ANIMAL FATS IN RABBIT FEEDING – A REVIEW

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Abstract

The purpose of this article is to overview the history of feeding rabbits with different types of animal fats, and to discuss their effects on rabbit performance and quality of their products. Other aspects of the inclusion of various animal fats in rabbit diets are also described. This article is based on the analysis of relevant scientific literature and presents animal fats fed to rabbits, such as beef tallow, butter, pork lard, poultry fat, fish oil, krill oil, oil extracted from insect larvae, mixtures of various animal fats, and mixtures of animal and vegetable fats. The reported papers describe the effect of fats on growth performance, lactation, rearing performance, meat quality, and health status of rabbits. It is notable that in many cases, various animal fats were often an integral part of numerous diets or were included in control diets. The presented information demonstrates that animal fat can be fed to rabbits at 2–4% of the diet without negative effects on reproductive performance, growth performance and quality of meat obtained. Rabbits were used as model animals in many studies in which fat was added to balance the diets and to increase their energy value, especially when investigating various cardiovascular and obesity-related diseases.

Key words: rabbits, feeding/nutrition, mammalian fat, poultry fat, fish oil, insect fat

Fats are very energy dense raw materials. They are present in many feedstuffs. Fats are one of the most important components of diets. Fat supplements play a major role in improving the absorption of fat soluble vitamins and reducing the dustiness of feed. Natural fats consist of fatty acids and glycerol components. Fatty acids are usually classified as saturated (SFA), unsaturated (USFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Among the polyunsaturated fatty acids group, linoleic (LA) and linolenic (LNA) fatty acids are the essential fatty acids. There is considerable evidence indicating that the fatty acid composition of animal diets influences the fatty acid profile of the tissues of the animals fed with these diets (Wiseman, 1984). Lin et al. (1991) demonstrated that the fatty

acids of dietary fats may significantly affect adipose fatty acid composition in rabbits. According to Trebušak et al. (2011), dietary supplementation of vegetable oils may have a beneficial influence on the fatty acid profile of rabbit meat. In the cited study, linseed oil added to diets reduced SFA concentrations in meat. The present findings differ from the results of studies investigating higher animals, mammals and birds. Andrade et al. (2018), who analysed the efficacy of different vegetable and animal fats in rabbits, found that neither animal performance nor meat composition were affected by dietary lipid sources. Beef tallow and poultry fat contributed to a lower proportion of MUFAs and PUFAs in meat compared with vegetable oils. The above authors concluded that the dietary inclusion of soybean oil was advantageous because it increased the PUFA content of rabbit meat. Interestingly, the effect of fish oil was different and similar to that exerted by insect-derived lipids. Kowalska and Bielański (2009) reported that dietary supplementation with fish oil led to a highly significant increase in the levels of *n*-3 PUFAs, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the lipid fraction of rabbit leg muscles.

It is now thought that fat should account for 2 to 4% of rabbit diets due to pelleted feed production technology (Gidenne et al., 2017). In the conventional, low-level feeding of rabbits, this nutrient was most often deliberately ignored or fatty components, such as those from oilseeds, were used (Gugolek et al., 2008; Daszkiewicz et al., 2012). In early studies on the addition of various fats to rabbit diets, the animals were commonly fed with pelleted feeds, which can be easily enriched with fat, or fat can be added during the production process. According to Maertens (1998), fat feeding of rabbits is limited by the need to maintain proper pellet hardness, reduce the intake of energy dense feed and also by economic factors associated with the price and availability of fat in local feed markets. The same author believes that the improvement of rabbit performance due to supplementation of dietary fat is not as pronounced as in other species of animals, notably pigs and poultry. Several attempts have been made to sum up the current state of knowledge on the role of fats in rabbit nutrition. Studies by Fortun-Lamothe (1997), Maertens (1998), Fernández-Carmona et al. (2000), Lebas (2004), De Blas and Mateos (2010), which focused on supplementing rabbits with both animal and vegetable fats, are considered the most interesting and comprehensive. Up until the 1970s, rabbit diets were most often supplemented with vegetable fats or plant feeds rich in fat. Later on scientists began to explore the possibility of feeding animal fats to these animals. The major interest for the use of fats is to increase the energy concentration of diet without reducing the fibre content, especially during intensive rabbit production (Maertens, 1998).

Given that feeding animal fats to rabbits has received less study and raises some concern, the aim of this review article was to outline the history of feeding various animal fats to rabbits and to discuss their effect on rabbit performance and quality of the products obtained. Other aspects of supplementing rabbit diets with different animal fats will also be described.

The paper is based on the analysis of relevant scientific literature from the Web of Science and Scopus databases, as well as other publications collected by the present authors. Information about the use of animal fats in publications concerning rabbit

nutrition is presented thematically and chronologically with data for the amounts of dietary fats as well as the principal aims and results of the reported studies.

Animal fats in rabbit feeding

Beef tallow in rabbit feeding

The initial research on the animal fat feeding of rabbits concentrated on beef tallow and pork lard. Beef tallow, due to its high melting point and low level of polyunsaturated fatty acids, is considered a less valuable fat not suitable for direct human consumption, yet it has often found application in livestock feeding (Gunstone, 2000).

In the 1970s and 1980s, several further experiments on the possibility of feeding tallow to rabbits were carried out. Raimondi et al. (1974) studied the effect of dietary tallow on production of meat rabbit, focusing their attention on growth, food consumption and killing out percentage in growing rabbits. Raimondi et al. (1976), who supplemented growing rabbits with beef tallow at 4 to 8% of the diet as an alternative to groundnut oil, concluded that the optimum level of dietary fat for rabbits is 4%. Also Reid et al. (1980) supplemented rabbit diets with 1 to 5% tallow to study the relationship between the diet's energy content and fat and protein content. In turn, Maertens et al. (1986) examined different fats, including tallow, for the digestibility and level of digestible energy in growing rabbits. Partridge et al. (1986) compared the suitability of different vegetable and animal fats (soybean oil and tallow) in the diets of growing rabbits. Santomá et al. (1987) compared tallow and lard with sunflower oil, by-products from the oil-refining industry, soy lecithin, and their mixtures. In general, the studies reported above showed no effect of the type of fat on the analysed production parameters of growing rabbits with the exception of sunflower oil, the large quantities of which caused growth disturbances. The above studies concluded that beef tallow can be fed to rabbits alongside vegetable fats. Abdelhamid (1989), who supplemented rabbits' diets with beef tallow and cottonseed oil, found tallow to cause a significant increase in feed intake and growth rate. In addition, rabbits on this diet tended to show lower total nitrogen and cholesterol levels in blood, and lower vitamin A in the liver. The author concluded that the addition of beef tallow and cottonseed oil to rabbit diets could improve performance, and considered the possibility of using both fat types together. In the 1990s, the effect of the level and type of fat on nutrient digestibility and blood parameters in rabbits was investigated by Fekete et al. (1990 a, b). Rather than supplementing pure beef tallow, they fed rabbits with diets containing 5% mixed animal fats (50% beef tallow and 50% lard), which was added alone or with sunflower oil. The supplemental fat increased the digestibility of fat in the diets. The supplementation had no effect on plasma cholesterol levels, but modified the composition of fatty acids in the blood depending on the type of added fat. Also, Fernández and Fraga (1992) found positive results when replacing vegetable fat with beef tallow in diets for young rabbits. In turn, Cobos et al. (1993) determined the effect of alternative feeding of rabbits with diets containing 3% beef tallow, sunflower olein and soybean oil on chemical composition and fatty acids composition of rabbit meat. No significant differences in the meat chemical composition

were observed between groups. However, a large effect of the diet on the total and apolar lipid fatty acids was found, with C18:1 and C18:2 being the most affected fatty acids. The influence of the diets on the fatty acids of the polar fraction was less marked. It is concluded that the enrichment of the rabbit diet with either soybean and sunflower oleins or soybean oil allows the production of rabbit meat with a higher unsaturation degree than is obtained by using conventional diets, which constitutes an important nutritional benefit to the human being. Further research concerning the application of different tallow levels in rabbit feeding was presented by Fernández et al. (1994). They showed that the addition of tallow had no effect on nutrient digestibility except for ether extract, which increased in proportion to its dietary level.

