Czech University of Life Sciences Prague

Faculty of Tropical AgriSciences

Department of Animal Science and Food Processing

The effect of milk thistle (*Silybum marianum*) supplement on the reproduction of rabbit does

Master Thesis

Supervisor: Ing. Petra Silberová, Ph.D.

Author: Bc. Grey Estefanía Aguilera-Hidalgo

Declaration of Integrity

I hereby declare that this thesis entitled **“The effect of milk thistle (*Silybum marianum*) supplement on the reproduction of rabbit does”** is my own work and all the sources have been quoted and acknowledged by means of complete references.

In Prague, 24th April of 2015

Signature: ………………………………

Bc. Grey Estefanía Aguilera-Hidalgo

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Abstract

Milk thistle (*Silybum marianum*) has been used as galactogogue in rats, sows, cows and in recent years its effects on the reproduction of livestock are being studied. The present study was conducted to evaluate the effect of the Milk thistle (*Silybum marianum*) supplement on the reproduction of rabbit does. The research took place in the farm of Jiří Kočár in Ratibořice, Czech Republic, from December 2013 until December 2014. For the research were chosen 30 nulliparous does of the HYLA line between the ages of 4 to 5 months old. These were randomly divided into 3 groups: AV3 (n=10), Milk thistle (n=10) and Control group (n=10). The AV3 group received the AV3 supplement, which is a phyto-additive with milk thistle (*Silybum marianum*) and gingko (*Gingko biloba*). Milk thistle group was fed with milk thistle supplement at a dose of 0.2%. The present study shows that the use of both supplements increased the number of born and weaned kids and decreased the number of dead offspring in comparison with the control group, but there was not a statistical significant difference among the groups. The number of born and weaned kids in AV3 group was slightly higher than in Milk thistle group, this could be due to the anti-oxidative effect of AV3 supplement. Furthermore studies on does with higher concentrations of milk thistle are recommended.

Keywords: milk thistle, reproduction, rabbit, productive parameters, doe

Abstrakt

Ostropestřec mariánský (*Silybum marianum*) je používán k podpoře mlečnosti u krys, prasnic a krav, v posledních letech byl studován jeho efekt na reprodukci hospodářkých zvířat. Tato výzkumná práce byla provedena k vyhodnocení efektu doplňku ostropestřce mariánského (*Silybum marianum*) na reprodukci chovných králic. Sběr dat byl prováděn na farmě Jiřího Kočára v Ratibořicích v České republice v v roce 2014. K výzkumu bylo vybráno 30 chovných králic linie „HYLA" ve věku 4 až 5 měsíců. Tyto byly náhodně rozděleny do 3 skupin: skupina AV3, skupina ostropestřce mariánského (n=10) a kontrolní skupina (n=10). Skupina AV3 dostávala doplňek AV3 - bylinný přídavek s ostropestřcem mariánským (*Silybum marianum*) a jinanem dvoulaločným (*Gingko biloba*). Skupině ostropestřce mariánského byla krmena spolu se standardní směsí pro kojící samice také doplněk ostropestřce mariánského v dávce 0.2%. Tato studie ukazuje, že oba doplňky zvýšily počet narozených a odchovaných králíčat a snížily mortalitu v porovnání s kontrolní skupinou, ale statisticky signifikantní rozdíly mezi skupinami nebyly nalezeny. Počet narozených a odchovaných králíčat byl vyšší v AV3 skupině než ve skupině ostropestřce mariánského, což může být způsobeno antioxidačním účinkem doplňku AV3. Proto doporučujeme další výzkum s vyšší koncentrací ostropestřce mariánského v přídavku pro chovné králice.

Kličová slova: ostropestřec mariánský, reprodukce, králík, reprodukční ukazatele, králice

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1 Introduction

The first records of controlled rabbit breeding date back to the sixteenth century, when they were bred together with other small livestock (Lebas et al. 1986). Since then its management has been focused on producing more and heavier rabbits for the production of meat for human consumption and also the production of hides or wool. In the current years the demand of rabbit meat has been increasing, because it is high in proteins and low in fat and cholesterol (Lumpkin and Seidensticker, 2011) and due to its quality properties.

In tropical countries its popularity spreads not only because it is a source of meat, but also due to the easiness of rabbit management, fast growth and short reproductive cycle. A good reproduction is one of the main goals of any breeder focused in livestock production. This is why the aim is to obtain several successful pregnancies and parturitions throughout the year, with a very promising number of newborns and weaned kids. It is important to point out that feed plays a significant role in the reproduction of any animal, therefore the quality and quantity of raw materials for the production of adequate feed mixtures for each animal category is of great importance. Each category of animal should receive a balanced diet that will meet all the energy as well as nutrient requirements that are essential and needed for the maintenance of reproduction and also to keep an optimal growth and health. In the recent years researchers have focused their interests on how to increase the reproductive performance of animals with the use of medicinal plants.

Milk thistle (*Silybum marianum*) is a weed native to the Mediterranean region, which for centuries was used as a medicinal plant to treat liver disease (Flora et al., 1998), hormonal alterations and digestive problems (Martínez-Morán et al., 2011). It is rich in natural compounds called flavonolignans, which are important plant pigments and have anti-allergy, anti-inflammatory, antiviral, antifungal and antioxidant properties (Bhattaram et al., 2002; Siegel and Stebbing, 2013). Silymarin, the biologically active compound, is mainly found in the mature seeds (Alemardan et al., 2013; Milic et al., 2013; Biedermann et al., 2014). According to Balszuweit et al. (2013) the compound is non-toxic to mammals. Due to its properties and low toxicity, *Silybum marianum* has been used in human as well as in veterinary medicine to treat different health problems or enhance a positive effect on the function of the body. In human milk thistle is used to: treat cancer, increase lactation, promote cell regeneration, treat liver poisoning with green death cap mushrooms and sulfur mustard poisoning, protect the skin against UV radiation, etc. In animals was also proved that milk thistle improves the lactation in cows (Capasso et al., 2009) and in dogs was demonstrated that it could be used against poisoning (Desplaces et al., 1975; Stickel and Schuppan, 2007). The objective of this study was to analyze the effect of the milk thistle (*Silybum marianum*) supplement on the reproduction of rabbit does.

2 Literature review

2.1 Rabbit breeding

Today's rabbit belongs to the family Leporidae; it was domesticated from the European wild rabbit (*Oryctolagus cuniculus*) (Weisbroth et al., 1974). It is an herbivore that has a high reproduction capability with multiple gestations throughout the year (Skřivan et al., 2007) and who can efficiently convert fodder into food having the capacity of turning 20 % of the protein they eat into meat (Lebas et al., 1997). Rabbit meat consumption depends on cultural, traditional and religious beliefs (Hernández and Dalle-Zotte, 2010). It is important to say that in some countries, rabbits represent a pest and in others it is a key prey species as a source of food (Tablado et al., 2009).

Lebas et al. (1986) explained that the first controlled breeding records, date back to the sixteenth century when rabbits were kept with chickens in the garden of houses and their diet was based on green forage, hay, and some grains. The same authors said that in the late 19^{th} and early 20^{th} centuries the breeding of these herbivores reached its peak and during that time, breeding, selection and hygiene techniques were improved and breeders associations formed, this all meant that rabbits were not raised with other animals anymore, but were rather divided into groups. Later on rabbits started being bred for meat, skin, fur, and as laboratory animals for the testation of medicine and production of hormonal prepares (Skřivan et al., 2007).

Skřivan et al., 2007 described that the normal weight of an adult rabbit is between 4 - 4.5 kg, their skeleton is formed from 212 bones representing 9 – 10 % of the total body weight, their digestive tract is around 4.5 to 5 m and the intestines' length represents 8 to 12 times the length of the rabbit's body. This author also stated that adult animals have a total of 28 teeth, while young animals have 16. The dental formula of the rabbit is I 2/1, C 0/0, PM 3/2, M 3/3 (Fraser and Girling, 2009). According to Lebas et al. (1986), the small intestine is about 3 m long and the colon, which is divided into proximal and distal colon, measures 1.5 m.

Rabbits have a distinguishing feature in the function of the proximal colon, they produce two types of manure hard and soft (Scanes, 2011). Both types are also known as pellets; the latter ones also as night pellets or cecotrophs (Gendron, 2000). Fox et al. (1984) and Manning et al. (1994) explained that the hard pellets are expelled, but soft pellets are recovered directly from the anus and swallowed without chewing; they also remarked that cecotrophs are rich in water, vitamins, minerals and nitrogen and its consumption highly depends on the composition and productive state of the animal. Soft pellets can be recycled once, twice or even 3 to 4 times (Lebas et al., 1997). This behavior, known as cecotrophy, starts at an age of 3 weeks, when young kids start to consume solid feed (Fox et al., 1984).

2.2 Rabbit products

2.2.1 Meat

The rabbit meat has specific characteristics and its quality highly depends on the rabbit's nutrition. This meat belongs among the white or diet meat. Hernández and Dalle-Zotte (2010) explained that rabbit meat is rich in: amino acids such as lysine, threonine, phenylalanine, leucine; vitamins B_3, B_6, B_{12} and E; they also stated that rabbit meat compared to other livestock species has a low content of iron, zinc and sodium, but it is rather high in phosphorus. Skřivan et al. (2007) pointed out that this meat has a low percentage of fat with less content of stearic and oleic acids in comparison to other species. Cholesterol content in rabbit meat is on average 53 mg/100 g of fresh meat (Dalle-Zotte, 2002).

According to its texture rabbit meat could be compared to hare, lamb and road deer (Hernández and Dalle-Zotte, 2010), it has a good palatability (Warren, 2002) although it does not have a very strong flavor, it can be comparable to chicken meat (Lumpkin and Seidensticker, 2011; Scanes, 2011). Rabbit meat is easy to digest (Parker, 2010) with a percentage of digestibility of 95 % (Skřivan et al., 2007). Its tenderness varies according to age; the younger the rabbit is, the more tender the meat will be, but flavor and juiciness on the other hand develop with age and highly depend on the content of fat (Lebas et al., 1997).

Table 1: Chemical composition and energy value of meat for 100 g of edible fraction (Dalle-Zotte, 2002).

	Pig	Beef	Veal	Chicken	Rabbit
Water (g)	70.5	69.1	73.5	72.2	70.8
Protein (g)	18.5	19.5	20.5	20.1	21.3
Lipid (g)	8.7	9	4	6.6	6.8
Energy (kJ)	639	665	493.5	586	618

The carcasses' presentation widely depends on the market where they will be sold; this varies greatly among different countries. For instance in some places people may accept just a bled and gutted carcass while in others the animals must be skinned, have the thoracic viscera, offal and head or be without neither head nor viscera. Lebas et al. (1997) and Skřivan et al. (2007) said that dressing yields vary between countries due to the presentation of the carcass, they also expounded that it varies among breeds, age, diet, genotype, housing, feeding and generally it is not influenced by sex. The carcass yield for rabbits ranges from 50 to 65 % (Abington et al., 1992; Dalle-Zotte, 2002).

Skřivan et al. (2007) explained that the slaughter of broiler rabbits could be done during early morning, or evening; first the animal is stunned, then the jugular is cut and left to bleed for 8 minutes. After slaughter the carcass is controlled for its quality and afterwards it is cooled.

2.2.2 By-products

As by-products of rabbit breeding their skin, fur, wool, and manure are widely used. The fur of rabbits is used for the manufacturing of garments, clothes, toys, hats and gloves (Warren, 2002). Angora wool is mainly used for the production of textiles.

The most desirable breeds for pelt production are The Rex and the Satin breeds (Parker, 2010).

Lebas et al. (1986) explained that for the treatment of the pelts, the coat must be mature; it means it should come from animals after moulting in winter, when the coat is stable and homogeneous. They also manifested that summer coat is not usually used, because it is thinner than winter coat and that the optimum age for the production of hides is 7 – 8 months. Furthermore they pointed out that adult rabbits moult twice a year (in spring and autumn); normally this process starts from the neck and goes back to the hindquarters. The same authors also made it clear that in most of the cases rabbits with white fur are used, but not broilers, because they are slaughtered at an age when the fur is not mature. Skřivan et al. (2007) particularized that a hide of good quality does not have residues of feed, and is not be contaminated with feces and urine. The latter authors detailed the process of removing the skin as follows: first the skin is removed soon after slaughter while the body is still warm; it is pulled off starting from the hindquarters to the head. After separating the skin, the excess meat and fat are removed. This operation should be done carefully to avoid any damage to the skin such as: mutilation, knife marks, grease or bloodstains, which would reduce its value. The skin is then dried at 20 – 30 °C for 36 hours. They also stated that it is important that drying starts immediately after the skin has been removed to prevent the action of enzymes and bacteria in the derma which attack the hair root and cause the hair to fall.

