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The Impact of Milk Thistle (*Silybum marianum*) Supplement in Feed Ration on the Rabbit Metabolism and Health Status

Master Thesis

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Declaration

I hereby declare that this thesis entitled "The Impact of Milk Thistle (*Silybum marianum*) Supplement in Feed Ration on the Rabbit Metabolism and Health Status" is my own work and all the sources have been quoted and acknowledged by means of complete references.

In Prague, 15th of April 2015

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Alice Procházková

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Abstract

The milk thistle (*Silybum marianum*) is a herb well known mainly for its hepatoprotective and antioxidative properties, which shows the positive results not only in human medicine, but also in veterinary medicine and animal feeding.

In total, 420 HYLA broiler rabbits were observed and tested. They were divided into 4 feeding groups. The control group was fed a complete feeding mixture without an addition of the milk thistle, the 1st experimental group was fed a feeding mixture with 0.2 ml/kg of AV3, the 2nd experimental group was fed a feeding mixture with 0.2 % of untreated milk thistle pomace and the 3rd experimental group was fed a feeding mixture with 1 % of untreated milk thistle pomace.

The experiment started at the rabbits´ age of 42 days and finished with the achievement of 2600g of live weight or at the rabbits´ age of 84 days by slaughter. 126 blood samples were processed and investigated by VetTest® chemistry analyzer (IDEXX Laboratories). Alanine aminotransferase, albumin, alkaline phophatase, amylase, asparate aminotransferase, calcium, cholesterol, globulins, glucose, phophorus, total bilirubin, total protein and urea were measured. A significant differences occured only in total protein, globulins, cholesterol and prosphorus, comparing feeding groups. But the obtained values do not advantage any of the experimental groups. In the third experimental group (1 %) occured the lowest mortality. The first (AV3) and the second (0.2 %) feeding groups showed the best results in the growth and gain parameters. But the usage of milk thistle supplement was counterproductive in the number of stunted rabbits.

A positive effect on the broiler rabbits was not demonstrated, but neither was a negative one, like deterioration of liver parameters, mortality or yields. It could be meaningful to feed does producing broiler rabbits, older and sick animals or pet animals milk thistle.

Key words: domestic rabbit, *Oryctolagus cuniculus f. domestici*, feeding, *Silybum marianum*, blood biochemistry, liver metabolism

Abstrakt

Ostropestřec mariánský je bylina známá především pro své hepatoprotektivní a antioxidační vlastnosti, které mají pozitivní výsledky nejenom v lidské medicíně, ale také v medicíně veterinární a v krmení zvířat.

Celkem bylo pozorováno a testováno 420 HYLA brojlerových králíků. Ti byli rozděleni do čtyř krmných skupin. Kontrolní skupina byla krmena kompletní krmnou směsí bez přidaného ostropestřce mariánského, první pokusná skupina byla krmena kompletní krmnou směsí s 0.2 ml/kg AV3, druhá pokusná skupina byla krmena kompletní krmnou směsí s 0.2 % neošetřených výlisků z ostropestřce a třetí pokusná skupina byla krmena kompletní krmnou směsí s 1 % výlisků z ostropestřce.

Pokus byl započat, když králíkům bylo 42 dní a ukončen porážkou při hmotnosti 2600g živé váhy, nebo po 84 dnech života. Zpracováno bylo 126 krevních vzorků, které byly vyšetřeny chemickým analyzátorem VetTest® (IDEXX Laboratories). Měřeny byly následující parametry: alaninaminotrasferáza, albumin, alkalická fosfatáza, amyláza, aspartátaminotransferáza, vápník, cholesterol, globuliny, glukóza, fosfor, celkový bilirubin, celková bílkovina a močovina.

Při porovnání krmných skupin, se statisticky významné rozdíly vyskytovaly pouze u celkové bílkoviny, globulinů, cholesterolu a fosforu. Ovšem získané hodnoty značně neznevýhodňovaly ani jednu krmnou skupinu. Nejnižší mortalita byla zaznamenána ve třetí pokusné skupině (1 %). První (AV3) a druhá (0.2 %) pokusná skupiny vykazovaly nejlepší růstové a výnosové výsledky. Ale užití doplňku z ostropestřce bylo kontraproduktivní ve vyhodnocování nedorostlých králíků.

Pozitivní vliv na brojlerové králíky nebyl potvrzen, ale potvrzen nebyl ani značný efekt negativní, jako například zhoršené jaterní parametry, úmrtnost či výnosy.
Využití krmného doplňku z ostropestřce mariánského může být smysluplné u chovných samic pro produkci brojlerových králíků, starších či nemocných zvířat nebo domácích mazlíčků.

Klíčová slova: králík domácí, *Oryctolagus cuniculus f. domestici*, krmení, *Silybum marianum*, krevní biochemie, jaterní metbolismus

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1. Introduction

The United Nations Food and Agriculture Organization estimates that about 805 million people in the world (one in nine) were suffering from a chronic undernourishment in 2014 (FAO, 2015). Rabbits are a great source of good quality and healthy meat, fur and manure. Due to their small size, short generation time and high reproductive potential, they appear to be the perfect laboratory animals as well. The utilization of non-competitive feeds, also plays an essential role. Like ruminants, rabbits can be successfully raised on grain-free diets, based on forages and by-products (Cheeke, 1986). Rabbit husbandry is very lucrative even for landless, small farmers. It provides work for women, children and also for handicapped people all over the world (Lebas et al., 1997). A great advantage is also the fact that there is very little, if any, cultural biases or religious prohibitions against the consumption of rabbit meat (Cheeke, 1986). However, it is well known that rabbits are quite sensitive and susceptible to many disease or disfunctions, which can make the husbandry less profitable.

Liver is the main organ managing the immunity and the resistance of the body (Racanelli and Renemann, 2006). When we support the liver functions, we suppose to have healthy, prosperous animals.

Milk thistle is well known mainly for its hepatoprotective and antioxidative properties especially in human medicine. Its friuts have been used for 2000 years in herbal remedies (Shokrpour et al., 2008), and the derivatives and the preparations from its main active component silymarin have been used in human medicine for almost 200 years (Kvasnička et al., 2003).

Milk thistle or its extracts were also tested on the animals such as mice (Abenavoli et al., 2010), rats (Jain et al., 2011; Sharifi et al., 2012), dogs (Flora et al., 1998; Abenavoli et al., 2010), thick tail gerbils (Bouderba et al., 2014) or cows (Abenavoli et al., 2010). Most of these researches used silymarin as a medicament for sick or poisoned animals. There are only few experiments done on the utilization of milk thistle in animal feeding. Most of them were done on the broiler chickens (Schiavone et al., 2007; Suchý et al., 2008; Li et al., 2013) and it brought possitive effects on carcass, reduction of the abdominal fat, modification of the biochemical parameters and an improvement of the immune response (Schiavone et al., 2007; Suchý et al., 2008; Li et al., 2013).

Can feeding the milk thistle bring any advantages for broiler rabbits as well? This thesis tries to answer this question and find the advantages of of milk thistle feeding for health status and metabolism of broiler rabbits.

2. Literary review

2.1. Milk Thistle (*Silybum marianum*): botanical description and characteristics

Silybum marianum L. Gaertn. (synonym: Carduus marianus L.) is a member of the Asteraceae family (Flora et al., 1998; Karkanis et al., 2011; García-Herrera et al., 2014). The genus Silybum contains two species: S. marianum and S. eburneum Coss. & Dureu (Hetz et al., 1995). It is an annual to biennial plant (Gresta et al., 2006). Milk thistle is a long-day plant (Karkanis et al., 2011), occuring mainly in the Mediterranean and East parts of Europe (Abenavoli et al., 2010) S. marianum was also naturalized in the North and the South America as well as in the South Australia (Abenavoli et al., 2010). The plant grows in warm, dry soil and blooms from July to August (Abenavoli et al., 2010).

