Effect of level of soluble fiber and n-6/n-3 fatty acid ratio on performance of rabbit does and their litters[1]

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ABSTRACT: The aim of this work was to study whether the dietary supplementation with soluble fiber (SF) and the reduction of the n-6/n-3 fatty acid ratio or the combination of both influence the survival, body and milk composition, and reproductive performance of rabbit does during the first four parturitions. Four diets in a 2×2 factorial arrangement were used with two levels of SF (7.8 vs. 13.0, on DM basis; HSF and LSF) and two different n-6/n-3 fatty acid ratio (13.4/1 vs. 3.5/1). Nulliparous does (24/diet) were inseminated 11 d after parturition. Body chemical composition and energy content of rabbit does and their performance, litter growth and milk production were measured between birth and weaning (25 d) along four parturitions, and milk composition and fecal digestibility were also recorded. The proportion of total removed does decreased in HSF respect to LSF groups (22.9 vs. 50.0%; P = 0.005) and it tended to decrease in LSF groups when the n-6/n-3 ratio increased and in HSF groups when the n-6/n-3 ratio decreased (P = 0.059). The increase of the level of SF reduced the digestible CP/DE ratio (by 4%; P < 0.001) and improved the digestibility of all fibrous fractions (P < 0.001). The reduction of the n-6/n-3 ratio reduced the total dietary fiber digestibility in rabbit does fed LSF diets but it had no effect in those fed HSF diets (P = 0.043). Treatments had no effect on ADFI among parturitions (P = 0.16), but the digestible CP intake among parturitions was lower in HSF compared with LSF groups (P = 0.003). Treatments had no effect on the total number of kits born, litter or average kit weight at birth or litter size at weaning, fertility, feed efficiency, total milk production and body chemical composition and energy content of rabbit does ($P \geq$ 0.29). The average weight of kits at weaning of LSF_$\mathrm{H}_{\text{n-6/n-3}}$ and HSF_$\mathrm{L}_{\text{n-6/n-3}}$ groups decreased by 6% compared with those from the other two groups (P = 0.030). The reduction of the dietary n-6/n-3 ratio increased the milk fat content by 12% with no effect on protein and DM content (P = 0.031). The proportion of milk odd fatty acids and SFA increased in rabbit does fed the HSF diets compared with those fed LSF diets ($P \leq$ 0.037) with no effect of the n-6/n-3 fatty acid ratio. In conclusion, SF reduced the replacement rate of rabbit does with no effect of the n-6/n-3 ratio, while both dietary factors modified milk composition and fatty acid profile with minor influence on litter productivity.

Key words: body composition, milk, n-6/n-3 fatty acid ratio, performance, soluble fiber, rabbit does

INTRODUCTION

The increase of soluble fiber **(SF)** in diets for rabbits, using sugar beet pulp, improves the health status of young rabbits after weaning (Trocino et al., 2013). It exerts positive effects on gut barrier function, growth performance and reduces mortality (Gómez-Conde et al., 2007). However, the increase of SF in high insoluble fiber diets for rabbit does reduced their feed and DE intake, milk production and consequently the weight of kits at weaning (Martínez-Vallespín et al., 2011). These effects might be related to the cecal filling effect produced by the high dietary NDF level and/or the inclusion of sugar beet pulp (Carabaño et al, 1997) that might limit both feed and DE intake (García et al., 2002).

The inclusion of n-3 fatty acids might enhance animal health and productivity, however studies in rabbits are not always consistent with this relationship and there are no defined recommendations (Casado et al., 2013). The reduction of the dietary n-6/n-3 fatty acid ratio from 4.1 to 1.0 with extruded linseed oil increased milk fat content of rabbit does and reduced kit mortality after weaning (Maertens et al., 2005). Also, the reduction of n-6/n-3 ratio from 7.3 to 2.2 using salmon oil tended to increase kit weight and survival rate at birth (Rebollar et al., 2014). This situation is similar to that found with sow productivity where the main positive and consistent effect of n-3 fatty acid supplementation was to improve piglet vitality (Thange and De Smet, 2013), although a positive effect of linseed oil supplementation on sow prolificacy was recently reported (Tanghe et al., 2014). On the opposite, the supplementation of rabbit does with marine algae PUFA impaired kit weight at birth and at weaning (Mordenti et al., 2010).

The aim of the present work is to study the long-term effects of combining two levels of SF (and a low NDF level) with two n-6/n-3 fatty acid ratio on the productivity and milk composition of rabbit does and their litters along four parturitions.

MATERIALS AND METHODS

Diets

Four diets in a 2×2 factorial arrangement were used with two levels of SF (7.8 vs. 13.0, on DM basis; **LSF** and **HSF** diets) and two different n-6/n-3 fatty acid ratio (13.4/1 vs. 3.5/1; **Hn-6/n-3** and **Ln-6/n-3** diets. Table 1). The combination of n-3 fatty acids with soluble fiber was planned to look for their potential synergistic effect on the rabbit doe and litter performance and health considering they have apparently different mechanisms of action. A control diet was formulated to meet nutrient requirements of rabbit does (De Blas and Mateos, 2010) with a low SF level and a high n-6/n-3 fatty acid ratio obtained with a blend of standard and high oleic sunflower oil (**LSF_Hn-6/n-3**). The high n-6/n-3 fatty acid ratio was selected considering that in rabbit diets with low alfalfa hay content (< 20%) and no added fat this ratio is usually above 15/1 (Santomá et al., 1987). The increase of soluble/fermentable fiber was obtained by replacing wheat straw and bran from control diet by sugar beet pulp (**HSF_Hn-6/n-3**). The level of inclusion of sugar beet pulp was maximized in order to obtain the highest dietary soluble fiber content, but under the threshold of 20% to limit the potential reduction of ADFI (Carabaño et al., 1997). In these two diets the n-6/n-3 fatty acids ratio was decreased by replacing high oleic sunflower oil and part of the standard sunflower oil by linseed oil (**LSF_Ln-6/n-3** and **HSF_Ln-6/n-3**). It resulted in the replacement of oleic acid by linolenic acid, maintaining constant linoleic acid (Table 2). Levels of CP and NDF were 16.4 and 30.9 % DM, respectively, while the level of starch content was higher in LSF than in HSF diets (22.4 vs.18.3 % DM).

Lactation trial

Ninety six nulliparous 130 d old rabbit does New Zealand White × Californian (line V from Universidad Politécnica de Valencia, Spain) were randomly assigned to the four treatments (24 rabbit does/diet) one week before the first AI. Rabbit does weighted on average 3.86 ± 0.02 kg BW and their body composition and energy content was (g/kg BW mean ± standard error): 595 ± 1.50 moisture, 177 ± 0.34 protein, 157 ± 1.47 fat, 30.6 ± 0.076 ash and 11.4 ± 0.063 MJ/kg BW gross energy. After birth, does were inseminated 11 d after parturition corresponding to a theoretical kindling-to-kindling interval of 42 d and weaning was set at 25 d of lactation. Adoptions were made after parturition among rabbit does belonging to the same diet groups resulting an average litter size of 10.5 ± 0.2 kits. The does were injected i.m. 25 IU eCG (Segiran, Lab. Ovejero, León) 48 h before insemination to synchronize estrus (Rebollar et al., 2006) and 1 μg of buserelin Suprefact® (Hoechst Marison Roussel, S.A., Madrid) the day of insemination. Buserelin is a Gonadotropin-releasing hormone agonist (GnRH agonist) used to induce ovulation in rabbit does (Quintela et al., 2004). The semen came from rabbit males of the R line (Universidad Politécnica de Valencia, Spain). Rabbit does that failed to get pregnant in two consecutive inseminations were excluded from the experiment.