Other known product from rabbit production is Angora wool specifically obtained from the angora breed. Garments made from this wools are softer, lighter, warmer, and finer than those made from sheep wool (Warren, 2002). It is also highly appreciated for its lightness, softness, length of the fiber, isolating and anti-rheumatic properties (Lebas et al., 1997; Skřivan et al., 2007). The fiber has a diameter of 13 – 15 microns and a length of 2.5 to 10.6 cm (Hiatt, 2012). Shearing of rabbits is a process that can be done with special scissors, electrical shearing machines or depilation. Rabbits can be sheared 3 to 4 times per year (Parker, 2010). Before the shearing the animal's wool is combed to eliminate any dirt. After shearing the wool is classified according to 4 quality classes depending on the length of the hair, softness, color and cleanness. From one animal can be obtained 1 – 1.5 kg of wool per year. The hair from the female is considered to be better than that of the male (Petrie, 1995). Females' production of wool is affected by the heat, gestation period and lactation.

2.3 Reproduction

2.3.1 Reproduction of males

Males become sexually mature at 4 – 7 months of age depending on the mature body weight of the strain; and are capable of breeding for several years under natural mating conditions (Morrell, 1995). It is important to keep in mind that sexual behavior, sperm production and quality vary with genotype, age, season, rearing conditions, reproductive rhythm, nutrition, social environment, health status and body weight (Castellini, 2008; Rodríguez-De Lara et al., 2015).

Testes start to descend to the scrotum on the twelve week of age (Gendron, 2000) and are placed cranial to the penis (Lumpkin and Seidensticker, 2011; Suckow et al., 2012). Young male kids start to show their first attempts of riding the female at an early age of 60 – 70 days (Lebas et al., 1986), 80 days (Skřivan et al., 2007), but the first ejaculation occurs at about 100 days of age (Skřivan et al., 2007). At this time the viability of the sperm is weak and it is better to wait until they reach an age of 135 – 140 days (Lebas et al., 1986). Manning et al. (1994) and Morrell (1995) explained that some males during mating may emit a characteristic noise followed by a collapse of the male which is typical when he has ejaculated.

Castration is normally practiced in rabbits between 2.5 and 4 months of age; it is mainly important in the production of angora wool, because their wool has higher quality (Skřivan et al., 2007). Vasectomized males can be used to induce ovulation after the artificial insemination procedure, but their use is limited due to certain reasons.

Morrell (1995) described some of the factors that limit the benefits of the usage of these type of males. He clarified that the first and the most important reason is that vasectomized males may transmit certain diseases to the rest of the flock; in addition he explained that females have a tendency to exhibit preferences concerning which male they will allow to mate with them and he also manifested that males could develop anti-sperm antibodies due to the alteration of the blood-testis circulation or simply the buck could mount the female without inducing a successful ovulation leading to pseudo-pregnancy.

Castellini (2008) expressed that rabbit semen has a large presence of granules produced by the prostate gland and these granules maybe implicated in the synchronization between ovulation time and the acrosome reaction; in this way the premature capacitation and acrosome reaction is delayed and the possibilities that the ovulated oocyte would meet a spermatozoa are increased. The volume of ejaculated semen is about 0.5 to 1.5 ml (Manning et al., 1994) with a concentration of 150 - 500 x 10^6 sperms per ml (Lebas et al., 1986). After semen collection seminal characteristics such as: volume of ejaculate, pH, motility and vitality of sperm and proportion of ejaculate with the presence of the gel fraction are evaluated. Lebas et al. (1997) pointed out that collection frequency tend to change the concentration and volume of the ejaculate, this means that the first one will be more voluminous, but less concentrated, and the second one will be more concentrated with less volume. The same authors said that concentration of spermatozoa in the ejaculate also depends on how often the buck is used; if used twice or more times per day, the ejaculate will only have half of the normal concentration of sperms.

Rodríguez-De Lara et al. (2015) found out that the buck's weight has an influence on the libido, semen and sperm characteristics and has some effect on the number of doses for artificial insemination, all resulting in a lesser reproductive potential as the body weight increases. They also stated that the reduction of reproductive performance due to overweight may by associated with the fact that a great amount of fat is deposited in the scrotum and testis. In other research Rodríguez-

De Lara et al. (2010) studied the effects of controlled doe exposure on the reproductive performance of bucks; the overall reproductive potential was greater in those males which were bio-stimulated than in those which were not. Stimulated bucks had a better libido (7%), increased semen volume (40%), increased sperm motility (29%), sperm per ejaculate (31%), normal alive motile sperm (65%) and number of semen doses (64%). They also found that season has some impact on the sperm production; sperm collected in spring had more motility (17%) and concentration per ml (10%) than the sperm collected during winter; on the other hand the winter semen had more normal live sperm (5%), less normal dead sperm (30%) and fewer abnormal dead sperm (45%) than spring semen. It was also explained that during spring more semen doses could be obtained than in winter. Because of these reasons they concluded that doe exposure is a bio-stimulation method that enhances libido, sperm production and semen quality.

Sperm cryopreservation is a great challenge, since many sperm are irreversibly damaged or present altered functionality after the whole process. Although components of extenders for sperm cryopreservation are quite similar between species, sperm from each of the species present peculiarities making it necessary to optimize the extenders and protocols for each species in particular (Moce and Vicente 2009).

2.3.2 Reproduction females

Female rabbits have good reproductive traits such as short sexual maturity age and gestation period and existence of a post-partum oestrus (Tablado et al., 2009). They are also capable of carrying out several pregnancies throughout the year, or can carry simultaneous pregnancies. Rabbit does are mainly selected for litter size (Castellini et al., 2010) and their productivity is evaluated by the number of young per doe per unit of time (Lebas et al., 1997), this depends on: the interval between kindling; litter size at birth; and the survival of the kids.

Does' reproductive tract is characterized by oval-shaped ovaries with a size of 1 – 1.5 cm; a uterus with 2 cervixes (bicornuate uterus - *uterus bipartitus*) of 7 cm each (Lumpkin and Seidensticker, 2011) and the length of the vagina is 6 – 10 cm. Females have between 6 to 8 nipples (Lumpkin and Seidensticker, 2011).

Female rabbits are capable of mating from an early age of 10 to 12 weeks, but without a successful ovulation. Suckow et al. (2012) explained that females' sexual maturity depends more on the reached body weight rather than the age. Skřivan et al. (2007) made clear that females generally reach puberty when they grow to 70 – 75 % of their adult body weight, but they recommended that is better to breed them until they have reached 80 % of body weight.

2.3.2.1 Oestrus cycle and ovulation

The first heat appears at an age of 3 – 4 months, which has a duration of 3 – 5 days and it repeats every 5 – 7 days (Skřivan et al., 2007). A female rabbit in oestrus presents the following behavior: easily accepts to be serviced, has a red swallowed vulva (Manning et al., 1994; Suckow et al., 2012), restlessness, rub their chin against the cage, pen or feeder, and in some cases loss of appetite (Gendron, 2000). Manning et al. (1994) and Lebas et al. (1997) explain that when a doe is in heat, she assumes a characteristic pose, called lordosis, during which the back is arched downwards and hindquarters are raised; and if the female is not in heat she tends to exhibit aggression towards the buck. During the oestrus cycle the doe may show variation in sexual receptivity, having various waves of follicular growth and regression within the ovaries (Morrell, 1995).

Rabbit does do not have a regular ovulatory cycle in which the oocytes are spontaneously released after certain amount of days as in other females like: cow, mare, sow, ewe and goat. They are so called induced ovulators (Morrell, 1995; Gendron, 2000; Majzlík, 2006; Skřivan et al., 2007); this means that is necessary to stimulate the vagina for ovulation to occur, but in some cases ovulation may occur spontaneously (Morrell, 1995). This stimulation happens naturally during mating, but can be induced artificially by mechanical stimulation of the vagina (Lebas et al., 1997), mating with a vasectomized buck (Morrell, 1995) and injections of gonadotropins such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), analogues of gonadotropins: Equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG), GnRH and its analogues which are known promoters of rabbit ovarian follicular growth and ovulation (Sirotkin et al., 2014). Ovulation usually occurs 10 – 12 hours after mating (Manning et al., 1994; Gendron, 2000) during which 3 – 9 oocytes are released form each ovary (Skřivan et al., 2007).

2.3.2.2 Gestation, kindling, lactation and weaning

During copulation the sperm is deposited in the upper part of the vagina (Lebas et al., 1986) and fertilization takes place in the ampulla of the oviduct approximately 1.5 hours after mating (Skřivan et al., 2007). In the following days the embryo migrates to the uterus and implantation happens at 7 days. Gestation in rabbits lasts between 28 to 35 days (Morrell, 1995; González-Mariscal et al., 2007; Castellini et al., 2010). Each fetus has its own fetal membranes to protect them, allow them to move and change position and to ensure the connection with the uterus and the formation of the placenta, which in rabbits is a hemochorial type (Fraser and Girling, 2009).

Pregnancy is confirmed at day 14 with palpation of the abdomen, day 11 with radiography and day 7 with ultrasonography (Suckow et al., 2012). Pseudo-pregnancy or phantom pregnancy, is a state caused by unfertilized oocytes, sterile mating or spontaneous ovulation (Morrell, 1995). This state may last for 15 to 17 days (Gendron, 2000). After this period the corpus luteum starts to luteolyze because there is not enough production of progesterone typical for gestation (Lebas et al., 1986). During true gestation the progesterone levels start to increase from the third day after mating or insemination, then during pregnancy it remains stable and it drops rapidly before parturition (Lebas et al., 1997). Their feed intake drops during the last stage of pregnancy and usually some females tend to refuse solid feed before parturition. After kindling the feed and water intake tends to increase.

Before the onset of birth the doe starts preparing herself for the parturition, a behavior known as “maternal behavior”. Under natural conditions, wild and domestic rabbit does dig a burrow, which they line with plant material and hairs plucked from their chest (Baumann et al., 2005; González-Mariscal et al., 2007). In controlled breeding systems, females cannot fulfil this behavior, so a nest box must be provided from day 26 – 28 (Manning et al., 1994; Gendron, 2000) after mating. This box should be of at least 50 cm x 25 cm x 25 cm (Lebas et al., 1986) or 30 cm x 30 cm x 30 cm (Baumann et al., 2005). Lebas et al. (1986) pointed out the following functions of the nest box in the breeding of rabbits:

- Should reproduce the conditions in the natural burrow and protect the kids against the outside environment.
- Should keep the young healthy, warm at a temperature of at least 30 – 35 °C.
- Should keep the young from leaving the nest too early and make it easy for them to get back if they do get out.
- Should prevent dampness from the animals' urine.
- Should allow the breeder to: monitor the litter, remove dead animals, introduce kids to be fostered, change the bedding material easily.

González-Mariscal (2001) explained that hormones such as estrogen, androgens, progesterone and prolactin regulate and promote the expression of maternal behavior during late pregnancy, this behavior is related with nest-building factors such as: digging, straw-carrying and hair-plucking; she also described that pregnant does with shaved bodies tend to collect the alternative hair (animal or synthetic) that is provided in their cages. In addition she stated that this function relies greatly on the stimuli from the litter. In other study González-Mariscal et al. (2007) described that digging begins around pregnancy day 21 and reaches its maximum at days 25 to 27, when levels of estradiol and progesterone are high 60 pg/ml and 9 ng/ml respectively; straw-carrying begins 3 to 1 days before kindling as the levels of progesterone decline and estradiol increases to 75 pg/ml; and finally hair-plucking is expressed before parturition to various degrees of intensity when the concentrations of testosterone and prolactin are elevated 308 pg/ml and 160 ng/ml.