The stem of a milk thistle is 40–200 cm high, glabrous or slightly downy, erect and branched in the upper part of the plant (Montemurro et al., 2007). The basal leaves are alternate, large and glabrous with spiny margins (Karkanis et al., 2011). The leaves are 50–60 cm long and 20–30 cm wide and the stem leaves are smaller (Karkanis et al., 2011). The main characteristic of milk thistle is the milk white veins in the leaves (Gresta et al., 2006).

Each stem ends in a flower head about 5 cm in diameter (Montemurro et al., 2007), of a red-purple colour, rarely white. The inflorescences are surrounded by spiny bracts. The florets are hermafrodite (Karkanis et al., 2011). Since the outcrossing rate is only about 2% on an average, milk thistle is predominantly a self-pollinator (Hetz et al., 1995).

Seeds are achened, 5–8 mm long, with long white pappus and a color ranging from black to brown (Karkanis et al., 2011). The weight of 1000 milk thistle seeds is 28–30 g (Andrzejewska et al., 2011 in Karkanis et al., 2011). Each flower head produces about 190 seeds, with an average of 6350 seeds per plant (Karkanis et al., 2011). In the soil, seed can remain viable for up to nine years (Karkanis et al., 2011). The seeds show little or no dormancy (Karkanis et al., 2011).

An interesting fact is that interspecific hybrids of these 2 species of S. marianum produce more fruits in comparison with their parents (Hetz et al., 1995). And fruits are the key product because it is used in a healthcare. Fruits contain approximately 70–80%

silymarin flavonolignans and approximately 20–30% chemically undefined fraction – usually polymeric and oxidized polyphenolic compounds (Křen and Walterová, 2005). The active lipophilic extract of milk thistle is silymarin (Flora et al., 1998; Kapoor et al., 2009). Silymarin is a complex mixture of substances isolated from fruits of milk thistle (Kvasnicka et al., 2003). It is a mixture of flavonolignans: isosilybin (5.1%), silydianin (5.9%), silychristine (15.7%), and silybin (36.6 %) (Saller et al., 2001; Šeršeň et al., 2006), which is biologically the most active part (Flora et al., 1998; Kapoor et al., 2009; Abenavoli et al., 2010). Silybin, has been reported in many previous studies, to work as antioxidants scavenging free radicals, inhibiting lipid peroxidation and to have hepatoprotective properties (Kapoor et al., 2009). Studies also suggest that silybinin prevents from genomic injury, increases synthesis of hepatocyte protein, decreases the activity of tumor promoters, chelates iron, stabilizes mast cells, slows down calcium metabolism and helps slow down the extent of diabetic retinopathy (Flora et al., 1998; Kapoor et al., 2009).

The highest concentration of silymarin is in the fruit of the plant, but considerably high is also in the seeds and leaves (Flora et al., 1998). The typical extraction is done with 95% ethyl alcohol, yielding a bright yellow fluid (Flora et al., 1998). This extract usually contains 70% silymarin (Willard, 1992 in Flora et al., 1998)

Another important elements found in fruits are betaine, trimethylglycine, and essential fatty acids, which may contribute to hepatoprotective and anti-inflammatory effects of silymarin (Luper, 1998).

2.2. Utilization of milk thistle as a medical plant

Silymarin, derived from the milk thistle plant, Silybum marianum, has been used for centuries as a natural remedy for diseases of the liver and biliary tract including hepatitis, cirrhosis and jaundice, and as a protection of the liver against poisoning from chemical and environmental toxins, including snakebites, insect stings, mushroom poisoning and alcohol (Flora et al., 1998; Abenavoli et al., 2010).

Dioscorides, a Greek herbalist, was the first man who wrote about curing with milk thistle. He suggested preparing it as a tea ‘for those that are bitten of serpents’(Abenavoli et al., 2010). Therefore, we know that it has been used as medical

plant since the ancient Greece (Flora et al., 1998). Later, it has been discovered that Indian and Chinese medicines used milk thistle in clinical practice too (Abenavoli et al., 2008).

Big flourish in the use of a milk thistle came at the turn of the 20th century. When a school of medical herbalists called the "Eclectics" was using milk thistle extracts for "liver congestion," varicose veins, menstrual disorders, and abnormalities of the spleen and kidneys (Flora et al., 1998).

The commercial drug originates principally from the cultivated sources, primarily from China, Argentina, Romania, and from few Mediterranean countries and partly from Germany (Abenavoli et al., 2010). (Morales et al., 2014) describes that we can also eat milk thistle boiled and fried in olive oil with garlic or raw in salads. But silybum marianum has a low amount of vitamin C and a high concentration of oxalic acid especially in the basal leaves (Morales et al., 2014). Oxalic acid can present a problem for peole suffering from adnex calculus disorders, but its content can be greatly reduced by a cooking process (García-Herrera et al., 2014; Morales et al., 2014).

One of the main problems is low bioavailability of silibinin (Abenavoli et al., 2010), which depends on several factors such as the content of the accompanying substances with a solubilizing character (other flavonoids, phenol derivates, amino acids, proteins, tocopherol, fat, cholesterol and other substances found in the preparation) and the concentration of the preparation (Abenavoli et al., 2010). Several attempts have been done to discover how to increase the bioavailability. The bioavailability can be enhanced by adding solubilizing substances to the extract (Saller et al., 2001), and also by the complexation with phosphatidylcholine or β-cyclodextrin and possibly by the choice of the kapsule material (Morazzoni et al., 1992). Other inconsiderable issue in the treatment and the use of silymarin is the lack of standardization of preparations and its dosing. It is very important to take care of potential interactions with other herbal or chemical drugs as well , furter studies are necessary (Ingram et al., 2000). On the other hand, it is possitive that silymarin has not been shown to have any adverse side effects (Flora et al., 1998; Abenavoli et al., 2010). But it is good to be careful, because at higher doses of more than 1500 mg/day silymarin may produce a laxative effect with an increased bile flow and secretion (Abenavoli et al., 2010). Preclinical data has not documented acute toxicity for sylimarin and silybin. Silymarin, given orally to mice, rats and dogs did not cause adverse effects or mortality (Abenavoli et al., 2010). But milk thistle preparations are

contraindicated for individuals with hypersensitivity to Asteraceae (Abenavoli et al., 2010).

2.2.1. The utilization of milk thistle for humans (in human medicine)

Marketing and common interest in herbal products is still rapidly incerasing and many people prefer natural products to synthetic drugs. Milk thistle is the most researched plant for the treatment of liver disease (Karkanis et al., 2011), and silymarin has been used for the treatment of liver diseases for centuries (Flora et al., 1998). All these facts makes milk thistle preparations well acceptable by people.

Nowadays, it has been found that silymarin and silybinin really exert hepato-protective, antioxidant, antiinflammatory and antifibrotic properties; in addition, they stimulate protein biosynthesis and liver regeneration, increase lactation and possess immuno-modulation activity (Abenavoli et al., 2010), thus silymarin is a suitable candidate for treating iatrogenic and toxic liver diseases (Abenavoli et al., 2010). (Morales et al., 2014) add it can also be used to treat haemorrhoids. The modern use of silymarin complex in human medicine is extended not only to the liver and gastrointestinal tract but also in the treatment of kidney, prostate and lung problems, as well as in dermatology. (Kvasnička et al., 2003) states, that the antioxidant and membrane stabilizing capabilities of the silymarin complex can protect cells and organs of the body. Antioxidant properties of silymarin can also help patients suffering from diabetes type II (Huseini et al., 2006). Sylimarin can also be administered locally, and can rapidly heal burns and morphological changes in the skin (Toklu et al., 2007).

