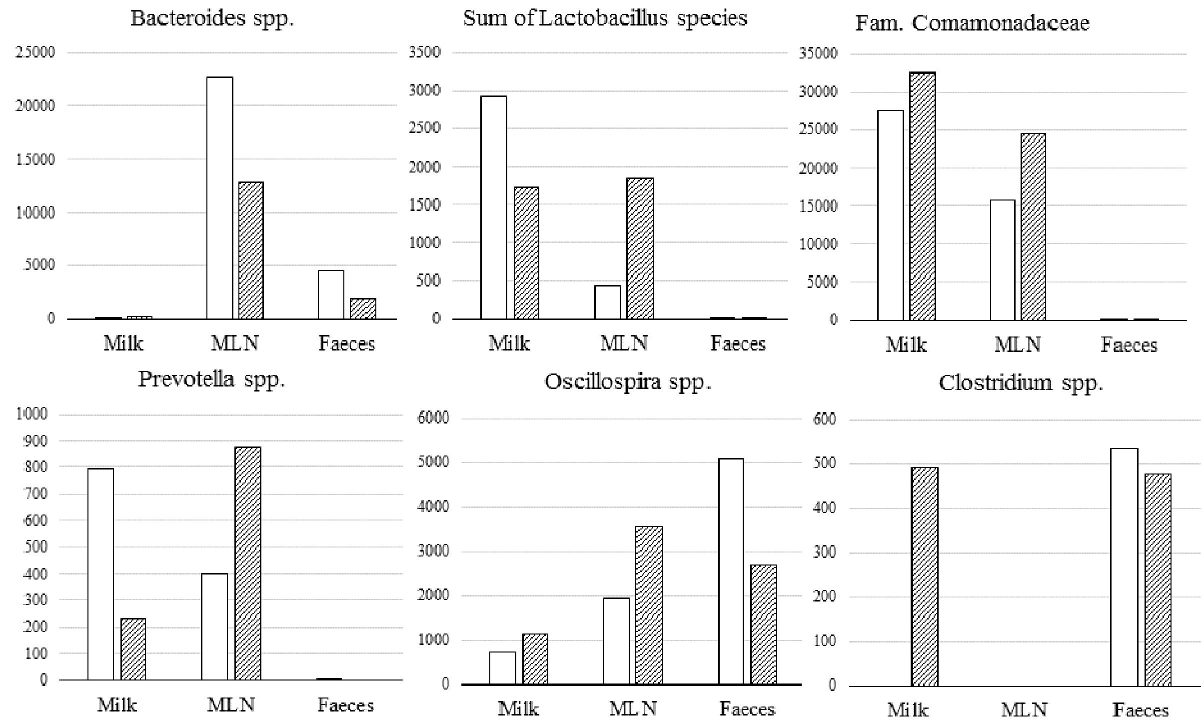

Figure 4: Abundance of different operational transfer units (number of 16s DNA sequences in 400 mg of fresh sample) in mesenteric lymph nodes (MLN) of 6-d old kits and maternal milk and faeces at 6-d of lactation (Delgado *et al*., unpublished). [□ high soluble fibre diet ▨ low soluble fibre diet]

REFERENCES

Abad-Guamán R., Larrea-Dávalos J.A., Carabaño R., García J., Carro M.D. 2018. Influence of inoculum type (ileal, caecal and faecal) on the in vitro fermentation of different sources of carbohydrates in rabbits. *World Rabbit Sci*. 26, 227-240.

Abecia L., Fondevila M., Balcells J., McEwan N.R. 2007. The effect of lactating rabbit does on the development of the caecal microbial community in the pups they nurture. *J. Applied Microbiol*. 53, 557-564.

Alfonso C., Saiz A., Terreros E., Fernández B., Marco M., Lizaso J. 2014. Efecto de dietas específicas de periparto en parámetros reproductivos y de crecimiento en conejas y camadas. *In Proc. VI Jornadas de Cunicultura de ASPOC, pp. 53-55. 2014 October, Aveiro, Portugal.*

Álvarez J.L., Margüenda, I., García-Rebollar P., Carabaño R., De Blas C., Corujo A., García-Ruiz A.I. 2007. Effects of type and level of fibre on digestive physiology and performance in reproducing and growing rabbits. *World Rabbit Sci*. 15, 9-17.

Achard C.S., Dupouy V., Siviglia S., Arpaillange N., Cauquil L., Bousquet-Mélou A., Zemb O. Variability of the Ability of Complex Microbial Communities to Exclude Microbes Carrying Antibiotic Resistance Genes in Rabbits. Front. Microbiol. 10, 1503.

Argente M.J., Santacreu M.A., Climent A., Blasco, A. 2003. Relationships between uterine and fetal traits in rabbits selected on uterine capacity. *J. Anim. Sci*. 81, 1265-1273.

Arias-Álvarez M., García-García R.M., Rebollar P.G., Nicodemus N., Revuelta L., Millán P., Lorenzo P.L. 2009. Effects of a lignin-rich fibre diet on productive, reproductive and endocrine parameters in nulliparous rabbit does. *Livest. Sci.* 123, 107-115.

Arias-Álvarez M., García-García R.M., Lorenzo P.L., Gutiérrez-Adán A., Sakr O.G., González-Bulnes A., Rebollar P.G. 2013. Embryo gene expression in response to maternal supplementation with glycogenic precursors in the rabbit. *Anim. Reprod. Sci.* 142, 173-182.

Arnau-Bonachera A., Cervera C., Blas E., Larsen T., Martínez-Paredes E., Ródenas L., Pascual J.J. 2018a. Long-term implications of feed energy source in different genetic types of reproductive rabbit females. I. Resource acquisition and allocation. *Animal*, 12, 1867-1876.

Arnau-Bonachera A., Savietto D., Pascual J.J. 2018b. Long-term implications of feed energy source in different genetic types of reproductive rabbit females. III. Fitness and productivity. *Animal*, 12, 1886-1894.

Bae I., Foote R.H., 1975. Carbohydrate and amino acid requirements and ammonia production of rabbit follicular oocytes matured in vitro. *Exp. Cell Res.* 91, 113-118.

Belhassen T., Simon E., Potel A., Auclair E., Bergaoui R. 2016. Effect of diet supplementation with live yeast (Saccharomyces cerevisiae) on performance of rabbit does and their progenies. *World Rabbit Sci*. 24, 77-82.

Bird I.M., Zhang L., Magness R.R., 2003. Possible mechanisms underlying pregnancy-induced changes in uterine artery endothelial function. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 284, 245-258.

Blasco A., Bidanel J.P., Bolet G., Haley C.S., Santacreu M.A. 1993. The genetic of prenatal survival of pigs and rabbits: a review. *Livest. Prod. Sci*. 37, 1-21.

Brecchia G., Bonanno A., Galeati G., Federici C., Maranesi M., Gobbetti A., Zerani M., Boiti C. 2006. Hormonal and metabolic adaptation to fasting: effects on the hypothalamic–pituitary–ovarian axis and reproductive performance of rabbit does. Dom. *Anim. Endocrinol*. 31, 105-122.

Bienertova-Vasku J., Bienert P., Zlamal F., Tomandl J., Forejt M., Tomandlova M., Vavrina M., Kudelková J., Splichal Z., Vasku A. 2012. B-cell activating factor (BAFF). A new factor linking immunity to diet? *Centr. Eur. J. Med.* 7, 273-285.

Carabaño R., Motta-Ferreira W., de Blas J.C., Fraga M.J., 1997. Substitution of sugarbeet pulp for alfalfa hay in diets for growing rabbits. *Anim. Feed Sci. Technol*. 65, 249-256.

Carney E.W., Bavister B.D. 1987. Stimulatory and inhibitory effects of amino acids on the development of hamster eight-cell embryos in vitro. *J. In Vitro Fertil. Embryo Transfer* 4, 162-167.

Cartuche L., Pascual M., Gómez E.A., Blasco A. 2014. Economic weights in rabbit meat production. *World Rabbit Sci*. 22, 165-177.

Chen W., Mi J., Lv N., Gao J., Cheng J., Wu R., Ma J., Lan T., Liao X. 2018. Lactation stage-dependency of the sow milk microbiota. *Front. Microbiol*. 9, 945.

Combes S., Gidenne T., Cauquil L., Bouchez O., Fortun-Lamothe L. 2014. Coprophagous behavior of rabbit pups affects implantation of cecal microbiota and health status. *J. Anim. Sci.* 92, 652-665.

Coutelet G. 2015. Résultats technico-économiques des éleveurs de lapins de chair en France en 2014. *In Proc. 16émes Journées de la Recherche Cunicole, pp. 193-195. Le Mans, France.*

Damico J., Torres C., Argente M.J., García M.L. 2013. Study of uterine and foetal traits of the rabbit female at 12 days of gestation. *World Rabbit Sci*. 21, 209-210.

De Andrés J., Jiménez E., Chico-Calero I., Fresno M., Fernández L., Rodríguez J.M. 2018. Physiological translocation of lactic acid bacteria during pregnancy contributes to the composition of the milk microbiota in mice. *Nutrients* 10, 14, doi:103390/nu10010014.

De Blas J.C., Mateos G.G., 2010. Feed Formulation. In: De Blas C., Wiseman J. (Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford Oxon, UK, 222-232.

De Blas J.C., Taboada E., Mateos G.G., Nicodemus N., Méndez J., 1995. Effect of the substitution of starch for fiber and fat in isoenergetic diets on nutrient digestibility and reproductive performance in rabbits. *J. Anim. Sci*. 73, 1131–1137.

Delgado, R., Badiola, I., Abad-Guamán, R., Nicodemus, N., Villamide, M.J., Pérez de Rozas, A., Menoyo, D., Carabaño, R., García, J., 2015. Effect of level of soluble fibre and omega-6/omega-3 ratio on microbiota colonisation in suckling rabbits. *World Rabbit Sci*. 23, 131.

Delgado R., Abad-Guamán R., Carabaño R., García J., Nicodemus N. 2017a. La movilización de grasa entre la segunda inseminación y el destete está positivamente relacionada con el éxito reproductivo en conejas primíparas. Proceedings of the XLII Symposium de Cunicultura de ASESCU, pp. 56-60, Murcia, Spain.

Delgado R, Abad-Guamán R, De la Mata E, Menoyo D, Nicodemus N, García J, Carabaño R. 2017b. Effect of dietary supplementation with arginine and glutamine on the performance of rabbit does and their litters during the first three lactations. *Anim. Feed Sci. Technol*. 227, 84-94.

Delgado R., Abad-Guamán R., Nicodemus N., Villamide M.J., Ruiz N., Carabaño R., Menoyo D., García J., 2018a. Effect of level of soluble fiber and n-6/n-3 fatty acid ratio on performance of rabbit does and their litters. *J. Anim. Sci*. 96, 1084-1100.

Delgado R., Nicodemus N., Abad-Guamán R., Sastre J., Menoyo D., Carabaño R., García J., 2018b. Effect of dietary soluble fibre and n-6/n-3 fatty acid ratio on growth performance and nitrogen and energy retention efficiency in growing rabbits. *Anim. Feed Sci. Technol*. 239, 44–54.

Delgado R., Nicodemus N., Abad-Guamán R., Menoyo D., García J., Carabaño R. 2019a. Effect of arginine and glutamine supplementation on performance, health and nitrogen and energy balance in growing rabbits. *Anim. Feed Sci. Technol*. 247, 63-73.

Delgado R., Menoyo D., Abad-Guamán R., Nicodemus N., Carabaño R., García J., 2019b. Effect of level of soluble fibre and n-6/n-3 fatty acid ratio on digestion and health in growing rabbits. *Anim. Feed Sci. Technol.* 96, 1084-1100.