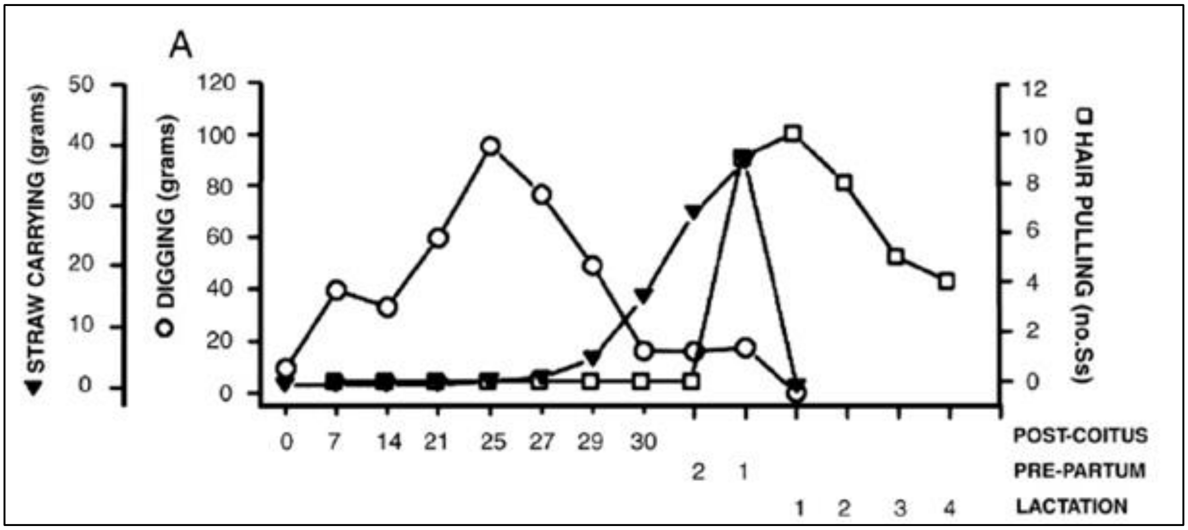

Figure 1: Straw, digging and hair pulling patterns in rabbit does (González-Maríscal et al., 2007).

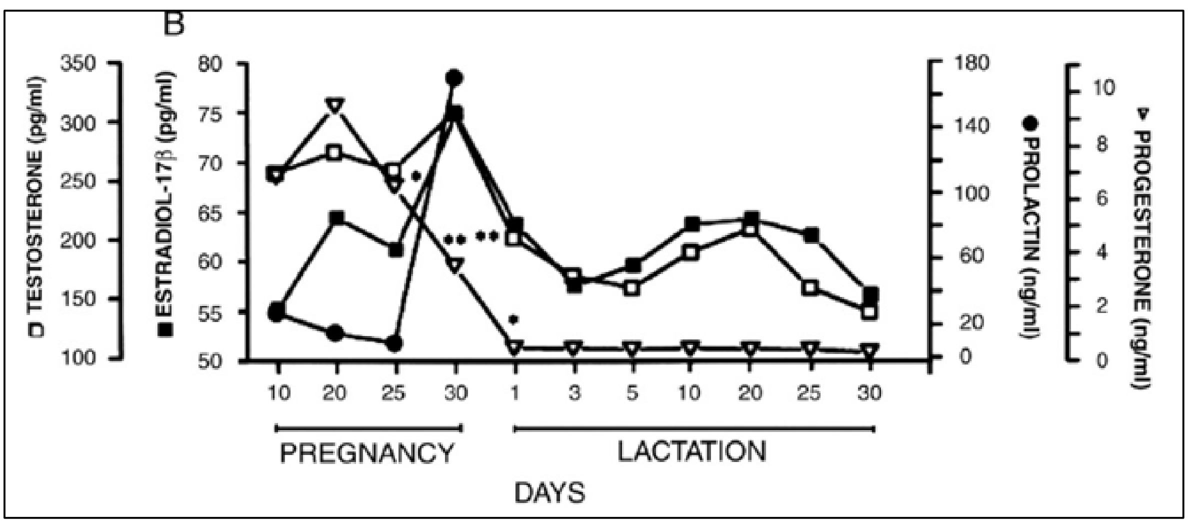

Figure 2: Testosterone, Prolactin and Progesterone levels during pregnancy and lactation in rabbit does (González-Mariscal et al., 2007).

The doe usually gives birth during the early morning (Castellini et al., 2010) or late night (Skřivan et al., 2007). Kindling or parturition in rabbits lasts in average 30 minutes (Manning et al., 1994). This depends on the number of young, which varies from 1 to 20 kids, being the average 7 – 9 young in normal rabbit production (Lebas et al., 1997). After the birth of each kid the doe eats the afterbirths. Pups are altricial type, this means that they are born blind, deaf and hairless (Gendron, 2000). Despite of blindness, kids can locate the mother's nipples and suckle milk due to an olfactory signal that is emitted from the mother; this signal is called "nipple pheromone", which its production is stimulated by estrogen, progesterone and prolactin (González-Mariscal, 2001). Kids open their eyes at 10 days of age (Suckow et al., 2012). Following kindling the uterus undergoes *puerperium* or regression very fast and it reaches its normal size just 2 days after birth and is ready for copulation and become pregnant again (González-Mariscal et al., 2007; Skřivan et al., 2007).

Lactation in rabbits lasts 4 – 5 weeks (Castellini et al., 2010). Does feed their young once or twice a day (Selzer et al., 2004; Fraser and Girling, 2009; Castellini et al., 2010) for only 2 – 3 minutes (Lebas et al., 1997). This function is regulated by oxytocin and prolactin (González-Mariscal, 2001). In domestic rabbits, more than 80% of nursing activity takes place between dusk and dawn having its peak 1 hour after dusk (Selzer et al., 2004). Doe's milk is rich in proteins, fats and poor in lactose. As described by Skřivan et al. (2007) the rabbit milk contains: 30 % dry matter, 15 % protein, 12 % fat, 1 % lactose and 2.5 % of minerals. Milk production increases from 30 - 50 gr after parturition to 200 – 250 gr at the end of the third week of lactation (Lebas et al., 1997). Females in this phase must be fed rich concentrated feed, because they have few reserves of energy compared to the demand that is put on them.

The good care of the kids during this period is crucial as kids are born without fur, it is important to keep a constant temperature of the surroundings. This must range between 28 – 32 °C (Lebas et al., 1997). In some cases if some females have too many kids it is possible to put them with other female. Lebas et al. (1997) and Skřivan et al. (2007) remark that three important aspects should be taken into account: fostering should take place within 3 days after kindling, there should not be fostered more than 4 young rabbits and that the maximum difference between the foster doe's litter and the fostered young should be 2 – 3 days.

Weaning is the period during which the young starts to give up milk for solid feed (Lebas et al., 1997). In breeding systems it is also considered the time when the breeder separates the young from their mother (Skřivan et al., 2007) and are identified and put in separate cages according to age and sex. Now a days in intensive systems weaning is divided into standard weaning 32 days, late weaning 46 days (Alfonso-Carrillo et al., 2014).

Lebas et al. (1986) described the process of weaning in different breeding systems as follows: in extensive systems the young are weaned at 42 – 56 days and the doe mated soon after weaning so does are serviced every 2.5 months; in semi-intensive breeding pups are weaned at 28 – 35 days and the doe is serviced at 10 – 20 days after kindling and finally in intensive systems the doe is mated just after kindling while she is still in heat, weaning takes place 26-28 days after parturition.

2.4 Artificial insemination (AI)

Morrell (1995) stated that artificial insemination allows a more controlled management of reproduction and better planning than conventional mating. He also expressed that this method presents several benefits to the breeding of animals such as: control of genetic diversity, rapid upgrading of stock, establishment of pregnancies in females who refuse to mate, and avoidance of the spread of diseases. Other author Rodríguez-De Lara et al. (2015) pointed out that the success of artificial insemination in rabbits depends greatly on the health and reproductive performance of males at the time of semen collection, being of grand importance factors such as: libido, sperm volume, motility and viability of sperm and sperm abnormalities. Besides this, the sexual response of the females at a certain time plays a significant role. It is important that the doe is receptive at the time of artificial insemination, therefore oestrus synchronization is needed to obtain high fertility rates (Castellini et al., 2010).

Nowadays many synchronizations are carried out with the use of hormones and others with the application or use of bio-stimulants, which are a natural and less expensive alternative to improve the reproduction and reduce the negative impact on the environment. According to González-Mariscal et al. (2007) and Castellini et al. (2010) bio-stimulants used for the oestrus synchronization of does include change of cage, doe-litter separation, flushing, alteration of the light management, placement of a buck close to the females, and shearing the does in the hot season. In males bio-stimulation such as controlled doe exposure mainly enhances sexual drive and improves sperm production and quality (Rodríguez-De Lara et al., 2010).

As in many other farm animals, the artificial vagina is by far the most used method of collection of semen. Morrell (1995) described that the equipment required for semen collection includes an artificial vagina, rubber liners and a small collecting tube. Furthermore he explained that the vagina is put together by inserting the rubber liner into the rigid outer case and turning the ends of the liner over the top and bottom edges, then the collecting tube is placed in the bottom hole and the space between the outer case and the liner is filled with hot water with a temperature of 39 °C, to resemble the normal temperature of the female tract. Morrell (1995) and Lebas et al. (1997) explained that for the collection of semen is needed to have either a rabbit skin or a female teaser for the ejaculation to take place.

Following semen collection, semen should be diluted with a buffer medium at the same temperature of the semen to avoid heat or cold shocks (Castellini C, 2008). Normally the semen is diluted in physiological solution in rates 1:5 or 1:10 (semen: solution) and packed in 0.5 ml pellets (Lebas et al., 1997). Insemination with fresh semen requires the use of physiological solution and addition of antibiotics (Morrell, 1995). Insemination dose should contain 20 to 50 X 10^6 spermatozoa in 0.3 to 0.7 ml of sperm (Manning et al., 1994).

There are two techniques to provide insemination. The first with the insemination gun and the second with the glass pipette which can have a slight bend of 8 cm on one end. For insemination the female is placed in a restraining box, when the female exhibits lordosis then the tail is lifted gently, and the lubricated insemination gun or pipette is inserted at an angle of approximately 45 ° into the vagina (Morrell, 1995).

2.5 Factors affecting reproduction of does

Reproductive performance of livestock is affected greatly by factors such as: genetic value, physical environment, stress, nutrition and poor management of the flock (Smith and Akinbamijo, 2000; Baumann et al., 2005). Stress can impair reproduction by disrupting the endocrine events that control the estrous cycle and ovulation (Sirotkin et al., 2014), it also reduces the immune response and increases the risk of diseases (Castellini et al., 2010). Stress affects the frequency and amplitude of luteinizing hormone (LH) pulses, therefore depriving the ovarian follicle of adequate LH support (Mungai et al., 2009). It is also important to keep in mind that reproductive parameters such as length of breeding season, proportion of pregnant does, age of first reproduction, and number and size of litters may vary in different regions. (Tablado et al., 2009).

According to Fortun-Lamothe (2006), Cardinali et al. (2008) and Castellini et al. (2010), the leading cause in the decrease of reproductive performance resulting in low fertility rates is the competition that exists between the mammary glands and the gravid uterus for nutrients to maintain lactation and pregnancy, resulting in negative energy balance, which is mainly negative in nulliparous does, because the energy requirement must maintain lactation, pregnancy and growth until they reach adulthood. The last author manifested that the overlap among lactation and gestation also leads to hormonal antagonism affecting the sexual receptivity, ovulation, fertilization, implantation and embryo survival. The problem is greater in intensive breeding systems, because the doe is usually inseminated at 11 days postpartum; Castellini et al. (2010) found out that does at this time showed at artificial insemination low sexual receptivity (37.2%), poor body condition score (71.2%) and low fertility rates (50.9%).

They also explained that the mobilization of greater amounts of fat during lactation is associated with the higher output of energy which is not compensated by feed intake.

Smith and Akinbamijo (2000) pointed out that a good nutrition and feed intake is very important to obtain high rates of fertility; also was stated that poor nutrition affects all the reproductive stages to different degrees. The same authors expressed that a delayed puberty, altered oestrus and ovulation, lower conception rates, perinatal mortality, poor neonatal performance and poor lactation are accounted to nutrition. They explained that the major problem in tropical countries is low feed intake due to feed poor in nutrients. The same authors remarked that lack of vitamin A and selenium (Se) in feed have negative effects on the litter like small litter size, weak and blind offspring and impaired reproductive performance of both females and males.

Other factors affecting reproduction such as light and temperature were described by Lebas et al. (1986). They explained that light affects in different ways both males and females. It was manifested that females obtained good reproduction rates when exposed to light for 8 hours a day but that a 12 hour light management is required to obtain average results. They also pointed out that high temperatures and heat restricts the feed intake in females and leads to low sperm motility and reduced libido in males.