Studies done on humans have shown a rapid absorption of silybin into the bloodstream after an oral dose. The highest plasma concentrations were reached after 2 h and the elimination time is approximately 6 hours (Barzaghi et al., 1990). From 3 to 8% of an oral dose is excreted in the urine (Barzaghi et al., 1990).

Silymarin is very effective when it comes to improving the clinical courses of acute and chronic viral, drug-, and toxin-induced and alcoholic hepatitis in humans (Flora et al., 1998). Studies on humans with acute viral hepatitis suggested that therapy with silymarin decreases complications, hastens recovery and shortens hospital stays (Flora et al., 1998). Silymarin can probably also protect from histologic changes found in the livers of women who are pregnant or on birth control pills (Flora et al., 1998). Silymarin also

shows very positive effect on treating or normalizing alcoholic liver injury. Especially standardizing of AST and ALT blood levels (Flora et al., 1998).

The future and new prospective of the use of silybum marianum is in cancer and chemopreventive role of milk thistle (Abenavoli et al., 2010). Particularly, the protective effects of silymarin and its major active constituent, silibinin, studied in a variety of cancer models, including liver cancer, suggest that they should be established in therapies as the adjuncts in the clinical application for these patients to prevent or reduce chemotherapy as well as radiotherapy-induced toxicity (Abenavoli et al., 2010).

2.2.2. The utilization of milk thistle in veterinary medicine and animal feeding

The positive hepatoprotective effect of Silybum marianum has also been tested on some animal species. Noteworthy are mice (Abenavoli et al., 2010), dogs (Flora et al., 1998; Abenavoli et al., 2010) thick tail gerbils (Bouderba et al., 2014), broiler chickens (Schiavone et al., 2007; Suchý et al., 2008; Li et al., 2013) and carps (Jia et al., 2013). It was reported, that milk thistle increased lactation in cows (Abenavoli et al., 2010).

In rats the efficacy of milk thistle was demonstrated in the speed of wound healing (Sharifi et al., 2012). Abenavoli et al. (2010) states that milk thistle significantly increased circulating prolactin levels in rats. (Jain et al., 2011) tested the utilization of silymarin on rats, and they confirmed a hepatoprotective effect against arsenite-induced cytotoxicity in rats.

Most of the experiments examining the utilization of milk thistle in animal feeding were done on broiler chickens (Schiavone et al., 2007; Suchý et al., 2008; Li et al., 2013). Administration of milk thistle to broiler chickens improved the properties of the carcass, including the reduction of abdominal fat, modification of biochemical parameters and improvement of the immune response (Schiavone et al., 2007; Suchý et al., 2008; Li et al., 2013). The musculature is also more resistant to oxidative stress (Schiavone et al., 2007). With concentrations of 40 and 60 ppm of the plant in the feed mixture there is no inhibition of growth (Suchý et al., 2008), but (Schiavone et al., 2007) recorded reduction of slaugtering yields and reduction of lipid deposition in both muscular tissue and abdominal fat pad. This is probably a direct consequence of reduced feed intake which negatively affected energy balance (Schiavone et al., 2007).

Biochemical tests show damage to the chicken broiler's liver during their fattening to heavier weights, and confirm the hepatoprotective effect of silymarin contained in the cakes from the seeds of *Silybum marianum* (Suchý et al., 2008). (Suchý et al., 2008) also add, that the hepatoprotective effect of *Silybum marianum* seed cakes added to the chickens' feed mixtures resulted in lower cholesterol levels. On the other hand (Schiavone et al., 2007) states, that in their experiment silymarin did not show any specific hepatoprotective effect, according to the tested blood parameters.

2.3. The Domesticated European rabbit (*Oryctolagus cuniculus f. domesticus*) as a model animal for testing of milk thistle

The rabbit was chosen as a model animal for testing of milk thistle because it is small animal and thus easy to handle, housed and transported. They are readily available commercially (Jeklova et al., 2009). Great plus for rabbit is also extended acceptability of its meat and no refusion of religion or other reasons. Also, the taste of the meat, which is comparable (but not identical) to chicken, without very strong flavor, is one of the advantages (Lebas et al., 1997). Reasons for having rabbit as a model animal are also its high rate of reproduction and early maturity, short life cycle, rapid growth rate, high genetic selection potential, efficient feed and land space utilization.

The European rabbit (*Oryctolagus cuniculus*) occurs in three forms: wild, feral and domestic (Fox et al., 2002). These tree forms can be found only on the European continent, because the wild type envolved in the Iberian Peninsula and spread to other regions (Lebas et al., 1997; Fox et al., 2002). The feral rabbit is the domestic type, which was returned back into the wild nature (Fox et al., 2002). Many examples can be found in America or Australia. The domestic form is characteristic by a great variety of strains and breeds (Arrington and Kelley, 1976). These forms are used for meat, fur, as pets or as laboratory animals.

Rabbits, hares and pikas were originally classified as Rodents (Rodentia). For this order it is characteristic to have four incisor teeth, but rabbits have six (Fox et al., 2002). The additional pair is reduced and placed behind the large pair in the upper jaw. Based on this diference, rabbits were placed in a separate order Lagomorpha (Arrington

and Kelley, 1976). The wild rabbit *Oryctolagus cuniculus* of southern Europe and North Africa was probably discovered by Phoenicians when they reached the shores of Spain about 1000 BC. The rabbit was the emblematic animal of Spain in Roman times. Later, the rabbit was spread by Romans throughout the Roman Empire as a game animal. Although this is still not a domestication, Varron (116 to 27 BC) suggested that rabbits were kept in enclosures called leporaria (Lebas et al., 1997). These were stone-walled pens or parks, with hares and other wild species for hunting. These leporaria were the origin of the game parks that were subsequently developed in the Middle Ages. The true domestication can be traced to the late Middle Ages (Arrington and Kelley, 1976). The rabbits were probably domesticated by monks, since it provided them a more delectable dish than the tougher wild rabbit (Lebas et al., 1997). It is also known that monks used to eat laurices (foetuses or newborn rabbits) during Lent as they were considered "an aquatic dish" (Lebas et al., 1997).

2.3.1. Life cycle and reproduction

Kittens (baby rabbits) are born with closed ears and eyes, and are scantily furred. In their first three weeks of life, they are totally dependent on their mothers. In four to five weeks, they are self-sufficient. Within two to three months, they are already sexually mature and able to breed and initiate the cycle again. Their lifespan is typically five to ten years, maximally thirteen years (Arrington and Kelley, 1976).

Rabbit males can also be called bucks. The sexual maturity is defined as a moment when daily sperm production ceases to increase. They reach it approximately at 32 weeks (Theau-Clément et al., 1998). However, (Lebas et al., 1997) states we can use bucks for reproduction from the age of twenty weeks. The first manifestations of sexual behaviour appears from 60 to 70 days of age (Arrington and Kelley, 1976; Lebas et al., 1997). Coitus may occur for the first time at about 100 days, but the viability of the sperm cells is very weak or nil in the first ejaculates. So first mating is recommended for age among 135 and 140 days (Lebas et al., 1997). The onset of puberty varies from breed to breed. But feeding and conditions in the rabbitry play essential role and can influence the time of puberty (Theau-Clément, 2000). The volume of ejaculated semen is circa 0.3 to 0.6 ml (Lebas et al., 1997; Theau-Clément et al., 1998). Concentration is evaluated at 150 to

500 x 106 spermatozoa per ml (Lebas et al., 1997). Maximum spermatozoa production can be obtained by using the buck regularly, once a day (Theau-Clément, 2000) .