Xiccato et al. (1995) studied the effect of different dietary energy levels on reproductive performance in pregnant and lactating rabbit does supplemented with 2.5% mixed fat containing 40% beef tallow and 60% lard. The diets influenced the digestible energy intake and the milk production. The high-energy diets significantly modified the final body composition. The maternal diet significantly influenced the weight of the sucking litters at 20 days. The pregnant and lactating does showed lower energy intake, milk yield and litter weaning weights than non-mated or non-pregnant lactating does. Between the first and the second parturition, the pregnant and lactating does had a loss of empty body weight and a negative protein balance, while the non-mated or non-pregnant does had an increase in live weight and empty body weight and a positive protein balance.

In the next two publications, Fernández and Fraga (1996 a, b) described the effect of diets containing oleins, beef tallow and soybean oil in addition to toasted whole soybeans (to increase dietary ether extract) on growth, carcass characteristics, chemical composition, efficiency of digestible energy and protein utilization. There were no differences in the utilization of animal and vegetable fats, and only for some traits (average daily gain, feed intake and kidney weights) did the diets with soybean oil result in better responses than those with oleins. The results indicate that fat addition to fibrous diets extends the rabbit's growing period without impairing the feed conversion ratio. The joints of rabbits slaughtered at heavier weights have more desirable weights for marketing the carcass on a retail cut basis.

A thematically similar study was conducted by Falcão-e-Cunha et al. (1996), who determined the effect of the level of dietary fat (beef tallow) and fibre on performance, nutrient digestibility and digestive function in growing rabbits. Increasing dietary fat reduced feed intake, daily gains and feed conversion ratio. The addition of fat reduced dry matter, organic matter and energy digestibilities but increased the digestibilities of neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose and cellulose. The digestibilities of fat and crude protein were not affected by fat level. Fernández-Carmona et al. (1996 a) investigated the effect of vegetable fat and animal fat (8.5% of commercial tallow) on reproductive performance of rabbit does kept at 30°C. More favourable results for litter weight at 21 days and 35 days were observed in the fat-supplemented groups. Fernández-Carmona et al. (1996 b) fed tallow to rabbits to determine the energy value of diets varying widely in fibre content. On the whole, many publications demonstrated that fat is added to the feed

to increase the level of digestible energy, without reducing the fibre level. This approach serves to reduce the antagonism between performance and health risk.

Slim et al. (1996) investigated the effect of diets supplemented with vegetable and animal fats, including beef tallow, on oxidative stress and glutathione metabolism in rabbit liver tissues. The study was designed to determine whether the type of dietary fat would impact fatty acid composition and oxidative status of tissues. It was concluded that high levels of dietary unsaturated fat should be avoided if oxidative stress is a critical issue in nutrition-related diseases.

Cervera et al. (1997) determined the effect of adding various fats, including tallow, on the growth of young rabbits maintained at different temperatures. They concluded that fat supplements may positively affect the growth of animals at high temperatures, which are detrimental to rabbit breeding. Pla and Cervera (1997) compared three diets given to three groups of does and their offspring: a standard commercial diet (control group), vegetable fat enriched (first experimental group), and animal fat (beef tallow) enriched (second experimental group). Animals receiving fat-enriched diets had a better feed conversion efficiency ratio, a lower drip loss and a dressing yield substantially better than the conventionally fed rabbits. Carcass colour was not affected by the diet but rabbits given vegetable fat diet showed less pale meat. Differences in the three colour parameters were found for the three diets for hot and chilled carcass fat colour. Muscular pH measured in the *longissimus dorsi* and *biceps femoris* was slightly higher for diets with added fats. Water-holding capacity of raw meat from rabbits given experimental diets was higher than from rabbits given the control diet. Protein and moisture content of the meat of a hind leg was almost the same for the three diets but fat content was much higher in animals given experimental diets than in the control group.

Beef tallow is also combined with lard and poultry fat, which were treatment factors in the experiment of Oliver et al. (1997). Beef tallow accounted for 25% of the supplemented mixture. The aim of this study was to examine the effect of adding vegetable and animal fat to the diet of rabbits, on the quantity and nutritional quality of the perirenal fat and the organoleptic quality of the meat. The obtained results suggest that from a human health point of view, the use of a diet supplemented with animal fat is useful on the basis of the nutritional index. However, the amount of fat added to the diets with vegetable fat and animal fat should be less than used in this study if the carcass is sold whole in order to avoid excessive perirenal fat. From an organoleptic point of view, the greater aniseed and grass flavour of the group with vegetable fat compared to the group with animal fat may give meat of better sensory quality.

A study on the effect of diets with different lucerne and energy proportions was conducted by Fernández-Carmona et al. (1998). Tallow was added to the diets to increase their energy value. Tallow used at 8.9% of the diet in one experimental group increased ether extract in the ration to 11.1%. In the control groups animal fat was added at 1% of the diet and its dietary level was 4.0–4.2%. The authors concluded that the addition of fat to the diet of growing rabbits increased dressing out percentage and dissectible fat of carcasses, although no differences in growth rate were detected.

Pascual et al. (1998, 1999) supplemented rabbit does diet with a mixture of animal fats in which beef tallow, alongside lard and poultry fat, accounted for 25%. This fat mixture was added at 8.5% of the diet and beef tallow constituted 2.12%. The authors concluded that high-fat diets were related to the high milk yield and energy content of milk, allowing a higher litter weight gain and a lower mortality of sucking pups. Type of fat did not have a significant effect, but there was a correlation between the fatty acid composition of the diet and doe milk.

In the study by Kritchevsky et al. (1998), tallow and lard were used as representative animal fats in experimental diets. They compared their respective atherogenic properties by feeding each fat (14%) as part of a semi-purified diet containing 0.5% cholesterol. The lard containing diet was significantly more atherogenic than the diet containing tallow. Lard and tallow both contain approximately 24% palmitic acid (16:0) but the palmitic acid is distributed differently in their component triglyceride molecules. Over 90% of 16:0 of lard is at the SN2 position, whereas only approximately 15% of the 16:0 of tallow is in that position. The fats were randomized so that every component fatty acid was present at each triglyceride carbon to approximately one third of its total concentration. Randomized tallow and lard had 8.5% and 7.6% of their component 16:0 at the SN2 position, respectively. The atherogenicity of diets containing either randomized lard or tallow was virtually the same and significantly lower than the atherogenicity of the diet containing native lard. This study confirms earlier studies from that laboratory which suggest that the level of 16:0 at the SN2 position of a triglyceride influences its atherogenicity.

Saricicek et al. (1998) determined the effects of different fat sources (beef tallow animal fat – beef tallow and vegetable oil – sunflower oil) and different fat levels on various blood parameters of rabbits. It was determined that blood parameters such as LDL-C, total cholesterol, phospholipids, HDL-C, triglycerides, free cholesterol, were influenced by different fat sources and levels in different manner and animal fats increased these blood parameters.

The research of Oliver et al. (1997) was followed up by Hernández et al. (2000), who investigated the effect of different fat types (animal vs vegetable fat) on meat quality. They fed rabbits a diet with a commercial mixture of animal fats which included 25% beef tallow, 65% lard and 10% poultry fat. The experiment showed a higher content of oleic acid in the muscles of the animals receiving the animal fat diet. Moreover, the diets used in this experiment had only a slight influence on the organoleptic characteristics of rabbit meat.

In another of their studies, Pascual et al. (2002 a) reported the effect of dietary energy source on the performance and perirenal fat thickness evolution of primiparous rabbit does. It was concluded that the addition of dietary fat, especially of animal origin, seems to improve the utilization of energy for milk production, while the use of a high level of starch could decrease the negative balance of primiparous rabbit does during lactation. Like in other studies by the same team, this study used a commercial mixture of animal fats consisting of beef tallow, lard, and poultry fat. In turn, in a lucerne-based feeding programme for rabbits, Pascual et al. (2002 b) used a 1–2% animal fat supplement to balance the diets.

Many of the subsequent experiments with rabbits no longer explored the possibility of feeding dietary tallow or other fats, instead they were mainly concerned with the effect of these fats on multiple aspects of rabbit meat quality. For example, performance, fatty acid profile and oxidative stability of meat of rabbits fed different lipid sources, such as soybean oil, sunflower oil, cottonseed oil, beef tallow and poultry fat, were investigated by Andrade et al. (2018). There was no effect of dietary lipid sources on animal performance, carcass traits and meat composition. Dietary inclusion of beef tallow resulted in a lower proportion of polyunsaturated fatty acids in meat compared to vegetable oils. The values of thiobarbituric acid-reactive substances indicated higher oxidation in the frozen meat of the animals fed with sunflower oil, cottonseed oil and bovine tallow in their diets. They confirm that vegetable oils and animal fats can be included in growing rabbit diets without impairing performance and meat quality parameters. Dietary inclusion of soybean oil is more advantageous because it promotes the incorporation of polyunsaturated fatty acids and greater lipid stability of refrigerated and frozen rabbit meat.