In their research Baumann et al. (2005) focused on the effects of different type of accesses to the nest in farmed rabbits. They discovered that free access to the nest leads to an altered nursing behavior with more than 3 visits per day to the nest. They also pointed out that pup mortality from birth to weaning can be more than 20 % and also manifested that the most common reasons of pup mortality are hypothermia, injuries, starvation and weakness due to low individual birth weights, lack of milk and behavioral problems of the does such as: nest soiling, insufficient nest building, crushing or scattering of pups and cannibalism.

In other study Mugnai et al., (2009) focused on understanding the effects of limited space in the cage and access to nest in single and colony groups of females.

They concluded that the restriction of space leads to atypical behaviors, frustration, boredom and skeletal anomalies because does are not allowed to assume positions such as lying, sitting and standing-up on hind legs. They also explained that the reproductive performance of females in colony groups was worst that those put in single cages having lower sexual receptivity, fertility, live-born pups, milk production and weaned pups. In the same study it was also found that females in colonies tended to behave more aggressive and also in these groups was found a higher rate of pup mortality due to the free access of does to other doe's nest. Szendro and McNitt (2012) also explained some of the advantages and disadvantages of individual-housing and group-housing of rabbit does (see Tab. 2).

Table 2: Comparison of benefits and costs of individual and group housing of rabbit does, adapted from Szendro and McNitt (2012).

Group-housing	Benefits	Larger place for movement Wider behavioral repertoire Social contact
	Costs	Higher rate of aggressiveness (stress and injuries) Competition for nest sites (infanticide, injuries) Higher mortality of kits Poor condition Lower productivity Increased probability of infections Shorter lifespan
Individual-housing	Benefits	Lower rate of aggressiveness (stress and injuries) No competition for nest sites (infanticide, injuries) Lower mortality of kits better body condition Higher Productivity Lower probability of infections Longer Lifespan
	Costs	Less space for movement Restricted behavioral repertoire Less opportunity for social contact

2.6 Milk Thistle (*Silybum marianum*)

Milk thistle (*Silybum marianum L. Gaertn*. or *Carduus marianus L*.) also known as Marian thistle (Cardile and Mbuy, 2013) is an important medicinal weed (Alemardan et al., 2013; Siegel and Stebbing, 2013), which has been used for centuries in the treatment of liver disease (Flora et al., 1998; Karkanis et al.; 2011; Sadowska et al., 2011). Other usages of milk thistle in traditional medicine was as treatment of hormonal alterations in women, digestive tract problems, acne, and *psoriasis* (Martínez-Morán et al., 2011). It was first described under the name “Pternix”, and was also mentioned in “*Materia Medica*” by Diocurides in the 1st century A.D. (Karkanis et al., 2011).

Its name derives from the characteristic spiked leaves with white veins (Flora et al., 1998). It belongs to the family Asteraceae, which also includes sunflowers and daisies (Siegel and Stebbing, 2013). This family is known for containing natural compounds called “flavonolignans”. The genus Silybum contains two species: *S. marianum* and *S. eburneum*. This plant is native to the Mediterranean countries (Capasso et al., 2009), but nowadays it is spread throughout the world.

2.6.1 Description of the plant

Milk thistle is an annual plant that can successfully grow on diverse soil types due to its adaptability to poor soils, different growing conditions and its tolerance to various pH (5.5 – 7.6); but it is susceptible to a wide variety of pests, insects and diseases (Karkanis et al., 2011). The chemical composition of the plant is mainly affected by the weather, rather than by the agro-technical conditions (Sadowska et al., 2011). Karkanis et al. (2011) and Eskanda-Nasrabadi et al. (2014) described the plant of *S. marianum* as slightly downy, erect and branched in the upper part, with a total length of 40 – 250 cm; its large prickly edged leaves covered with white veins can reach 50 – 60 cm long and 20 – 30 cm wide, the flowers’ head are of about 5 cm in diameter of white or red-purple color; each head can produce an average of 190 seeds, which are achenes of 5 – 8 mm long. Their color varies from black to brown. It also has an abundance of stout spines, which local people use as a substitute for barbed wire (Flora et al., 1998).

Milk thistle plants have a four-staged growing pattern; this phases include: vegetative (germination), elongating (growth period), flowering (pollination and fertilization) and seed maturation stage (Eskanda-Nasrabadi et al., 2014). Germination takes place at a temperature of 15 °C; blossom of flowers and maturation of seeds, which is uneven due to the dispersion of the flowering heads, occur from April to May and even during July respectively (Karkanis et al., 2011). After harvesting and cleaning, the seeds should be dried at 50 °C to 8 % of moisture content.

2.6.2 Milk thistle properties

In spite of that the whole plant could be used for the extraction of the compounds, a special focus is given to Silymarin, which is the biologically active component of the weed (Cardile and Mbuy, 2013). This extract is mainly obtained from the mature seeds of milk thistle (Alemardan et al., 2013; Milic et al., 2013; Biedermann et al., 2014). The Silymarin content often ranges from 4 – 6 % in the ripe seeds (Bhattaram et al., 2002), but can exceed 8 % (Karkanis et al., 2011); this is because different genotypes of milk thistle have variable amount of Silymarin (Alemardan et al., 2013). Standard extracts of milk thistle contain in average 70 to 80 % of Silymarin (Ross, 2008).

Silymarin is a flavonoid complex that consists of different compounds including silybin (silybinin), isosylibinin, silydianin, and silychristin (Flora et al., 1998; Stickel and Schuppan, 2007; Eskanda-Nasrabadi et al., 2014). Silymarin consists of a mixture of 2 diastereoisomers, silybin A and silybin B in a ratio 1:1 (Jacobs et al., 2002; Ward et al., 2013). Silybin content rages from 50 – 70 % (Bhattaram et al., 2002). Silymarin is known to be very safe and non-toxic to mammals; for example in rabbits and dogs the lethal dose (LD50) is 140 mg per kg (Balszuweit et al., 2013). Ward et al. (2013)

reported that the amounts used of Silymarin for clinical trials range from 420 – 480 mg per day. It is excreted from the body mainly by the biliary route (Bhattaram et al., 2002).

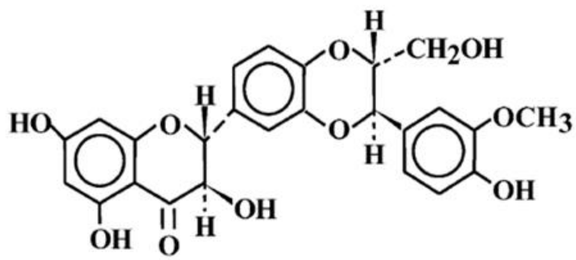

Figure 3: Chemical structure of Silybin (Singh and Agarwal, 2004).

2.6.3 Use in human medicine

Karkanis et al. (2011) and Milic et al. (2013) explain that *Silybum marianum* could be used in the treatment of Alzheimer's disease, Parkinson's disease, sepsis, burns, osteoporosis and diabetes. Its compounds have an anti-oxidant, anti-viral, anti-carcinogenic, anti-inflammatory potential (Bhattaram et al., 2002; Siegel and Stebbing, 2013). Furthermore the same authors described milk thistle as a strong antioxidant which promotes liver cell regeneration, reduces blood cholesterol and inhibits the binding of toxins to cell membranes. It was also found that due to its low toxicity and fetoprotective properties, milk thistle is safe to use during pregnancy and lactation (Capasso et al., 2009; Milic et al., 2013). It has been used as a galactogogue, to stimulate milk production in women (Ross SM, 2008). *Silybum marianum* as reported by Cardile and Mbuy (2013) and Jacobs et al. (2002) has antiviral activity against certain strains of viruses. They found that when Silymarin and silibinin are administered intravenously there is a significant activity against Herpes virus and human immunodeficiency virus (HIV).

Capasso et al. (2009) studied the effects of Silymarin BIO-C extract on prolactin levels on female rats and suggested that milk thistle could stimulate milk production in nursing mothers or women with lactation insufficiency. Di Piero F et al. (2008) analyzed the role of Silymarin as a galactogogue for human. The dose of micronized Silymarin used was 420 mg/day during 63 days. They discovered that women, who were treated with Silymarin showed an increase in milk production by 85.94 % compared to the control group 32.09 % and reported no undesirable side effects, concluding that Silymarin is a safe herbal product that could be used by women after parturition.

Silymarin has also been used in the treatment against liver poisoning with "green death cap mushrooms" (*Amanita phalloides*) (Flora et al., 1998; Jacobs et al., 2002; Siegel and Stebbing, 2013). This mushroom is characterized for having various types of toxins, but alpha–amanitin is the main hepatotoxin (Ward et al., 2013). Per oral application of a derivate silibinin-bis-succinate (SIL-BS) is used as a treatment in non-acute poisoning cases, but intravenous administration of the compound is applied in case of acute poisonings (Balszuweit et al., 2013); besides the previous mentioned derivate of silybin, Legalon SIL, is indicated in many countries for the treatment of death cup intoxication (Jacobs et al., 2002). Silybin inhibits the action of OATP (Organic anion-transporting poly-peptide) transporters, thus preventing the uptake of the toxin by the liver cells (Balszuweit et al., 2013). Besides this, Stickel and Schuppan (2007) explained that Silymarin interrupts the entero-hepatic recirculation of the toxin, inhibits of toxins to hepatocyte membranes and competes with the toxin for transmembrane transporters. Furthermore *Silybum marianum* has a potential as an antidote to sulfur mustard poisoning. This chemical warfare causes skin blistering, ulceration, impaired wound healing and permanent lesions. Balszuweit et al. (2013) found that the use of a derivate SIL-BS helped in the reduction of necrosis in the cells that were exposed to the sulfur mustard, but suggested that more studies, using cellular co-cultures, animal models and possibly multidrug treatment, need to be performed to affirm the protective efficacy of silybin against sulfur mustard toxicity.

In addition, in the last century researches have been focused on cosmeceutical preparations from herbal origin, because in most cases they are non-poisonous and have a strong antioxidant activity. Singh and Agarwal (2009) manifested that according to some studies silybin can interfere with signaling pathways which are altered by toxic compounds, UV radiation and decrease the apoptosis of cells in skin exposed to UVB or arsenic compounds. They also expressed that in some studies topic silybin protected hairless mice skin from sunburn. Therefore they suggested that silybin has an enormous potential to be an ideal compound for cosmeceutical preparations.

Despite the safety of the milk thistle's extracts in human medicine it was reported a case of a 45-year-old patient with an acute generalized *exanthematous pustulosis* (AGEP). This disease is uncommon and may be caused by certain drugs, antibiotics, fungi, or trigged by infections, pregnancy, allergens, tumors and some herbal medicinal products. Martínez-Morán et al. (2011) explained that the patient had been taking infusions of milk thistle one week prior to the skin eruption, but could not conclude that milk thistle was the trigger. They also explain that it was important to report the case as it illustrates the relevance of herbal medicines, which are often considered harmless and in many cases these can cause skin lesions, gastrointestinal discomfort and diarrhea. Post-White J et al. (2007) explained that milk thistle can have a mild laxative effect causing gastrointestinal discomfort. Also Flora et al. (1998) and Ward et al. (2013) respectively reported that milk thistle may slow calcium metabolism and cause allergic reactions in those people that have allergy to other plants of the Asteraceae family. Geier J et al. (1990) reported a case in which the patient had an allergic reaction to milk thistle causing him to collapse and suffer from edema, respiratory distress and decrease in blood pressure.

2.6.4 Use in animals

There have been some studies focused on the potential of milk thistle as an additive to animal feeds. Sadowska et al. (2011) reported that the whole milk thistle's fruit can be used only in ruminants, due to its high content of cellulose-lignin fraction. Schiavone et al. (2007) studied the effects of different doses of Silymarin in diet on the performances of broilers and their quality and concluded that Silymarin did not affect the growth performance, but to some extent affected the slaughter yields and that the treatment reduced the lipid content in the breast and thigh. Muhammad et al. (2012) also analyzed the possibilities of milk thistle as a hepatoprotectant in chicken fed with aflatoxin B_1. They reported that 10 gr/kg of milk thistle in feed helped with body weight gain, feed conversion ratio and minimized the harmful effects of toxin-contaminated feed; they concluded that milk thistle has a good potential as a mycotoxin binder. In other research focused on the possible hepatoprotective effect of milk thistle in dairy cows, Tedesco et al. (2004) reported that *Silybum marianum* has neither a harmful nor protective effect on the liver of lactating cows. In a different study Weisbroth et al. (1974) concluded that 50 % of milk thistle cake meal in the feed of rabbit helps improve the production performance and immunity in rabbits.