Rabbit females, called does, are able to mate first at 10 to 12 weeks, but as a rule this will not produce ovulation. The onset of puberty varies greatly with the breed and body development. Sexual precocity is more developed in small or medium breeds (four to six months) than in large breeds (five to eight months). In Europe, does are usually mated at 120 to 130 days. (Lebas et al., 1997) states, that does fed ad lib reach puberty three weeks earlier than other does of the same strain receiving only 75 percent of the same daily feed. Generally, does reach puberty when they have grown to 70 to 75 % of their mature weight (Lebas et al., 1997).

Does do not have an oestrus cycle with regular periods of heat during which ovulation will occur spontaneously (Theau-Clément, 2000). Does are considered to be in oestrus more or less permanently. Ovulation is normally induced by the stimuli associated with coitus and occurs ten to twelve hours after mating (Lebas et al., 1997). So, a doe is considered to be in heat when she accepts service and in dioestrus when she refuses (Boiti, 1998). The reproduction cycles of the European wild rabbit are strongly influenced by the season (Arrington and Kelley, 1976). Does breed from the end of the winter until the early summer. The lenght of the gestation is about 31 days (Lebas et al., 1997; Theau-Clément et al., 1998). In the end of the gestation the doe makes a nest for the litter from its own fur and other available materials such as straw and shavings (Arrington and Kelley, 1976). Sometimes the does do not make nests or kindle outside the nesting box. Kindling lasts from 15 to 30 minutes, according to the size of the litter (Theau-Clément et al., 1998). Size of the litter can vary very much, as much as from one to twenty youngs (Arrington and Kelley, 1976; Lebas et al., 1997). But most litters range between three and twelve youngs (Theau-Clément, 2000). After parturition the uterus retracts very quickly, losing more than half its weight in less than 48 hours.

Nowadays, in European countries, rabbit artificial insemination is commonly practiced (Boiti, 1998; Theau-Clément et al., 1998; Theau-Clément, 2000). Very discussed is the topic of biostimulation methods in opposition to hormone use, to improve does receptivity and thus their fertility and reproductivity (Theau-Clément et al., 1998; Theau-Clément, 2000).

2.3.2. Digestive tract

Digestive tract begins in the oral cavity with teeth. Rabbit´s dental formula is i2/1, c0/0, p3/3, m2-3/3 x 2 = 26 or 28 teeth (Fox et al., 2002). The total length of the alimentary canal is 4.5 to 5 m (see Figure 1) (Lebas et al., 1997). After a short oesophagus there is a simple stomach which stores about 90 to 100 g of a rather pasty mixture of feedstuffs, which has an acid environment there (Arrington and Kelley, 1976). The feed remains in the stomach for three to six hours and it undergoes little chemical change.

The content is then injected from the stomach to the small intestine (3 m long and 0.8 to 1 cm in diameter) (Lebas et al., 1997; Fox et al., 2002). Then the content is moved to the small intestine, where it is diluted by the flow of bile, the first intestinal secretion and finally the pancreatic juice (Arrington and Kelley, 1976). This enzymatic action allows that the elements which can easily be broken down are freed and can pass through the intestinal wall to the blood stream (Arrington and Kelley, 1976). The other particles which are not broken down (after one and a half hour in the small intestine) enter the caecum (40-45 cm long and 3 or 4 cm in diameter (Lebas et al., 1997). The caecum is not only a a blind pouchreservoir, but it has its own important role in the digestion. The content circulate there from the base to the tip along the wall (Arrington and Kelley, 1976). The content remains there from two to twelve hours, while it is breaking down by bacterial enzymes. Elements which can be broken down (mainly volatile fatty acids) are freed through the wall of the caecum into the bloodstream. Caecum ends with the caecal appendix (10 to 12 cm) (Fox et al., 2002). The content can be dividend into two halves. One consists of small and large food particles, which still have not been broken down. The other half consists of bacteria that have developed in the caecum, using the enrgy from the matter of the small intestines (Lebas et al., 1997).

The colon (1.5m) is the next important part of the rabbit´s digestive system (Lebas et al., 1997). It begins very near the end of the small intestine, at the entrance to the caecum. We can recognize two part of the colon. The first is wrinkled and dented (proximal colon) and the second is smooth (distal colon) (Lebas et al., 1997). Uniqueness of the rabbit´s digestive tract lies in the dual function of the proximal colon (Abecia et al., 2007). If the content of caecum enters the colon in the early mening, it undergo few biochemical changes. The pellets are formed by the contractions of caecal walls and are

gradually enveloped of the mucus produced by the colon wall (Arrington and Kelley, 1976). These pellets are called caecotrophes and are congregated in elongated clusters (also soft or night pelets) (Lebas et al., 1997). If the content of caecum enters the colon at another time of day, the response of the proximal colon is different. The content is moved normally by consecutive waves of contractions, but then it is pushed back to the caecum. (Lebas et al., 1997). Back to the caecum is pushed the liquid part, which contain soluble products and partices of less than 0.1 mm (Fox et al., 2002). Large particles (over 0.3mm) are the main components of the solid part, which forms hard pellets which are then expelled. The soft pellets are eaten again by the rabbit. They usually take it directly after they are expelled from the anus. From then the soft pellets follow the same digestive process as normal feed (Arrington and Kelley, 1976). The soft pellets consist half of incompletely broken-down food residues and of residua from the gastric secretions and half of bacteria (Arrington and Kelley, 1976; Lebas et al., 1997). The bacterial content contain a large amount of high-value proteins and watersoluble vitamins (Fox et al., 2002). The practice of caecotrophy therefore has a certain nutritional value (Lebas et al., 1997). This is why rabbits are called pseudoruminants (Arrington and Kelley, 1976). Caecotrophy starts in young rabbits at the age of about three weeks, when they start eating solid feed in addition to mother's milk (Lebas et al., 1997; Abecia et al., 2007).

Digestion of rabbits is widely affected by bacterial colonization of the cecum (Arrington and Kelley, 1976; Abecia et al., 2007). The composition of bacterial colonization in the intestinal ecosystem of rabbits has not yet been fully clarified (Abecia et al., 2007). Rabbits old between 17 and 42 days are most sensitive to digestive disorders, which are caused by changes in nutrition when young rabbits begin to take solid food and water, and turn from the animal protein contained in milk to rabbit food containing the vegetable protein (Abecia et al., 2007). Viability of weaned rabbits is affected by nutrition before weaning (Abecia et al., 2007).

Two major glands secrete into the small intestine: the liver and pancreas. Bile from the liver contains bile salts and many organic substances which aid digestion, but has no enzymes. On the other hand, pancreatic juice contains a considerable quantity of digestive enzymes which can breakdown proteins (trypsin, chymotrypsin), starch (amylase) and fats (lipase) (Arrington and Kelley, 1976; Lebas et al., 1997).