Research concerning the application of beef tallow in rabbit diets was conducted from the early 1970s to the early 21st century. Initially beef tallow was added to the diets to determine whether animal fats can be fed to rabbits and to determine their optimum levels. It was also used to increase dietary energy level and to balance diets in the experiments dealing with other problems. The second decade of the 21st century saw an interest in the effect of dietary beef tallow and other fats on rabbit meat quality, which was probably dictated by growing consumer awareness.

Butter in rabbit feeding

Butter is another bovine fat, in addition to tallow, fed to rabbits. Milk fat, which is 97 to 98% triacylglycerols, is a source of energy and nutrients and an important food ingredient that provides desirable textural and flavour characteristics. The fatty acid composition of milk fat typically comprises 70% saturated fatty acids, 25% monounsaturated fatty acids, and 5% polyunsaturated fatty acids (Grummer, 1991; Jensen, 2002). However, rather than serving as a treatment factor, this fat was used to increase the amount of dietary fat in many studies concerning the effect of high-fat diets on rabbit health. In these experiments, rabbits were most often used as model animals.

Funch et al. (1960), Krogh et al. (1961) and Moore and Williams (1963) were among the first to use butter in rabbit diets. Slim et al. (1996) studied the effect of the type of dietary fat on fatty acid composition and oxidant status in tissues. The results support the hypothesis that diets rich in milk fat provide a lipid environment with low susceptibility to oxidative stress. Next, Kang et al. (1997) studied the effect of diet induced hypercholesterolemia and selenium supplementation on nitric oxide synthase activity. In another publication, Kang et al. (2000) investigated the correlation between selenium and hyperlipidemia in rabbits supplemented with 10% dietary butter. Mehta et al. (2002 a) studied apoptosis in experimental atherosclerosis induced by a diet with 10% table butter and how it was influenced by selenium supplementation. In a study of rabbit aortic wall following high-fat diet feeding and

selenium supplementation, Mehta et al. (2002 b) added 10% butter to increase the level of dietary fat.

Bansal et al. (2002) examined heat shock protein in rabbits with experimental atherosclerosis. In this experiment, table butter was given to animals at 10% of the diet for even several months. Another study concerning the effect of high-fat diet on the pharmacokinetics of phenytoin and carbamazepine in rabbits fed a butter-supplemented diet was conducted by Sidhu et al. (2004). Purohit and Vyas (2005) used a mixture of butter and hydrogenated fat to increase the level of fat in a high-fat diet in a study on the effect of adding *Capparis deciduas* fruit to the diet of growing rabbits. Other publications by the same team from France also used butter in rabbit diets. In general, dairy fat contains high amounts of saturated fatty acids, which are associated with cardiovascular disease risk. In the study by Faulconnier et al. (2006), experimental butters with a high content of trans-18:1 fatty acids and/or cis-9,trans-11-18:2 were fed to investigate their effects on adipose tissue (AT) and liver lipogenic activities. The ingestion for 6 weeks had little or no effect on liver and AT lipogenesis. The ingestion for 12 weeks decreased perirenal AT weight, lipogenic enzyme and lipoprotein lipase activities, without affecting liver lipid concentration or lipogenic activities except for a decrease in glycerol-3-phosphate dehydrogenase activity. A subsequent study by Bauchart et al. (2007) found that butters varying in trans 18:1 and cis-9 trans-11 conjugated linoleic acid modify plasma lipoproteins in the hypercholesterolemic rabbit. In turn, Roy et al. (2007) studied the effect of butters rich either in trans-10-C18:1 or in trans-11-C18:1 plus cis-9, trans-11 CLA on plasma lipids and aortic fatty streak in experimental atherosclerosis in rabbits.

Three groups of rabbits were used in the experiment of Elrufai et al. (2015). One group was fed conventional rabbit feed, the second group was fed modulated feed by adding extra 7% fat from animal source (fresh butter extracted from raw milk), and the third group received modulated feed of extra 7% fat from plant source origin (sunflower oil). The study showed significant increase in the plasma level of cholesterol, phospholipids and triglycerides in both fat fed groups compared to the control group; the difference was significant between the plant fed and animal fat fed groups with the latter showing great increase. The authors believe that future studies should emphasize on using different concentrations of either plant or animal fat.

In summing up the information presented above, it is stated that rather being the main experimental factor, butter was added to increase the fat and energy levels in rabbit diets. This allowed for the investigation of atherosclerosis and other diseases induced by high-fat diets in rabbits as model animals. Furthermore, butter was used to assess the effects of different sources of fat on selected antioxidant profile in rabbits.

Pork lard in rabbit feeding

Lard is a commonly used dietary fat with a high saturated fatty acid content and no trans fat.

Fekete et al. (1990 a, b) studied the effect of fat on nutrient digestibility. The added fat was composed of 50% lard and 50% beef tallow. This study was described above in the subsection on beef tallow. Also Wills (1980) fed rabbits with lard, her-

ring oil or corn oil mixed with starch and casein to study lipid peroxide formation in irradiated synthetic diets and the effects of storage after irradiation.

The publication by Santomá et al. (1987), which was already mentioned in the beef tallow subsection, compared tallow and lard with sunflower oil, by-products from the oil-refining industry, soy lecithin, and their mixtures. The type of fat was found to have no effect on rabbit growth. These results suggest the possibility of feeding rabbits with animal fats (including pork lard) in addition to vegetable oils.

In another study, Fraga et al. (1989) investigated the suitability of lard in diets for lactating does. Supplemental fat improved milk yield, litter weight at 21 days and survival of young rabbits, but had no effect on doe fertility and chemical composition of milk.

In many experiments described above, lard was not an experimental factor; it was added to the diets to improve their energy value, or was a standard ingredient of the diet. By way of example, the study by Bertomeu et al. (1990), which used lard as a dietary component, examined vascular synthesis in rabbits used as a model species. In turn, Gillis et al. (1992) fed rabbits with 10% of lard, fish oil or safflower oil in a study concerning the influence of dietary fat on the pharmacodynamics of propafenone in isolated, perfused rabbit hearts. Likewise, in the study by de Blas et al. (1995) lard was not an experimental factor but was used to balance rabbit diets for energy when investigating crude fibre fractions.

During the research on the effect of different dietary energy levels on reproductive performance in pregnant and lactating does, Xiccato et al. (1995) supplemented 2.5% of mixed fat containing 60% lard and 40% beef tallow. Likewise, Slim et al. (1996) supplemented rabbit diets with lard as one of the fats to investigate the effect of fat on oxidative stress. Lard accounted for 65% in the mixtures of animal fats added to rabbit diets by Oliver et al. (1997) and Pascual et al. (1998, 1999).

Large amounts of lard (14%) were added to rabbit diets, together with beef tallow, by Kritchevsky et al. (1998). The results of studies reported in the above paragraph were discussed in the subsection discussing beef tallow. Lard was also used in obesity and hypertension studies performed in rabbits (Carroll, 1999; Carroll et al., 1999; Dwyer et al., 2000). These studies used 10% fat consisting of 1/3 lard and 2/3 corn oil. In another study, concerned with physiology of postprandial lipid responses in rabbits, rabbit diets contained 7.5% lard (Juhel et al., 1999). Antic et al. (1999) studied cardiovascular and renal function of rabbits ingesting a high-fat diet containing 10% fat, in which lard was mixed with soybean oil at a 1:1 ratio. In their next studies, the same team examined the sympathetic nervous system during the development of obesity-induced hypertension in rabbit (Antic et al., 2000) and loss of nocturnal dipping of blood pressure and heart rate in obesity-induced hypertension in rabbits (Antic et al., 2001) fed a high-fat diet with a 10% mixture of soybean oil and pork fat. Polichetti et al. (2000) added 5% lard to rabbit diets when studying cholesterolemia in hypercholesterolemic rabbits.

Lard formed 65% of the mixture of fats added to rabbit diets by Hernández et al. (2000) who investigated the effect of three diets of different fat type and content (animal fat and vegetable fat) on meat quality. The same fat mixture was fed to rabbits by Pascual et al. (2002 a, b) in an experiment designed to determine the effect

of dietary energy source on the performance and perirenal fat thickness evolution of primiparous rabbit does, which was reported in the subsection on beef tallow.

Yin et al. (2002) reported that a diet rich in saturated fat (with 10% lard) and sucrose alters glucoregulation and induces aortic fatty streaks in rabbits. In their next study on glucose and lipid metabolism in rabbits, the same authors used lard in high-fat and high sucrose diets (Yin et al., 2003).