Delgado R., Abad-Guamán R., Nicodemus N., Díaz-Perales A., García J., Carabaño R., Menoyo D. 2019c. Effect of pre- and post-weaning dietary supplementation with arginine and glutamine on rabbit performance and intestinal health. BMC Vet. Res. 15:199. https://doi.org/10.1186/s12917-019-1945-2

Deltoro J., López A.M. 1985. Allometric changes during growth in rabbits. *J. Agric. Sci*.105, 339-346.

Denker H.W. 1977. Implantation. The role of proteinases, and blockage of implantation by proteinase inhibitors. *Adv. Anat. Embryol. Cell Biol.* 53, 3–123.

Donnet-Hughes A., Pérez P.F., Doré J., Leclerc M., Levenez F., Benyacoub J., Serrant P., Segura-Roggero I., Schiffrin E.J., 2010. Prebiotics and probiotics usefulness against pathologies. Potential Role of the intestinal microbiota of the mother in neonatal immune education. *Proc. Nutr. Soc.* 69, 407-415.

Duncan S.L.B., 1969. The partition of uterine blood flow in pregnant rabbit. *J. Physiol.* 204, 421-433.

Eiben C.S., Kustos K., Kenessey Á., Virág Gy., Szendrő Zs. 2001. Effect of different feed restrictions during rearing on reproduction performance in rabbit does. *World Rabbit Sci.* 9, 9-14.

Fernández-Carmona J., Bernat F., Cervera C., Pascual J.J. 1998. High lucerne diets for growing rabbits. *World Rabbit Sci.* 6, 237-240.

Fernández L., Langa S., Martín V., Maldonado A., Jiménez E., Martín R., Rodríguez J.M. 2013. The human milk microbiota: Origin and potential roles in health and disease. *Pharmacol. Res.* 69, 1-10.

Fernández L., Cárdenas N., Arroyo R., Manzano S., Jiménez E., Martín V., Rodríguez J.M. 2016. Prevention of infectious mastitis by oral administration of *Lactobacillus salivarius ps2* during late pregnancy. *Clin. Infec. Dis.* 62(5):568–73.

Ferrian S., Guerrero I., Blas E., García-Diego F.J., Viana D., Pascual J.J., Corpa J.M. 2012. How selection for reproduction or foundation for longevity could have affected blood lymphocyte populations of rabbit does under conventional and heat stress conditions. *Vet. Immunol. Immunopathol.* 150, 53-60.

Fortun-Lamothe L. 1998. Effect of pre-mating energy intake on reproductive performance of rabbit does. *Anim. Sci*. 66, 263–269.

Fortun-Lamothe L., Lacanal L., Boisot P., Jehl N., Arveux P., Hurtaud J., Perrin G. 2005. Influence de la stratégie alimentaire autour du sevrage sur les performances de reproduction des lapines et la santé des lapereaux: effets de l'origine et de la tener en énergie d l'aliment. In: *Proc. 11émes Jorunées de la Recherche Cunicole, November, Paris, France. Pp 129-132.*

Fortun-Lamothe L., 2006. Energy balance and reproductive performance in rabbit does. *Anim. Reprod. Sci.* 93, 1-15.

Friggens N.C. 2003. Body lipid reserves and the reproductive cycle: towards a better understanding. *Livest. Prod. Sci.* 83, 219-236.

García, J., Gidenne, T., Falcao e-Cunha, L., De Blas, J.C., 2002. Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Anim. Res.* 51, 165-173.

García-García R.M., Sakr O., Velasco B., Aguado F., Lorenzo P.L., Rebollar P.G. 2010. La duración del suministro de propilenglicol a conejas primíparas afecta al peso y viabilidad de sus camadas. *Boletín de Cunicultura*, 163, 39-46.

García-Quirós A., Arnau-Bonachera A., Penadés M., Cervera C., Martínez-Paredes E., Ródenas L., Selva L., Viana D., Corpa J.M., Pascual J.J. 2014. A robust rabbit line increases leukocyte counts at weaning and reduces mortality by digestive disorder during fattening. *Vet. Immunol. Immunopathol.* 161, 123-131.

Gerencser Zs., Matics Zs, Nagy I., Scendrö Zs. 2011. Effect of feeding program before weaning on the production of rabbit does and their kits. *World Rabbit Sci*. 19, 217-223.

Guerrero I., Ferrian S., Blas E., Pascual J.J., Cano J.L., Corpa J.M. 2011. Evolution of peripheral blood lymphocyte populations in multiparous rabbit does with two reproductive management rhythms. *Vet. Immunol. Immunopathol*. 140, 75-81.

Gidenne T., 1993. Measurement of the rate of passage in restricted-fed rabbits: effects of dietary cell wall level on the transit of fibre particles of different sizes. *Anim. Feed Sci. Technol*. 42, 151–163.

Gidenne T., Poncet C., Gomez L., 1987. Effet de l'addition dun concentré riche en fibres dans une ration a base de foin, distribuee a deux niveaux alimentaires chez la lapine adulte. 1. Temps de sejour moyen des aliments. *Reprod. Nutr. Develop*. 27, 733-743.

Gómez-Conde M.S., García J., Chamorro S., Eiras P., García-Rebollar P., Pérez de Rozas A., Badiola I., De Blas J.C., Carabaño R., 2007. Neutral detergent-soluble fibre improves gut barrier function in twenty-five-day-old weaned rabbits. *J. Anim. Sci*. 85, 3313-3321.

Gwatkin R.B.L., Haidri A.A., 1973. Requirements for the maturation of hamster oocytes in vitro. *Exp. Cell. Res*. 73, 1-7.

Harper M.J. 1961. The time of ovulation in the rabbit following the injection of luteinizing hormone. *J. Endocrinol.* 22, 147–152.

Huneau-Salaün A., Bougeard S., Balaine L., Eono F., Le Bouquin S., Chauvin C. 2015. Husbandry factors and health conditions influencing the productivity of French rabbit farms. *World Rabbit Sci*. 23, 27-37.

Iñigo M.A., De Blas J.C., Cachaldora P., García-Rebollar P. 2011. Effect of starch substitution with crude glicerol on growing rabbit and lactating rabbit doe performance. *World Rabbit Sci.* 19, 67-74.

Jeklova E., Leva L., Knotigova P., Faldyna M. 2009. Age-related changes in selected haematology parameters in rabbits. *Res. Vet. Sci.* 86, 525-528.

Jiménez E., Marín M.L., Martín R., Odriozola J.M., Olivares M., Xaus J., Fernández L., Rodríguez J.M. 2008. Is meconium from healthy newborns actually sterile? *Res. Microbiol.* 159, 187-193.

Klein-Jöbstl D., Quijada N.M., Dzieciol M., Feldbacher B., Wagner M., Drillich M., Schmitz-Esser S., Mann E. 2019. Microbiota of newborn calves and their mothers reveals possible transfer routes for newborn calves' gastrointestinal microbiota. *Plos One* 14 (8): e0220554.

Kovács M., Szendrõ Z., Milisits G., Biro-Nemeth E., Radnai I., Posa R., Bónai A., Kovács F., Horn. P, 2006. Effect of nursing method and faeces consumption on the development of bace-troides, lactobacillus and coliform flora in the caecum of the newborn rabbits. *Reprod. Nutr. Dev*. 46, 205–210.

Li P., Kim S.W., Li X.L., Datta S., Pond W.G., Wu G. 2009. Dietary supplementation with cholesterol and docosahexaenoic acid affect concentrations of amino acids in tissues of young pigs. *Amino Acids* 37, 709-716.

Luzi F., Barbieri S., Lazzaroni C., Cavani C., Zecchini M., Crimella C. 2001. Effects de l'addition de propylene glycol dans l'eau de boisson sur les performances de reproduction des lapines. *World Rabbit Sci*., 9, 15-18.

Maertens L. 1992. Rabbit nutrition and feeding: a review of some recent developments. *J. Appl. Rabbit Res*. 15, 810-818.

Maertens L., Van Renterghem R., De Groote G. 1994. Effects of dietary inclusion of Paciflor® (Bacillus CIP 5832) on the milk composition and performances of does and on caecal and growth parameters of their weanlings. *World Rabbit Sci*., 2, 67-73.

Maertens L., Aerts J.M., de Brabander D.L. 2005. Effect d´un aliment riche en acides gras omega-3 sur les performances et la composition du lait des lapines et la viabilité de leur descendance. In: *Proc. 11 èmes Journées de la Recherche Cunicole. Melle, Belgium. pp. 205-208.*

Manal A.F., Tony M.A., Ezzo O.H. 2010. Feed restriction of pregnant nulliparous rabbit does: consequences on reproductive performance and maternal behaviour. *Anim. Reprod. Sci*. 120, 179-186.

Martín R., Langa S., Reviriego C., Jiménez E., Marín M.L., Olivares M., Boza J., Jiménez J., Fernández L., Xaus J., Rodríguez J.M. 2004. The comensal microflora of human milk: new perspectives for food bacteriotherapy and probiotics. *Trends in Food Sci. Technol.* 15, 121-127.

Martínez-Paredes E. 2008. Efecto de diferentes modelos de alimentación para recría y gestación de conejas nulíparas sobre su vida productiva. *Master Thesis. Master in Animal Production. Universitat Politècnica de València. Spain*, pp 14.

Martínez-Paredes E., Ródenas L., Martínez-Vallespín B., Cervera C., Blas E., Brecchia G., Boiti C., Pascual J.J. 2012. Effects of feeding programme on the performance and energy balance of nulliparous rabbit does. *Animal* 6, 1086-1095.

Martínez-Paredes E., Ródenas L., Pascual J.J., Savietto D. 2018. Early development and reproductive lifespan of rabbit females: implications of growth rate, rearing diet and body condition at first mating. *Animal* 12, 2347-2355.

Martínez-Paredes E., Savietto D., Ródenas L., Cervera C., Blas E., Brecchia G., Boiti C., Pascual J.J. 2019. Consequences of rearing feeding programme on the performance of rabbit females from 1st to 2nd parturition. *Animal*, 13: 2173-2182.

Martínez-Vallespín B., Martínez-Paredes E., Ródenas L., Cervera C., Pascual J.J., Blas E. 2011. Combined feeding of rabbit female and young: Partial replacement of starch with acid detergent fibre or/and neutral detergent soluble fibre at two protein levels. *Livest. Sci*. 141, 155-165.

Mateo R.D., Wu G., Bazer W.B., Park J.C., Shinzato I., Kim S.W. 2007. Dietary L-Arginine supplementation enhances the reproductive performance of gilts. *J. Nutr*. 137, 652-656.

Mateo R.D., Wu G., Moon H.K., Carroll J.A., Kim W. 2008. Effects of dietary arginine supplementation during gestation and lactation on the performance of lactating primiparous sows and nursing piglets. *J. Anim. Sci*. 86, 827-835.

Matics Z.S., Dalle Zotte A., Radnai I., Kovacs M., Metzger S.Z., Szendrő Z.S. 2008. Effect of restricted feeding after weaning on the productive and carcass traits of growing rabbits. In: *Proc. 9th World Rabbit Congress, Verona, Italy*, 741-745.

Mattioli S., Dal Bosco A., Maranesi M., Petrucci L., Rebollar P.G., Castellini C. 2019. Dietary fish oil and flaxseed for rabbit does: fatty acids distribution and Δ6-desaturase enzyme expression of different tissues. *Animal* 13, 1934-1942.