Experimental period begun at first successful insemination and finished at weaning of the fourth cycle of inseminations. First insemination was carried out when rabbit does were 130 d old. Live weight, feed intake, reproductive traits and body composition was recorded at insemination, just after parturition and at weaning, and only rabbit does that completed at least two lactations were considered in the analysis. The bioelectrical impedance analysis (BIA) technique was used to determine the chemical body composition and energy content of rabbit does (Pereda, 2010). Measurements of resistance and reactance were taken in rabbit does with a body composition analyzer (Model Quantum II, RJL Systems, Detroit, MI, USA) few hours after parturition, at insemination and at weaning (always after suckling), according to Romero et al. (2011). Multiple regression equations according to Nicodemus et al. (2009) were used to estimate water, protein, ash, fat and energy proportions with respect to the body weight of rabbit does. Fertility was expressed as 100/number of scheduled inseminations (every 42 days) performed until fertile kindling. Prolificacy (total number of rabbits born alive and dead) and mortality of rabbit does were also recorded. Mortality of young rabbits was controlled daily during lactation and was calculated as the percentage of rabbits dead with respect to the number of rabbits per litter once homogenized, using the litter as experimental unit. Milk production was estimated daily from weight loss of does during suckling (10 min, once a day). Litters were moved to another cage at 20 d of age. They were offered *ad libitum* the same feed as their mothers and water, and suckled once a day for 10 min until weaning age (25 d). One day after weaning, in vivo body and carcass chemical composition and energy content was estimated using the BIA technique in 40 rabbits/diet, weaned from multiparous does and weighing 467 ± 6.6 g. Measurements of resistance and reactance were taken in rabbits with a body composition analyzer (Model Quantum II, RJL Systems, Detroit, MI, USA). Multiple regression equations according to Saiz et al. (2013a, 2013b and 2017) were used to estimate water, protein, ash, fat and energy proportions both in the body and in the carcass. During the first gestation until 14 d before parturition, rabbit does were restricted to approximately 150 g/d but afterwards diets were offered *ad libitum* along the experiment. Only does that failed to get pregnant were also restricted between weaning and the next insemination.

Milk composition trial

A total of 36 rabbit does (9/diet) weighing 4.30 ± 0.05 kg and with an average litter size of 10.6 ± 0.1 rabbits, were used to determine the milk composition of DM, ash, CP, fat and fatty acids profile. Five days after the first parturition, the area of the proximal mammary glands were shaved, disinfected with ethanol right after 0.3 ml oxitocin (Oxiton, Laboratorios Ovejero, León, Spain) were injected i.v. in the ear and at least 8 ml of milk were collected in sterile tubes (scrapping the first jets). Samples were freeze-dried and stored frozen to determine milk composition.

Fecal digestibility trial

A total of 24 rabbit does (6/diet) weighing 4.04 ± 0.04 kg and with an average litter size of 10.2 ± 0.3 kits were used to determine the fecal apparent digestibility of DM, GE, CP, ether extract, total dietary fiber, NDF and SF. Total fecal output was collected for each doe during four consecutive days (from day 15 to 19 of the third lactation). Feed intake (*ad libitum* access) was recorded during this period. Feces were stored at -20 ºC and later dried at 80ºC for 48 h and ground to 1 mm for further analysis.

Housing

Rabbit does were housed individually in flat-deck cages (600 × 500 × 320 mm) throughout the trial. An external nest box (355 × 230 × 360 mm high) with wood shavings was provided three days before parturition. Housing conditions were controlled during the whole experimental period with a 16-h light: 8-h dark cycle and temperature conditions maintained between 18 and 24°C. All the experimental procedures used were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid, and were in compliance with the Spanish guidelines for care and use of animals in research (BOE, 2013).

Chemical analysis

Procedures of the AOAC (2000) were used to determine DM (method 934.01), ash (method 942.05), CP (method 968.06), ether extract (920.39), starch (amyloglucosidase-α-amylase method; method 996.11), and total dietary fiber (985.29). Sugars were analyzed according to Yemm and Willis (1954). Dietary NDF was determined using the filter bag system (Ankom Technology, New York) according to Mertens et al. (2002), and a thermo-stable amylase without any sodium sulphite added. It was corrected for its own ash and protein as indicated for total dietary fiber. Dietary ADF and ADL were analyzed according to Goering and Van Soest (1970). The soluble fiber was calculated by difference as TDF–NDF. Gross energy was determined by adiabatic calorimetry. Fatty acids were extracted and quantified by the one-step procedure as described by Sukhija and Palmquist (1988) in experimental diets. Total lipids were extracted from milk samples by the method of Segura and López-Bote (2014). Previously, lyophilized samples (300 mg) were homogenized in dichloromethane: methanol (8:2, by vol.) using a mixer mill (MM400, Retsch technology). The final biphasic system was separated by centrifugation. The extraction was repeated three times. Solvent was evaporated under nitrogen stream and lipids were dried by vacuum desiccation. Total lipid content was determined gravimetrically. Fatty acid methyl esters were prepared from total lipids by transesterification using a mixture of methanol: toluene: H_2SO_4 (88:10:2, by vol.). Samples were heated for 2 h at 80 °C as in Ruiz-López et al. (2015). After cooling, fatty acid methyl esters were recovered with hexane and separated and quantified by GC-FID. Fatty acid methyl esters were separated using a gas chromatograph (HP 6890 Series GC System) equipped with flame ionization detector. Separation was performed with a J&W GC Column, HP-Innowax Polyethylene Glycol (30 m × 0.316 mm × 0.25 µm). Nitrogen was used as a carrier gas. After injection at 170 °C, the oven temperature was raised to 210 °C at a rate 3.5 °C/min, then to 250 °C at a rate of 7 °C/min and held constant for 1 min. The flame ionization was held at 250 °C. Fatty acid methyl ester peaks were identified by comparing their retention times with those of authentic standards (Sigma–Aldrich, Alcobendas, Spain). Diets were analyzed in triplicate, and milk in duplicate.

Statistical analysis

The results obtained for fecal digestibility (DM, GE, CP, total dietary fiber, NDF, and SF) and milk composition (DM, ash, CP, fat, and fatty acid profile) were analyzed using a mixed model considering the level of SF, n-6/n-3 ratio and their interaction as the main sources of variation. Data from total removed, dead and culled does were analyzed using a logistic regression (GENMOD procedure of SAS, considering a binomial distribution) including the same variables in the model, and the results were transformed from the logit scale. Data from the lactation and body composition trial were analyzed in a factorial arrangement repeated for four parturitions by the mixed procedure of SAS (Littell et al., 1996) including in the model the level of SF, n-6/n-3 ratio, parity order and their interactions, and the rabbit doe as a random effect. The number of kits in each period was used as covariate in the model for milk production. Initial BW, body composition (protein, fat, moisture, ash) and energy content at 130 d of age were used as covariates in the model used for body composition and energy content along time. A compound symmetry structure was fitted as it showed the lowest value of the Schwarz Bayesian criterion (Littell et al., 1998). It assumes that measures over time on the same animals had the same variance and that all pairs of measures on the same animal had the same correlation. The data are presented as least-squares means. When interactions were significant ($P < 0.05$) comparisons among all the treatment means were made using a t-test.

RESULTS

Dry matter intake during the digestibility period was higher ($P = 0.038$) in rabbit does fed $L_{n\text{-}6/n\text{-}3}$ diets than in $H_{n\text{-}6/n\text{-}3}$ groups (Table 3). Treatments did not affect fecal DM and GE digestibility, but CP digestibility tended to decrease in HSF compared with LSF groups ($P = 0.072$; Table 3) and led to a reduction of the digestible CP/DE ratio (by 4%; $P < 0.001$). Fecal digestibility of total dietary fiber, NDF and SF increased in rabbit does fed HSF diets respect to does fed LSF diets (by 17, 12 and 22 percentage units, respectively; $P < 0.001$). The reduction of the n-6/n-3 ratio reduced the total dietary fiber digestibility in rabbit does fed LSF diets but it had no effect in those fed HSF diets, resulting in an interaction SF × n-6/n-3 ratio ($P = 0.043$). A similar trend was observed for NDF digestibility ($P = 0.091$). Fecal digestibility of ether extract tended to be higher in does fed LSF_Hn-6/n-3 and HSF_$L_{n\text{-}6/n\text{-}3}$ diets ($P = 0.092$).

Feed intake during the first gestation and during the lactation period was lower in rabbit does fed with HSF compared with those fed LSF diets (by 10 and 6%, respectively; $P \leq 0.047$. Table 4). However, treatments had no effect on ADFI among parturitions ($P = 0.16$). It resulted in a minor effect on DE intake along the experiment (only a trend to reduce it in the first gestation in HSF groups), but in a reduction of digestible CP intake among parturitions (by 7%) and throughout the experiment in does fed HSF diets ($P \leq 0.005$, and $P = 0.079$ for the weaning-parturition period). The dietary n-6/n-3 ratio had a minor influence on ADFI, DE and digestible CP intake. Feed intake from the third weaning to the fourth parturition tended to decrease in $L_{n\text{-}6/n\text{-}3}$ groups, leading to a reduction of DE and digestible CP intake in this period (interaction n-6/n-3 ratio × parity; $P \leq 0.054$; data not shown). On the opposite, ADFI, DE and digestible CP intake tended to increase in $L_{n\text{-}6/n\text{-}3}$ groups at the beginning of lactation (parturition-insemination) from the second to the third and fourth parturitions ($P \leq 0.081$; data not shown).