Aguilera et al. (2002, 2003) fed rabbits with a diet containing 95.7% standard chow, 3% lard and 1.3% cholesterol to provoke an atherosclerotic process. Lard was not an experimental factor but was used to elevate the dietary fat level. In the first study (Aguilera et al., 2002), animals were subsequently fed a basal diet supplemented with sunflower oil, virgin olive oil and fish oil. The experiment demonstrated that the replacement of a high cholesterol-saturated fat diet by another cholesterol free-unsaturated fat diet did not regress atherosclerosis in rabbit. However, sunflower oil provoked a significant progression in lesion development, whereas diet enrichment with extra virgin olive oil and, to a lesser extent, fish oil, stopped this progression. In the second study (Aguilera et al., 2003), the same team showed that virgin olive and fish oils enhance the hepatic antioxidant defence system in atherosclerotic rabbits. The intake of cholesterol- and lard-enriched diet led to a high impairment in the hepatic antioxidant defence system. However, the replacement of that diet by other unsaturated fat-enriched diets using virgin olive, sunflower and fish oils enhanced hepatic antioxidant defence system, virgin olive and fish oil diet providing the best results.

As pointed out before, lard was used by many authors, also during the research concerning atherosclerosis in rabbits. Mesa et al. (2003), who fed the experimental group with 3.5% lard and 1.5% cholesterol, found that turmeric extract inhibits erythrocyte and liver microsome membrane oxidation in rabbits fed with an atherogenic diet. Chen et al. (2003) supplemented diets with 3% lard in a study concerning the effect of *Hibiscus sabdariffa* extract on rabbits with experimental atherosclerosis. In this case, lard also served to increase the dietary fat level. The situation was similar in the study by Lapenna et al. (2004).

Kitajima et al. (2004) fed transgenic rabbits with a mixture of lard and corn oil (1:2 ratio) to study dysfunctions of lipoprotein lipase. The results showed that systemically increased lipoprotein lipase activity improves insulin resistance and reduces adipose accumulation in transgenic rabbits, indicating that systemic elevation of lipoprotein lipase may have potential benefits for the treatment of insulin resistance and obesity. A similar mixture of fats was used by Carroll et al. (2005), who evaluated the effect of a high-fat diet on the loss of diurnal rhythms of blood pressure and heart rate in rabbits. To increase the energy value, the experimental diets of rabbits were supplemented, as previously, with a 10% mixture of lard and corn oil. Also Soler et al. (2004) described the effect of digestible fibre/starch ratio and animal fat level in diets around weaning on mortality rate of rabbits. According to these results, in order to reduce the mortality rate, it seems advisable to increase digestible fibre/starch ratio instead of increasing the animal fat content in diets for rabbits around weaning, but further research is necessary to develop adequate feeding programmes to maintain reduction of mortality during the rest of growing period.

Aguilera et al. (2005) studied the effect of adding not only lard but also fish oil to the rabbit diet, which is why this study will be discussed in the subsection on fish oil.

Chen et al. (2005) evaluated the effect of mulberry extract on the development of atherosclerosis in cholesterol-fed rabbits after adding 3% lard to the diet of experimental rabbits. The objective of the study by Chen and Li (2008) was to assess the effects of different dietary fat varieties and levels on growth performance, nutrient digestion and caecal fermentation of 2- to 3-month-old meat rabbits. The diets contained 2 to 6% of either lard or soybean oil. Compared with the control group, the lard addition decreased the average daily intake and improved the feed conversion rate. Compared with the control group, the fat addition increased the coefficients of total tract apparent digestibility of ether extract and decreased the pH and acetic acid/propionic acid ratio in the caecum, but did not affect the digestibility of crude fibre and energy. When the dietary fat level increased, pH and acetic acid/propionic acid ratio decreased and butyric acid content in the caecum increased.

Aragno et al. (2009) reported that in rabbits fed a high-fat diet (10% fat, of which 1/3 was lard), cardiac impairment is counteracted by dehydroepiandrosterone supplementation. Wu et al. (2009) investigated the changes in knee cartilage morphology of New Zealand White rabbits fed with high-sugar and high-fat diet and explored the effect of high-sugar and high-fat diet on articular cartilage degeneration. The authors concluded that long-term high-fat diet or high-sugar and high-fat diet may induce or aggravate the disorder of articular cartilage, suggesting that it may contribute to the development of osteoarthritis. In turn, Zheng et al. (2009) studied fat and cholesterol diet induced lipid metabolic disorders and insulin resistance in rabbit. They fed the animals an experimental diet with 10% fat (a mixture of 6.7% corn oil and 3.3% lard). The results showed that the high-fat diet induced insulin resistance, increased adipose accumulation and atherosclerotic lesions in rabbits, suggesting that the experimentally fed rabbits can serve as a model for the research on human insulin resistance and lipid metabolism abnormalities.

Obesity in angora rabbits was also studied by Dhungel et al. (2009), who fed the animals with diets containing 10% mixed fat (70% corn oil and 30% animal fat). They found that a high-fat diet may cause obesity in rabbits, which can be used as a model for other animals and for humans.

Casado et al. (2010) investigated the effects of the dietary inclusion of fats of different origin (lard or vegetable oil), fatty acid profile (linseed or sunflower), oxidation level and vitamin E supplementation on the rabbit diet apparent digestibility. Contrast analysis between groups of diets showed that lard, characterized by a greater saturated fatty acid content, compared with vegetable oils, rich in unsaturated fatty acid, reduced the apparent digestibility of ether extract. However, there were no significant differences in the nutrient digestibility when linseed or sunflower oils (rich in ω -3 or in ω -6 polyunsaturated fatty acids, respectively) were compared. The oxidation degree of the sunflower oil and the supplementation with 100 ppm of vitamin E to the diets did not modify the apparent digestibility values of any dietary fraction. Likewise, Zhou et al. (2012) supplemented diets with 5% lard and 1% cholesterol in a study of high-fat diet-induced atherosclerosis in rabbits.

Another publication by Casado et al. (2013) concerned the effect of dietary inclusion of different fatty acid combinations (saturated, *n*-3 and *n*-6 polyunsaturated fatty acids; PUFA) on caecal fermentation activity, feed intake, growth rate and feed efficiency of young rabbits around weaning and fattening rabbits. Five diets were formulated using the same raw materials and including 3% of lard diet (rich in saturated fat), sunflower oil (diet rich in *n*-6 PUFA), linseed oil diet (rich in *n*-3 PUFA), sunflower oil and lard at a 1:1 ratio, or linseed oil and lard at a 1:1 ratio. The use of lard or vegetable oil and the richness in *n*-3 or *n*-6 PUFA of vegetable oils had no significant effect on the feed intake (130 g dry matter/d) and growth rate of fattening rabbits, but the inclusion of sunflower oil in the diet improved feed conversion rate. Mortality rate was lower when vegetable oils were included in the diet. Feed intake and growth rate increased quickly from 17 to 44 d, but only small occasional differences were recorded in growth rate of young rabbits, in favour of rabbits receiving animal fat from 17 to 21 days or sunflower oil from 28 to 30 days. Caecal traits of rabbits at 30 and 44 d of age were similar for the different dietary groups, although butyric acid concentration in caecum content at 44 d was the lowest with linseed oil diet.

In the study by Hu et al. (2013), 5% lard was added to rabbit diets to increase fat and energy levels. In many experiments with rabbits, lard was added to the control diets. This was the case in the study by Rebollar et al. (2014), who fed rabbit does with a commercial supplement containing 50% ether extract and 35% of total fatty acids (FAs) as PUFA *n*-3 and determined its effect on reproductive long term effects.

Also in the study of Juśkiewicz et al. (2014), the experimental factor was not lard (which served to increase the energy level of rabbit diets), but inclusion of black currant (*Ribes nigrum*). The situation was similar in the study of Jurgoński et al. (2014), who evaluated the effect of blackcurrant pomace on the intestinal tract and serum lipid changes induced by a high-fat diet in rabbits. Likewise, in the study by Zou et al. (2014) concerning the effects of serum lipid smoothness on the progression and vulnerability of atherosclerotic plaque in rabbits, lard was used to elevate the level of dietary fat. In turn, Song and Xin (2014) added as much as 20% lard in research concerning the effect of high-fat diet on rabbit knee articular cartilage. Similarly in the other studies on high-fat and high cholesterol diet-induced atherosclerosis and non-alcoholic steatohepatitis in rabbit, lard served to increase the level of fat (Liaw et al., 2014).