Menchetti L., Brecchia G., Cardinali R., Polisca A., Boiti C. 2015. Food restriction during pregnancy: Effects on body condition and productive performance of primiparous rabbit does. *World Rabbit Sci*. 23, 1-8.

Menchetti L., Canali C., Castellini C., Boiti C., Brecchia G. 2018. The different effects of linseed and fish oil supplemented diets on insulin sensitivity of rabbit does during pregnancy. *Res. Vet. Sci*. 118, 126-133.

Minuti A., Bani P., Piccioli-Cappelli F., Uboldi O., Bacciu N., Trevisi E. 2015. Metabolic and biochemical changes in plasma of the periparturient rabbit does with different litter size. *Animal 9:4, 614-621.*

Minuti, A., Gallo, A., Lopreiato, V., Bruschi, S., Piccioli-Cappelli F., Uboldi, O., Trevisi, E. 2020. Effect of litter size on prepartum metabolic and aminoacid profile in rabbit does. *Animal* 14, 2109-2115.

Moncada S., Higgs E. 1995. Molecular mechanisms and therapeutic strategies related to nitric oxide. *FASEB J*. 9, 1319-1330.

Moreno-Grua E., Pérez-Fuentes S., Muñoz-Silvestre A., Viana D., Fernández-Ros A.B., Sanz-Tejero C., Corpa J.M., Selva L. 2018. Characterization of livestock-associated methicillin-resistant *Staphylococcus aureus* isolates obtained from commercial rabbitries located in the iberian península. *Frontiers Microbiol*. 9, 1812.

Mordenti A.I., Sardi L., Bonaldo A., Pizzamiglio V., Brogna N., Cipollini I., Tassinari M., Zaghini. G. 2010. Influence of marine algae (Schizochytrium spp.) dietary supplementation on doe performance and progeny meat quality. *Livest. Sci*. 128, 179-184.

Naturil-Alfonso C., Lavara R., Vicente J.S., Marco-Jiménez F. 2016. Effects of female dietary restriction in a rabbit growth line during rearing on reproductive performance and embryo quality. *Reprod. Dom. Anim.* 51, 114-122.

Naturil-Alfonso C., Peñaranda D.S., Vicente J.S., Marco-Jiménez F. 2017. Feed restriction regime in a rabbit line selected for growth rate alters oocyte maturation manifested by alteration in MY2 gene expression. *Reprod. Dom. Anim.* 52, 976-984.

Nicodemus N., Carabaño R., García J., Méndez J., De Blas J.C., 1999a. Performance response of lactating and growing rabbits to dietary lignin content. *Anim. Feed Sci. Technol.,* 80, 43-54.

Nicodemus N., Mateos J., de Blas C., Carabaño R., Fraga M.J. 1999b. Effect of diet on amino acid composition of soft faeces and the contribution of soft faeces to total amino acid intake, through caecotrophy in lactating doe rabbits. *Anim. Sci.* 69, 167-170.

Nicodemus N., Carabano R., Garcia J., De Blas J.C. 2004. Performance response of does rabbit to Toyocerin® (Bacillus cereus var. toyoi) supplementation. *World Rabbit Sci*., 12 109-118.

Nicodemus N, Gómez-Conde M.S., Chamorro S., Rodríguez-Granados J.D., García J., De Blas, J.C. 2005. Efecto de la adición de propilenglicol en el pienso sobre los rendimientos de conejas reproductoras. *In Proc. XXX Symposium de Cunicultura. pp. 107-113. Mayo, Valladolid, Spain.*

Nicodemus N., García J., Carabaño R., De Blas, J.C. 2006. Effect of a reduction of dietary particle size by substituting a mixture of fibrous by-products for lucerne hay on performance and digestion of growing rabbits and lactating does. *Livest. Sci.* 100, 242-250.

Nicodemus N., García J., Carabaño R., De Blas J.C. 2007. Effect of substitution of a soybean hull and grape seed meal mixture for traditional fiber sources on digestion and performance of growing rabbits and lactating does. J. Anim. Sci. 85, 181-187.

Nicodemus N., Redondo R., Pérez-Alba L., Carabaño R., de Blas J.C., García J., 2010. Effect of level of fibre and type of grinding on the performance of rabbit does and their litters during the first three lactations. *Livest. Sci.* 129, 186–193.

Nicodemus N., Abad-Guamán R., Delgado R., Ocasio-Vega C., Guenaoui M., Menoyo D., Carabaño R., García J. 2015. Evolución de la presencia de heces en el nidal durante la lactación en conejas multíparas. In *Proc. XL Symposium de Cunicultura de ASESCU, Santiago de Compostela, Spain. pp. 97-99.*

Nielsen N.I., Ingvartsen K.L. 2004. Propylene glycol for dairy cows. A Review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. *Anim. Feed Sci. Technol*. 115, 191-213.

Nizza A., Di Meo C., Esposito L. 1997. Influence of the diet used before and after the first mating on reproductive performance of rabbit does. *World Rabbit Sci*. 5, 107-110.

Oger M.A., Lebas F., Laplace J.P. 1978. Le transit digestif chez le lapin: variations peripartum du component alimentaire et de l'excrétion fécale chez la lapine multipare. *Ann. Zootech*. 27, 519-532.

Parigi-Bini R., Xiccato G. 1993. Recherches sur l'interaction entre alimentation, reproduction et lactation chez la lapine, une revue. *World Rabbit Sci.* 1, 155-161.

Partridge G.G. 1986. Meeting the protein and energy requirement of the commercial rabbit for growth and reproduction. In: *Proc. 4th World Congress of Animal Feeding, Madrid, Spain*, 9, 271-556.

Pascual J.J., Cervera C., Blas E., Fernández-Carmona J. 1999. Effect of high fat diets on the performance, milk yield and milk composition of multiparous rabbit does. *Anim. Sci*. 68, 151-162.

Pascual J.J., Cervera C., Fernández-Carmona J. 2002. A feeding program for young rabbit does based on all lucerne diets. *World Rabbit Sci.* 10, 7-13.

Pascual J.J., Cervera C., Blas E., Fernández-Carmona J. 2003. High-energy diets for reproductive rabbit does: effect of energy source. *Nutr. Abs. Rews*. (Series B), 73, 27-39.

Pascual J.J., Blanco J., Piquer O., Quevedo F., Cervera C. 2004. Ultrasound measurements of perirenal fat thickness to estimate the body condition of reproducing rabbit does in different physiological states. *World Rabbit Sci*. 12, 7-31.

Pascual J.J., Xiccato G., Fortun-Lamothe L. 2006. Strategies for does' corporal condition improvement –relationship with litter viability and career length. In: Maertens L., Coudert P. (Eds.), Recent Advances in Rabbit Science, ILVO, Merenbeke, Belgium. pp. 221-227.

Pascual J.J., Savietto D., Cervera C., Baselga M. 2013. Resources allocation in reproductive rabbit does: a review of feeding and genetic strategies for suitable performance. *World Rabbit Sci*. 21, 123-144.

Penades M., Arnau-Bonachera A., García-Quirós A., Viana D., Selva L., Corpa J.M., Pascual J.J. 2018. Long-term implications of feed energy source in different genetic types of reproductive rabbit females. II. Immunologic status. *Animal* 12, 1877-1885.

Pereda N.L. 2010. Evaluación de la técnica del análisis de impedancia bioeléctrica para predecir la composición corporal: aplicación en conejas sometidas a diferentes sistemas de alimentación durante la recría. *Ph.D. Thesis. Universidad Politécnica de Madrid. Spain*, pp. 196.

Pereda N.L., Nicodemus N., Rebollar P.G. 2009. Evaluación de la técnica de análisis de impedancia bioeléctrica (BIA) para estimar la composición corporal en conejas reproductoras. *Boletín de Cunicultura* 159, 14-21.

Petters R.M., Johnson B.H., Reed M.L. Archibong A.E. 1990. Glucose, glutamine and inorganic phosphate in early development of the pig embryo in vitro. *J. Reprod. Fert.* 89, 269-275.

Pinheiro V., Mourão J.L., Jimenez G. 2007. Influence of Toyocerin® (Bacillus cereus var. toyoi) on breeding performance of primiparous rabbit does. *World Rabbit Sci*. 15, 179-188.

Quevedo F., Cervera C., Blas E., Baselga M., Costa C., Pascual J.J. 2005. Effect of selection for litter size and feeding programme on the performance of young rabbit females during rearing and first pregnancy. *Anim. Sci*. 80, 161-168.

Quevedo F., Cervera C., Blas E., Baselga M., Pascual J.J. 2006a. Long-term effect of selection for litter size and feeding programme on the performance of reproductive rabbit does 1. Pregnancy of multiparous does. Anim. Sci. 82, 739-750.

Quevedo F., Cervera C., Blas E., Baselga M., Pascual J.J. 2006b. Long-term effect of selection for litter size and feeding programme on the performance of reproductive rabbit does 2. Lactation and growing period. Anim. Sci. 82, 751-762.

Rebollar P.G., Pereda N., Schwarz B.F., Millán P., Lorenzo P.L., Nicodemus N. 2011. Effect of feed restriction or feeding high-fibre diet during the rearing period on body composition, serum parameters and productive performance of rabbit does. *Anim. Feed Sci. Technol*. 163, 67-76.

Rebollar P.G., García-García R.M., Arias-Alvarez M., Millán P., Rey A.I., Rodríguez M., Formoso-Rafferty N., de la Riva S., Masdeu M., Lorenzo P.L., García-Rebollar P. 2014. Reproductive long-term effects, endocrine response and fatty acid profile of rabbit does fed diets supplemented with n-3 fatty acids. *Anim. Reprod. Sci*. 146, 202-209.

Read T., Combes S., Gidenne T., Destombes N., Bébin K., Balmisse E., Fortun-Lamothe L. 2016. Influence of feeding strategy and diet for reproductive rabbit does on intake, performances, and health of young and females before and after weaning. *J. Anim. Sci*., 94, 4848-4859.

Rodríguez M., García-García R.M., Arias-Álvarez M., Formoso-Rafferty N., Millán P., López-Tello J., Lorenzo P.L., González-Bulnes A., Rebollar P.G. 2017. A diet supplemented with n-3 polyunsaturated fatty acids influences the metabolic and endocrine response of rabbit does and their offspring. *J. Anim. Sci*. 95, 2690-2700.

Rodríguez M., García-García R.M., Arias-Álvarez M., Millán P., Febrel N., Formoso-Rafferty N., López-Tello J., Lorenzo P.L., Rebollar P.G. 2018. Improvements inthe conception rate, milk composition and embryo quality of rabbit does after dietary enrichment with n-3 polyunsaturated fatty acids. *Animal* 12, 2080-2088.

Romero C., Nicodemus N., Martínez de Morentin C., García A., de Blas C. 2011. Effect of grinding size of barley and dehydrated alfalfa on performance and body composition of does during their early reproductive cycles. *Livest. Sci*. 140, 55-61.

Rommers J.M., Kemp B., Meijerhof R., Noordhuizen J.P.T.M. 2001. Effect of litter size before weaning on subsequent body development, feed intake and reproductive performance of young rabbit does. *J. Anim. Sci*. 79, 1973-1982.

Rommers J.M., Meijerhof R., Noordhuizen J.P.T.M., Kemp B. 2002. Relationships between body weight at first mating and subsequent body development, feed intake and reproductive performance of rabbit does. *J. Anim. Sci*. 80, 2036-2042.

Rommers J.M., Meijerhof R., Noordhuizen J.P.T.M., Kemp B. 2004a. Effect of feeding program during rearing and age at first insemination on performances during subsequent reproduction in young rabbit does. *Reprod. Nutr. Develop*. 44, 321-332.