Treatments had no a relevant effect on total number of kits born (either alive or dead), litter or average kit weight at birth (Table 5). Once homogenized litter size the does fed $L_{n\text{-}6/n\text{-}3}$ diets showed a higher initial number of kits (+0.5; $P = 0.039$) due to the artificial reduction of the standard error, whereas those fed HSF diets tended to reduce it (P = 0.091). Rabbit does from LSF_H$_{n\text{-}6/n\text{-}3}$ and HSF_L$_{n\text{-}6/n\text{-}3}$ tended to reduce gradually kit mortality during lactation from the first to the fourth lactation (from 14 to 0%; Figure 1), whereas those fed LSF_L$_{n\text{-}6/n\text{-}3}$ and HSF_H$_{n\text{-}6/n\text{-}3}$ showed a constant mortality along lactations (10.5% on average), resulting in an interaction SF × n-6/n-3 ratio × parity (P = 0.007). However, treatments had no effect on litter size at weaning ($P \geq 0.29$). The average weight of kits at weaning from LSF_H$_{n\text{-}6/n\text{-}3}$ and HSF_L$_{n\text{-}6/n\text{-}3}$ groups decreased by 6% compared with those from the other two groups (P = 0.030). Furthermore, only in the fourth lactation kit weight at 20 and 25 d and litter feed intake from 20 to 25 d was higher in LSF compared with HSF groups (data not shown; $P \leq 0.047$). Feed efficiency of rabbit does did not differ among different experimental groups ($P \geq 0.11$).

The weight of rabbit does at parturition and at were not modified by the experimental diets, but those fed L$_{n\text{-}6/n\text{-}3}$ diets tended to be heavier than those from H$_{n\text{-}6/n\text{-}3}$ group the day of insemination (P = 0.10. Table 6). Fertility and number of parturitions averaged 94.4% and 3.5, respectively, and dietary treatments did not influence them ($P \geq 0.11$). The increase of dietary SF tended to reduce the milk production from 10 to 20 d of lactation (P = 0.089), but had no effect on total milk production (P = 0.12). The body chemical composition and body energy content of rabbit does at parturition, AI and weaning were not affected by the treatments (Table 7). Only at weaning body protein decreased from the first to the fourth weaning in rabbit does from all treatments but in those from LSF_H$_{n\text{-}6/n\text{-}3}$ group it remained constant, leading to an interaction SF × n-6/n-3 fatty acid ratio × parity (P = 0.049. Data not shown). The proportion of total removed does decreased in HSF respect to LSF groups (22.9 vs. 50.0%; P = 0.005), due to the trend to reduce the culled and dead rabbit does ($P \leq 0.059$. Table 8). The proportion of total removed does tended to decrease in LSF groups when the n-6/n-3 ratio increased and in HSF diets when the n-6/n-3 ratio decreased (P = 0.059). The causes of mortality were reproductive (mainly abortions), digestive troubles and other causes that could not be established.

The milk DM and CP contents did not differ among rabbit does fed different dietary treatments ($P \geq 0.13$. Table 9). The reduction of the dietary n-6/n-3 ratio increased the milk fat content by 12% ($P = 0.031$), with no effect of the level of SF. The proportion of milk odd fatty acids and SFA increased in rabbit does fed the HSF diets compared with those fed LSF diets (by 11 and 3%, respectively; $P \leq 0.037$), whereas the proportion of short and medium chain fatty acids tended also to increase in HSF groups ($P = 0.080$). The sum of all milk odd-chain fatty acids tended to be positively correlated with the fecal digestible total dietary fiber (r = 0.91; P = 0.089), but it was not observed for the proportion of milk short and medium chain fatty acids (r = 0.89; $P = 0.11$). The dietary n-6/n-3 fatty acid ratio had no effect on these proportions.

However, the reduction of the dietary n-6/n-3 ratio reduced the milk C18:1n9 (by 19%; $P < 0.001$) and increased the C18:3n3 proportion (by 306%; $P < 0.001$). The C18:1n9 and C18:3n3 milk concentrations were closely correlated to their concentrations in the diet ($r = 0.95$ and $P = 0.046$ for C18:1n9; $r = 0.99$ and $P = 0.002$ for C18:3n3). Dietary linolenic acid also was positively correlated with milk C18:4n3, C20:5n3 and C22:1n9 contents ($r \geq 0.98$; $P \leq 0.022$). The milk C18:2n6 proportion decreased by 4% in rabbit does fed HSF diets ($P = 0.025$) and increased by 4% in does fed $L_{n\text{-}6/n\text{-}3}$ diets ($P = 0.022$), but it did not correlate with the dietary concentration of C18:2n6. The reduction of the n-6/n-3 ratio in the diet increased the milk concentrations of C18:4n3 (by 710%), C20:3n3 (by 122%), C20:4n3 (by 22%) and C20:5n3 (by 105%) ($P \leq 0.027$) and decreased C20:1n9 concentration ($P = 0.010$). These changes were reflected in a reduction of the milk n-6/n-3 ratio and an increase of PUFA when the dietary n-6/n-3 ratio decreased ($P < 0.001$), with no effect of the level of SF. These changes in milk composition had minor effects on the body and carcass composition of weaned rabbits (Table 10). The reduction of the n-6/n-3 ratio slightly increased the body protein and moisture but decreased the carcass protein proportion, while the level of SF reduced minimally the body energy content.

The weight of rabbit does at parturition, their ADFI, DE and digestible CP intake, and milk production increased progressively from the first until the fourth parturition (Tables 4 and 6; $P < 0.001$). Fertility rate was higher in the first and fourth parturition compared with the second and third ones ($P < 0.001$). Total number kits born per litter and at weaning increased between the first and the second parturition, decreasing from the second to the fourth parturition (Table 5; $P < 0.008$). Weight of litter at birth and at weaning increased in the second parturition respect to the first parturition and remained constant onwards ($P < 0.001$). However, average weight of kits at birth and at weaning increased from the first to the fourth parturition ($P < 0.001$). Feed efficiency increased in the second and third parturition respect to the first parturition ($P < 0.001$).

Body protein proportion at parturition increased in multiparous does compared with nulliparous does ($P < 0.001$. Table 7), whereas no change was found for the fat content. At first insemination rabbit does had a lower body protein and higher body fat content than multiparous does ($P < 0.001$), although body protein decreased at fourth parturition. At weaning the body protein remained constant until the fourth parturition where it decreased, while body fat content increased successively from the first to the fourth parturition ($P < 0.001$). The temporal evolution with the productive/reproductive events (five reproductive cycles) of body composition, energy content and weight of all the rabbit does considered in this study is showed in Fig 2.A (time effect: $P < 0.001$ for all traits). In Fig 2.B is represented this evolution but only for rabbit does that had five successful and consecutive inseminations (n = 18). These highly productive rabbit does did not differ from the whole group in the initial BW, protein, fat and energy content ($P \geq 0.26$. Figure 2A and 2B). Highly productive primiparous does tended to mobilize a higher proportion of fat from the second insemination until the first weaning compared with the standard does ($P = 0.10$).

DISCUSSION

Dietary SF has demonstrated to reduce the mortality rate of young rabbits after weaning affected by epizootic rabbit enteropathy (Martínez-Vallespín et al., 2011; Trocino et al., 2013). The soluble fiber exerts positive effects on the jejunal mucosa and on the ileal starch digestibility in young rabbits (Gómez-Conde et al., 2007). It also increases the ileal flow of mucins in adult rabbits (Abad-Guamán et al., 2015), which might indicate a better gut barrier function in the small intestine. In this study, the increase of the level of SF also reduced the proportion of the rabbit does removed as it tended to decrease the mortality and the culling rate. The higher fecal NDF and SF digestibility in rabbit does fed HSF diets might promote a change in the intestinal microbiota profile. In fact, Delgado et al. (2015) found that the fecal microbiota of these rabbit does differed according to the dietary SF content. If changes in the intestinal microbiome because of a higher amount of fermented fiber are behind the positive effects on rabbit does health deserves future attention.

Despite the fact that the level of sugar beet pulp inclusion was not high the increase of SF affected ADFI negatively during the first pregnancy and along the lactation period where ADFI is maximal. This might be explained by the accumulation of digesta in the cecum when sugar beet pulp is included substituting other sources of fiber (Carabaño et al., 1997; Falcao-e-Cunha et al., 2004; Gómez-Conde et al., 2009) reducing the rate of passage of the digesta through the gut (Gidenne et al., 1987). The reduction of ADFI in HSF groups did not affect DE intake, due to the increase of fiber digestibility, but reduced the digestible CP intake throughout the experiment. This effect was also due to the negative effect on CP digestibility of CP-NDF content in HSF diets. However, the performance of rabbit does fed HSF diets did not impair (milk production, litter size and weight at weaning, or body condition) although the initial litter size at the beginning of lactation (once homogenized) tended to be lower. In contrast, the substitution of sugar beet pulp for wheat in highly fibrous diets decreased ADFI, CP and DE intake in rabbit does leading to a reduction of milk production and litter weight at weaning (Martínez-Vallespín et al, 2011).