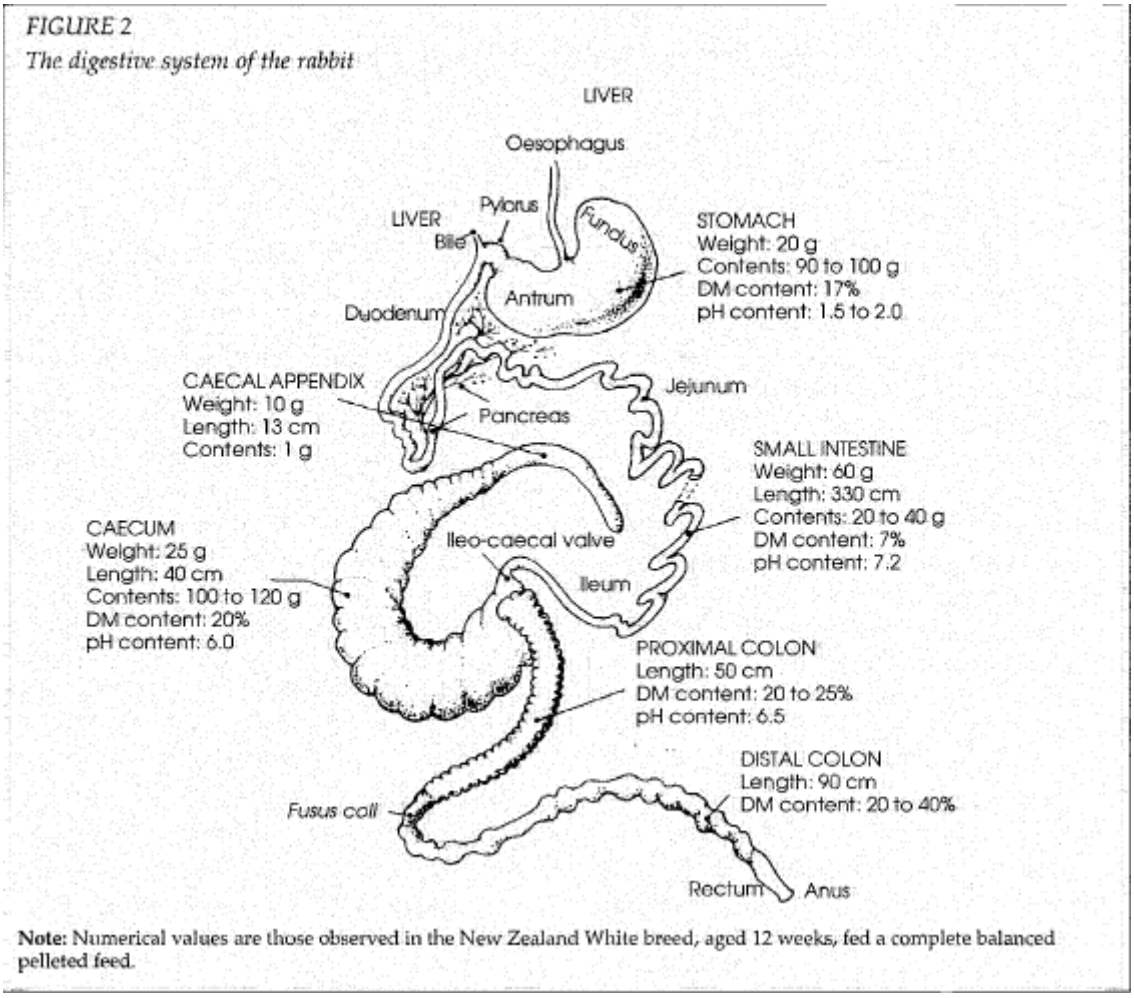

Figure 1: The digestive system of the rabbit (Lebas et al., 1997).

The water content can vary markedly from one segment to the next, depending on secretions and water absorption.

2.3.3. Nutrition of broiler rabbits

The production of rabbits is strongly dependent on adequate and proper feeding. The quantity of provided feed is very important, but the quality is equally or mor important.

The rabbit's energy expenditure is dependend on surrounding temperature. Feed coping with energy needs is therefore linked to temperature. Laboratory tests on growing rabbits have shown that at temperatures between 5°C and 30°C the intake of pelleted feed

dropped from. 180 to 120 g a day and the water intake rose from 330 to 390 g (Lebas et al., 1997).

Rabbits with access to drinking water but no solid feed can survive for three or four weeks. Sodium chloride in the water (0.45%) reduces this high intake (Lebas et al., 1997). The rabbit is therefore very resistant to hunger and relatively resistant to thirst. Feeding preferences of rabbits are usually unpredictable (Lebas et al., 1997). But acording Cheeke et al. (1977) they prefere bitter taste.

A mixed feeds are the most common, the cheapest and the easiest feedstuffs for intensive European rabbitries. They can be found in the form of pellets or granules and they are prepared to cover the nutritional needs of all the rabbit categories.

There are four main components in feedstuffs. First there are standards on proteins and its composition (distribution of amino acids) (Lebas et al., 1997). Proteins are the main elements to build or rebuild rabbit bodies. The proportion of indigestible fibre is necessary and essential for the proper functioning of the digestive tract (Lebas et al., 1997). Also the corresponding proportion of fibre can be estimated by the acid detergent fibre. The energy is necessary for regulation of the body temperature as well as for the general functioning of the body (Lebas et al., 1997). For minerals and vitamins, which serve as building blocks for certain parts of the animal (skeleton, etc.) and for the enzymes which use energy to build and rebuild the body proteins continually (Lebas et al., 1997). Rabbits are (as ruminants) herbivorous and some microbial fermentation also occurs in the intestine. Thus we can expect that rabbits may also utilize nonprotein nitrogen (Arrington and Kelley, 1976). Rabbit requires eleven essentials aminoacids: arginine, histidine, isoleucine, methionine, phenylalanine, threonine, tryptophane, valine, leucine, lysine and for the rapid growth glycine (Arrington and Kelley, 1976).

The potential problem of gastroinestinal tract diseases in intensive rabbit production led to the frequent use of antibiotics, either as therapeutics or for growth-promoting purposes (Abecia et al., 2007). Administration of fodder antibiotics does not necessarily lead to degradation of microbes in the digestive tract of herbivores, while some types of fodder antibiotics verifiably improve digestion of rabbits (Falcão-e-Cunha et al., 2007). (Abecia et al., 2005) add, that antibiotics not only reduce the risk of potential infections, but may also affect the symbiotic bacterial population of the digestive tract, potentially improving nutrient utilization in growing rabbits. Since 1 January 2006 antibiotics usage in fodder mixtures has been prohibited, except for coccidiostatics and

histomoniostatics (Directive no. 1831/2003). Nowadays, there are efforts to replace the fodder antibiotics and other chemicals by natural products.

2.3.4. Health problems of broiler rabbits

Rabbits can mask signs of illness or show few or confusing clinical signs (Melillo, 2007). It can be problem while setting the right diagnosis, which is crucial for successfull treatment and prevention of the remaining animals.

Pasteurellosis, the illness caused by the bacterium *Pasteurella multocida*, is the principle disease of domestic rabbits (Arrington and Kelley, 1976; Thompson, 1976; Jenkins, 2008). It is a highly contagious, persistent infection of rabbits world-wide (Thompson, 1976). Clinical signs vary with the part of the body affected by the organism. The most frequent manifestation of disease is upper respiratory tract disease involving the nasolacrimal ducts, nares, nasal sinuses, and nasopharynx (Arrington and Kelley, 1976; Jenkins, 2008). Clinical signs usually include sneezing, matted forepaws, nasal discharge, shaking of the head, and abnormal respiratory sounds as sneezing and coughing (Jenkins, 2008). A second form of pasteurellosis is conjunctivitis (Thompson, 1976). Typical sign is a white mucopurulent exudate at the medial canthus with reddening of the conjunctiva (Arrington and Kelley, 1976). The fur under the eyes may become wet and matted due to epiphora (Arrington and Kelley, 1976). Otitis media is also a result of *P. multocida* as the bacteria ascends the eustachian tube to colonize the middle ear. Clinical signs in this case are absent (Thompson, 1976). Reproductive problems can also be linked to pasteurellosis. This is a result of infection through copulation or a septicemia. Vaginal and penile discharges may be absent or show as yellowish-grey pus (Manning et al., 1994). Treatment of all forms of pasteurellosis includes a good management and close watching of the colony. Penicillin and streptomycin have shown good results if given for 7-10 days (Manning et al., 1994). The main preventive measure of this and most rabbit diseases is management and housing. Isolation for 30 days of all the infected rabbits and newly purchased stock is a must (Thompson, 1976). Prevention of colony exposure to disease is of a primary importance.