Milk thistle has been also tested in reproduction and lactation of some farm animals having in some cases positive results. Kummer et al. (2000) reported that a concentration of 4.1 % of Silymarin should not be fed more than 4 to 5 weeks after calving, because it can cause some adverse effect on the cow's fertility. They also stated that this concentration has a positive effect on the involution of the uterus, and is preferably to be given to cows with low levels of estrogens. Some studies demonstrated that milk thistle increases lactation in cows Capasso et al. (2009). The same authors analyzed the effects of the Silymarin BIO-C extract on the prolactin levels on female rats. It was found out that 14 days after the treatment, rats had increased their total body weight as well as the circulating levels of prolactin. Moreover they observed that this increase was at the Silymarin BIO-C's doses of 50, 100 and 200 mg/kg and noted that the stimulatory action of the extract on prolactin levels remained for up to 66 days after the discontinuation of the treatment. On the other hand Loisel F et al. (2013) reported that Silymarin extract at doses 1, 2 and 4 gr/day did not increased the concentrations of prolactin or affected the progesterone, estradiol-17β and leptin levels in cycling sows.

There have been researches focused on the protection of milk thistle against *Amanita phalloides* toxin. Stickel and Schuppan (2007) demonstrated that dogs which were treated with milk thistle against poisoning with amanita toxin reported no cases of fatalities compared to those which were not given the preparation. In other study Desplaces et al. (1975) reported the hepatoprotective effect of Silymarin in rats and explained that Silymarin inhibits the toxin when given in a dose of 15 mg/kg 60 minutes before or 100 mg/kg 10 minutes after the poisoning with phalloidine. They also remarked that if Silymarin is given 30 minutes after the administration of the toxin, it has no curative effect.

Silybum marianum has a potential as a new non-edible feedstock for biodiesel, due to its content of oil (Takase et al. 2014), from which could be obtained around 25 % of oil (Karkanis et al., 2011).

3 Aims of the thesis

The aim of the thesis was to analyze the possible effect of the milk thistle's (*Silybum marianum*) supplement on the productive parameters of the female rabbits.

- H_1: Milk thistle supplement will positively affect the reproduction of does.
- H_2: The number of born offspring will be greater in the milk thistle group.
- H_3: The number of weaned kids will be higher in milk thistle group.

4 Material and methods

4.1 Methodology

Writing of the literature review based on scientific studies from different databases (Web of Knowledge, Science direct) and books.

The research took place in the farm of Jiří Kočár in Ratibořice, located in the south east of the Czech Republic. The research was divided into 4 experiments, which were carried out during one year. The first experiment started on December 2013 and the last experiment ended on December 2014. Each experiment consisted of the insemination procedure, gestation period, parturition and weaning of the litter. Later on the litters of these does were used by students of the Faculty of Tropical AgriScieces for fattening experiments focused also on the effect of milk thistle. In total thirty nulliparous females of the line “HYLA” were chosen for the research. The does were 4 to 5 months old. These were randomly divided into 3 groups and does were numbered. Each experimental group was of ten animals. Throughout the whole experiment 2 of the groups were given a specific supplement while the third group was the control group.

During the research period, the following data was collected: number of born and number of dead offspring, number of dead does, and number of weaned offspring. The recorded data were evaluated from January to March 2015.

4.2 Feeding management

All does received a complete feed mixture produced by BIOKRON (s.r.o.) feed. Two experimental groups were fed an herbal supplement as follows:

a. Group 1 was given the AV3 supplement; an herbal phyto-additive with antioxidant properties. The supplement was extracted from milk thistle (*Silybum marianum*) and gingko (*Gingko balboa*). The extract was produced by Manghebati SAS Company. The concentration was 0.36 ml/kg.

b. Group 2 was fed the 0.2 % milk thistle (*Silybum marianum*) supplement. The Silymarin extract was obtained from fruits of milk thistle plant.
c. Group 3 was the control group, therefore it did not receive any supplement.

Gestating females were fed the complete feed mixture for breeding does while lactating females received the complete feed mixture for lactating does. Does in gestation had a restricted feeding to avoid obesity; each of them was given 150 grams of feed mixture per day. During gestation and lactation periods water was given ad libitum. Besides the mentioned supplements, the three experimental groups received Emanox PMX and Probiostan E10 at doses 0.25 g/kg and 2.5 g/kg respectively.

Table 3: Composition of the complete feed mixture for lactating rabbit does produced by Biokron (s.r.o.).

Analytical constituents	(%)
Crude Fiber	13.5
Ashes	8.0
Crude oil and fat	2.5
Crude Protein	17.5
Calcium	1.1
Phosphorus	0.6
Sodium	0.35

4.3 Housing and environmental conditions

Each female was individually housed in Italian type cages for breeding does with the following measurements: 60 cm long x 33 cm high x 40.5 cm wide (see Fig. 4, 5). Three days before the expected day of parturition the nest box was provided with straw to assure that the doe would have enough time to build the nest. The nest box had the following measurements: 25 cm long x 24.5 cm high x 40.5 cm wide (see Fig.

4, 5). The females had free access to the nest during the whole time of the experiment. The nest was removed shortly after the weaning of the litter, at 32 – 35 days after kindling.

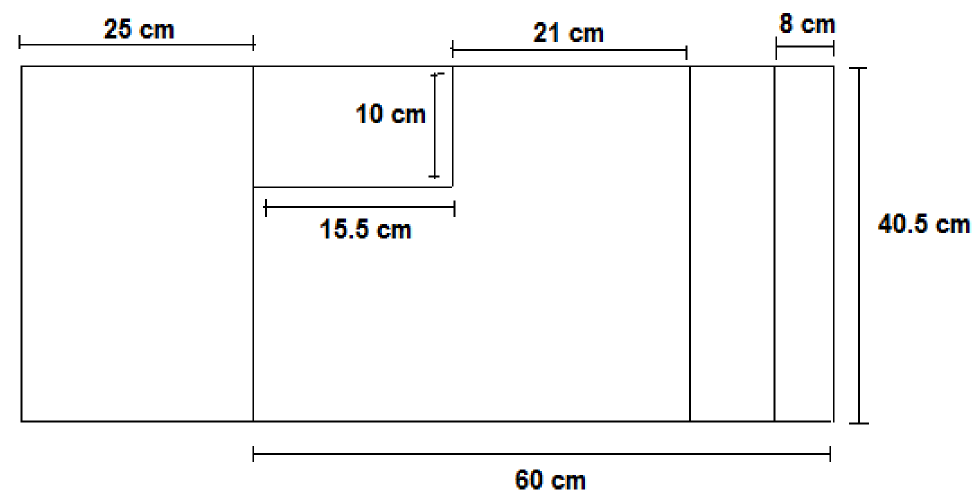

Figure 4: Italian type cage with nest box for breeding does - view from above (Aguilera-Hidalgo, 2015).

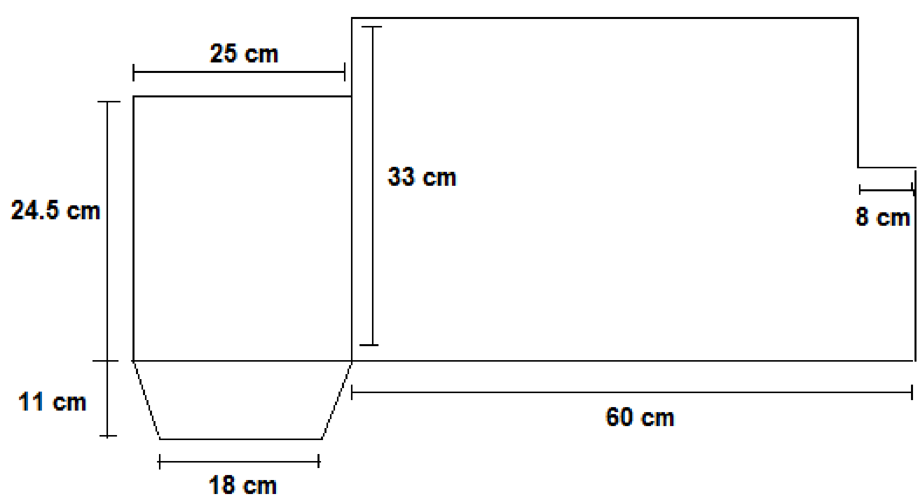

Figure 5: Italian type cage with nest box for breeding does - view from the side (Aguilera-Hidalgo, 2015).

The light regime was always changed 10 days before insemination or mating, the artificial light was increased during the evening to create longer days. The constant temperature was kept at: 18 – 20 °C and the exchange of air was through normal ventilation system (open windows).

4.4 Insemination procedure

Females were artificially inseminated with an insemination gun. During the research were provided 4 inseminations per doe. The process of insemination:

a. Take the female out of her cage and fixate her.
b. Then the tail was grabbed between two fingers and lifted up, and afterwards the insemination gun was inserted into the vagina and the semen was released once the uterine horn was found.
c. Immediately after the insemination, Supergestran, a commercial hormonal product, was administrated to induce the ovulation. It was injected perpendicularly to the tight of the doe. The semen and Supergestran dose were 0.5 cm^3 per female.
d. If a female did not get pregnant she was left resting until the next experiment started. They were not re-inseminated after 10 days, because during the research, the aim was to have a synchronized parturition with a difference of maximum +/- 2 days.
e. If a female could not get pregnant after the second insemination she was eliminated from the experiment.
f. For the next experiment females were inseminated between 32 - 35 days after parturition i.e. immediately after weaning.

4.5 Statistical analysis

Data collected was processed and analyzed by statistical software STATISTICA 12. The data was analyzed by ANOVA method.

5 Results

The research started with 30 nulliparous does of the HYLA line. From the original number of does only 10 finished the four experiments. The number of does that completed the study at the end of each treatment AV3, Milk thistle and Control was 4, 2 and 4 respectively. After the first, second and third parturition in total 3, 3 and 14 does died or were eliminated from the experiment (see Fig. 6). The major causes of death were exhaustion or injuries after parturition and death by Pasturellosis. The data obtained of the number of dead females was not taken into consideration for the statistical analysis.

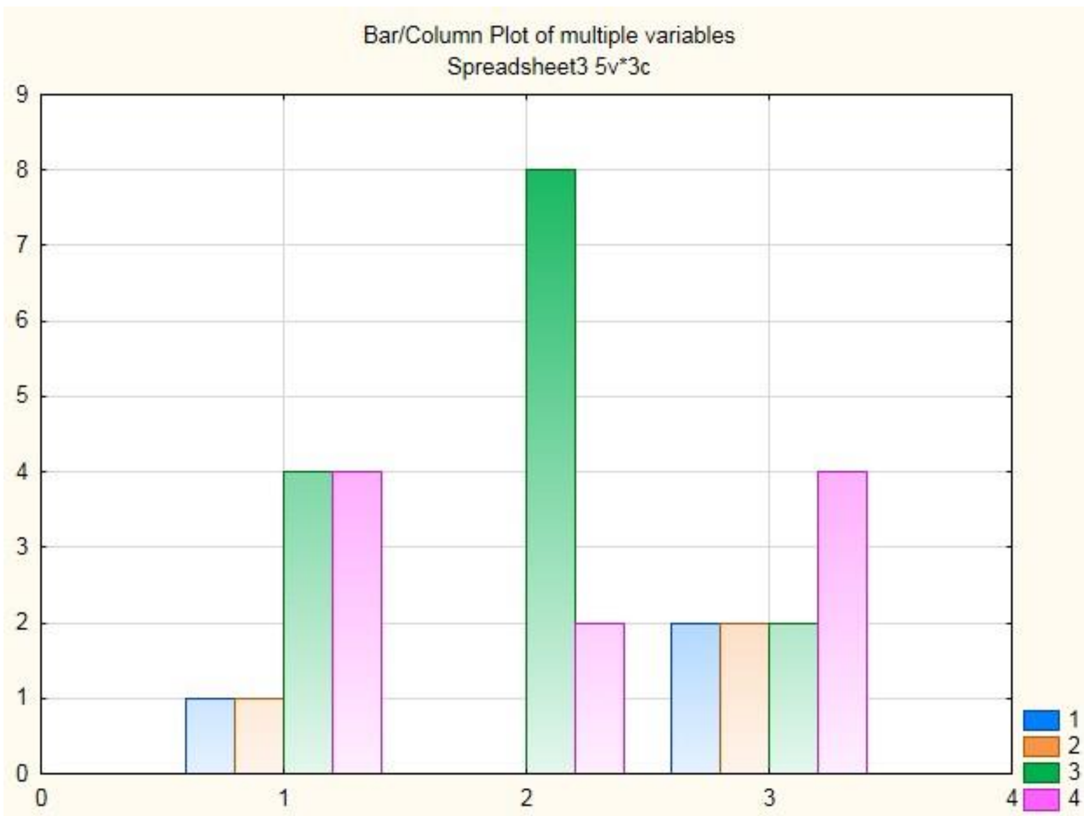

Figure 6: Number of eliminated or dead does according to treatment. AV3 (1), Milk Thistle (2), Control (3). Colors represent the 1st (blue), 2nd (orange), 3rd (green) and 4th (pink) parturition.