The reduction of the dietary n-6/n-3 fatty acid ratio did not influence the proportion of removed rabbit does and their performance (ADFI, DE and digestible CP intake, milk production, fertility, litter size and weight at birth and at weaning, and body condition). It indicates that in this study there was no relevant and practical effect of n-6/n-3 ratio on fetal development during pregnancy (prolificacy o average kit weight at birth) or fertility, although in the latter the effect might be masked by the protocol used for estrus synchronization and ovulation induction that rendered high fertility values. Only, the number of rabbits per litter at the beginning of the lactation increased, once homogenized the litters, due to the artificial reduction of the variability of this trait. These results might suggest a potential minor effect of the reduction of this ratio on rabbit prolificacy, similar to that reported in sows (Tanghe et al., 2014), although it should be confirmed using a higher number of rabbit does. In contrast, when the reduction of the n-6/n-3 ratio was obtained by the supplementation of fish oil a punctual reduction of kit mortality and increase of kit size at birth was found (Rebollar et al., 2014; Rodríguez et al., 2017), which might suggest an influence of the type PUFA profile on these traits. These studies (Rebollar et al., 2014; Tanghe et al., 2014; Rodríguez et al., 2017) used a higher dose dietary vitamin E and other antioxidants than those used in this work, which might have enhanced the effects of the n-3 fatty acids, although this effect would require confirmation.

The level of SF and the n-6/n-3 fatty acid ratio interacted and influenced the litter performance during lactation. The ADG of kits during lactation tended to decrease with the reduction of n-6/n-3 ratio in the HSF groups, whereas no effect was found in LSF groups. By contrast, the mortality during lactation of litters from LSF_H$_{n\text{-}6/n\text{-}3}$ and HSF_ L$_{n\text{-}6/n\text{-}3}$ groups was reduced along the successive lactations compared with the other two groups. There is no clear explanation for these effects, and they do not seem to be related to differences in milk production and/or composition, ADFI before weaning or body composition at weaning. Besides, in the literature exists some discrepancy regarding the effects of milk fatty acid profile on suckling rabbits survival and performance (Pascual et al. 1999). While for some authors fatty acids such as C8:0 and C10:0 exert a positive effect on pup survival and growth others claim this effect for C18:2 and C18:3 (Pascual et al. 1999). In addition, it is important to take into account the microbial environment (i.e. fecal and milk microbial profile of rabbit does) on the pup health and performance (Delgado et al., 2015). In fact, the gut microbiota seem to play an important role in the interaction between dietary fermentable carbohydrates (fructooligosaccharides) and n-3 PUFA on growth rate or cecal weight in adult mice (Pachikian et al., 2011). Moreover, both SF level and the n-6/n-3 ratio of rabbit does diets affected the microbial profile observed in the mesenteric lymph nodes of 5-d old kits (Delgado et al., 2015). Therefore, we can´t exclude the potential effect of the experimental diets on the microbial environment and hence on the suckling rabbits health.

Both dietary factors, level of SF and n-6/n-3 fatty acid ratio, significantly changed the fatty acid profile of milk. However, the observed changes on fatty acid profile did not affected litter performance and survival as already commented. As expected, the dietary n-6/n-3 ratio modified the major fatty acids with 18 carbon atoms or more, having a minor influence the SF on this fraction. It has been previously reported the direct relationship between the fatty acid profile of the dietary fat source and the milk fatty acid composition in rabbit does (Pascual et al. 1999). Therefore higher C18:1n-9 and C18:3n-3 concentrations were observed in the milk of rabbit does fed the Hn-6/n-3 and Ln-6/n-3 respectively. Besides, the increase of dietary C18:3n3 enhanced the milk C20:5n3 (EPA) content, confirming its capacity to elongate this fatty acid, although no DHA was detected. In this regard, milk C20:4n6 was the predominant fatty acid among those of C20 group, with no effect of treatments due to their similar dietary C18:2n6 content. It might be due to the much higher dietary content of C18:2n6 than C18:2n3, and a potential preference for C18:2n6 than for C18:3n3 desaturation. The latter result was already reported for fattening rabbits from a fast-growing strain (Castellini et al., 2016), and might partially account for the limited effect of the dietary n-6/n-3 reduction on the rabbit does performance observed in this study. It was also noticeable the effect of dietary SF on C18:2n-6 concentration which decreased in the milk of does fed the high SF diets. The higher PUFA content in the Ln-6/n-3 diets might account for the higher milk fat content in these groups. This is in agreement with Pascual et al. (1999) and Maertens et al. (2005), and the result of a higher efficiency of long chain PUFA incorporation from blood to milk as suggested by Pascual et al. (1999). However, other authors obtained different results (Volek et al., 2014) probably because the influence of other factors (like the level of added fat).

Rabbit does fed HSF diets increased the proportion of milk odd-chain fatty acids that constitutes a minor proportion of milk fat, with no effect of the n-6/n-3 ratio. Odd-chain fatty acids are synthetized by the intestinal microbiota through the elongation of propionate and valerate, minor volatile fatty acids derived from microbial fermentation in rabbits, and in ruminants are used as a biomarker of ruminal function (Vlaeminck et al., 2006). In this study, the sum of all milk odd-chain fatty acids tended to be positively correlated with the fecal digestible total dietary fiber, that is another indicator of microbial activity although it would not represent the whole organic matter fermented (Abad-Guamán et al., 2015). These fatty acids would be transferred to milk through the digestion of microbial fat recycled through cecotrophy or by the direct transference of intestinal bacteria to the milk (Donnet-Hughes et al., 2010; Delgado et al., 2015). The increase of SF also tended to increase the proportion of milk short and medium chain fatty acids, but it was not clearly correlated with digestible total dietary fiber, and with no influence of the n-6/n-3 ratio. This might be explained because short and medium fatty acids are mainly synthetized from glucose and acetate (Jones and Parker, 1981), and HSF diets supplied a higher digestible fiber content (and probably of acetic) but lower starch content.

In conclusion, SF reduced the replacement rate of rabbit does with no effect of the n-6/n-3 ratio, while both dietary factors modified milk composition and fatty acid profile with minor influence on litter productivity.

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Table 1. Ingredient and chemical composition of experimental diets.

	Low soluble fiber		High soluble fiber	
Item	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_ Hn-6/n-3	HSF_ Ln-6/n-3
Ingredient, % as fed basis				
Wheat bran	28.0	28.0	13.0	13.0
Wheat straw	10.0	10.0	5.00	5.00
Beet pulp	0.00	0.00	18.0	18.0
Wheat	22.7	22.7	21.7	21.7
Dehydrated alfalfa	15.0	15.0	15.0	15.0
Soybean meal	8.00	8.00	8.00	8.00
Sunflower meal	9.97	9.97	12.97	12.97
High oleic sunflower oil	0.85	0.00	0.85	0.00
Standard sunflower oil	2.15	2.00	2.15	2.00
Linseed oil	0.00	1.00	0.00	1.00
L-lysine HCl	0.44	0.44	0.44	0.44
DL-methionine	0.08	0.08	0.06	0.06
L-threonine	0.31	0.31	0.32	0.32
Calcium carbonate	1.20	1.20	0.70	0.70
Sodium chloride	0.30	0.30	0.31	0.31
Calcium phosphate	0.50	0.50	1.00	1.00
Mineral/vitamin premix[1]	0.50	0.50	0.50	0.50
Analyzed chemical composition, % DM				
DM	90.8	90.6	90.8	91.0
Ash	7.08	7.29	6.75	6.72
CP	16.7	16.4	16.5	16.5
CP-Total dietary fiber	9.29	9.87	9.65	9.78
CP-NDF	5.83	5.70	7.86	7.86
Total dietary fiber	39.1	38.0	44.2	43.8
NDF	30.7	30.9	31.2	30.8
ADF	16.5	16.7	18.5	18.7
ADL	3.10	3.10	3.30	3.40
Soluble fiber	8.40	7.19	13.0	13.0
Starch	22.6	22.2	18.2	18.4
Ether extract	5.38	4.91	4.87	5.00
Sugars	7.99	8.23	8.17	8.44
GE, MJ/kg MS	18.4	18.2	18.3	18.1

[1] Provided by Trouw Nutrition (Madrid, Spain). Mineral and vitamin composition (per kg of complete diet): 20 mg of Mn as MnO; 59.2 mg of Zn as ZnO; 10 mg of Cu as $CuSO_4$ $5H_2O$; 1.25 mg of I as KI; 0.495 mg of Co as $CoCO_3$ H_2O H_2O; 76 mg of Fe as $FeCO_3$; 8375 UI of vitamin A; 750 UI of vitamin D_3; 20 UI of vitamin E as DL-α-tocopherol acetate; 1.0 mg of vitamin K; 1.0 mg of vitamin B1; 2 mg of vitamin B2; 1 mg of vitamin B6; 20 mg of Niacin; 54.1 mg of Betaine; 137.5 mg of Choline chloride; 66 mg of robenidine; 50 mg of ethoxyquin.