Hepatic coccidiosis is another common and chronic infection of mostof the rabbit colonies cased by *Eimeria stiedae* (Arrington and Kelley, 1976; Thompson, 1976). It can be found world-wide in both wild and domestic breeds of rabbits. Ingestion of the sporulated oocyst

starts the cycle which includes excysting of the oocyst in the duodenum. The sporozoites penetrate the intestinal mucosa and travel to the liver via the blood and lymf systems (Thompson, 1976; Manning et al., 1994). The oocysts pass down the bile duct, exit in the feces and sporulate outside the body (Manning et al., 1994). In 2-3 days the sporulated oocysts are infectious and are transmitted by fecal contamination of feed and water (Arrington and Kelley, 1976; Manning et al., 1994). Clinical signs in the adult do not exist or are negligible, because they can be immune from non-lethal infections at a young age or can carrier a low grade infections (Thompson, 1976). Young rabbits (in 5-12 weeks of age) are the most often and most severely affected (Thompson, 1976). Anorexia, debilitation, diarrhea or constipation, icterus, enlargement of the liver, and death are described as clinical signs, but they are rarely seen (Manning et al., 1994). Treatment is usually done by one of several sulfonamides (Arrington and Kelley, 1976; Thompson, 1976).

Other quite common disease in rabbits is ear cancer. *Psoroptic otoacariasis* is the ectoparazite, which causes an expensive disease especially of laboratory rabbits (Thompson, 1976). This might occur world-wide and can infect also horses, goats, sheep, donkeys, and mules. Not only ears, but also the top of the head, distal paws and claw areas of both front and hind feet can be infected. Clinical signs are drooping ears, scratching, shaking of the head, selfmutilation and white-grey crust in the ear at the base of the concha. Treatment should be done by any of the number of otic solutions that smother the mites and prevent secondary bacterial infections (Thompson, 1976). All rabbits in colony should be prophylacticly treated at three- month intervals with newly developed and old recovered cases (Thompson, 1976).

A malocclusion is a misalignment between the teeth of upper and lower jaws. Proper occlusion means the upper incisors are anterior to the lower ones and in good contact. In rabbit it is caused by constantly growing teeth. The upper incisors can grow from 10-13 centimeters while the lower incisors may grow 13-20 centimeters in a year (Thompson, 1976). This problem is hereditary and thus it can be solved by elimination of rabbits with this recessive gene from the breeding herd (Thompson, 1976). Clinicall signs are anorexia, loss of weight, the lower incisors may protrude from the mouth, lower jaw may be wet or buccal and tongue lesions with abscesses may occur. Treatment requires the clipping of the teeth every 3 to 4 weeks (Thompson, 1976).

2.4. Blood profile of the rabbit

2.4.1. Hematology

The blood volume of a healthy rabbit is approximately 55 to 65 mL/kg, and 6% to 10% of the blood volume may be safely collected (Mellilo, 2007). Ear artery, the marginal auricular vein ort he cephalic vein are the most common locations to obtain a blood sample of the rabbit(Jenkins, 2008). Other common site for bleeding rabbit especially in the veterinary clinic is the jugular vein (Jenkins, 2008). Hematological parameters can yield information about the red blood cell population and leukocyte response to stress and pathogens (Mellilo, 2007).

One of the most important diagnostic tool after obtaining a through history and physical examination is the complete blood cells count (see Table 1) (Jenkins, 2008).

Rabbit erythrocytes (red blood cells) are biconcave discs averaging 6.7 to 6.9 μm in diameter and 2.15 μm in thickness (Jenkins, 2008). Rabbit erythrocytes are larger than erythrocytes of a cat, a horse, or a cow, with diameters averaging 5.8 μm, 5.5 μm, and 5.7 μm, respectively, and smaller than those of a dog, averaging 7.0 μm. Rabbit erythrocytes are reported to have a mean life span of 57 days and a potential life span of 67 days (Melillo, 2007; Jenkins, 2008). Erythrocyte life spans can be linked to metabolic rate. This can be illustrated by the average life span of the different domestic species: cat, 70 days; dog, 120 days; horse, 145 days; and cow, 170 days (Jenkins, 2008). Red blood cells counts and hematocrit vary with age, sex, and breed (Melillo, 2007). Females and younger animals tend to have lower values than males and older animals (Jenkins, 2008).

Rabbit platelets occur singularly or in clumps. They can be seen as pale blue cytoplasm with a small cluster of azurophilic granules. Most are 1 to 3 μm in diameter, but larger forms may also occur.

Leucocytes (white blood cells) counts in rabbits are slightly lower or similar than other domestic animals. Leucocytes counts vary with age, sex, and breed as well as season (Jenkins, 2008). Red and also white blood cells gradually increased with the time after birth in rabbits (Jeklova et al., 2009). They are significantly reduced in rabbits up to four weeks of age (Jeklova et al., 2009). (Melillo, 2007) states, that the total white blood cells counts can be used to further characterize acute stress from chronic stress.

Lymphocytes are the most common rabbit white blood cells and make 30% to 80% of the total (Jenkins, 2008). They are predominantly small, 7 to 10 μm and they are very similar to those of other mammals. The nucleus is round to oval and dark purple-blue with Romanowsky stains. Lymphocytes of a healthy rabbit consist of 39% B lymphocytes, 44% T lymphocytes, and 8% null cells (Jenkins, 2008). The primary role of the lymphocytes is to respond to those activities that stimulate the immune system (Melillo, 2007). Rabbits with an acute infection may present with 60% or more neutrophils and 30% or less lymphocytes (Jenkins, 2008). In rabbits, lymphocytes are primarily found in the blood, spleen, bone marrow, lymph nodes and the lymphatic tissues in the gastrointestinal tract (Melillo, 2007).

Monocytes are the largest cells in the peripheral blood of the normal healthy rabbit. They have 15 to 18 μm in diameter (Jenkins, 2008). Monocytes have a large, variably shaped nucleus with chromatin. Monocytes comprise 1% to 4% of the normal rabbit white blood cells (Jenkins, 2008). (Melillo, 2007) states, that monocyte counts within the normal range are a common finding in rabbits with osteomyelitis due to dental disease.

Circulating basophils make approximately 2% to 5% of the total white blood cells count in rabbit (Jenkins, 2008). The diameter of rabbit basophil measures 8 to 12 μm (Melillo, 2007). As in other species, the function of the rabbit basophil is not fully understood (Melillo, 2007). (Jenkins, 2008) states, that the number of circulating basophils is inversely proportional to the number of tissue mast cells an animal has.

In rabbits, as well as in other animal species, hematological and biochemical parameters are significantly influenced by the breed of the rabbits, gender, diurnal variation, physiological condition, such as pregnancy and age (Jeklova et al., 2009). So we have to keep on mind, that reference ranges can markedly vary. (Melillo, 2007) describes, that caged rabbits, fed on commercial mixes or those, who suffered from dental disease had consistently lower packed cell volumes, red blood cells counts, hemoglobin values, and lymphocyte counts in comparison with rabbits kept outside with a more natural diet and exercise.

Table 1: Normal hematological values for the domestic rabbit (Jenkins, 2008).

Hematological parameters values for the domestic rabbit	
Erythrocytes (count)	5.4 - 7.6 x 10^6/mm^3
Hematocrit/Packed cell volume	33 - 50 %
Hemoglobin	10.0 - 17.4 g/dL
Mean corpuscular volume	60 - 69 μm^3
Mean corpuscular hemoglobin	19 - 22 pg
Mean corpuscular hemoglobin concentration	30 - 35 %
Leukocytes	5.2 - 12.5 x 10^3/mm^3
Lymphocytes	30 - 85 %
Neutrophils	20 - 75 %
Eosinophils	1 - 4 %
Basophils	1 - 7 %
Monocytes	1 - 4 %
Platelets	250 - 650 x 10^3/mm^3

2.4.2. Blood biochemistry

Blood chemistry values of the rabbit have been investigated for a long time. Changes in these chemistries are helpful in the diagnosis of many diseases (Jenkins, 2008). They can also show the overall health status of the animal. Biochemistry evaluation can be used to investigate liver, kidney, and other organ function (Mellilo, 2007).