Rommers J.M., Boiti C., Brecchia G., Meijerhof R., Noordhuizen J.P.T.M., Decuypere E., Kemp B. 2004b. Metabolic adaptation

and hormonal regulation in young rabbit does during longterm caloric restriction and subsequent compensatory growth. *Anim. Sci.* 79, 255-264.

Rosell J.M. 2000. Enfermedades de menor presentación. Enfermedades metabólicas. In: *Enfermedades del conejo (ed. JM Rosell), Mundiprensa, Madrid, Spain. pp. 399–454.*

Rosell J.M., de la Fuente L.F. 2009. Culling and mortality in breeding rabbits. *Prev. Vet. Med*. 88, 120-127.

Saidj D., Ainbaziz H., Iles I., Dahman Y., Hornick J.L., Moula N. 2019. Productive performance, metabolic and hematologic parameters of pregnant nulliparous rabbit does according to dietary protein level. *J. Adv. Vet. Res*. 6, 18-24.

Savietto D., Friggens N.C., Pascual J.J. 2015. Reproductive robustness differs between generalist and specialist maternal rabbit lines: the role of acquisition and allocation of resources. *Genet. Sel. Evol*. 47, 2.

Savietto D., Marono S., Martinez I., Martínez-Paredes E., Ródenas L., Cervera C., Pascual J.J. 2016. Patterns of body condition use and its impact on fertility. *World Rabbit Sci*. 24, 39-45.

Sakr O.G., Crespo R., Velasco B., García-García R.M., Arias-Álvarez M., García-Rebollar P., Lorenzo P.L., Rebollar P.G. 2011. Composición corporal y datos productivos de conejas primíparas suplementadas con propilenglicol durante largos periodos de tiempo. In: *Proc. 36 Symposium de Cunicultura de ASESCU, May, Peñíscola, Spain. pp. 104-112.*

Szendrő Zs., Gyovai M., Maertens L., Biró-Németh E., Radnai I., Matics Zs., Princz Z., Gerencsér Zs., Horn P. 2006. Influence of birth weight and nutrient supply before and after weaning on the performance of rabbit does to age of the first mating. *Livest. Sci.* 103, 54-64.

Taghouti M., Macchiavelli R., García J., Demey J., Nicodemus N. 2011a. Relación entre la composición química corporal, la fertilidad y la prolificidad en conejas primíparas. Proceedings XXXVI Symposium de Cunicultura de ASESCU, pp. 85-88, Peñíscola, Spain.

Taghouti M., García J., Macchiavelli R., Demey J., Nicodemus N. 2011b. Relación entre la composición química corporal, la fertilidad y la prolificidad en conejas nulíparas. Proceedings XIV Jornadas sobre Producción Animal AIDA, Tomo I, pp. 335-337, Zaragoza, Spain.

Theilgaard P., Sánchez J.P., Pascual J.J., Friggens N.C., Baselga M. 2006. Effect of body fatness and selection for prolificacy on survival of rabbit does assessed using a cryopreserved control population. *Livest. Sci*. 103, 65-73.

Uhlírova L., Volek Z. 2019. Effect of dehulled White lupine seeds on the milk production and milk composition in rabbit does and the growth performance of their litters before weaning. *J. Anim. Feed Sci.* 28, 291-297.

Urao M., Moy J., Van Camp J., Drongowski R., Altabba M., Coran A.G., 1995. Determinant of bacterial translocation in the newborn: small bowel versus large bowel colonization. *J. Pediatr. Surg*. 30, 831-836.

Urao M., Teitelbaurn D.H., Drongowski R.A., Coran A.G. 1996. The association of gut-associated lymphoid tissue and bacterial translocation in the newborn rabbit. *J. Ped. Surg*. 31, 1482-1487.

Verdelhan S., Bourdillon A., David J.J., Huirtaurd J.J., Lédan L., Renouf B., Roulleau X., Salaun J.M. 2005. Comparaison de deux programmes alimentaires pour la préparation des futures reproductrices. In: *Proc. 11émes Journées de la Recherche Cunicole, 29-30 November, 2005. Paris, France, 119-122.*

Vicente J.S., Llobat L., Viudes-de-Castro M.P., Lavara R., Baselga M., Marco-Jiménez F. 2012. Gestational losses in a rabbit line selected for growth rate. *Theriogenology* 77, 81-88.

Viudes-de-Castro P., Santacreu M.A., Vicente J.S. 1991. Effet de la concentration énergétique de l'alimentation sur les pertes embryonnaires et foetales chez la lapine. *Reprod. Nutrit. Develop*. 31, 529-534.

Volek Z., Marounek M., Volková L., Kudrnová E. 2014 Effect of diets containing whole lupin seeds on rabbit doe milk yield and milk fatty acid composition as well as the growth and health of their litters. *J. Anim. Sci*. 92, 2041-2049.

Volek Z., Ebeid T.A., Uhlírová L. 2018. The impact of substituting soybean meal and sunflower meal with a mixture of white lupine seeds and rapeseed meal on rabbit doe milk yield and composition, and the growth performance and carcass traits of their litters. *Anim. Feed Sci. Technol.* 236, 187-195.

Wu G.Y. 2009. Amino acids: metabolism, functions, and nutrition. *Amino Acids*. 37, 1-17.

Wu G.Y., Morris S.M., 1998. Arginine metabolism: nitric oxide and beyond. *Biochem. J*. 336, 1-17.

Wu G., Ott T.L, Knabe D.A., Bazer F.W. 1999. Amino acid composition of the fetal pig. J. Nutr. 129, 1031-1038.

Trocino A., García J., Carabaño R, Xiccato G. 2013. A meta-analysis on the role of soluble fibre in diets for growing rabbits. *World Rabbit Sci.* 21, 1-15.

Wathes D.C., Abayasekara D.R.E., Aitken R.J., 2007. Polyunsaturated fatty acids in male and female reproduction. *Biol. Reprod. 77, 190–201*.

Willyard C. 2018. Baby's first bacteria. *Nature* 553, 264-266.

Xiccato G. 1996. Nutrition of lactating does. In. Proc. 6th World Rabbit Congress. Vol 1, pp. 29-47. Toulouse, France.

Xiccato G., Trocino A. 2010. Energy and protein metabolism and requirements. In: De Blas C., Wiseman J. (Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford Oxon, UK, 83-118.

Xiccato G., Parigi Bini R., Dalle Zotte A., Carazzolo A., Cossu M.E., 1995. Effect of dietary energy level, addition of fat and physiological state on performance and energy balance of lactating and pregnant rabbit does. *Anim. Sci*. 61, 387– 398.

Xiccato G., Bernardini M., Castellini C., Dalle Zotte A., Queaque P.I., Trocino A. 1999. Effect of postweaning feeding on the performance and energy balance of female rabbits at different physiological states. *J. Anim. Sci.* 77, 416-426.

Xiccato G., Trocino A., Nicodemus N. 2006. Nutrition of the Young and growing rabbit: a comparative approach with the doe. In: Maertens L., Coudert P. (Eds.), Recent Advances in Rabbit Science, ILVO, Merenbeke, Belgium. pp. 239-246.

Zhang Y.K., Cui H.X., Sun D.F., Liu L.H., Xu X.R. 2018. Effects of doe-litter separation on intestinal bacteria, immune response and morphology of suckling rabbits. *World Rabbit Sci*. 26, 71-79.

CHALLENGES IN RABBIT DOES FEEDING, INCLUDING THE YOUNG DOE

Martínez-Paredes E., Nicodemus N., Pascual J.J., García J.

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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npact of rabbit female nutrition on the far

However, it's the ke component of farm profitability

ose to 70%

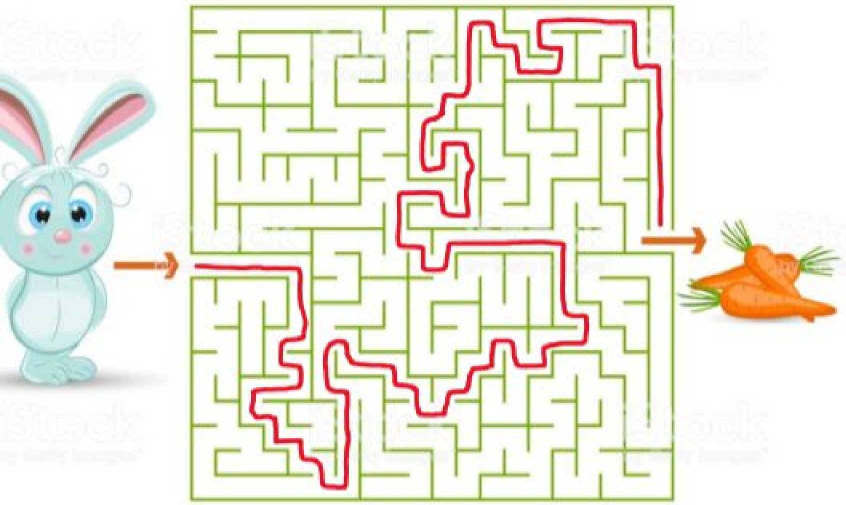
only 1/3

prolificacy

life span

farm health

w can we improve rabbit females' nutritio



Rearing period

Reproductive period

J.J. Pascual

J. García

What should we do during rearing?

DECUATE DEVELOPMENT ⇒ **ADECUATE MATURITY**

ascual et al., 2013; Martínez-Paredes et al., 2018)

Great variability

Fertility and prolificacy

Resilience

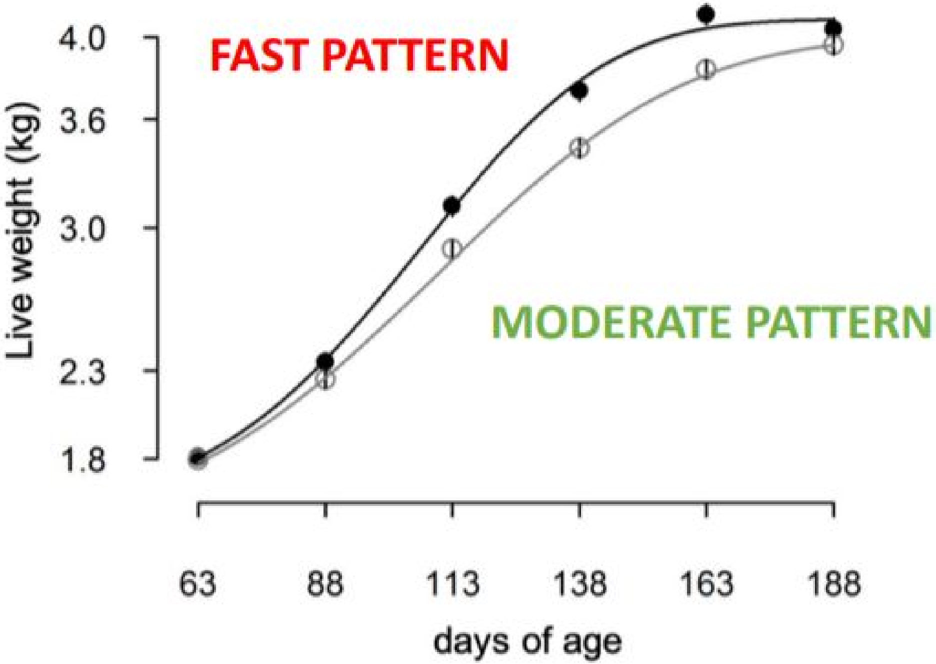
Kits growth and health

Indicators for adequate development

1. **Growth pattern**
2. **Body condition**
3. **Blood traits**

Moderate growth pattern during the rearing

Martínez-Paredes *et al.* (2018)