Sun et al. (2014) studied the effect of a high fluoride and high-fat diet (including 5% lard) on serum lipid levels and oxidative stress in rabbits. In summary, high fluoride and high fat increased serum total cholesterol and low density lipoprotein cholesterol levels individually and synergistically, which caused and aggravated hypercholesterolemia in rabbits. At the same time, high fluoride and high fat both made the accumulation of product of oxidative stress in experimental animals. Animal fat was also used by García-Quirós et al. (2014) to diversify energy sources in rabbit diets. The authors evaluated how a rabbit line selected for robustness and two other lines selected for productive criteria, could have affected the physiological maturity and blood leukocyte counts of young rabbits at weaning, as well as their possible effect on the subsequent performance and health status during the growing period. In

conclusion, the authors stated that the foundation of a line for reproductive longevity, which has been previously reported to give greater robustness (low environmental sensitivity) to their reproductive stock, could have also conferred a greater immunological development at weaning to their offspring, as well as a better ability to confront digestive disorders as compared to other lines founded or selected exclusively for productive criteria.

5% lard was added to the experimental diet by Liang et al. (2015) in a study in which rabbits were an abdominal aortic atherosclerosis model, and by Zhang et al. (2015) and Bao et al. (2015) in the studies of atherosclerosis in rabbits.

The aim of the study by Burgos et al. (2015) was to investigate the consequences of a diet high in cholesterol and saturated fat on the exocrine pancreas. Control rabbits were fed a hypercholesterolemic diet containing 95.7% standard chow, 3% lard, and 1.3% cholesterol. The hypercholesterolemic diet increased monounsaturated fatty acids and decreased saturated fatty acids in pancreatic plasma membranes compared with the standard chow. These results suggest a homeostatic adaptation of the pancreas to an atherogenic diet and a greater resistance to the development of lesions than exists in other organs. According to the authors, further studies are needed, given the lack of research on this issue.

Arnau-Bonachera et al. (2018 a) hypothesized that it is possible to modulate acquisition and allocation of animals from different genetic types by varying the main energy source of the diet. To test this hypothesis, they used rabbit does belonging to three genetic types: a maternal line characterized by hyper-prolificacy; a maternal line characterized by functional hyper-longevity; a paternal line characterized by growth rate. Does were fed with two isoenergetic and isoprotein diets differing in energy source: animal fat (6% lard) enhancing milk yield and cereal starch promoting body reserves recovery. Results from this work indicate that the resource acquisition capacity and allocation pattern of rabbit does is different for each genetic type. Moreover, it seems that by varying the main energy source of the diet it is possible to modulate acquisition and allocation of resources of the different genetic types. However, the response of each one depends on its priorities over time. In the next experiment by the same team (Penadés et al., 2018), where rabbits received the same diets as in the experiment of Arnau-Bonachera et al. (2018 a), the results indicated that there are detectable variations in the leucocyte profile depending on the reproductive stage of the animal (parturition, weaning or none of them). Moreover, foundation for reproductive longevity criteria allows animals to be more capable of adapting to the challenges of the reproductive cycle from an immunological viewpoint. In turn, Arnau-Bonachera et al. (2018 b) concluded that genetic types prioritized different fitness components and that diets could affect them. In this sense, it seems that more specialized genetic types were more sensitive to diets than the more generalist type.

Rodríguez et al. (2019 a) fed control rabbits with lard at 0.75% of the diet in a study on the supplementation of diets with fish oil, which will be discussed in the subsection on fish oil.

Lard was undeniably the most popular animal fat used in rabbit feeding. In the initial stage of the research, it was used as an experimental factor, just like beef tallow. The effect of lard on performance of rabbits from different age groups was

investigated. However, at a later stage it occurred in pure form or was mixed with other animal or vegetable fats mainly as a factor increasing the dietary fat level in experiments concerning the health status of rabbits on high energy diets. These studies prove an important role of rabbits as experimental model animals for human medicine.

Poultry fat in rabbit feeding

It is notable that pure poultry fat was less often used in rabbit feeding than other animal fats. A high level of this fat (up to 9%) was only used in the experiment of Hemid et al. (1995) on the effects of dietary fat and/or oil on rabbit productive performance under intensive meat production. However, such a high level of poultry fat adversely affects pellet quality. A 5% level of fat was considered optimal. Furthermore, Slim et al. (1996) supplemented rabbit diets with poultry fat among other fats, when studying the effect of fat on oxidative stress.

Poultry fat occurs more often in mixtures of fat. In the experimental mixtures of animal fats used by Oliver et al. (1997) and Hernández et al. (2000), poultry fat accounted for 10% of the mixed fats added to rabbit diets in studies on the effect of different fat types (animal and vegetable) and contents on meat quality. Poultry fat also accounted for 10% of the animal fat mixtures supplemented to diets by Pascual et al. (1998, 1999, 2002 a) during studies on the effect of fats on reproductive performance of rabbits, and by Pascual et al. (2002 b) in a study investigating a lucerne-based feeding programme for rabbits. These issues were described in previous subsections.

The aim of the study by Klinger et al. (2017) was to evaluate the performance of growing rabbits fed on dry mash with different lipid sources. The animals were fed diets with 2.5% pork lard, sunflower oil and soy oil addition. It was observed that the lipid source did not significantly alter the performance, carcass weight, liver weight and skin weight.

Andrade et al. (2018) evaluated the effect of adding poultry fat, beef tallow and vegetable fats to the diets on rabbit meat quality. The poultry fat was added at 2.5% of the diet. The results of this publication were presented in an earlier subsection on beef tallow.

Fish oil in rabbit feeding

Fat in the form of fish oil is among the various types of fats fed to rabbits. It should be noted as a preliminary point that this fat was included in the diet not just for its energy value. Over the last decades, the potential role of fish oil (rich in omega-3 polyunsaturated fatty acids) in preventing various diseases, has become the centre of attention worldwide. In rabbit feeding, fish oil not only provides the energy, it can also be a carrier of fatty acids which have positive effects on the overall health and reproduction of these animals, and on the quality of their meat, including the health-promoting benefits for consumers. Most of the studies presented below were performed in these directions. In many experiments, rabbits were model animals and the obtained results may be relevant to human health.

As early as 1980, herring oil, lard and corn oil mixed with starch and casein were added to rabbit diets during studies investigating lipid peroxide formation in

irradiated synthetic diets and the effects of storage after irradiation (Wills, 1980). In the early 1990s, dietary fish oil was administered to rabbits as model animals by Bertomeu et al. (1990), who investigated the association between fish oil diet and decreased thrombosis, as related to changes in platelet and vessel wall prostanoid synthesis. Moreover, fish oil as a carrier of some fatty acids with potential benefits in reproductive problems of rabbit does was described by Lin et al. (1991). Among others, they reported a high increase of docosahexaenoic acid (DHA) in newborn rabbits whose mothers were fed fish oil, suggesting placental transfer of DHA and incorporation into retinal lipids.

Zhu et al. (1988) conducted research on inhibition of atherosclerosis by fish oil in cholesterol-fed rabbits and showed that dietary fish oil can attenuate the development of aortic and pulmonary atherosclerosis in cholesterol-fed rabbits. The increased bleeding time appeared to confirm the known anti-platelet effects of fish oil.

Gillis et al. (1992), during the above-mentioned study concerning the influence of dietary fat on the pharmacodynamics of propafenone in isolated, perfused rabbit hearts added 10% fish oil to the diet. The authors observed that dietary fat significantly altered the fatty acid composition of the phospholipids in sarcolemma. Propafenone effects on ventricular conduction time and ventricular excitability were significantly influenced by the type of dietary fat.

Ritskes-Hoitinga et al. (1998) studied the association of increasing dietary concentrations of fish oil with hepatotoxic effect and higher degree of aorta atherosclerosis in rabbits. Similar research was performed by Ramírez-Tortosa et al. (1998). The aim of this study was to investigate the composition of plasma lipoproteins and their susceptibility to oxidation in rabbits with experimental atherosclerosis provoked by the intake of a diet rich in cholesterol and saturated fat as well as the influence of the intake of four diets differing in their lipid profiles (fish, refined olive, virgin olive and sunflower oils) on plasma lipoprotein composition and susceptibility to oxidation of these atherosclerotic rabbits. Diets rich in monounsaturated and *n*-3 polyunsaturated oils showed the same efficacy in restoring the normal lipid profiles in atherosclerotic rabbits provided the fish oil was adequately stabilized with antioxidants.