Blood chemistry values of the rabbit have been investigated for a long time. Specific subgroups of one specie can differ and have speciffically different numbers of some blood values. These groups might occur on the basis of different age, breed, gender, pregnancy status or type of husbandry. It is a very expensive and time-consuming task to establish reference range, ranges for such subdivisions are usually not established. Thus,

we have to follow single reference ranges for all animals of given specie (Thrall et al., 2006). Most reference ranges are from experimental laboratory studies that are run on homogeneous groups of rabbits belonging to the same breed, strain, age, and environmental conditions (Melillo, 2007). So it is improtant to consider these characteriscis, when we are evaluating values outside these ranges,especially mildly abnormal values (Thrall et al., 2006). But what does it mean mildly abnormal? It must be consider for each value separatelly.

Other problematic fact is that reference ranges from different authors significantly differs (see table 2).

Blood biochemistry values of most of the parametres of rabbits can be detected from whole blood, serum, plasma or urine. We investigated rabbit blood serum. Serum is defined as the fluid that separates from clotted whole blood or blood plasma that is allowed to stand (Cork and Halliwell, 2002). Rabbit blood clots very easily at room temperature and will coagulate quickly if it is not mixed with anticoagulant (Melillo, 2007). The best way is to mix blood with anticoaguant during collection. Heparin is a suitable anticoagulant because it does not alter biochemical parameters (Melillo, 2007). It can be problematic to establish the anticoagulant/blood ratio, which is not always optimal (Mellilo, 2007). In our research we focused mainly on blood parameters, which detect the liver diseases and liver health. Healthy liver is well known as a synonym for the health of whole organism (Meredith and Rayment, 2000; Jenkins, 2008).

Other problem is, that there is a little information describing the effect of clinical disease on the blood parameters in rabbits available. Or those describing the effect on the use of blood tests as diagnostic and prognostic indicators (Melillo, 2007).

Table 2: Plasma Biochemical Values in Rabbits.

Parameters	Units	IDEXX Laboratories, 2015	Jenkins, 2008	Thrall et al., 2006	Laboklin, 2015	Inlab medical s.r.o., 2015	Meredith and Rayment, 2000	Melillo, 2007
ALT	U/L	31-53	27,4-72,2	<100	< 61	19,4-106,5	55-260	45-80
ALB	g/L	27-56		30-43	36-57	26-46	27-36	27-50
ALKP	U/L	70-145		<120	< 397	17,6-125,3	12-96	12-96
AMYL	U/L		212-424		< 459	113-334	400-3600	200-400
AST	U/L	42-98	10-78	<100	< 28		33-99	35-130
Ca	mmol/L	1,40-3,00	2,2-3,9	3,25-3,75	3,1-3,9	3,13-4,20	2,17-4,59	2,75-3,50
CHOL	mmol/L	0,90-1,37		0,6-1,8	0,3-1,7	0,28-2,1	0,62-1,68	0,26-2,07
GLOB	g/L	15-28				15-46	24-33	15-27
GLU	mmol/L	4,17-8,06	5,5-8,2	4,9-8,0		5,5-8,6	6-8,9	4,16-8,6
PHOS	mmol/L	0,39-1,58	1,0-2,2	1,8-2,9	0,8-3,2	0,55-2,13	1,0-2,2	1,3-2,1
TBIL	µmol/L	5-14	2,6-17, 1	0-12		1,7-5,1	4,3-12,8	0-12
TP	g/L	55-72		50-85	49-74	53-85	49-71	54-75
UREA	mmol/L	3,6- 8,6	4,6-10,7	5,0-8,2	2,1-8,4	3,6-11,4	9,1-22,7	7,14-16,07

Blood biochemistry parameters included in our research

Alanine aminotransferase (ALT)

Alanine aminotransferase, also known as glutamic pyruvic transaminace, has little tissue specifity, so it is only of a limited use for evaluation damage of liver in the rabbit (Meredith and Rayment, 2000). On the other hand, in humans, dogs, cats and rats it is valuable in the assesment of hepatic damage (Jenkins, 2008). Melillo (2007) adds it can be also influenced by the half-life, which is in rabbits around 5 hours and 45-60 hours in dogs. Because ALT concentrations are not affected by restraint it can be used as a diagnostic tool (Melillo, 2007). Slightly increased ALT levels are common for apparently healthy rabbits, but they have been attributed to the exposure to low concentration of toxic substances, such as resins in the wood-based litter or aflatoxins in food (Melillo, 2007). Significantly higher ALT (especially together with ALP, bilirubin and glutamyltransferase) can be found in the conditions of hepatic damage and necrosis, or hepatic coccidiosis and hepatic lipidosis (Meredith and Rayment, 2000; Melillo, 2007). Differences in ALT levels between individuals of the same species may be caused by protein content in the diet (Jenkins,

2008). This may also be the reason, why herbivorous species have lower levels of ALT activity (Jenkins, 2008). ALT in rabbit liver and heart muscle is similar (Jenkins, 2008).

Albumin (ALB)

Approximately 40% to 60% of total protein is albumin (Thrall et al., 2006). Female rabbits tend to have higher albumin concentrations than male rabbits (Thrall et al., 2006).

Alkaline phophatase (ALKP)

Alkaline phosphatase is a widely distributed enzyme, which originates from many tissues, including bone, intestine, kidney, placenta and liver. The highest levels are in the intestine and kidney (Meredith and Rayment, 2000; Melillo, 2007; Jenkins, 2008). The highest levels of ALKP (sometimes also AP or ALP) activity in the liver are found in the membranes bordering the bile canaliculi, and levels increase in conditions of biliary stasis (Meredith and Rayment, 2000). Young animals have higher plasma ALKP activity than adults because of osteoblastic activity (Thrall et al., 2006; Melillo, 2007). Rabbit ALKP significantly differs from that of many other species. Rabbits are the only species shown to have three ALKP isoenzymes. They have an intestinal and two liver/kidney forms compared with the intestinal and liver/kidney/bone forms found in mammals other than primates (Thrall et al., 2006). Jenkins (2008) add, that these two liver isoenzymes are produced from two separate genes. The fact, that serum ALKP concentration is the sum of these 3 isoenzymes, may explain why many reference ranges are unclear, wide and why raised ALKP levels in clinically healthy animals are a common finding (Melillo, 2007). Increases of ALKP as a result of liver necrosis are minimal. Increases as a result of biliary stasis, and result from increased ALKP synthesis resulting from an increased bile acid level are more frequent (Jenkins, 2008). ALKP does have a significant diagnostic value because it is not altered by restraint and thus is considered a good indicator of real tissue damage (Melillo, 2007).

Amylase (AMYL)

Rabbits have very little or no content of amylase in their salivary glands, intestinal tissue and liver (Jenkins, 2008). In rabbits amylase there is an almost pure pancreatic enzyme (Melillo, 2007). Normal amylase values are low compared with those of other animals (Jenkins, 2008). Amylase can be used to diagnose pancreatic disease especially . Significant increases in amylase are observed in the rabbit with pancreatitis, pancreatic

duct obstruction, peritonitis, or abdominal trauma (Melillo, 2007; Jenkins, 2008) . Increased serum amylase levels may also result from renal disease and treatment with corticosteriods, whereas hemolysis can lower it (Melillo, 2007; Jenkins, 2008).