Table 2. Fatty acid profile of experimental diets (g/100 g of total fatty acids).

	Low soluble fiber		High soluble fiber	
Item	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_Hn-6/n-3	HSF_Ln-6/n-3
C14:0	0.22	0.27	0.26	0.35
C15:0	0.09	0.09	0.11	0.14
C16:0	11.0	11.6	10.7	11.4
C16:1n7	0.19	0.15	0.16	0.16
C16:1n9	0.18	0.21	0.15	0.16
C18:0	2.98	3.20	3.17	3.33
C18:1n9	30.3	20.1	31.4	20.1
C18:1n7	0.66	0.70	0.67	0.75
C18:2n6	48.9	48.0	47.2	46.8
C18:3n3	2.85	12.4	2.70	13.3
C18:3n6	0.07	0.07	0.09	0.10
C18:4n3	0.10	0.08	0.12	0.11
C20:0	0.41	0.41	0.45	0.46
C20:1n9	0.41	0.45	0.39	0.36
C20:3n6	0.00	0.15	0.14	0.18
C20:5n3	0.00	0.13	0.13	0.18
C22:1n9	0.78	0.60	0.81	0.70
Unidentified	0.78	1.36	1.34	1.34
SFA	14.7	15.6	14.7	15.7
MUFA	32.5	22.2	33.6	22.3
PUFA	51.9	60.8	50.4	60.7
n-6	49.0	48.2	47.4	47.1
n-3	3.4	13.0	3.8	14.0
n-6/n-3	14.4	3.71	12.5	3.36

Table 3. Effect of level of soluble fiber and n-6/n-3 fatty acids profile on DMI and fecal apparent digestibility in rabbit does.

	Diets				SEM		*P*-value		
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_Hn-6/n-3	HSF_Ln-6/n-3	SF and n-6/n-3	SF×n-6/n-3	SF	n-6/n-3	SF×n-6/n-3
N	8	8	8	8					
DMI, g DM/d	318	368	319	340	11.5	16.3	0.43	0.038	0.37
Fecal apparent digestibility, %									
DM	62.3	61.4	62.6	64.0	0.85	1.21	0.23	0.83	0.34
GE	62.7	60.7	62.4	63.3	0.86	1.21	0.34	0.64	0.25
CP	69.5	69.2	65.9	66.6	1.17	1.65	0.072	0.90	0.76
Ether extract	73.2	70.3	71.2	74.2	1.17	1.66	0.57	0.98	0.092
Total dietary fiber	29.0[b]	23.2[a]	42.3[c]	43.8[c]	1.21	1.74	<0.001	0.22	0.043
NDF	24.3	20.0	33.1	35.4	1.31	1.85	<0.001	0.60	0.091
Soluble fiber	46.4	36.8	64.4	63.8	2.30	2.49	<0.001	0.13	0.18
DE, MJ/kg DM	11.5	11.0	11.3	11.4	0.16	0.22	0.69	0.26	0.19
Digestible CP, % DM	11.7	11.2	11.0	11.0	0.19	0.28	0.11	0.51	0.37
Digestible CP/DE, g/MJ	10.1	10.2	9.70	9.71	0.09	0.12	<0.001	0.61	0.69

[a-c] Diet mean values in the same row with a different superscript differ, P < 0.05

Table 4. Effect of level of soluble fiber and n-6/n-3 fatty acids profile and parity order on DE and digestible CP intake of rabbit does[1].

	Diets				Parity order				SEM				*P*-value					
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_ Hn-6/n-3	HSF_ Ln-6/n-3	1	2	3	4	SF and n-6/n-3	SF× n-6/n-3	Parity	SF ×Parity and n-6/n-3×Parity	SF	n-6/ n-3	SF× n-6/n-3	Parity	SF ×Parity	n-6/n-3× Parity
N	15	18	22	20	75	75	62	40										
ADFI, g/d																		
AI 1-Parturition 1	147	139	131	126	–	–	–	–	4.99	7.06	–	–	0.042	0.38	0.80	–	–	–
Parturition-AI	292	310	289	279	232^{a}	300^{b}	314^{bc}	324^{c}	5.57	7.85	6.49	9.13	0.034	0.60	0.069	<0.001	0.60	0.081
Lactation	340	347	327	324	297^{a}	335^{b}	346^{bc}	361^{c}	6.19	8.73	6.57	9.27	0.047	0.81	0.55	<0.001	0.37	0.90
Weaning-Parturition	200	196	200	190	180^{a}	198^{b}	211^{b}	–	4.42	6.22	4.66	6.56	0.60	0.23	0.65	<0.001	0.89	0.051
Among parturitions	270	276	268	260	239^{a}	273^{b}	294^{c}	–	4.52	6.37	4.76	6.71	0.16	0.83	0.28	<0.001	0.67	0.76
DE intake, MJ/d																		
AI 1-Parturition 1	1.57	1.42	1.40	1.33	–	–	–	–	0.05	0.07	–	–	0.074	0.14	0.58	–	–	–
Parturition-AI	3.12	3.17	3.09	2.93	2.44^{a}	3.14^{b}	3.30^{bc}	3.41^{c}	0.06	0.08	0.07	0.10	0.11	0.48	0.20	<0.001	0.65	0.075
Lactation	3.63	3.54	3.49	3.40	3.12^{a}	3.52^{b}	3.63^{bc}	3.80^{c}	0.06	0.09	0.07	0.10	0.13	0.32	0.98	<0.001	0.38	0.88
Weaning-Parturition	2.14	2.00	2.14	1.99	1.89^{a}	2.08^{b}	2.22^{b}	–	0.05	0.07	0.05	0.07	0.94	0.028	0.99	<0.001	0.88	0.047
Among parturitions	2.89	2.81	2.87	2.73	2.51^{a}	2.87^{b}	3.10^{c}	–	0.05	0.07	0.05	0.07	0.43	0.11	0.65	<0.001	0.68	0.70
Digestible CP intake, g/d																		
AI 1-Parturition 1	16.0	14.6	13.5	12.9	–	–	–	–	0.53	0.75	–	–	0.005	0.18	0.57	–	–	–
Parturition-AI	31.5	32.6	29.8	28.4	24.3^{a}	31.4^{b}	32.8^{bc}	33.8^{c}	0.59	0.83	0.68	0.96	<0.001	0.86	0.15	<0.001	0.50	0.072
Lactation	36.8	36.4	33.6	33.1	31.1^{a}	35.0^{b}	36.1^{bc}	37.8^{c}	0.65	0.92	0.69	0.97	<0.001	0.61	0.92	<0.001	0.33	0.88
Weaning-Parturition	21.7	20.5	20.6	19.3	18.8^{a}	20.7^{b}	22.1^{b}	–	0.46	0.64	0.48	0.68	0.079	0.067	0.93	<0.001	0.90	0.054
Among parturitions	29.3	29.0	27.6	26.5	25.0^{a}	28.5^{b}	30.8^{c}	–	0.47	0.66	0.50	0.70	0.003	0.29	0.54	<0.001	0.63	0.77

[a-c] Parity order mean values in the same row with a different superscript differ, P<0.05.

[1] No significant differences were found for the interaction SF×n-6/n-3×Parity (P ≥ 0.50).

Table 5. Effect of level of soluble fiber and n-6/n-3 fatty acids profile and parity order on performance of rabbit does and their litters.