Fish oil added at 10% was found to adversely affect cortical bone morphology and biomechanics in growing rabbits. This level of supplementation in the presence of modest (too low) vitamin E supplementation can have detrimental effects on skeleton of rapidly growing rabbits (Judex et al., 2000).

Hsu et al. (2001) studied the effects of fish oil and vitamin E on the antioxidant defence system in diet-induced hypercholesterolemic rabbits. The diets of experimental groups contained 10% refined edible fish oil. The results indicated that a high fat and cholesterol diet attenuates blood glutathione levels and plasma antioxidant enzyme activities, which may account for some of its atherogenic properties. Consumption of fish oil enhances antioxidative defences against the oxidative stress imposed by hypercholesterolemia, and vitamin E further enhances these beneficial effects.

Aguilera et al. (2002) induced an atherosclerotic process in rabbits with a high-fat diet. Next, the animals were fed a standard chow diet with sunflower oil, virgin olive oil and fish oil. The replacement of a high cholesterol-saturated fat diet by another

cholesterol free-unsaturated fat diet did not regress atherosclerosis in rabbit. However, sunflower oil provoked a significant progression in lesion development, whereas diet enrichment with extra virgin olive oil and, to a lesser extent, fish oil, stopped this progression. In the next publication, the same research team reported that virgin olive and fish oils enhance the hepatic antioxidant defence system in atherosclerotic rabbits (Aguilera et al., 2003). The intake of cholesterol- and lard-enriched diet led to a high impairment in the hepatic antioxidant defence system. However, the replacement of that diet by other unsaturated fat-enriched diets using virgin olive, sunflower and fish oil enhanced hepatic antioxidant defence system, virgin olive and fish oil diet providing the best results. In another study, Aguilera et al. (2005) investigated whether administration of diets differing in fatty acid profiles (omega-3 PUFA, omega-6 PUFA, or MUFA) influence the content of biliary lipids, the lithogenic index of gallbladder bile, and the development of hepatic fibrosis in hypercholesterolemic rabbits. There was a beneficial effect of MUFA and omega-3 PUFA on hepatic fibrosis in hypercholesterolemic rabbits because both dietary fats led to recovery from hepatic lesions. However, because intake of omega-3 PUFA provoked lithogenic bile in rabbits, MUFA intake would be more advisable. The same year, Torres et al. (2005) described another study concerning atherosclerosis in rabbits fed with fish oil diets.

Verkerk et al. (2009) evaluated the effect of adding fish oil on reducing pacemaker and heart rate in rabbits. The fish oil diet prolonged the sinus cycle length because of, at least in part, a reduction in cardiac pacemaker I(f) density. The results showed that fish oil induced heart rate reduction and suggested fish oil as an alternative or adjunct to I(f)-lowering drugs.

A number of studies on the effect of fish oil in rabbit diets were conducted in Poland by Kowalska and her team (Kowalska and Bielański, 2009; Kowalska et al., 2011, 2015; Kowalska, 2015), which are discussed chronologically below. Kowalska and Bielański (2009) concluded that 3% fish oil supplemented to rabbit diets had a beneficial effect on the composition of the meat lipid fraction. Relative deposition of *n*-3 PUFA increased and the decrease in the *n*-6/*n*-3 PUFA ratio was beneficial from the standpoint of modern human dietetics. Analysis of the mean cholesterol content of meat showed a significant reduction in cholesterol in animals from the experimental groups. Fish oil made the fat of stored meat slightly more susceptible to oxidation, but the dietary vitamin E supplement prevented lipid oxidation. The study confirmed that it is appropriate to use dietary antioxidants in rabbit feeds containing fats rich in unsaturated fatty acids. The fish oil supplement had a beneficial effect on reducing carcass fatness and improving the juiciness and tenderness of rabbit meat.

The study that followed described the effect of supplementing the diet with 3% linseed or fish oil as well as an antioxidant (α -tocopherol acetate) on the fatty acid profile, cholesterol and vitamin E content of rabbit meat lipids. The efficiency of transformation of fatty acids in dietary fats to fatty acids in muscle tissue indicates that long-chain fatty acids from fish oil are physiologically most effective in enriching the tissues with *n*-3 PUFA. The study also confirmed the appropriateness of using feed antioxidants when feeding diets high in unsaturated fatty acids (Kowalska et al., 2011).

A valuable review paper on the effect of fish oils rich in omega-3 fatty acids on male fertility in rabbits was published by Castellano et al. (2012). In conclusion, the authors stated that dietary fish oil may help to improve male fertility by increasing omega-3 polyunsaturated fatty acids in sperm cells and would be most beneficial with sperm of initial poor quality.

A study with neonatal rabbits as model animals for parenteral feeding was conducted by Zhu et al. (2014). They evaluated if supplementing a lipid emulsion containing fish oil rich in ω -3 acids may reduce signs of parenteral nutrition-associated liver disease. The researchers found that incorporating ω -3 fish oil in parenteral nutrition emulsion greatly prevented liver dysfunction and liver tissue damage in week-old rabbit kits, possibly by preventing endoplasmic reticulum stress.

An interesting study by El-Moghazy et al. (2014) was aimed to evaluate the possible effects of a partial replacement of soybean meal in control economic diet by different concentrations of fish oil on the possible harmful changes in histological structure of liver and kidney and blood parameters in rabbits. Results showed that feeding diet supplemented with fish oil significantly increased the percentages of hemoglobin, platelets, the mean corpuscular hemoglobin, white blood cell (WBCs) count, total proteins, albumin, albumin/globulin ratio, aspartate aminotransferase and testosterone and significantly decreased the total lipids, cholesterol and triglycerides. Fish oil is a good supplement for growing rabbits as it has no adverse effects on histological structure of their liver and kidney.

Rebollar et al. (2014) found a greater deposition of EPA and DHA in the periovarian adipose tissue than in the interscapular fat of pregnant rabbit does after a long-term dietary supplementation with a 15% preparation containing salmon fish oil, ether extract, *n*-6 and *n*-3 fatty acids, and vitamin E.

Tres et al. (2014) assessed the impact of the oxidative quality of fish oils in feeds on the composition and oxidative stability of chicken and rabbit meat. This study showed that not only the fatty acid composition of fish oils, but also their oxidative quality affects the final nutritional and stability properties of meat, liver and plasma. Both chicken and rabbit meat and tissues were enriched in *n*-3 fatty acids, but the amounts varied depending on the content of *n*-3 fatty acids in the fish oil, reaching values around 1000 mg/100 g in chicken and 230 mg/100 g in rabbit meat.

In an another study, Ivanova et al. (2015) supplemented fish oil and krill oil (*Euphausiacea*) in research on glucose tolerance in obese New Zealand White rabbits. The results showed that fish oil and krill oil supplementation decreases fasting blood glucose and improves glucose tolerance in obese rabbits. This could be ascribed to the ameliorated insulin sensitivity and insulin secretion and modified gene expressions of some key enzymes involved in β -oxidation and lipogenesis in liver and skeletal muscle.

Kowalska et al. (2015) compared sensory quality of rabbit meat from animals fed a diet supplemented with 2% fish oil and various concentrations of α -tocopherol acetate (0, 40 or 100 mg/kg diet) depending on packaging and storage methods (vacuum packing and cold storage for 14 days, or freezing in ziploc bags for 14 days). The varying vitamin E supplements in the diets had a significant effect on the aroma, flavour, juiciness, tenderness and sensory quality of the meat, and on thermal loss. The

packaging and storage method significantly affected cooking loss, aroma, flavour, juiciness and total sensory quality, and tenderness of the meat. Significant interactions (amount of vitamin E added $\times$ packaging and storage method) were found for aroma, flavour, tenderness, sensory quality and juiciness. Highest scores were given to the samples of vacuum packed meat from rabbits supplemented with 100 mg/kg vitamin E.

The aim of the next study by Kowalska (2015) was to modify the fatty acid composition of rabbit fat using complete pelleted diets with 2% rapeseed or fish oil. The use of rapeseed and fish oils in the diet had a favourable effect on the final body weight of the animals. As a result of supplementation with fish oil, the $n-6/n-3$ PUFA ratio in the adipose tissue of the rabbits decreased significantly, which is beneficial for the consumers of this meat. There was a considerable improvement in the thrombogenic index after adding fish oil to the rabbit diets.