Negative consequences of a fast pattern:

↓ 1.3 liveborn during the 1st cycle

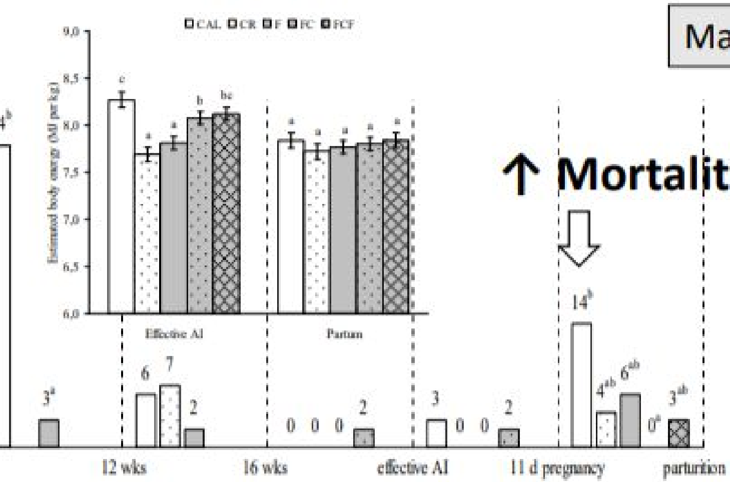
↓ 6.1 liveborn and ↓ 4.8 weaned

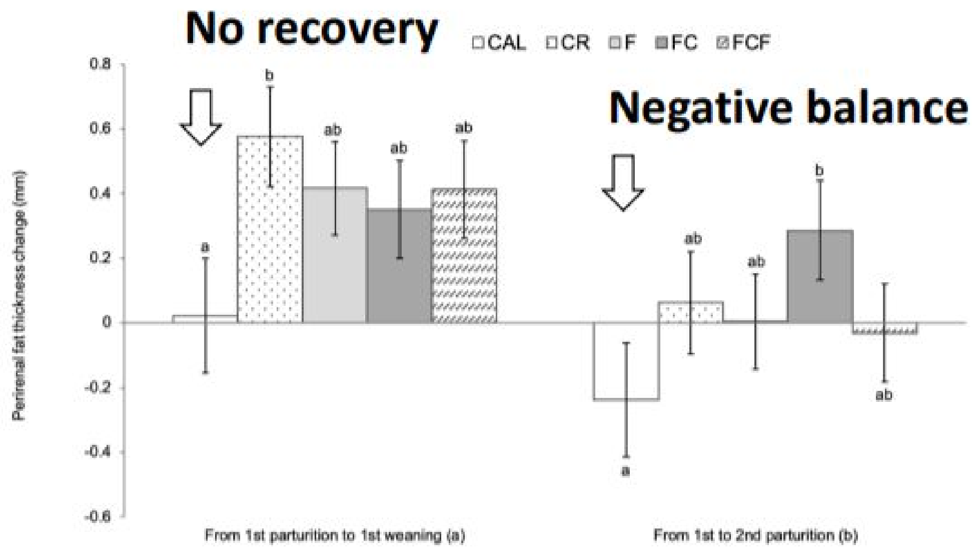
during the whole reproduction

(Savietto et al., 2016; Martínez-Paredes et al., 2018)

Avoid excessive fatness at 1st Insemination

Martínez-Paredes *et al.* (2012)





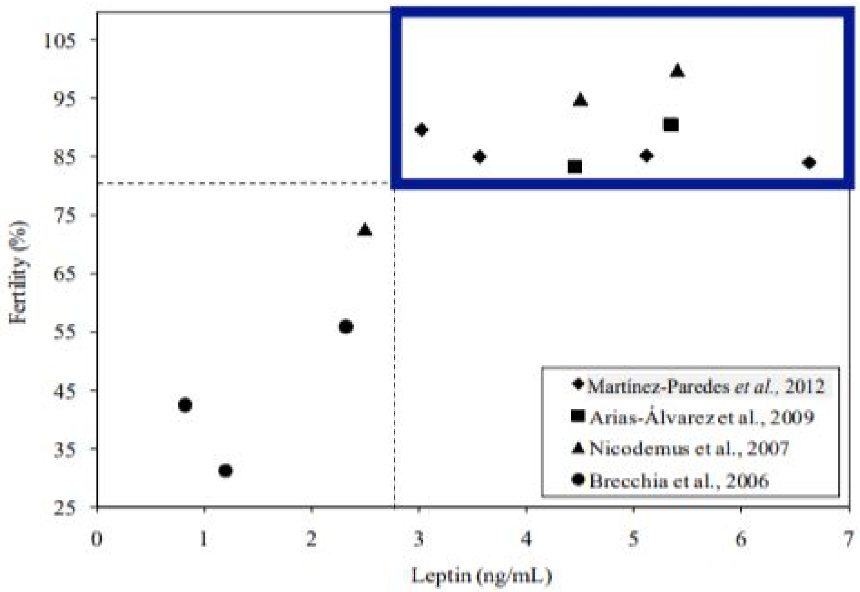
Risk of pregnancy toxemia:

Low pre-partum DE intake
High pre-partum mobilization
High NEFA and low glucose levels
Higher number of stillborn

Higher prenatal losses
(Vicente et al., 2012)
-3.2 liveborn and -3.0 weaned per 1 mm PFT
(Martínez- Paredes et al., 2018)

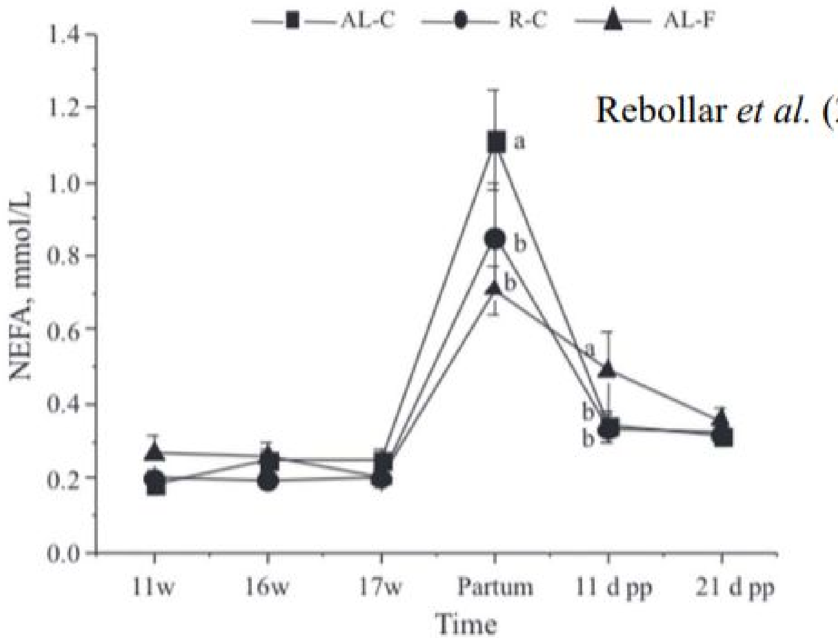
Blood traits during rearing

Leptine at 1st AI



Leptine threshold 2.8 ng/mL: +80% fertility

NEFA at 1st parturition



Excessive mobilization > Lower fertili

How can the young rabbit female achieve an appropriate level of maturity?

⇩

Rearing feeding programs

Rearing feeding programs: concentrate diets

Restriction vs. Ad libitum

- Avoid excessive fatness
- ↓ Risk of toxemia
- ↓ Perinatal mortality
- ↑ DM intake at early lactation
- ↓ Female maturity
- ↑ Feeding management

Martínez-Paredes *et al.*, 2012

	Ad libitum	*Restricted*
Body Energy (MJ/kg)	*8.3* [b]	*7.7* [a]
Mortality (%):		
Doe at late pregnancy	*14* [b]	*4* [a]
Litter at birth	*20.6* [b]	*8.3* [a]

↓ Fertility
↓ Litter size

9 10 **12** weeks of age

Pascual et al. 2002

Martínez- Paredes et al. 2012

Martínez- Paredes et al. 2019

Maturity delay

Rearing feeding programs: fibrous diets

Use of fibrous diets

- Ad libitum presentation
- Advantages of restriction

Martínez-Paredes *et al.*, 2012

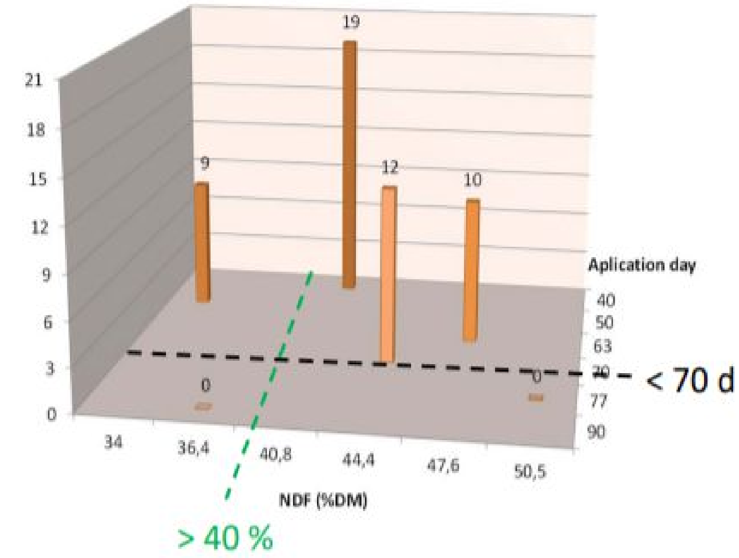
	Ad libitum	*Restricted*	*Fibrous diet*
Body Energy (MJ/kg)	*8.3 [b]*	*7.7 [a]*	*8,0 [ab]*
Mortality (%):			
Doe at late pregnancy	*14 [b]*	*4 [a]*	*3 [a]*
Litter at birth	*20.6 [b]*	*8.3 [a]*	*10,4 [a]*

Rearing feeding programs: fibrous diets

Use of fibrous diets

- Ad libitum presentation
- Advantages of restriction
- Stimulate feed intake

Increase of feed intake in lactation (g DM/d $M^{0.75}$)

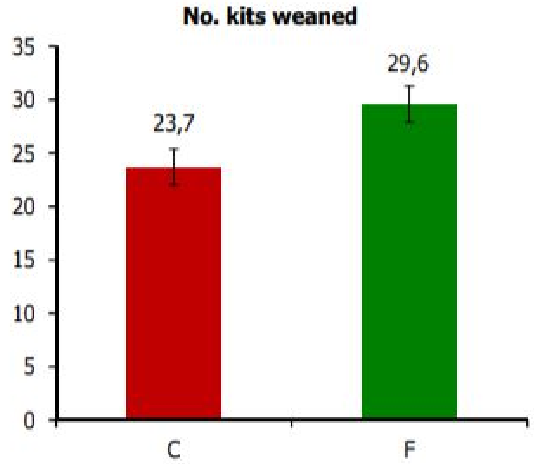


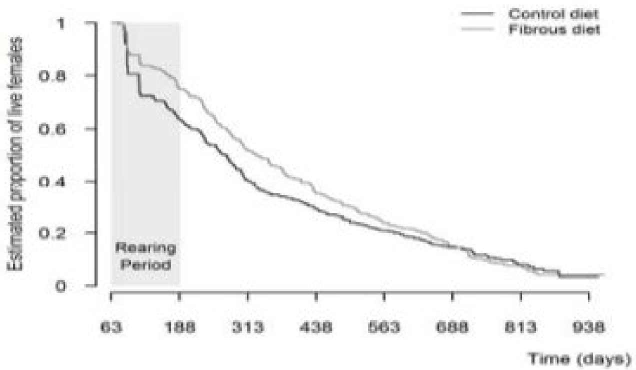
Introduce a diet with more than 40% NDF and 7.5% ADL before 70 days of life