Throughout the entire experiment there were born in total 840 kids from which 573 were weaned and 267 died. The weaned and dead animals represent 68.21 % and 31.79 % respectively. The total number of offspring born in treatment AV3 was 295 (35.12 %), in milk thistle treatment was 286 (34.05 %) and in control group were born 259 (30.83 %) animals. Total number of weaned animals in AV3 was 212 (71.86 %), in milk thistle group was 203 (70.98 %) and in control group the number was 158 (61.00 %). Total dead kids in AV3 and milk thistle groups was 83 kids each representing 28.14 % and 29.02 % respectively, and in control group the number of dead kids was 101 (39.00 %). The total results show that the best performance was from does from AV3 group followed by the performance of does from milk thistle group and the worst performance was from control group; the best and worst performances are respectively highlighted in blue and red color in Table 4.

Table 4: Comparison of number and percentage of born, weaned and dead kids between treatments and parturitions.

Parturition		1	2	3	4	Total
Born	**AV3**	83	95	82	35	295
	Milk Thistle	80	92	95	19	286
	Control	83	73	55	48	259
Dead	**AV3**	20	38	20	5	83
	Milk Thistle	28	28	21	6	83
	Control	38	25	25	13	101
% Dead	**AV3**	24.10	40.00	24.39	14.29	28.14
	MT	35.00	30.43	22.11	31.58	29.02
	Control	45.78	34.25	45.45	27.08	39.00
Weaned	**AV3**	63	57	62	30	212
	Milk Thistle	52	64	74	13	203
	Control	45	48	30	35	158
% Weaned	**AV3**	75.90	60.00	75.61	85.71	71.86
	MT	65.00	69.57	77.89	68.42	70.98
	Control	54.22	65.75	54.55	72.92	61.00

*blue color represents the best numbers and percentages, while red color represents the worst percentages

In terms of mean born kids among does, there was a significant difference between does from AV3 and control group. Does number 9 (AV3) and 27 (control) presented the lowest mean of born kids (2.00); doe number 2 (AV3) had the highest mean of born kids (11.5). In weaned and dead offspring, there were no significant differences (P>0.05) among the females. The highest number of weaned kids were from doe number 2 (AV3) and 3 (AV3) with a mean of 7.75 weaned kids per year. The lowest mean of dead kids was 0.25 from doe number 9 (AV3) and the highest mean was 5.00 from doe number 21 (control). From this data it is possible to see that the highest number of born and weaned kids and the lowest number of mortality in kids was within the group AV3.

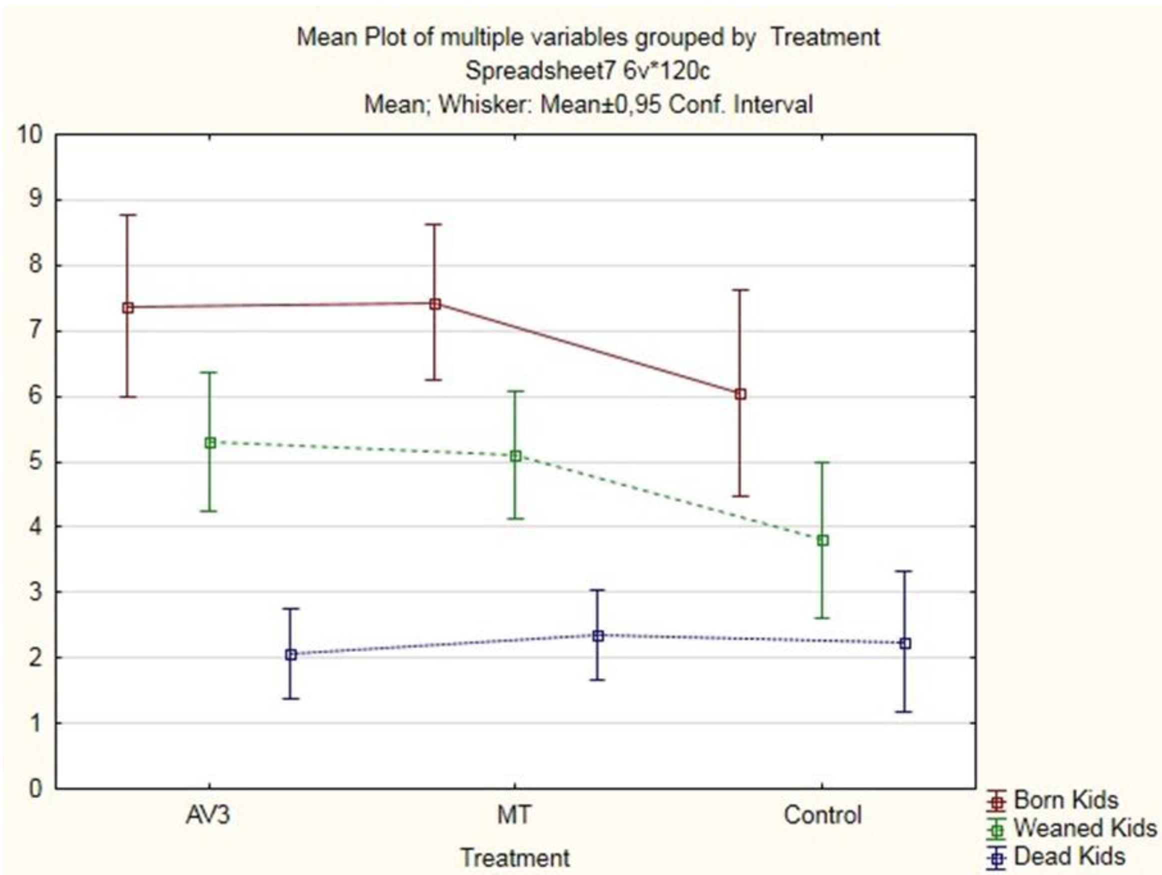

Figure 7: Mean of born, weaned and dead kids by treatment.

There was no significant difference (P>0.05) in the mean of born, weaned and dead kids among the experimental groups, the means were similar (see Fig. 7). The mean ± standard error for born kids was 7.38 ± 0.69 (AV3), 7.15 ± 0.61 (Milk thistle) and 6.47 ± 0.73 (control group); the average for weaned kids in AV3 group was 5.30 ± 0.52, for Milk thistle group 5.07 ± 0.50 and for control group 3.95 ± 0.55 kids and the mean for the number of dead kids was 2.07 ± 0.37 for AV3, 2.07 ± 0.37 for milk thistle and 2.52 ± 0.50 for control group. This data shows that the means of born and weaned kids was greater in AV3 than in milk thistle and control group and also demonstrates that the control group had the worst performance in terms of number of born and weaned kids compared to the other experimental groups, this may be due to the high mortality of kids within the group.

There were also found statistical differences (P<0.05) in the general mean of born, weaned and dead kids (see Tab. 5); in this case the treatment was not taken into consideration. The means of born and weaned kids in the fourth parturition presented the lowest mean with 3.06 ± 0.83 and 2.36 ± 0.66 kids respectively in comparison with the first, second and third parturitions. Likewise the previous results, the number of dead kids after the third and fourth parturition was low 2.30 ± 1.31 and 0.70 ± 0.24 (see Fig 8, 9, 10). The low number of born and weaned kids after the fourth parturition lead to a low mortality of kids in the same parturition (see Fig. 10).

Table 5: Total mean of born, weaned and dead kids according to the order of parturition.

	Born Kids	**Weaned Kids**	**Dead Kids**
1	8.20 ± 0.30^{a}	5.33 ± 0.46^{a}	2.86 ± 0.54^{a}
2	8.66 ± 0.61^{a}	5.63 ± 0.48^{a}	3.03 ± 0.40^{a}
3	8.06 ± 0.79^{a}	5.76 ± 0.62^{a}	2.30 ± 1.31^{ab}
4	3.06 ± 0.83^{b}	2.36 ± 0.66^{b}	0.70 ± 0.24^{b}

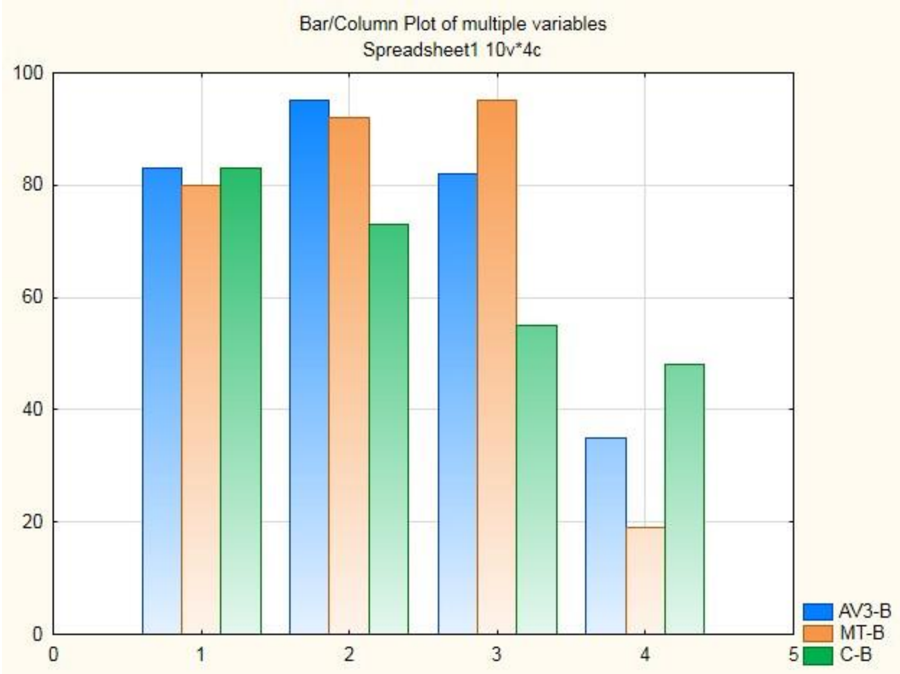

Figure 8: Variability of born kids in 1st, 2nd, 3rd and 4th parturition by treatment AV3 (blue), Milk thistle (orange) and Control group (green).

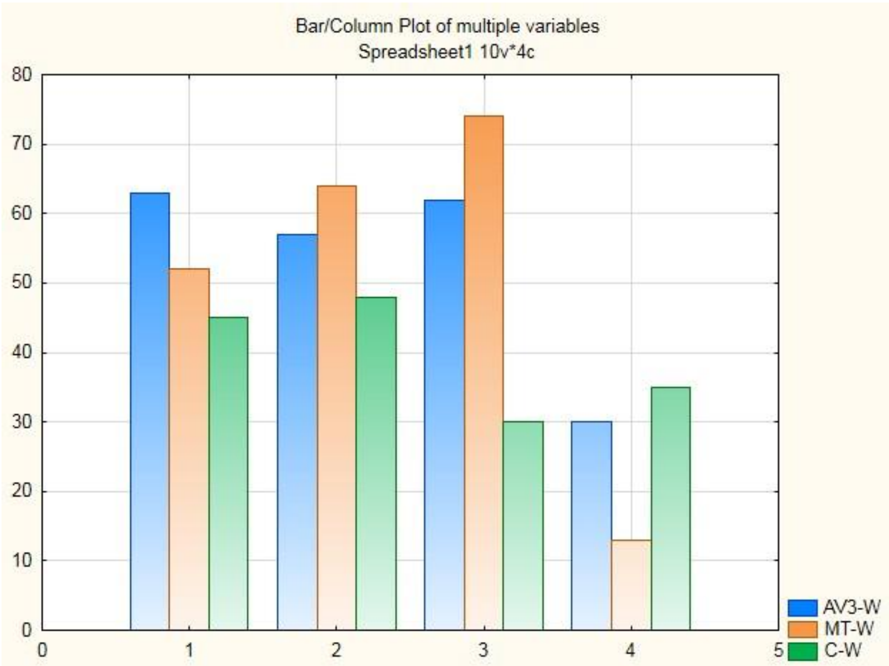

Figure 9: Variability of weaned kids after 1st, 2nd, 3rd and 4th parturition by treatment AV3 (blue), Milk thistle (orange) and Control group (green).