	Diets				Parity order				SEM				*P*-value[1]					
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_ Hn-6/n-3	HSF_ Ln-6/n-3	1	2	3	4	SF and n-6/n-3	SF× n-6/n-3	Parity	SF×Parity and n-6/n-3×Parity	SF	n-6/ n-3	SF× n-6/n-3	Parity	SF× Parity	n-6/n-3 ×Parity
N	15	18	22	20	75	75	62	40										
Number of kits per litter																		
Total born	11.4	11.6	11.0	11.3	11.8^{bc}	12.5^{c}	10.7^{b}	10.2^{a}	0.43	0.61	0.50	0.71	0.59	0.66	0.87	0.003	0.90	0.95
Born alive	10.3	11.0	10.0	10.5	10.7	11.4	10.0	9.74	0.44	0.63	0.51	0.72	0.55	0.36	0.90	0.073	0.46	0.97
Born dead	0.98	0.64	0.95	0.78	1.17	1.04	0.69	0.45	0.19	0.27	0.24	0.33	0.84	0.34	0.75	0.14	0.57	0.88
Initial number[2]	10.2	10.9	9.87	10.3	10.6^{bc}	11.0^{c}	10.3^{b}	9.36^{a}	0.19	0.27	0.25	0.35	0.091	0.039	0.65	0.001	0.26	0.71
20 d lactation	9.43	9.60	8.97	9.46	9.27^{a}	10.1^{b}	9.35^{a}	8.76^{a}	0.21	0.29	0.27	0.38	0.32	0.26	0.60	0.005	0.27	0.52
25 d (weaning)	9.37	9.55	8.91	9.38	9.22^{a}	10.0^{b}	9.27^{a}	8.70^{a}	0.21	0.29	0.27	0.38	0.29	0.27	0.61	0.008	0.31	0.61
Mortality at birth, %	8.59	7.09	11.0	5.91	9.45	9.50	6.28	7.41	2.04	2.89	2.26	3.18	0.83	0.25	0.53	0.56	0.11	0.83
Mortality during lactation, %	7.56	11.5	9.60	7.70	12.7	8.50	9.08	6.13	1.55	2.18	2.01	2.82	0.68	0.63	0.18	0.15	0.98	0.77
Weight of the litter, kg																		
Birth	0.551	0.572	0.554	0.562	0.479^{a}	0.620^{b}	0.570^{b}	0.570^{b}	0.19	0.26	0.23	0.32	0.90	0.60	0.82	<0.001	0.96	0.87
20 d	2.907	2.983	2.827	2.846	2.588^{a}	2.946^{b}	2.990^{b}	3.039^{b}	0.56	0.79	0.65	0.92	0.18	0.55	0.72	<0.001	0.045	0.64
25 d (weaning)	3.725	3.823	3.636	3.613	3.215^{a}	3.740^{b}	3.858^{b}	3.984^{b}	0.75	1.06	0.87	1.23	0.17	0.72	0.57	<0.001	0.046	0.67
Weight of the kits, g																		
Birth	55.1	54.6	55.1	55.9	46.4^{a}	56.6^{b}	58.1^{b}	59.6^{b}	1.12	1.59	1.36	1.91	0.66	0.92	0.67	<0.001	0.35	0.98
20 d	310	323	325	307	281^{a}	297^{b}	326^{c}	360^{d}	5.68	8.00	7.04	9.92	0.91	0.78	0.053	<0.001	0.90	0.11
25 d (weaning)	399^{ab}	414^{ab}	423^{a}	391^{b}	351^{a}	379^{b}	425^{c}	473^{d}	7.40	10.4	9.03	12.7	0.95	0.44	0.030	<0.001	0.94	0.16
ADG of kits 0-25, g/d	13.6	14.0	14.4	13.3	11.9^{a}	12.6^{b}	14.5^{c}	16.4^{d}	0.30	0.42	0.35	0.49	0.89	0.44	0.074	<0.001	0.91	0.25
Kits ADFI 20-25 d, g/d	6.31	6.24	5.99	5.66	6.48^{bc}	6.78^{b}	5.67^{ab}	5.26^{a}	0.29	0.41	0.37	0.51	0.28	0.63	0.76	0.016	0.20	0.35
Litter ADFI 20-25 d, g/d	59.0	62.1	54.0	53.4	60.3^{b}	68.4^{c}	54.0^{ab}	45.9^{a}	3.16	4.46	3.83	5.39	0.13	0.77	0.68	<0.001	0.047	0.46
Feed efficiency[3]	0.281	0.298	0.301	0.300	0.263^{a}	0.302^{b}	0.320^{b}	–	0.005	0.007	0.007	0.010	0.11	0.23	0.21	<0.001	0.50	0.98

[a-c] Parity order mean values in the same row with a different superscript differ. P < 0.05.

[1] The interaction SF×n-6/n-3×Parity only was significant for the kit mortality during lactation (P = 0.007; Figure 1), but no other significant differences were found (P ≥ 0.15).

[2] Initial number once homogenized

[3] Feed efficiency = kg weaned/ (kg feed doe among parturitions+ kits).

Table 6. Effect of level of soluble fiber and n-6/n-3 fatty acids profile and parity order on doe weight, number of parturitions, fertility and milk production of rabbit does.

	Diets				Parity order					SEM			*P*-value[1, 2]			
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_ Hn-6/n-3	HSF_ Ln-6/n-3	1	2	3	4	5	SF and n-6/n-3	SF× n-6/n-3	Parity	SF	n-6/n-3	SF× n-6/n-3	Parity
N	15	18	22	20	75	75	62	40	40							
Doe weight, g																
Parturition	3939	3952	3999	3995	3910^{a}	3942^{ab}	3979^{bc}	4055^{c}	−	47.9	67.8	40.0	0.45	0.95	0.90	0.002
AI (11 d)	3931	4111	4094	4174	4034	4069	3996	4132	4158	55.3	78.2	53.7	0.15	0.10	0.53	0.066
Weaning (25 d)	4167	4237	4229	4236	4161^{a}	4163^{a}	4240^{b}	4305^{b}	−	48.4	68.4	42.3	0.66	0.58	0.65	0.005
Number parturitions	3.07	3.72	3.77	3.65	−	−	−	−	−	0.17	0.24	−	0.19	0.27	0.11	−
Fertility, %	92.0	96.1	95.3	94.2	100^{b}	87.2^{a}	90.5^{a}	99.9^{b}	−	1.87	2.63	2.55	0.79	0.58	0.34	<0.001
Milk production, kg																
0-10 d	1.73	1.75	1.70	1.64	1.42^{a}	1.75^{b}	1.80^{c}	1.85^{b}	−	0.029	0.042	0.034	0.11	0.61	0.30	<0.001
10-20 d	2.63	2.69	2.57	2.52	2.35^{a}	2.51^{b}	2.69^{c}	2.86^{d}	−	0.045	0.064	0.053	0.089	0.93	0.38	<0.001
20-25 d	1.35	1.35	1.36	1.28	1.34	1.28	1.33	1.39	−	0.032	0.045	0.038	0.55	0.34	0.39	0.29
0-25 d	5.73	5.80	5.67	5.46	5.09^{a}	5.60^{b}	5.84^{c}	6.13^{c}	−	0.088	0.124	0.099	0.12	0.60	0.26	<0.001

[a-c] Parity order mean values in the same row with a different superscript differ, P<0.05.

[1] Number of kits at 10 d for 0-10 d milk production, number of kits at 20 d for 10-20 d milk production, number of kits at 25 d for 20-25 d and 0-25 d milk production were significant as covariates (P ≤ 0.001).

[2] No significant differences were found for the interactions SF× Parity, n-6/n-3×Parity and SF×n-6/n-3×Parity (P ≥ 0.15).

Table 7. Effect of level of soluble fiber and n-6/n-3 fatty acids profile and parity order on body chemical composition and energy content of rabbit does