Rearing feeding programs: fibrous diets

Use of fibrous diets

- Ad libitum presentation
- Advantages of restriction
- Stimulate feed intake
- Positive long term effects





Martínez-Paredes *et al.*, 2018

619 females

Until **880 days** of life

With fibrous diet:

+46.4 days of life

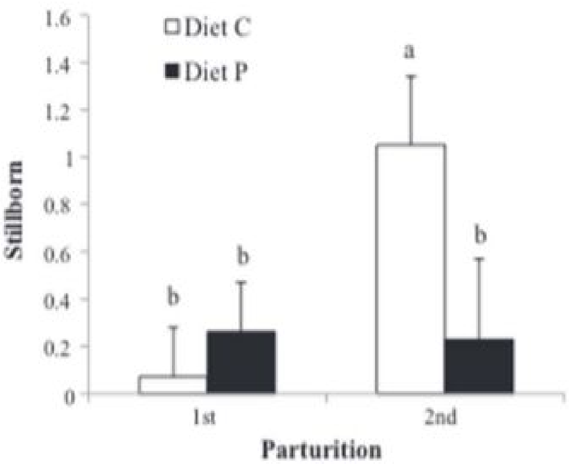
+5.1% at 4th partum

+7.4 born alive per female

Rearing feeding programs: PUFA

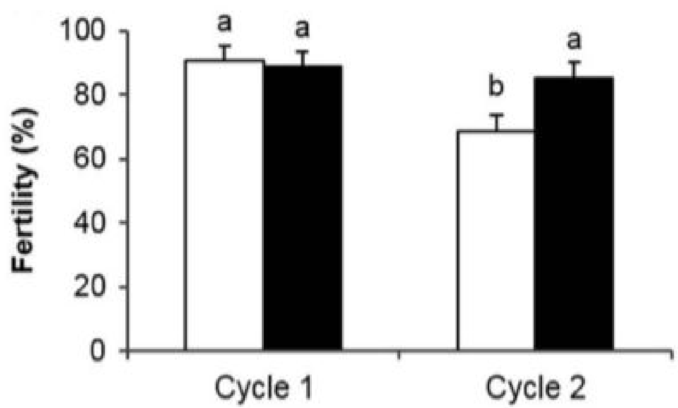
n-3 PUFA

Rebollar *et al.* (2014)



+3.2 g n-3 PUFA/kg

Rodriguez *et al.* (2018)



+14.3 g n-3 PUFA/kg

> 5 g n-3 PUFA/kg:

Increase plasma progesterone

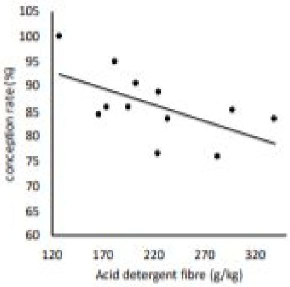
Reduce embryos apoptosis

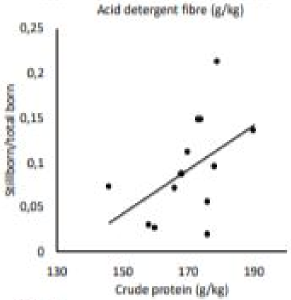
Reduce stillborns

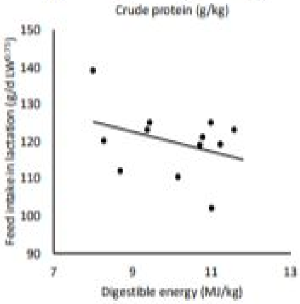
Maintain fertility rate

Modify milk fatty acid profile

Nutrient recommendations for rearing diets







Nutrient		Unit	Rearing diet	Reproduction diet
Digestible energy		kcal	8.5-9.5	11.0
Fiber	NDF	%	37.0-43.0	30.0-34.0
	ADF	%	25.0-35.0	15.0-18.0
	ADL	%	7.5	5.0
Starch		%	<12.0	15.0-21.0
n-3 PUFA		%	4.0-6.0	1.0-2.0
Protein	CP	%	13.0-15.5	16.3-19.8
	DP	%	9.1-10.9	11.4-13.9
	Lys	%	0.72	0.84
	Met+Cys	%	0.59	0.65
	Thr	%	0.63	0.70
Minerals	Ca	%	1.00	1.15
	P	%	0.50	0.60
	Na	%	0.20	0.22
	Cl	%	0.25	0.28

Management:

+ Ad libitum
+ Start: 63 days
+ Finish: 1 wk before AI
  1st partum day
+ Flushing recommended

Nutritional requirements and feeding strategies for rabbit does

⇨ Current standards that are working (De Blas and Mateos, 2020; Trocino and Xiccato, 2020):

– Ratio digestible [crude protein/energy]: 11.5 - 12.5 g/MJ - But litter size can be maximized with 10 g dCP/MJ DE (Delgado et al., 2018)

– NDF: 31 – 34 %

– Lignin: > 4 %

⇨ Current standards questioned (De Blas and Mateos, 2020):

Total Lys/DE:	0.76 g/MJ	Digestible Lys/DE:	0.60 g/MJ
Total Met + Cys/DE:	0.59	Digestible Met + Cys/DE:	0.45
Total Thr/DE:	0.63	Digestible Thr/DE:	0.43

- ☹ **Not updated for 20 years**
- ☹ **Faecal *vs.* ileal digestibility**
- ☹ **Apparent *vs.* real digestibility**
- ☹ **Missing other amino acids**

HEALTH > PRODUCTIVE ISSUES

New data regarding:

- Dietary levels:
  - Arginine and glutamine
  - ω-3 fatty acids
  - Fibre:
    - Particle size
    - Soluble fibre
  - New ingredients

- Feeding:
  - To maintain body condition
  - For the doe or the litter

- New opportunities:
  - Diet and microbiota
  - Diet and immune system

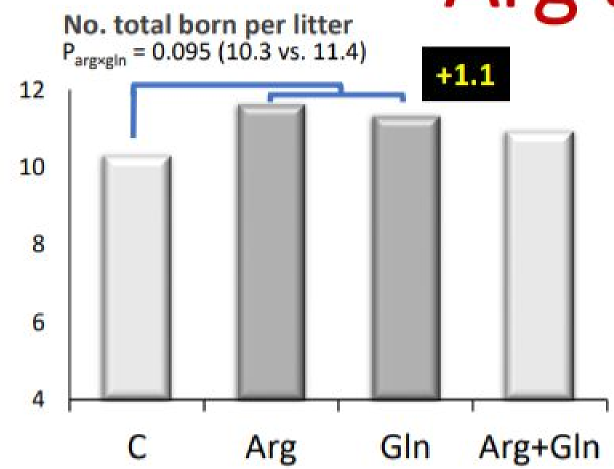
Arg and Gln supplementation

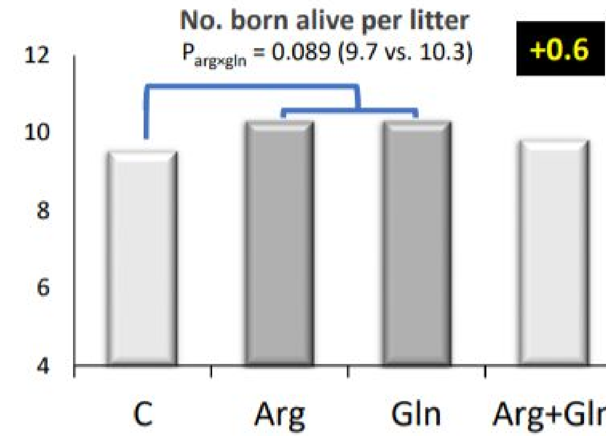
18.6 CP, 31 NDF (% DM), and 11.5 g dCP/DE

	Control 0.8 g Lys/MJ DE	**Arg** (+4 g/kg)	**Gln** (+4 g/kg)	**Arg + Gln** (+4 + 4 g/kg)
Arg/Lys	121	166	126	166
Gln/Lys	331	329	375	384

Delgado et al., 2017

Arg and Gln supplementation





Arginine: substrate for NO synthesis

Vasodilator

Regulates:

Blood flow → promoting: Nutrients, O_2 — Mother ⇅ Foetuses

Arginine supplementation (1-1.3%):

→ ↑ Litter size at birth in rats.

Survival rate at birth in sows

Mateo et al., 2007; Zeng et al., 2008

Glutamine: energy source?

→ follicular, foetal — fluids

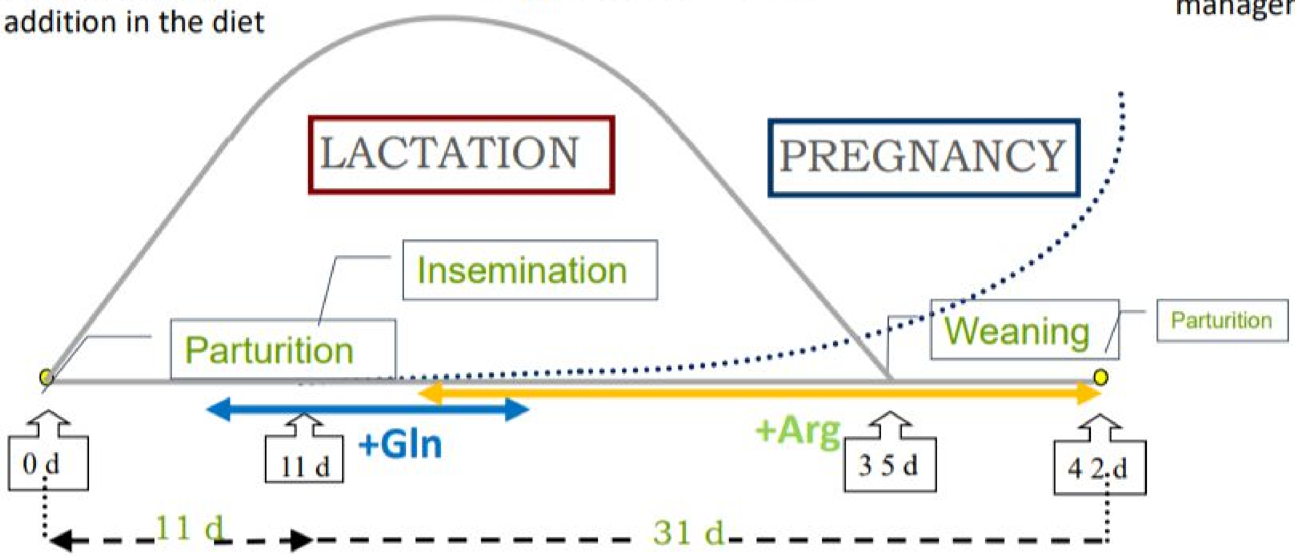
→ foetal — nutrition, growth

Delgado et al., 2017

How could the use of Gln and Arg be optimised?