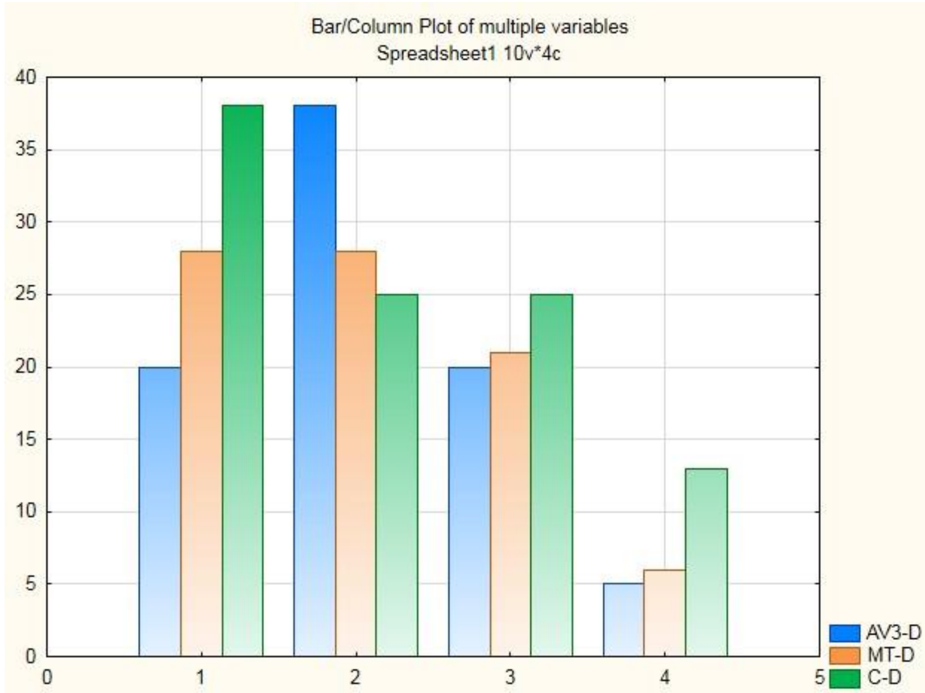

Figure 10: Variability of Dead kids in 1st, 2nd, 3rd and 4th parturition by treatment AV3 (blue), Milk thistle (orange) and Control group (green).

6 Discussion

Milk thistle (*Silybum marianum*) has been used for centuries in the treatment of liver disease (Flora et al., 1998; Karkanis et al.; 2011; Sadowska et al., 2011). In human medicine has been also used against amanita poisoning (Jacobs et al., 2002; Siegel and Stebbing, 2013), it has anti-oxidant, anti-viral, anti-carcinogenic, anti-inflammatory effects (Bhattaram et al., 2002; Siegel and Stebbing, 2013), as galactogogue, to stimulate milk production in women (Di Piero F et al., 2008; Ross, 2008), and in skin care products against UV radiation (Singh and Agarwal, 2009). In animal research has been used in broilers (Schiavone et al., 2007; Muhammad et al., 2012), cows (Kummer et al., 2000; Tedesco et al., 2004; Capasso et al., 2009), rabbits (Weisbroth et al., 1974), as galactogogue in sows (Loisel F et al., 2013) and rats (Capasso et al., 2009), against amanita poisoning in dogs (Desplaces et al., 1975; Stickel and Schuppan, 2007). There are still many studies being performed on animals to improve their reproductive performance.

The hypotheses of the study were that milk thistle supplement will positively affect the reproduction of does, the number of born kids and the number of weaned kids will be greater in the milk thistle supplement group. Form the data obtained and analyzed is possible to say that the use of both AV3 and Milk thistle supplements increased the number of born and weaned kids and decreased the number of dead offspring in comparison with the control group, but the statistical analysis proved that there was not a significant difference ($P>0.05$) among the three groups. This is in accordance with Hernán-Arcila et al. (2008), who found that silymarin improved the efficiency of hens during breeding. In other study performed on broilers, Muhammad et al. (2012) and reported that milk thistle in feed helped with body weight gain, feed conversion ratio and help binding aflatoxin B_1, therefore minimizing the harmful effects of toxin-contaminated feed. Capasso et al. (2009) also expressed that Silymarin improves the prolactin levels in cows and rats, but Loisel F et al. (2013) reported that Silymarin did not increased the prolactin levels in sows. It is important to mention that the fact that the number of born and weaned kids in AV3 group was slightly higher than in Milk thistle group, could be due the anti-oxidative properties of the AV3 and the low concentration of milk thistle used in the research.

In comparison with a similar study done for the conference XI. Kábrtovy Dietetické Dny at the University of Veterinary and Pharmaceutical Sciences Brno (VFU-Brno), was also found that milk thistle influenced the reproduction positively, but in like manner the results showed that there were no significant statistical differences among the experimental groups. Comparing the means of born and weaned kids in the three groups (see Tab. 6), their results were slightly higher than the ones found in this study. It was also found out that the percentage of weaned kids in milk thistle group was higher (76.05 ± 4.75 %) than AV3 group (73.03 ± 6.55 %); this differs from the data obtained in this study, the milk thistle group had lower percentage of weaned kids (70.98 %) than AV3 group (71. 86 %). In both studies the control group had the lowest percentage of weaned kids.

Table 6: Comparison of born, weaned kids of two different analysis.

		Born Kids	**Weaned Kids**	**% Weaned kids**
VFU, Brno	**AV3**	9.52 ± 0.61	6.84 ± 0.57	73.03 ± 6.55
	Milk thistle	8.81 ± 0.32	6.47 ± 0.37	76.05 ± 4.75
	Control	9.11 ± 0.42	5.79 ± 0.54	66.8 ± 6.68
Current study	**AV3**	7.38 ± 0.69	5.30 ± 0.52	71.86
	Milk thistle	7.15 ± 0.61	5.07 ± 0.50	70.98
	Control	6.47 ± 0.73	3.95 ± 0.55	61.00

Also in cooperation with the same university (VFU, Brno), there was performed the analysis of the biochemistry in the blood to evaluate the effect of milk thistle on the metabolism of the animal. The blood samples were taken from the 10 does that finished the 4 experiments. There was found that there were not significant differences in the metabolic profile among the experimental groups and moreover that the results obtained were similar to the referent values, this means that there was no serious disturbance to the metabolism of the doe. This result is a like with the findings of

Tedesco et al. (2004) and Kummer et al. (2000). The first author reported that *Silybum marianum* has neither a harmful nor protective effect on the liver of lactating dairy cows and the second author Kummer et al. (2000) also reported that Silymarin does not have an adverse effect on cows and could be fed in concentrations up to 4.1 % for no more than 5 weeks after calving.

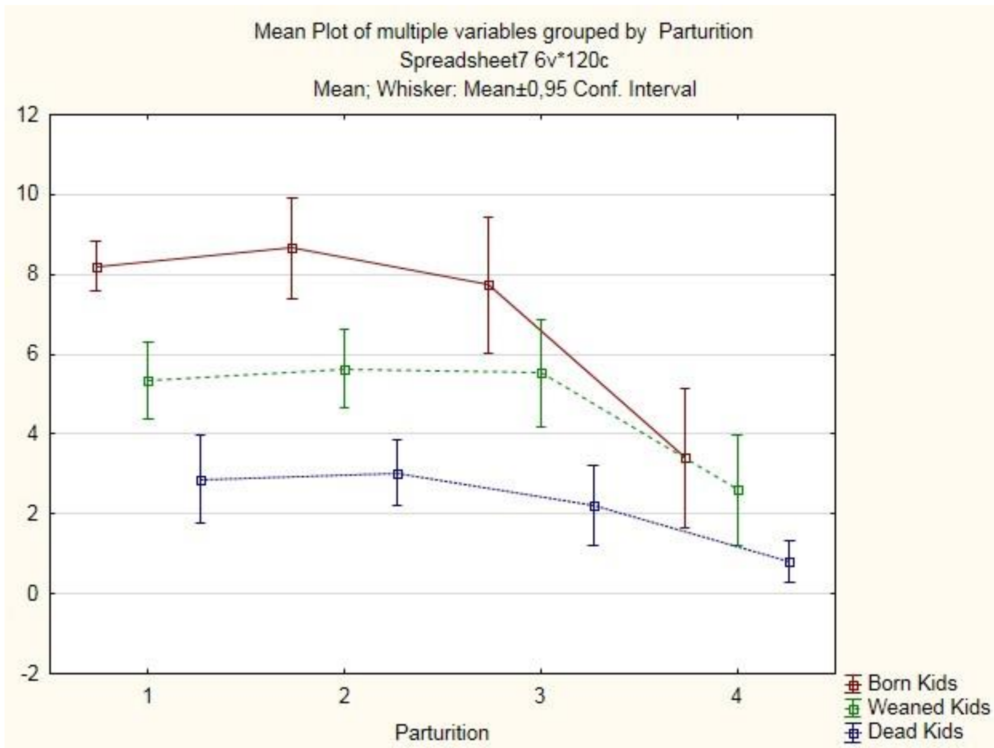

Figure 11: Total mean of born, weaned and dead kids according to parturitions.

From the general point of view the total mean number of born, weaned and dead kids in the first, second and third parturition was around the same value among the 3 experimental groups, but in the fourth parturition there was a significant drop in the mean of born and weaned kids (see Fig. 11). This low number of born and weaned kids after the fourth parturition may be due to the high mortality or poor conception performance of the does after the third parturition due to exhaustion or disease.

7 Conclusion

The aim of the thesis was to find any effect of the milk thistle (*Silybum marianum*) supplement on the reproduction of the rabbit does. The present study showed that both AV3 and Milk thistle supplements positively influenced the productive parameters of rabbit does. The parameters analyzed were number of born, weaned and dead kids, mortality and morbidity of rabbit does. Both groups had very promising numbers of offspring, but AV3 group had a slightly higher performance than milk thistle group, this may be due to a higher antioxidative effect in AV3 supplement. It is concluded that milk thistle could be used to improve the reproduction of does to certain degree, but furthermore more studies focused on the same topic on rabbit does should be performed with higher concentrations of milk thistle.

8 References

- Abington JB, Joshi BR, Shrestha RK, Dhaubhadel TS, Paudel KC, Tiwari BN, Ghimire SL, Clinch NL 1992. Sustainable Livestock Production in the Mountains Agro-ecosystem of Nepal, FAO-Animal Production and Health Paper 105, Food and Agricultural Organization, ISBN 92-5-10-3273-4.
- Alemardan AA, Karkanis A, Salehi R. 2013. Breeding objectives and selection criteria for milk thistle *Silybum marianum (L.) Gaertn*. Improvement. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 41: 340-347.
- Alfonso-Carrillo C, García-Rebollar P, De Blas C, Ibáñez MA, García-Ruiz AI. 2014. Effect of late weaning and use of alternative cages on performance of does, suckling and fattening rabbits under extensive reproductive management. Livestock Science 167: 425-434.
- Balszuweit F, John H, Schmidt A, Kehe K, Thiermann H, Steinritz D. 2013. Silibinin as a potential therapeutic for sulfur mustard injuries. Chem Biol Interact 206: 496-504.
- Baumann P, Oester H, Stauffacher M. 2005. Effects of temporary nest box removal on maternal behaviour and pup survival in caged rabbits (Oryctolagus cuniculus). Applied Animal Behaviour Science 91: 167-178.
- Bhattaram A, Graefe VU, Kohlert C, Veit M, Derendorf H. 2002. Pharmacokinetics and Bioavailability of Herbal Medicinal Products. Phytomedicine 9: 1-33.
- Biedermann D, Vavrikova E, Cvak L, Kren V. 2014. Chemistry of silybin. Natural Product Reports 31: 1138-1157.
- Capasso R, Aviello G, Capasso F, Savino F, Izzo AA, Lembo F, Borrelli F. 2009. Silymarin BIO-C, an extract from *Silybum marianum* fruits, induces hyperprolactinemia in intact female rats. Phytomedicine 16: 839-844.