The research of Zhu et al. (2014) was followed up by the study of Zhu et al. (2016), which hypothesized that soybean oil-based lipid emulsions (SOLE) and fish oil-based lipid emulsions (FOLE) may have distinctive effects on hepatocyte lipid droplets (LD) accumulation and endoplasmic reticulum (ER) stress. The study was performed with primary newborn rabbit hepatocytes. The authors revealed concluded SOLE, but not FOLE, damage the ER and mitochondria, resulting in LD accumulation and ER stress and, finally, hepatocyte injury. This ultimately induces hepatocyte injury, which likely explains the differential impacts of soybean and fish oils on parenteral nutrition-associated liver disease development and progression.

A significant contribution to the discussed area was made by a Spanish team in a series of publications (Rodríguez et al., 2017 a, b, 2018, 2019 a). The objective of the study by Rodríguez et al. (2017 a) was to investigate the effect of dietary supplementation with fish oil on growth performance, carcass characteristics and fatty acid profile of muscle and fat tissues, as well as caecal fermentation and ileal mucosa morphology of growing rabbits. Two isonitrogenous and isoenergetic diets, only differing in their fat source (lard or salmon oil), were formulated and provided each to rabbit does and their offspring during pregnancy and lactation. The control diet contained 4.59 g of $n-3$ per 100 g of total fatty acids (FA), and the enriched diet contained 14.9 g of $n-3$ per 100 g of total FA. There were no differences between groups in basic production parameters during the growing period. At slaughter, body weight, full gastrointestinal tract weight, carcass yield, meat colour and pH, drip loss percentage, content of scapular fat and tissue composition of the left hind leg were similar between groups, but perirenal fat was lower and skin weight and abdominal fat tended to be lower in enriched rabbits than in control ones. Total PUFA content in both *longissimus dorsi* muscle and perirenal fat was greater in enriched rabbits, which also showed lower $n-6/n-3$ ratios in *longissimus dorsi* muscle and perirenal fat than those fed the control diet. Caecal concentrations of total VFA were greater in enriched than in control group at 30, 45 and 60 d of age, but diet did not affect VFA profile, with the exception of a lower proportion of minor VFA (sum of isobutyrate, isovalerate, and valerate) in control group. Diet did not affect either pH and NH-N concentrations in the caecum or ileal morphology. In summary, the results showed that dietary fish oil supplementation enhanced beneficial long-chain $n-3$ FA and de-

creased $n-6/n-3$ ratio in rabbit meat and fat, being healthier for human consumption, without having negative effects on growth performance, caecal fermentation, and ileal morphology or carcass characteristics. Continuing their research, Rodríguez et al. (2017 b) presented the effect of the experimental factor examined in the previous study (Rodríguez et al., 2017 a) on metabolic and endocrine response of rabbit does and their offspring. The study showed that an increase of $n-3$ PUFA concentration in the diet of rabbit does and, consequently, of their offspring during a productive cycle alters their lipid profile and the indicators of oxidative stress, without major endocrine modifications or improvements in the productive variables. Rodríguez et al. (2018) confirmed the effect of enriching a diet with polyunsaturated fatty acids on mitigating reproductive performance issues in the second cycle. The control diet contained mixed fats and the experimental group diet was enriched with $n-6$ and $n-3$ fatty acids. Feed intake was similar in both groups. Plasma progesterone concentration was higher in does fed the enriched diet. Reproductive performance and litter parameters were similar in both groups. However, in the second production cycle reproductive performance of the does did not deteriorate as much as in the control group. Embryo apoptosis rate was higher in the control group than in the enriched group. In summary, enrichment of the does diets with PUFA over two production cycles improved plasma progesterone level during gestation, fertility, milk fatty acid profile and neonatal development, which is indicative of the beneficial effect of PUFA supplementation in rabbits.

Abdel-Raheem et al. (2018) presented a study in which they determined the effect of dietary inclusion of 3% animal fat or different oils (palm, sunflower and fish oil) on productive performance, milk yield and milk composition of rabbit does. The highest total number of litters born alive was recorded in the group fed on diets supplemented with fish oil and polyfat. Also the highest total number of litters at 21 and 30 days of age was recorded in the group fed diet supplemented with 3% fish oil. The lowest mortality rate was reported in groups fed diets supplemented with sunflower oil, palm oil and fish oil, in comparison with the control group. Milk yield of the groups fed diets containing 3% fish oil was higher than that recorded in the control group. Animal fat had no effect on the analysed parameters with the exception of milk yield, which was higher than in the control group.

In another study, Rodríguez et al. (2019 a) tested the hypothesis that salmon oil supplementation to early weaned rabbits would positively affect animal performance by reducing morbidity and/or mortality and improving meat FA profile. Therefore, the objective of this study was to analyse the influence of replacing lard with fish oil in the diet of rabbits weaned at 25 days of age on growth performance, meat quality, caecal fermentation, and ileal morphology. The experiment demonstrated that including fish oil (containing PUFA) in the diet of early weaned rabbits can reduce their morbidity and enhance the content of beneficial long-chain $n-3$ fatty acids in rabbit meat and fat, but it also slightly reduced their growth.

One of the most recent studies on fish oil in rabbit feeding is the publication of Mattioli et al. (2019). The aim of this study was to evaluate the effect of dietary supplementation with flaxseed or fish oil on PUFA metabolism in rabbit does. Feed consumption and milk production were not affected by the feeding regime. The fatty

acid composition of milk, plasma, liver and ovaries was widely influenced by diet, showing higher concentrations of *n*-3 long-chain PUFA (LCP) in does fed with *n*-3 enriched diets. FISH diet resulted in the highest *n*-3 LCP enrichment, whereas in the flaxseed group, this increase was lower. Blood and milk showed low levels of LCP, whereas liver and ovaries were the main sites of *n*-3 LCP synthesis and accumulation. Accordingly, although the liver is the main metabolic centre for LCP synthesis, ovaries also have a prominent role in LCP generation. FADS2 expression in liver and ovary tissue was downregulated by FISH administration. In conclusion, the enrichment of diets with *n*-3 PUFA could be an effective strategy for improving the reproductive performance of does.

Grigorova et al. (2019) conducted research on obesity in rabbits. Their study investigated the effect of fish oil supplementation and restricted feeding on body fat distribution and blood lipid profile in experimentally induced obesity in rabbits. The results of this study indicate that fish oil supplementation reduced obesity-associated abnormalities in lipid profile (high-density lipoprotein cholesterol to low-density lipoprotein cholesterol ratio and non-esterified fatty acids) and in body fat distribution in full-diet fed rabbits. Restricted feeding alone and the combination of restricted feeding and fish oil supplementation in particular, has a detrimental effect on the lipid profile despite the marked reduction in intra-abdominal fat.

Castellini et al. (2019) investigated the effect of different dietary sources of *n*-3 polyunsaturated fatty acids (PUFA) from extruded flaxseed and fish oil on reproductive traits using rabbit buck as the animal model. Results showed that dietary administration of *n*-3 PUFA improved the track speed of the sperm and increased the *n*-3 long-chain PUFA mainly confined in the sperm tail. Seminal plasma increased the thiobarbituric reactive substances by three times in the groups fed supplemental *n*-3, whereas the F2-isoprostanes (F2-IsoPs) and F4-neuroprostanes (F4-NeuroPs) were lower and higher, respectively, in both supplemented groups than in the control. The testes and sperm showed a higher DHA and EPA distribution in rabbits from the *n*-3 supplemented groups compared with the control. In conclusion, supplemental dietary *n*-3 PUFA improved sperm motion traits and resulted in an enrichment of membrane fatty acid in the sperm and testes of the rabbits. However, such an increased amount of PUFA negatively affected the sperm oxidative status, which was mainly correlated with the generation of F4-NeuroPs with respect to F2-IsoPs. Accordingly, the latter cannot be considered a good marker of oxidation when diets rich in *n*-3 PUFA are provided.