Total born **+1.1** → **Born alive** **+0.6** **Weaned** ***+0.7***

- **Dose?**
  - total Arg/Lys ratio of 2.0-2.6
  - No reference for Gln/Lys
  - > 0.4 % addition in the diet
- **Period of supplementation?**
- **Who?**
  - Very good nest management



Delgado et al., 2017

Soluble fibre and n-6/n-3 ratio

16.5 CP, 31 NDF, 5 fat (% DM), 10 g dCP/MJ DE

	↓SF ↑n-6/n-3	↓SF ↓n-6/n-3	↑ SF ↑n-6/n-3	↑SF ↓n-6/n-3
Soluble fibre,	8.4	7.2	13.0	13.0
n-6/n-3	13.4/1	3.5/1	13.4/1	3.5/1

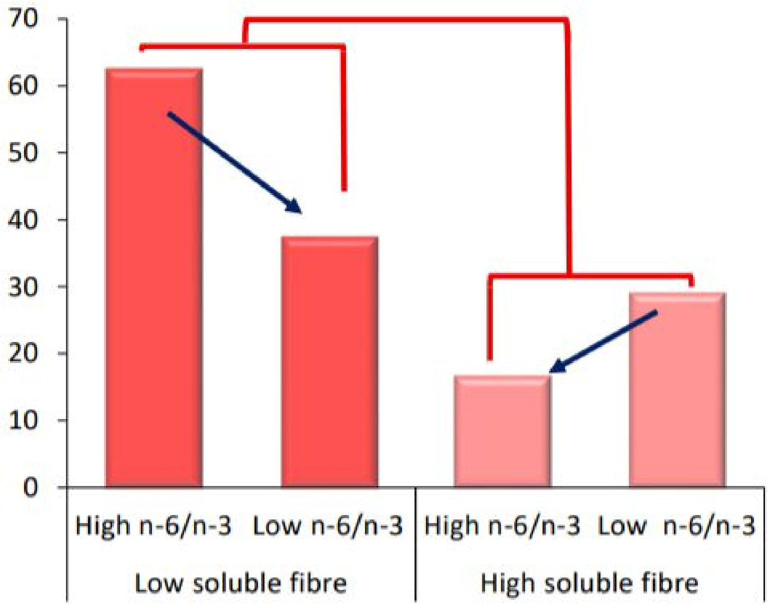
Delgado et al., 2018

Soluble fibre and n-6/n-3 ratio

Total does removed, %

$P_{soluble\ fibre} = 0.005$

$P_{soluble\ fibre \times ratio\ n\text{-}6/n\text{-}3} = 0.056$



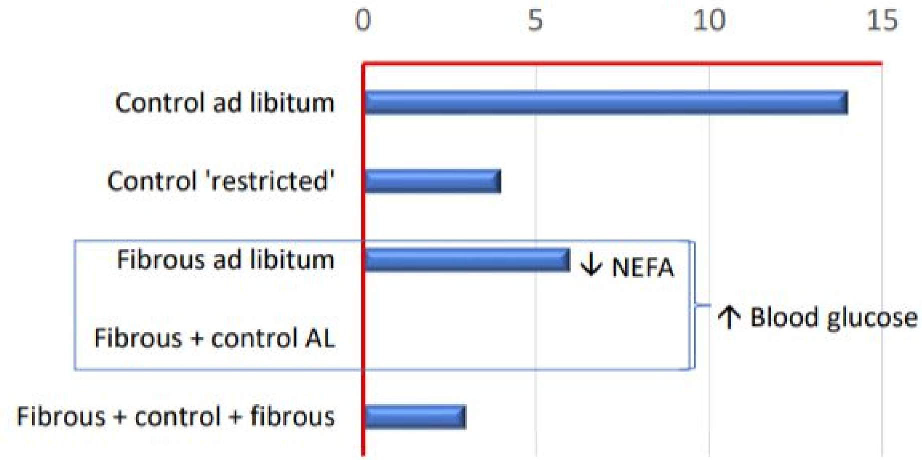
Delgado et al., 2018

Related to pregnancy toxemia?

Rearing: **Control vs. fibrous diet:**

48 NDF, ~11 soluble fibre (% DM)

Mortality at first parturition, %

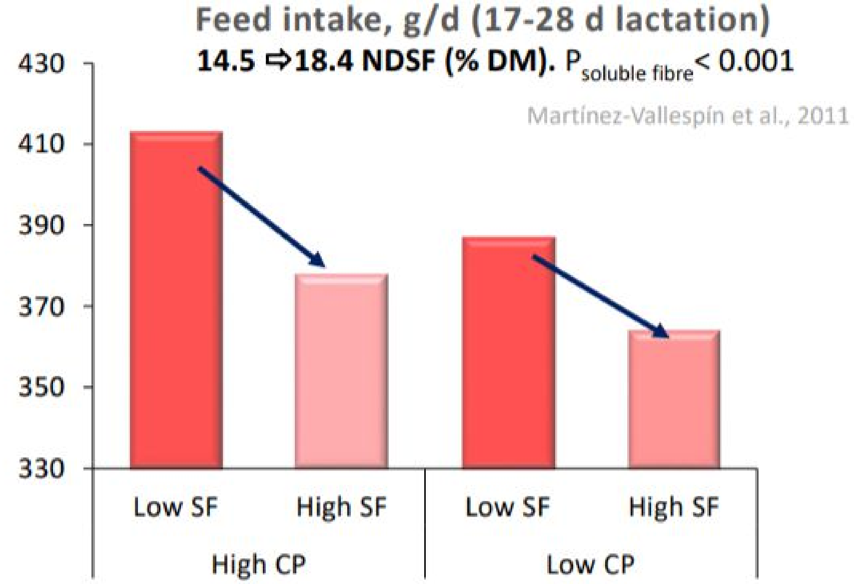


Martínez Paredes et al., 2012

Soluble fibre

⇨**Soluble fibre:**

- **Reduced 5% feed intake during lactation**
- **Reduced protein intake along the experiment**
- **No other relevant effects**



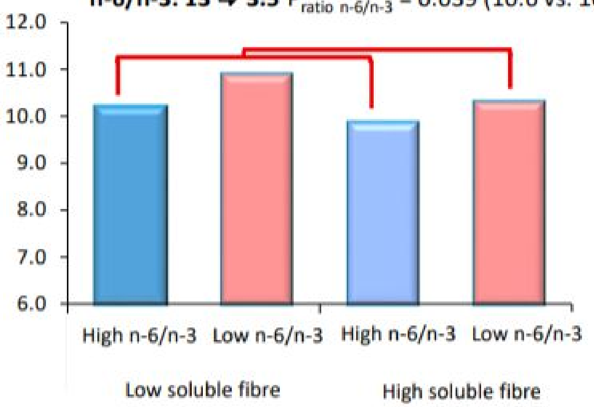
n-6/n-3 ratio

- ⇨ **Range: 4-15 in diets with no added fat**
- ⇨ **< 4 in diets enriched with n-3**
- ⇨ **Requirements ?**
- ⇨ **Litter size at birth: 6% improvement** (only 3% at weaning)

Litter size once homogenized Delgado et al., 2018

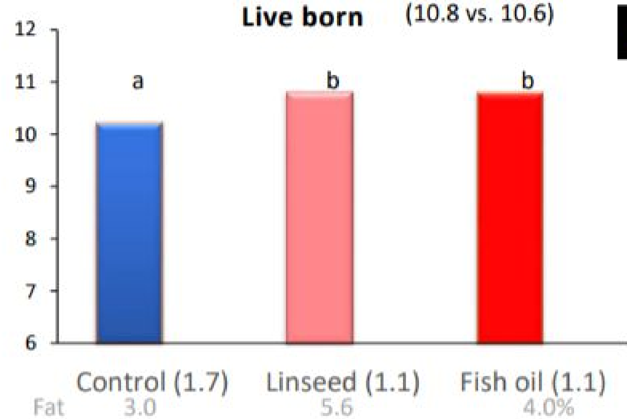
n-6/n-3: 13 ⇨ 3.5 $P_{ratio\ n\text{-}6/n\text{-}3}$ = 0.039 (10.6 vs. 10.0)

+0.6



Live born (10.8 vs. 10.6)

+0.6

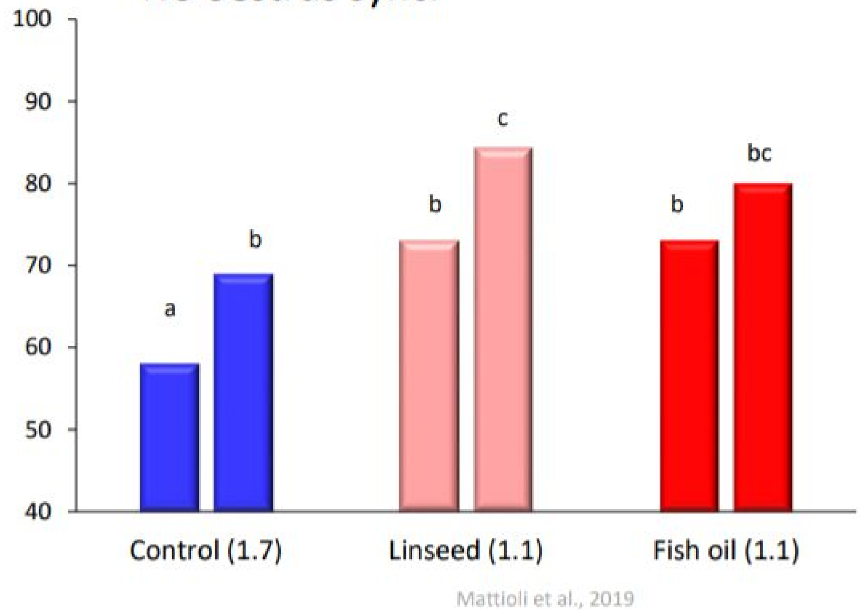


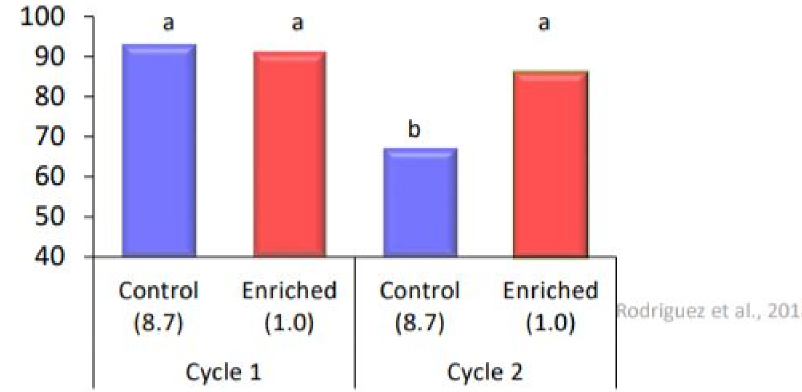
Mattioli et al., 2019

n-6/n-3 ratio

⇨ **Fertility, %**

- No oestrus sync.