	Diets				Parity order					SEM				*P*-value[1]				
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_ Hn-6/n-3	HSF_ Ln-6/n-3	1	2	3	4	5	SF and n-6/n-3	SF× n-6/n-3	Parity	SF×n-6/n-3 ×Parity	Cov[2]	SF	n-6/ n-3	SF× n-6/n-3	Parity
N	15	18	22	20	75	75	62	40	40									
Parturition																		
Moisture, g/kg	609	611	609	607	612	611	609	605	–	0.23	0.33	0.25	0.49	<0.001	0.48	0.93	0.53	0.20
Protein, g/kg	179	179	179	178	177[a]	179[b]	180[b]	179[b]	–	0.03	0.05	0.04	0.07	0.001	0.51	0.76	0.75	<0.001
Fat, g/kg	146	144	147	149	145	144	146	151	–	0.25	0.35	0.26	0.51	<0.001	0.44	0.91	0.52	0.25
Ash, g/kg	31.7	31.8	31.6	31.6	31.1[a]	31.7[b]	31.9[b]	31.9[b]	–	0.01	0.02	0.01	0.03	<0.001	0.38	0.86	0.88	<0.001
GE, MJ/kg BW	11.0	10.9	11.0	11.1	10.9	10.9	11.0	11.2	–	0.10	0.14	0.10	0.21	<0.001	0.47	0.93	0.53	0.19
AI (11 d)																		
Moisture, g/kg	597	596	593	589	580[a]	597[b]	599[b]	597[b]	597	0.29	0.41	0.36	0.72	<0.001	0.19	0.52	0.73	<0.00
Protein, g/kg	179	179	179	178	176[a]	182[b]	181[b]	178[a]	178[a]	0.05	0.06	0.07	0.14	<0.001	0.53	0.38	0.34	<0.001
Fat, g/kg	156	157	160	165	173[b]	155[a]	153[a]	158[a]	158[a]	0.28	0.40	0.34	0.67	<0.001	0.16	0.47	0.65	<0.001
Ash, g/kg	31.4	31.1	31.1	30.7	30.0[a]	30.8[b]	31.6[c]	31.3[c]	31.6[c]	0.01	0.02	0.02	0.04	<0.001	0.11	0.14	0.84	<0.001
GE, MJ/kg BW	11.4	11.5	11.6	11.8	12.1[b]	11.4[a]	11.3[a]	11.5[a]	11.5[a]	0.12	0.16	0.14	0.28	<0.001	0.16	0.47	0.77	<0.001
Weaning (25 d)																		
Moisture, g/kg	630	636	635	634	652[c]	639[b]	635[b]	610[a]	–	0.50	0.71	0.50	0.99	0.11	0.78	0.72	0.61	<0.001
Protein, g/kg	180	179	179	179	180[b]	180[b]	179[b]	178[a]	–	0.04	0.05	0.04	0.07	<0.001	0.74	0.58	0.61	0.005
Fat, g/kg	132	127	127	127	110[a]	123[b]	128[c]	152[d]	–	0.46	0.65	0.45	0.90	0.076	0.75	0.72	0.67	<0.001
Ash, g/kg	31.3	31.4	31.3	31.4	31.3	31.4	31.5	31.2	–	0.01	0.02	0.01	0.03	<0.001	0.97	0.67	0.79	0.36
GE, MJ/kg BW	10.3	10.0	10.0	10.1	9.32[a]	9.89[b]	10.1[b]	11.1[c]	–	0.20	0.29	0.20	0.40	0.095	0.78	0.70	0.62	<0.001

[a-c] parity order mean values in the same row with a different superscript differ, P<0.05.
[1] No significant differences were found for the interactions SF× Parity, n-6/n-3×Parity and SF× n-6/n-3 × Parity (P ≥ 0.12).
[2] Initial BW. Initial proportion of moisture, protein, fat, ash and energy were not significant as covariates (P ≥ 0.34).

Table 8. Effect of level of soluble fiber and n-6/n-3 fatty acids profile and parity order on the proportion of total removed, dead and culled does.

	Diets				Parity order				*P*-value			
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_Hn-6/n-3	HSF_Ln-6/n-3	1	2	3	4	SF	n-6/n-3	SF×n-6/n-3	Parity
N	24	24	24	24	96	78	64	61				
Total removed, %	62.5	37.5	16.7	29.1	18.7	17.9	4.69	0.00	0.005	0.75	0.056	0.11
Culled,%	20.8	20.8	4.17	12.5	7.29	8.97	0.00	0.00	0.059	0.37	0.37	0.96
Mortality,%	41.7	16.7	12.5	29.2	11.5	8.97	4.69	0.00	0.13	0.38	0.13	0.54

Table 9. Effect of level of soluble fiber and n-6/n-3 fatty acids profile and parity order on milk composition at the 7th day of lactation of primiparous does.

	Diets				SEM		*P*-value		
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_Hn-6/n-3	HSF_Ln-6/n-3	SF and n-6/n-3	SF×n-6/n-3	SF	n-6/n-3	SF×n-6/n-3
N	9	9	9	8					
DM,%	30.0	31.4	30.6	31.5	0.53	0.75	0.65	0.13	0.79
CP, %	9.74	8.98	9.43	9.11	0.37	0.53	0.87	0.31	0.68
Fat, %	14.5	17.1	15.8	16.9	0.58	0.82	0.52	0.031	0.36
Fatty acids, g/100g of total fatty acids									
C6:0	0.258	0.241	0.255	0.258	0.009	0.040	0.60	0.56	0.44
C8:0	13.31	12.50	14.08	13.70	0.55	0.78	0.22	0.45	0.79
C10:0	14.62	13.81	15.89	15.02	0.46	0.65	0.066	0.20	0.96
C12:0	2.593	2.461	2.909	2.668	0.11	0.16	0.12	0.26	0.74
C12:1	0.011	0.039	0.054	0.015	0.018	0.025	0.72	0.83	0.20
C14:0	1.893	1.966	1.870	1.830	0.06	0.08	0.36	0.85	0.52
C14:1	0.181	0.166	0.163	0.179	0.014	0.020	0.88	0.98	0.45
C15:0	0.359	0.373	0.427	0.422	0.006	0.009	<0.001	0.69	0.32
C15:1	0.042[a]	0.039[a]	0.056[b]	0.066[b]	0.001	0.001	<0.001	0.13	0.008
C16:0	14.90	16.02	14.23	14.49	0.46	1.29	0.10	0.30	0.51
C16:1n7	1.869	1.878	1.670	1.939	0.16	0.23	0.76	0.55	0.57
C16:1n9	0.218	0.203	0.207	0.192	0.007	0.009	0.28	0.13	0.99
C17:0	0.366	0.373	0.397	0.401	0.008	0.012	0.019	0.64	0.87
C17:1	0.184	0.197	0.177	0.195	0.009	0.013	0.75	0.27	0.86
C18:0	3.042	3.658	3.539	3.534	0.16	0.23	0.43	0.20	0.19
C18:1n9	21.53	17.40	20.44	16.44	0.45	0.64	0.12	<0.001	0.92
C18:1n7	0.668	0.716	0.644	0.755	0.02	0.03	0.80	0.010	0.29
C18:2n6	21.58	22.34	20.64	21.60	0.25	0.36	0.025	0.022	0.78
C18:3n3	1.202	4.361	1.113	5.020	0.16	0.23	0.22	<0.001	0.11
C18:3n6	0.210	0.228	0.245	0.213	0.030	0.040	0.78	0.85	0.51
C18:4n3	0.009	0.071	0.011	0.090	0.004	0.005	0.055	<0.001	0.097
C20:0	0.118	0.118	0.121	0.119	0.002	0.002	0.43	0.76	0.68
C20:1n9	0.195	0.178	0.183	0.167	0.004	0.006	0.059	0.010	0.94
C20:2	0.160	0.153	0.156	0.157	0.004	0.006	0.97	0.60	0.48
C20:3n6	0.063	0.063	0.084	0.059	0.005	0.007	0.26	0.098	0.11
C20:4n6	0.238	0.230	0.234	0.226	0.009	0.013	0.78	0.50	0.98
C20:3n3	0.017	0.032	0.015	0.039	0.002	0.003	0.43	<0.001	0.18
C20:4n3	0.015	0.021	0.022	0.024	0.001	0.002	0.009	0.027	0.18
C20:5n3	0.021	0.037	0.017	0.041	0.003	0.004	0.97	<0.001	0.31
C22:0	0.083	0.081	0.100	0.099	0.010	0.015	0.23	0.93	0.95
C22:1n9	0.037	0.043	0.036	0.042	0.002	0.003	0.83	0.087	0.92
SMCFA[1]	30.8	29.0	33.2	31.7	0.98	1.38	0.080	0.24	0.94
OFA[2]	0.95	0.98	1.06	1.08	0.021	0.030	0.002	0.37	0.96
SFA	51.55	51.59	53.83	52.54	0.52	0.74	0.037	0.40	0.37
MUFA	24.94	20.88	23.62	20.00	0.60	0.85	0.21	<0.001	0.80
PUFA	23.5	27.5	22.5	27.5	0.34	0.47	0.26	<0.001	0.34
n-6/n-3	17.5	5.91	18.0	4.26	0.50	0.69	0.43	<0.001	0.13
n-3	1.262	4.530	1.178	5.212	0.17	0.23	0.21	<0.001	0.11
n-6	22.10	22.86	21.19	22.09	0.23	0.33	0.017	0.019	0.84

[a-c] Diet mean values in the same row with a different superscript differ, *P* < 0.05

[1] Short and medium chain fatty acids included: C6:0, C8:0, C10:0, C12:0, and C12:1.

[2] Odd fatty acids included: C15:0, C15:1, C17:0, and C17:1.

Table 10. Effect of level of soluble fiber and n-6/n-3 fatty acid ratio on body and carcass chemical composition and energy content of rabbits one day after weaning (26 d of age).