The application of fish oil mainly comes from the conviction that it has health-promoting benefits, especially for the cardiovascular system, resulting from a considerable content of omega-3 unsaturated fatty acids. In addition, this oil contains many vitamins and fights inflammation. A valuable review article was published by Rodríguez et al. (2019 b), who presented current knowledge of *n*-3 PUFA sources for rabbits. This review compares the effects of different *n*-3 PUFA sources on biological activity, physiological/reproductive endpoints, and health implications with a special emphasis on a rabbit case study. Linoleic acid (LA) and α -linolenic acid (ALA) are members of two classes of PUFAs, namely the *n*-6 and *n*-3 series, which are required for normal human health. Both are considered precursors of a cascade of molecules

(eicosanoids), which take part in many biological processes (inflammation, vasoconstriction/vasodilation, thromboregulation, etc.). However, their biological functions are opposite and are mainly related to the form (precursor or long-chain products) in which they were administered and to the enzyme-substrate preference. ALA is widely present in common vegetable oils and foods, marine algae, and natural herbs, whereas its long-chain PUFA derivatives are available mainly in fish and animal origin products. Recent studies have shown that the accumulation of *n*-3 PUFAs seems mostly to be tissue-dependent and acts in a tissue-selective manner. Furthermore, dietary *n*-3 PUFAs widely affect the lipid oxidation susceptibility of all tissues. In conclusion, sustainable sources of *n*-3 PUFAs are limited and exert a different effect about the form in which they are administered, precursor or derivatives, their antioxidant protections and the purpose to be achieved – health improvement, physiological and reproductive traits, metabolic pathways, etc. The above review can be considered as a partial summary of the present subsection. Of major significance was also the research concerning the effect of fish fat and the fatty acids it contains on the quality of rabbit meat. It is also worth mentioning that many authors supplemented rabbits with dietary fish oil to increase the amount of fat in experimental diets during the study of obesity and cardiovascular diseases, notably atherosclerosis. This is because, according to Fan et al. (2015), rabbits are good models for the study of human atherosclerosis, especially for the translational medicine.

Insect and shellfish oil

First information about feeding invertebrate fat to rabbits was presented by Ivanova et al. (2015), who supplemented diets with krill oil and fish oil in a study of obesity and glucose tolerance in rabbits. The results indicated that krill oil and fish oil supplementation decreases fasting blood glucose and improves glucose tolerance in obese rabbits. This could be ascribed to the ameliorated insulin sensitivity and insulin secretion and modified gene expressions of some key enzymes involved in β -oxidation and lipogenesis in liver and skeletal muscle.

Comprehensive studies on the possibility of feeding rabbits with fat extracted from insect larvae were carried out and reported in a series of publication by an Italian research team (Gasco et al., 2017, 2019 a, b). They focused their interest on fat extracted from black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) larvae. Preliminary research was published during the INSECTA Conference in Berlin, Germany (Gasco et al., 2017), and subsequent studies in Gasco et al. (2019 a). This publication determined the effect of replacing soybean oil in diets of growing rabbits with two types of fat extracted from black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) larvae. The effects of insect fat supplementation on growth performance, nutrient digestibility, blood parameters, intestinal morphology and health of 200 rabbits divided into 5 groups were investigated. The control diet contained 1.5% soybean oil, and in the four experimental diets 50% or 100% of soybean oil was replaced with insect fats. No differences were observed between the control group and the experimental groups fed insect fats on performance, mortality and blood parameters. The addition of insect fats did not influence digestibility of dry matter, protein, ether extract, fibre fractions and gross energy. The

dietary inclusion of insect fats had no effect on gut morphometric indices and organ histopathology. The authors concluded that insect fats can replace soybean oil in rabbit diets without any detrimental effect on performance and morphometric indices.

Of paramount importance for the present discussion is another publication by Gasco et al. (2019 b). This study evaluated not only the effect of supplementing growing rabbit diets with *Hermetia illucens* and *Tenebrio molitor* fat on carcass composition, chemical composition of meat, lipid peroxidation and fatty acid profile, but also on the consumer acceptance of so produced meat. The experiment revealed that the dietary substitution had no effect on carcass traits and meat quality characteristics. The fat content of *longissimus thoracis* and *lumborum* muscles averaged 1.1%. The muscles of rabbits fed a diet with *Hermetia illucens* fat showed a higher proportion of saturated fatty acids and lower proportion of polyunsaturated fatty acids compared to rabbits fed a diet with *Tenebrio molitor* fat and soybean oil. It was also demonstrated that the meat of rabbits fed diets with insect fat was less prone to oxidation. Of interest is the information reported in the study about the scientifically confirmed consumer acceptance of meat from rabbits fed insect fats, which holds promise for this type of production.

Similar studies to those described above were conducted by a Portuguese and Italian research team and published in Martins et al. (2018) and Dalle Zotte et al. (2018).

Martins et al. (2018) studied the use of *Hermetia illucens* larvae fat in rabbit diets. Insect oils and extruded linseed were supplemented to the diets of growing rabbits at 30 g or 60 g/kg. The effects of experimental diets on mortality, dry matter intake, average daily gain, slaughter live weight, carcass, liver, perirenal fat, scapular fat, and digestive tract weights were evaluated. The values of the analysed parameters were unrelated to the dietary fat source and level. The digestibility of organic matter, ether extract and gross energy was lower in the insect fat diet than in the linseed diet, but this had no effect on growth performance and carcass yield. The authors concluded that *Hermetia* fat could be considered an alternative lipid source for growing rabbit diets.

The above study was followed up by Dalle Zotte et al. (2018), who evaluated the effect of including *Hermetia* fat or extruded linseed in rabbit diets on meat quality. The diets with insect oil reduced the intramuscular fatty acids but increased the C12:0 and C14:0 in meat compared to extruded linseed, whereas diets with linseed increased the C18:3 *n*-3 deposition in meat. Regressions between fatty acid intake and fatty acid meat concentration indicate that the deposition of C12:0 and C14:0 in the meat of insect oil-fed rabbits was lower than the deposition of *n*-3 PUFA in the meat of linseed-fed rabbits. Overall, lipid profiles of meat from insect oil-fed rabbits were less healthy, but meat from linseed-fed rabbits was more susceptible to oxidation.

The application of insect oil is the latest nutritional trend in rabbit diets. The results show that this type of fat can be used in rabbit feeding without negative effects on animal performance and meat quality. It should be noted, however, that further development of this nutritional trend will depend on the development of insect breeding, and thus on the price and availability of this raw material.

Conclusions

The scientific literature has described feeding of rabbits with beef tallow, butter, pork lard, poultry fat, fish oil, krill oil, insect larvae oil, mixtures of various animal fats and animal fats mixed with vegetable fats. Their effects on performance of growing rabbits, lactation, rearing performance, meat quality, and health of animals were evaluated. It is interesting to note that in many cases, various animal fats were often an integral part of numerous diets or were found in control diets. They were also added to the diets to balance them and to increase their energy value, especially during studies of various cardiovascular diseases and obesity, where rabbits served as model animals.

Beef tallow was initially added to rabbit diets to determine whether vegetable fats can be replaced with animal fats and to determine their optimum levels. It was also used to increase dietary energy level and to balance diets in the experiments dealing with other problems. Recent studies concentrated on the effect of dietary inclusion of beef tallow on rabbit meat quality, which fits in the field of studies concerned with production of functional foods. Butter, another bovine fat, was most often fed to rabbits not to determine its nutritional effects on these animals, but to increase the level of fat and energy in the experimental diets. This allowed for the study of atherosclerosis, obesity and many other diseases induced by high-fat diets in rabbits as model animals. Furthermore, butter was used to assess the effects of different sources of fat on selected antioxidant profile in rabbits. Lard was undeniably the most frequently described animal fat used in rabbit feeding alongside fish oil. In the initial stage of the research, just like beef tallow, it was used as an experimental factor to study the possibility of using animal fat in rabbit diets and their effects on performance. At a later stage it was most often a factor increasing the dietary fat level in many experiments concerning the health status of rabbits fed high energy diets. Interestingly, poultry fat, which is widely available in the feed market, was less often used in rabbit feeding than other animal fats. Poultry fat is most often mixed with other fats. Numerous studies concentrated on the application of fish oil in rabbit diets. This use mainly comes from the conviction that it has health-promoting benefits, especially for the cardiovascular system, resulting from a considerable content of omega-3 unsaturated fatty acids. In addition, this oil contains many vitamins and fights inflammation. Many studies evaluated the effect of this oil on reproductive performance, growth performance and health of rabbits. Later publications focused on the effect of supplemental fish oil and fish oil fatty acids on the quality of rabbit meat. It is also notable that many authors added fish oil to rabbit diets to increase the amount of fat in experimental diets when studying obesity and cardiovascular diseases, notably atherosclerosis, just as in the case of the other fats. The application of insect oil is the latest nutritional trend in rabbit diets. Research findings show that this type of fat can be used in rabbit feeding without detrimental effects on animal performance and meat quality. It should be noted, however, that further development of this nutritional trend will depend on the development of insect breeding, and thus on the price and availability of this raw material.

The information reviewed above points to the possibility of feeding rabbits with animal fat at 2–4% of the diet without adverse effects on reproductive performance,

productivity of growing animals and quality of their meat. It should also be highlighted that research on the dietary inclusion of various animal fats for rabbits contributes significantly to understanding the essence and mechanisms of many diseases induced by the consumption of high-fat diets in humans.

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