- Cardile AP, Mbuy GKN. 2013. Anti-herpes virus activity of silibinin, the primary active component of *Silybum marianum*. Journal of Herbal Medicine 3: 132-136.
- Cardinali R, Dal Bosco A, Bonanno A, Di Grigoli A, Rebollar PG, Lorenzo PL, Castellini C. 2008. Connection between body condition score, chemical characteristics of body and reproductive traits of rabbit does. Livestock Science 116: 209-215.
- Castellini C, Dal Bosco A, Arias-Álvarez M, Lorenzo PL, Cardinali R, Rebollar PG. 2010. The main factors affecting the reproductive performance of rabbit does: a review. Anim Reprod Sci 122: 174-182.
- Castellini C. 2008. Semen Production and Management of rabbit bucks, Reproduction, Ninth World Rabbit Congress, June 10 – 13.
- Dalle-Zotte A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality, Livestock production Science 75 (1): 11 – 32.
- Desplaces A, Chopin J, Vogel G, Trost W. 1975. The Effects of Silymarin on experimental phalloidine poisoning, Arzneimittel-Forschung 35 (1): 89-96.
- Di-Piero F, Callegari A, Carotenuto D, Mollo-Tapia M. 2008. Clinical efficacy, safety and tolerability of BIO-C (micronized Silymarin) as a galactagogue, Acta Biomed 79: 205-210.
- Eskanda-Nasrabadi S, Ghorbani R, Rezvani-Moghaddam P, Nassiri-Mahallati M. 2014. Phenological response of milk thistle (*Silybum marianum [L.] Gaertn*.) To different nutrition systems. Journal of Applied Research on Medicinal and Aromatic Plants 1: 148-151.
- Flora K, Hahn M, Rosen H, Benner K. 1998. Milk thistle (*Silybum marianum*) for the therapy of liver disease. The American Journal of Gastroenterology 93: 139-143.
- Fortun-Lamothe L. 2006. Energy balance and reproductive performance in rabbit does. Anim Reprod Sci 93: 1-15.

- Fox JG, Cohen BJ, Loew FM. 2013. Laboratory Animal Medicine, American College of Laboratory, Animal Medicine Series, Academic Press Inc., ISBN-0-12-263620-1, 766 p.
- Fraser MA, Girling SJ. 2009. Rabbit Medicine & Surgery for Veterinary Nurses, ISBN: 978-1-4051-4706-4, 231 p.
- Geier J, Fuchs T, Wahl R. 1990. Anaphylactic shock due to extract of *Silybum marianum* in a patient with immediate-type allergy to kiwi fruit, Allergologie 13 (10): 387- 388.
- Gendron K. 2000. The Rabbit handbook: Purchase, Care and Breeding, Understanding rabbit behavior, China, ISBN 0-7641-1246-5, 135 p.
- González-Mariscal G, McNitt JI, Lukefahr SD. 2007.Maternal care of rabbits in the lab and on the farm: Endocrine regulation of behavior and productivity, Hormones and Behavior 52: 86–91.
- González-Mariscal G. 2001. Neuroendocrinology of Maternal Behavior in the Rabbit, Hormones and Behavior 40: 125–132.
- He Y, Wang C, Li Y. 2014. Effects of milk thistle cake on production performance and immune organ of rex rabbit, Feed Industry 22.
- Hernán-Arcila V, Lizarazo LV, Serrano MY. 2008. Efecto de la silimarina sobre la mejora productiva durante el período de cría en ponedoras Hy-Line variedad W-36, Revista Speri Domus 4 (8): 7-11.
- Hernández P, Dalle-Zotte A. 2010. Influence of diet on meat quality, Carlos de Blas and Julian Wisemam 2nd edition, Nutrition of the Rabbit, ISBN-13: 9781845936693, 333p.
- Hiatt J. 2012. The Principles of Knitting, The Comprehensive & Timeless Guide, Methods and Techniques of Hand Knitting, ISBN 978-1-4165-3517-1, 712 p.
- Jacobs BP, Dennehy C, Ramirez G, Sapp J, Lawrence VA. 2002. Milk thistle for the treatment of liver disease: A systematic review and meta-analysis. The American Journal of Medicine 113: 506-515.

- Karkanis A, Bilalis D, Efthimiadou A. 2011. Cultivation of milk thistle (*Silybum marianum L. Gaertn*.), a medicinal weed. Industrial Crops and Products 34: 825-830.
- Kummer V, Mašková J, Zralý Z, Čanderle J. 2000. Side effects of feeding Silybum marianum seed cake to dairy cows, Veterinářství 50 (2): 55-58.
- Lebas F, Coudert P, De Rochambeau H, Thébault RG. 1997. The rabbit husbandry, health and production. FAO Animal Production and Health Series.
- Lebas F, Coudert P, Rouvier R, De Rochambeau H. 1986. The rabbit husbandry, health and production. FAO Animal Production and Health Series.
- Loisel F, Quesnel H, Farmer C. 2013. Effect of Silymarin (Silybum marianum) treatment on prolactin concentration in cyclic sows, Canandian Journal of Animal Science 93: 227-230.
- Lumpkin S, Seidensticker J. 2011. Rabbits: The Animal Answer Guide,The John Hopkins University Press, ISBN-13: 978-0-8018-9788-7, 272 p.
- Majzlík I. 2006. Chov zvířat I. Česká zemědělská univerzita v Praze, Prague, ISBN: 978-80-213-1253-1,239 p.
- Manning PJ, Ringler DH, Newcomer CE. 1994. The Biology of the Laboratory Rabbit, Second Edition, Academic Press Inc., ISBN-0-12-469235-4, 483 p.
- Martínez-Morán C, Espinosa-Lara P, Nájera L, Romero-Maté A, Córdoba S, Hernández-Núñez A, Borbujo J. 2011. Embolia Cutis Medicamentosa (Nicolau Syndrome) after glatiramer acetate injection. Actas Dermo-Sifiliográficas (English Edition) 102: 742-744.
- Milic N, Milosevic N, Suvajdzic L, Zarkov M, Abenavoli L. 2013. New therapeutic potentials of Milk Thistle (*Silybum marianum*). Natural Product Communications 8: 1801-1810.

- Moce E, Vicente JS. 2009. Rabbit sperm cryopreservation: a review. Anim Reprod Sci 110: 1-24.
- Morrell J. 1995. Artificial insemination in rabbits. British Veterinary Journal 151: 477-488.
- Mugnai C, Dal Bosco A, Castellini C, 2009. Effect of different rearing systems and pre-kindling handling on behaviour and performance of rabbit does, Applied Animal Behaviour Science 118: 91–100
- Muhammad D, Chand N, Khan S, Sultal A, Mushtaq M. 2012. Hepatoprotective role of milk thistle (Silybum marianum) in meat type chicken fed aflatoxin B1 contaminated feed, Pakistan Veterinary Journal 22 (3): 443-446.
- Parker K. 2010. The Rabbit Handbook, Barrow's Educational Series Inc., ISBN-13: 978-0-7641-4253-6, 152 p.
- Pechová A, Martinec M, Ondráček J, Buchta M. 2015. The Effect Of Herbal Extracts With Silymarin On Production Parameters And Selected Biochemical Profile In Rabbit Does, Conference XI. Kábrtovy Dietetické Dny, University of Veterinary and Pharmaceutical Sciences Brno (VFU-Brno),,
- Petrie OJ. 1995. Harvesting of Textile Animal Fibers, FAO Agricultural Services Bulletin No 122, Food and Agricultural Organization, ISBN 92-5-103759-0, 101 p.
- Post-White J, Ladas EJ, Kelly KM. 2007. Advances in the Use of Milk Thistle (*Silybum marianum*), Integrative Cancer Therapies 6 (2): 104-109.
- Rodríguez-De Lara R, Fallas-López M, Garcia-Muniz JG, Martínez-Hernández PA, Rangel-Santos R, Maldonado-Siman E, Cadena-Meneses JA. 2015. Sexual behavior and seminal characteristics of fertile mature New Zealand White male rabbits of different body weights. Anim Reprod Sci 152: 90-98.

- Rodríguez-De Lara R, Noguez-Estrada J, Rangel-Santos R, Garcia-Muniz JG, Martínez-Hernández PA, Fallas-Lopez M, Maldonado-Siman E. 2010. Controlled doe exposure as biostimulation of buck rabbits. Animal Reproductive Science 122: 270-275.
- Ross SM. 2008. Milk Thistle (Silybum marianum): An Ancient Botanical Medicine for Modern Times, Holistic Nursing Practice 22 (5): 299–300.
- Sadowska K, Andrzejewska J, Woropaj-Janczak M. 2011. Effect of weather and agrotechnical conditions on the content of nutrients in the fruit of milk thistle (*Silybum marianum L. Gaertn*.). Acta Scientiarum Polonorum-Hortorum Cultus 10: 197-207.
- Scanes C. 2011. Fundamentals of Animal Science, Delmar Cengage Learning, ISBN-13: 978-1-4283-6127-0, 448p.
- Schiavone A, Righi F, Quarantelli A, Bruni R, Serventi P, Fusari A. 2007. Use of *Silybum marianum* fruit extract in broiler chicken nutrition, influence on performance and meat quality, Journal of Animal Physiology and Animal Nutrition 91 (5-6): 256-262.
- Selzer D, Lange K, Hoy St. 2004. Frequency of nursing in domestic rabbits under different housing conditions, Applied Animal Behaviour Science 87: 317–324
- Siegel AB, Stebbing J. 2013. Milk thistle: early seeds of potential. The Lancet Oncology 14: 929-930.
- Singh RP, Agarwal R. 2004. A cancer chemopreventive agent silibinin, targets mitogenic and survival signaling in prostate cancer, Mutation Research 555: 21–32.
- Singh RP, Agarwal R. 2009. Cosmeceuticals and silibinin. Clin Dermatol 27: 479-484.
- Sirotkin AV, Chrenek P, Kolesarova A, Parillo F, Zerani M, Boiti C. 2014. Novel regulators of rabbit reproductive functions. Anim Reprod Sci 148: 188-196.

- Skřivan M, Tůmová E, Skřivanová V. 2007. Chov králíků a kožešinových zvířat. Česká zemědělská univerzita v Praze, Katedra speciální zootechniky, Prague, ISBN: 978-80-213-0955-5, 247 p.
- Smith OB, Akinbamijo OO. 2000. Micronutrients and reproduction in farm animals. Anim Reprod Sci 60-61: 549-560.
- Stickel F, Schuppan D. 2007. Herbal medicine in the treatment of liver diseases. Dig Liver Dis 39: 293-304.
- Suckow MA, Stevens KA, Wilson RP. 2012. The Laboratory Rabbit, Guinea-pig, Hamster and Other Rodents, First Edition, American College of Laboratory, Animal Medicine Series, ISBN 978-0-12-380920-9, 1268 p.
- Szendro Zs, McNitt JI. 2012. Housing of rabbit does: Group and individual systems: A review, Livestock Science 150:1–10.
- Tablado Z, Revilla E, Palomares F. 2009. Breeding Like Rabbits: global paterns of variability and determinants of European wild rabbit preproduction, Ecography 32: 310-320.
- Takase M, Feng WW, Wang W, Gu XY, Zhu Y, Li T, Yang LQ, Wu XY. 2014. *Silybum marianum* oil as a new potential non-edible feedstock for biodiesel: A comparison of its production using conventional and ultrasonic assisted method. Fuel Processing Technology 123: 19-26.
- Tedesco P, Domeneghini C, Sciannimanico D, Tameni M, Steidler S, GAlletti S. 2004. Silymarin a possible hepatoprotector in dairy cows: Biochemichal and histological observations, Journal of Veterinary Medicine Series 51 (2): 85-89.
- Theau-Clement M. 2007. Preparation of the rabbit doe to insemination: a review, World Rabbit Series 15: 61-80.
- Ward J, Kapadia K, Brush E, Salhanick SD. 2013. Amatoxin poisoning: case reports and review of current therapies. Journal Emerg Med 44: 116-121.

- Warren DM. 2002. Small Animal Care and Management, Second Edition, Thomsom Learning, ISBN: 0-7668-1424-6, 434 p.
- Weisbroth SH, Flatt RE, Kraus AL. 1974. The Biology of the Laboratory Rabbit, Academic Press Inc., ISBN-0-12-742150-5, 510 p.

Annexes

Photo 1: Litter of rabbits (Silberová, 2014)

Photo 2: BIOKRON (s.r.o.) feed (Silberová, 2014)

Photo 3: New born kid (Silberová, 2014)

Photo 4: Stable of rabbit does at farm Jiří Kočár (Silberová, 2014)

Photo 5: Italian type cage for breeding does with nest box (Aguilera-Hidalgo, 2015).