⇨ **Change of FA composition**

- Milk
- Body
- Ovaries

n-6/n-3 ratio

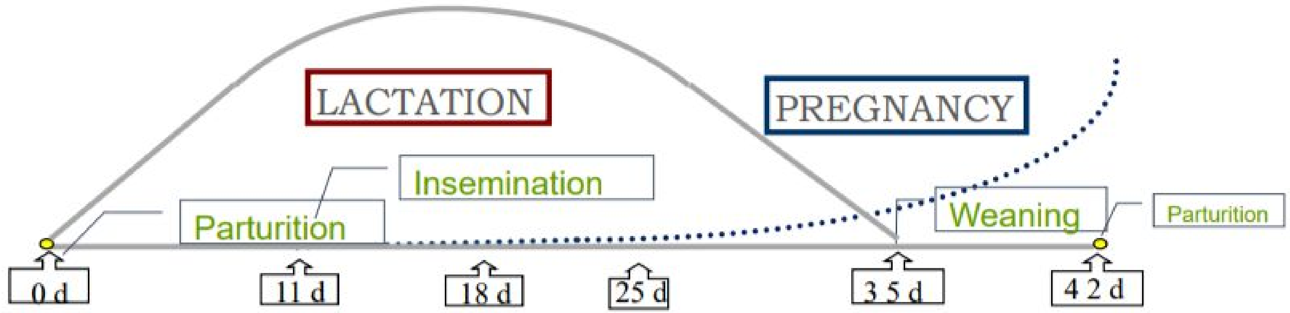
⇨ **Positive effects on fertility and litter size** (excellent nest management required)

⇨ **But effects widely variable** (Maertens et al., 2005; Menchetti et al., 2018)

- Fatty acid profile (competition between n-3 and n-6)
- Dietary antioxidant level
- Methodology differences (oestrus sync., supplementation period…)
- Interaction with microbiota

⇨ **No negative side-effects**

Different diet for the doe and the litter?



Fortune-Lamothe et al., 2005 ↓ Mortality in fattening

+NDF +fat –starch 30.5/5.5/9.5 — Does ☺

+NDF –fat --starch 32.5/3.2/9.5 — Does ☹ - fertility

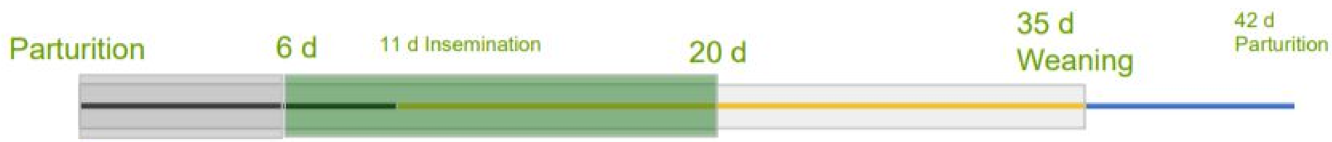
Martínez-Vallespín et al., 2011 ↓ Mortality in fattening

++NDF +SF +fat–starch --CP 50/18/6.9/3.0/14 — Does ☹ - body condition

Read et al., 2016 ↓ Mortality in fattening

+NDF –starch 41.5/7.0 9.7 g dCP/MJ ED — Does ☺

Diet and microbiota in the doe



Microbiota present in mesenteric lymph nodes of 6-d old kits → Influenced by microbiota { Milk / Hard faeces (birth canal?)

Urao et al., 1995, 1996

Influenced by diet

Birth canal

Nest material – skin around nipples

?

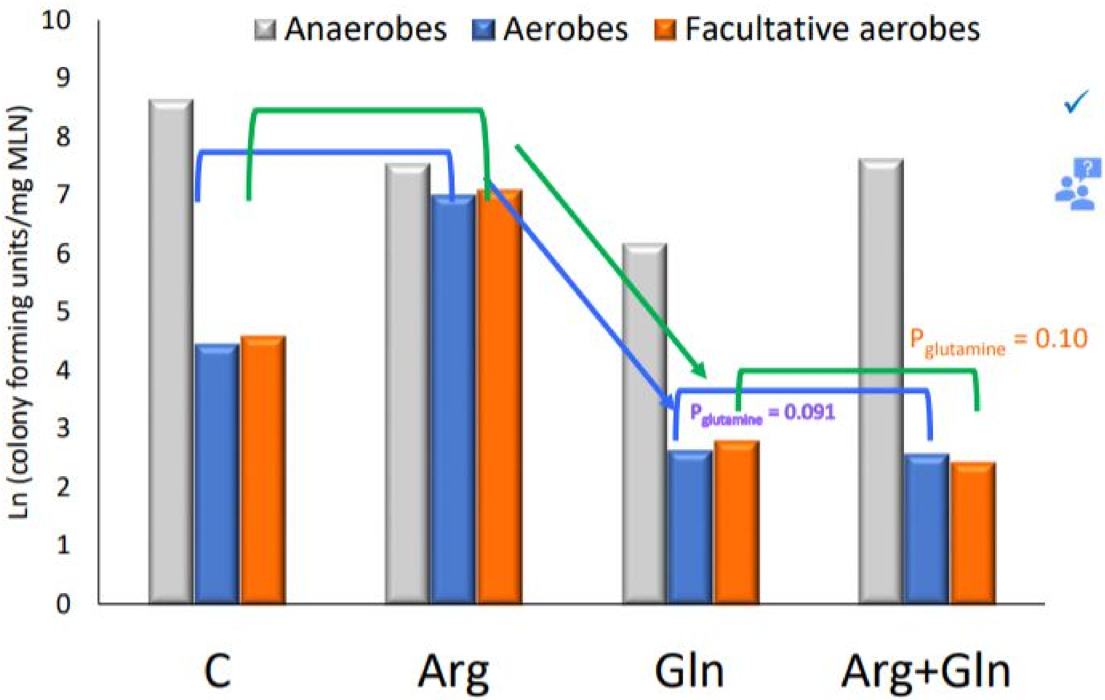
Milk

Hard faeces

Kovacs et al., 2006; Combes et al. 2014; Nicodemus et al., 2015

Diet and microbiota in the doe

- **Bacterial translocation to mensenteric lymph nodes at 6 d of age**



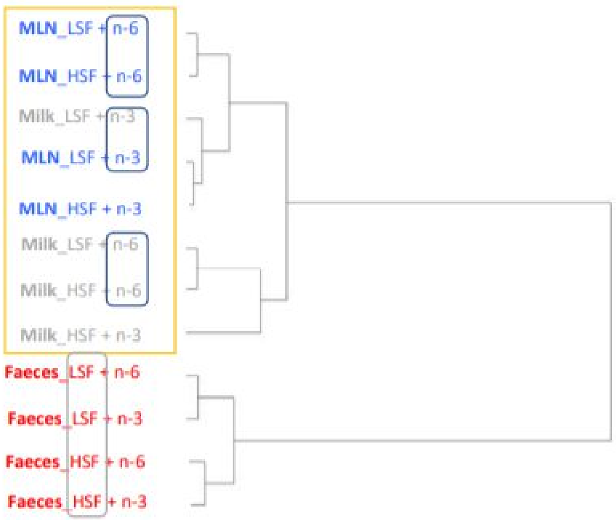
✓ Diet seems to have an influence.

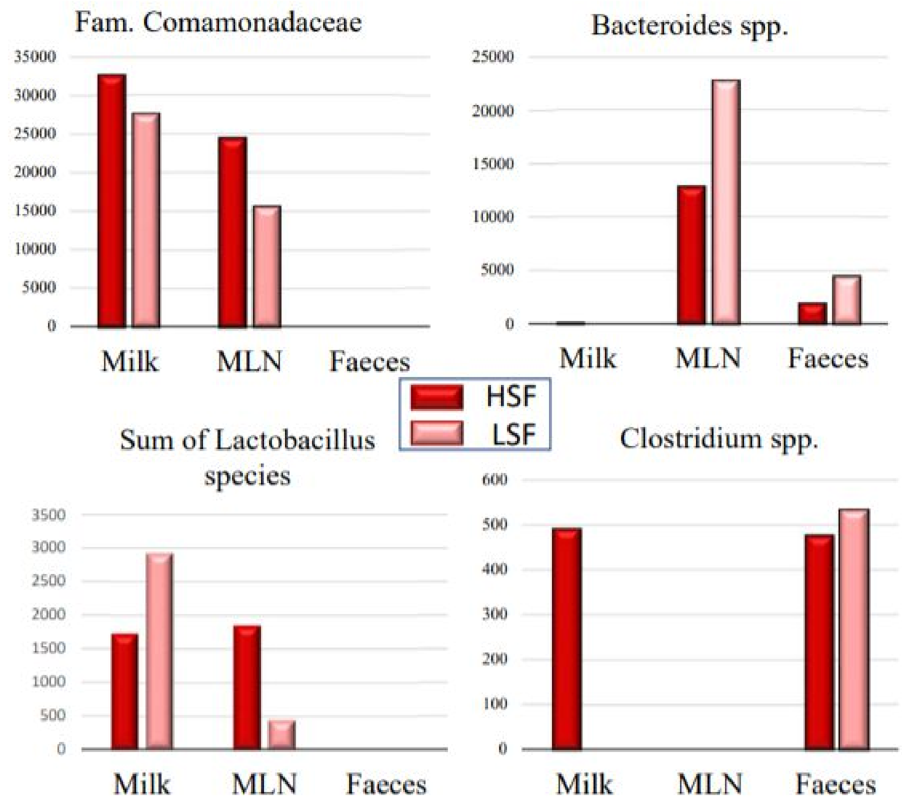
Meaning? Implications?

Delgado et al., 2019

Diet and microbiota in the doe

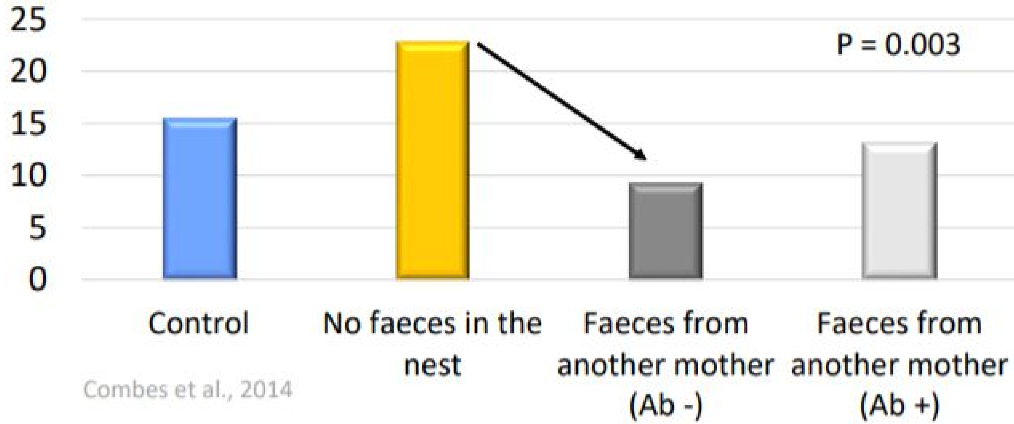
Soluble fibre and n-6/n-3 ratio





Diet and microbiota in the doe

- **Mother's hard faeces intake by the litter on its mortality from 1 to 80 d of age**



Combes et al., 2014

- Hard faecal excretion by the doe is highly variable
- Less faeces intake:
  - delayed the cecal bacterial community implantation.
  - Enhanced kit mortality.
    - No effect on mortality in other studies (Nicodemus et al., 2015)
- Microbiota inoculation: better with faeces than oral suspension (Archard et al., 2019)

Conclusions

Rearing: ⇨ **Minimal maturity at first insemination**

- 7.0 mm perirenal fat thickness, 2.8 ng leptin/mL plasma
- 18% body protein, 15-20% body fat

⇨**How?**

- Restricted feed intake from 12 wk of age to insemination
- Fibrous diet ad libitum from 60 d of age to parturition (<10.5 MJ DE/kg)

Lactating: ⇨ **Improvement of litter size**

- Increasing Arg/Lys and Gln/Lys…….level?
- Reducing n-6/n-3…….level?

⇨**Soluble fibre may reduce replacement rate**

⇨**Supply of glucose precusors before parturition**

⇨**Different diets for the doe and litter from 18 d onwards**

Future

⇨ Better definition of nutritional requirements for different physiological status.

⇨ Revision and extension of amino acid requirements

⇨ Expanding knowledge on early microbial colonization


Merci