	Diets				SEM		*P*-value		
	LSF_Hn-6/n-3	LSF_Ln-6/n-3	HSF_Hn-6/n-3	HSF_Ln-6/n-3	SF and n-6/n-3	SF× n-6/n-3	SF	n-6/n-3	SF×n-6/n-3
N	40	40	40	40					
BW, g	447[a]	496[b]	471[ab]	452[a]	8.83	12.5	0.42	0.23	0.007
Body composition									
Moisture, g/kg	695	703	696	700	2.41	3.41	0.70	0.068	0.51
Protein, g/kg	568	569	564	5.0	1.27	1.79	0.46	0.044	0.12
Fat, g/kg	259	25.8	25.9	25.6	1.12	1.58	0.59	0.16	0.33
Ash, g/kg	11.1	11.1	11.2	11.1	0.29	0.41	0.038	0.39	0.75
GE, MJ/kg BW	22.1	22.1	22.0	22.1	0.21	0.30	0.036	0.71	0.14
Carcass composition									
Moisture, g/kg	710	715	715	713	1.50	2.13	0.57	0.41	0.10
Protein, g/kg	619	613	622	616	1.94	2.75	0.30	0.021	0.95
Fat, g/kg	226	221	220	223	2.02	2.86	0.49	0.59	0.18
Ash, g/kg	154	159	160	158	2.01	2.84	0.39	0.61	0.15
GE, MJ/kg BW	21.6	21.2	21.2	21.3	0.13	0.18	0.37	0.66	0.20

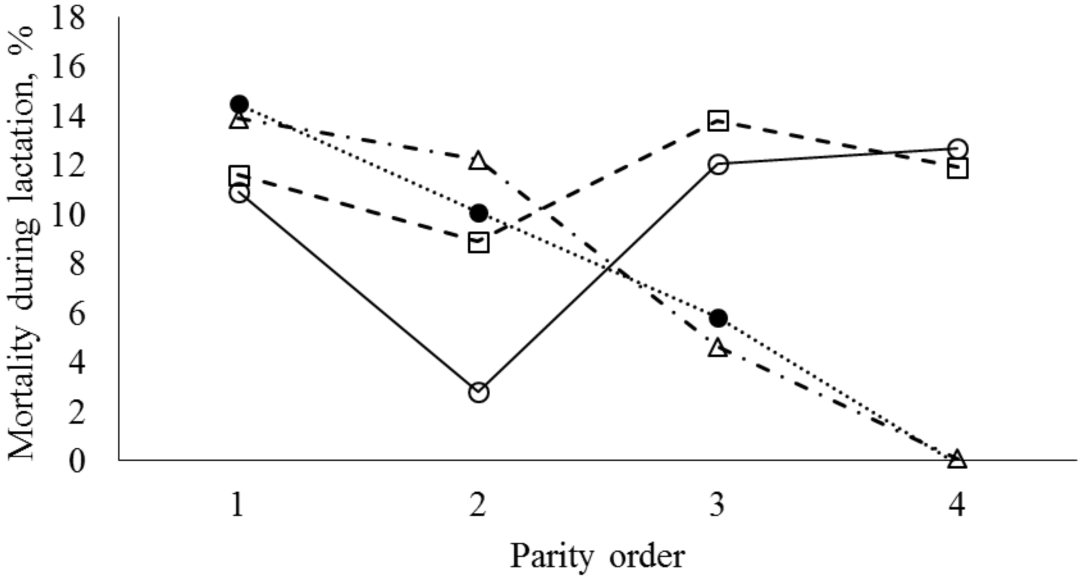

Figure 1. Effect of level of soluble fiber and n-6/n-3 fatty acids profile over parity order on litter mortality during lactation [LSF_Hn-6/n-3 ●; LSF_Ln-6/n-3 □; HSF_ Hn-6/n-3 ○; HSF_ Ln-6/n-3 Δ]. $P_{\mathrm{SF} \times \mathrm{n\text{-}6/n\text{-}3} \times \mathrm{Parity}} = 0.007$ (SEM = 2.01).

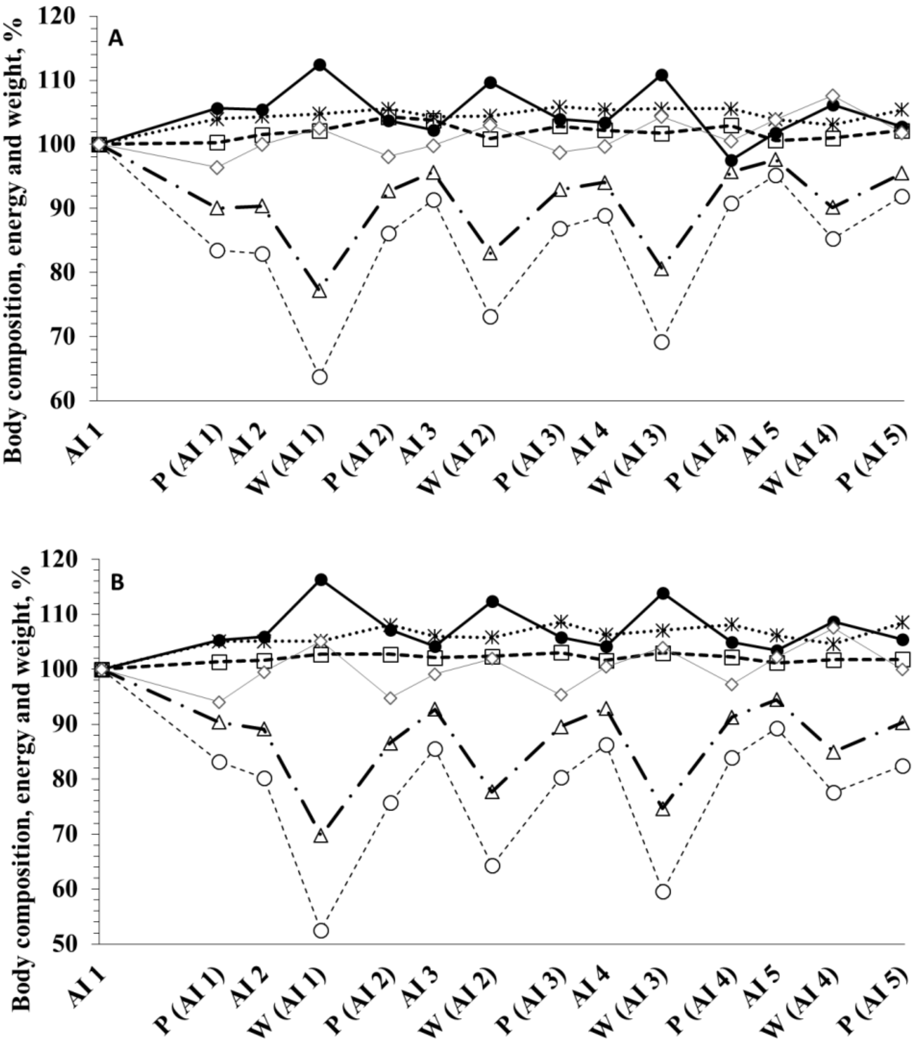

Figure 2. Evolution of body composition, energy and weight (Moisture ●, protein □, fat ○, ash *, energy Δ, and BW ◊) over time (AI, parturition –P- and weaning –W-) of all rabbit does considered for productive traits (n = 75. Fig. 2A) or only those that had five consecutive parturitions (n = 18. Fig. 2 B). Values obtained at first artificial insemination (AI 1) were considered as 100 (Fig. 2 A: Moisture: 596 ± 1.92, protein: 177 ± 0.46, fat: 157 ± 1.87; ash: 30.7 ± 0.10%; energy: 11.4 ± 0.082 MJ/kg BW; BW: 3.85 ± 0.025 kg. Fig. 2 B: Moisture: 596 ± 3.44, protein: 178 ± 0.90, fat: 156 ± 3.87; ash: 30.6 ± 0.21; energy: 11.4 ± 0.15 MJ/kg BW; BW: 3.90 ± 0.033 kg) and the values obtained later were expressed as percentage of the value obtained at AI 1. $P_{Time} < 0.001$ in both figures.

AI 1, AI 2, AI 3 and AI 4: 1st, 2nd, 3rd and 4th artificial insemination, respectively. P (IA 1), P (IA 2), P (IA 3), P (IA 4) and P (IA 5): Parturition corresponding to IA 1, AI 2, AI 3 and AI 4, respectively. W (IA 1), W (IA 2), W (IA 3) and W (IA 4): Weaning corresponding to IA 1, AI 2, AI 3 and AI 4, respectively.