Aspartate aminotransferase (AST)

Aspartate aminotransferase, formerly called serum glutamate transaminase, is found in the rabbit liver, cardiac and skeletal muscle, kidney, and pancreas, with the highest levels in the liver and skeletal muscle (Meredith and Rayment, 2000; Melillo, 2007; Jenkins, 2008). Elevation of AST serum activity in rabbit is usually observed with conditions causing hepatocellular necrosis (Meredith and Rayment, 2000). AST in rabbits has a short half-life (5 hours) (Melillo, 2007). Although higher AST levels may be found in rabbits with liver damage, difficulties during collection or hemolysis of the sample can also raise AST levels (Melillo, 2007).

Calcium (Ca)

Calcium can be found bounded to serum proteins or ionized (free) in the serum, so the total calcium value is the sum of bound and ionized calcium (Melillo, 2007). It is influenced by feed intake and diet, serum protein levels, and other metabolic conditions (Melillo, 2007). Blood calcium levels are higher, and the normal range is broader in rabbits than those in other species (Jenkins, 2008). Calcium metabolism in rabbits is different from other animals (Melillo, 2007; Jenkins, 2008). Blood calcium level is influenced more by the calcium content of the diet. Rabbits absorb calcium in proportion to the concentration of the ion in the gut, and the kidney eliminates the excess (Melillo, 2007). Rabbit can defend its serum ionized calcium concentration against hypocalcemia and hypercalcemia by rapid changes in parathyroid hormone secretion and calcitonin (Jenkins, 2008). In rabbits, the blood level of calcium at which it is moved to the bones is very high (Melillo, 2007). Growing youngs and pregnant does use more calcium, which is results in lower blood calcium levels. In these rabbits blood Ca rarely rise above 14 mg/dL, even when fed calcium-rich diets. Adult rabbits on a varied diet can show calcium levels up to 16 to 17 mg/dL (Melillo, 2007). Rabbits excrete about 45% to 65% of calcium through the urine, whereas most mammals do not excrete more than 2% of Ca in urine (Melillo, 2007). Due to this the rabbits are more succestible to having sludge and stones in the urinary system. Rabbits fed unbalanced diet are more at risk of renal failure. Hypercalcemia is also a consequence of renal disease in rabbits caused by the inability of kidneys to eliminate

surplus calcium (Melillo, 2007; Jenkins, 2008). Hypocalcemia is very rare. The most frequent cause of low blood calcium levels is caused by poor nutrition (Melillo, 2007).

Cholesterol (CHOL)

Cholesterol is a lipid molecule biosynthetised by many cells, but especially by hepatic cells. It can be obtained from the diet and it is a precursor of steroids (Melillo, 2007). It is metabolized by the liver and excreted in bile. Cholesterol level peak after a meal and fasting is needed for accurate measurement (Melillo, 2007). The normal cholesterol concentration in rabbits varies with age, breed, strain, and sex (Thrall et al., 2006). Normal adult male rabbits have twice the cholesterol concentration of adult female rabbits (Thrall et al., 2006). Cholesterol may increase in animals with extrahepatic biliary obstuction and hypercholesterolemia is often associated with fatty infiltration of many tissues (Thrall et al., 2006). Hypercholesterolemia has also been linked with pancreatitis, diabetes mellitus, nephrotic syndrome, and chronic renal failure (Melillo, 2007). Decreased cholesterol levels in rabbits might be found in cases of malnutrition, liver failure, and sometimes even in pregnancy (Melillo, 2007).

Globulins (GLOB)

The globulins are a family of globular proteins that have higher molecular weights and water solubility values than the albumins. Some globulins are produced in the liver, while others are made by the immune system. Very important for the rabbit immune system is a gamma-globulin (Porter, 1959).

Glucose (GLU)

Blood glucose determination is used to asses carbohydrate metabolism and so its value depends on feeding (Jenkins, 2008). Rabbits use volatile fatty acids produced by cecal flora as a primary energy source, and it is impossible to obtain a fasting blood sample, because rabbits ingest fecal pellets (Melillo, 2007). It has been shown that 4 days of starvation does not reduce blood glucose levels in rabbits (Melillo, 2007). In normal adult rabbits, glucose levels range from a low of 115 mg/dL 1 hour before feeding to a high of 113 to 145 mg/dL 3 hours postfeeding (Jenkins, 2008). Elevated blood glucose in rabbits is most often associated with stress (Thrall et al., 2006; Melillo, 2007). Heat stress, transportation, fear, and pain are common causes of the hyperglycemic rabbit. It can be aslo connected with diabetes mellitus, but it is very rare in rabbits (Melillo, 2007; Jenkins,

2008). Although hyperglycemia is a common finding and may be associated with glucosuria (Melillo, 2007). Hypoglycemia is a very important finding. In anorexic rabbits, it indicates that the rabbit is using fatty tissue and is at risk of developing hepatic lipidosis (Melillo, 2007). Hypoglycemia may occur in terminal mucoid enteropathy, liver failure, or other chronic diseases. Rabbits with acute sepsis may be hypoglycemic too (Melillo, 2007).

Phosphorus (PHOS)

Phosphorus´ main function is contributing to the proper formation of bones and teeth. Phosphorus is also involved in many enzymatic systems in rabbits (Melillo, 2007). The amount of phosphorus in the blood of rabbits is a subject to spontaneous (seasonal) variation, and so they may be affected by environmental conditions (Brown, 1928). Blood phosphate concentrations can easily be increased by hemolysis (sampling difficulties or spontaneous), because phosphorus is present inside blood cells. (Melillo, 2007) presents, that phosphate concentrations should always be evaluated with blood calcium levels to determine the mineral balance in rabbits diagnosed with urinary tract stones, dental disease, or other signs of nutritional secondary hyperparathyroidism (Melillo, 2007). Brown (1928) adds, that there might be found a relation between the variations in calcium and phosphorus and the susceptibility of rabbits to disease. Phosphate levels can be an indirect measurement of kidney function, because it is the main organ which is involved in phosphorus balancing by regulation of glomerular filtration and tubular reabsorption (Melillo, 2007). Hyperphosphatemia indicates chronical kidney failure - a loss of more than 80% of nephrons, and it may also be an indicator of a soft tissue trauma (Melillo, 2007).

Total Bilirubin (TB)

Rabbits secrete a large amount of bile (approximately 125 ml/kg/day), and the primary bile pigment is biliverdin rather than bilirubin (Meredith and Rayment, 2000). The main product of heme breakdown is biliverdin in the rabbit, although some is converted to bilirubin, which is present at measurable levels (Meredith and Rayment, 2000; Jenkins, 2008). Rabbits have very low biliverdin reductase activity, which cause that rabbits excrete 70% biliverdin and 30% bilirubin (Jenkins, 2008). Biliverdin assays are not commercially available (Meredith and Rayment, 2000). Serum bilirubin levels reflex hepatocellular and biliary tree function (Meredith and Rayment, 2000; Jenkins, 2008). In young rabbits,

hepatic coccidiosis is the most common cause of biliary obstruction (Melillo, 2007). In adult rabbits it is biliary neoplasia (Melillo, 2007). Visible icterus is a rare presenting sign in the rabbit. Significant hyperbilirubinemia is usually associated with biliary obstruction and cholestasis, such as neoplasia of the biliary tree and hepatic coccidiosis (Meredith and Rayment, 2000). Bile acids (cholic acid and chenodeoxycholic acid) are presumed to be a sensitive indicator of liver function too (Meredith and Rayment, 2000).

Total Protein (TP)

Total protein is a very important parameter in any animal specie and it is the sum of albumin and globulin (Melillo, 2007). Total protein levels vary in rabbits with breed, reproductive status and age (Meredith and Rayment, 2000). Hyperproteinemia in rabbits is mainly caused by dehydration (Melillo, 2007). Higher protein may also indicate a chronic infectious or metabolic process in the body (Melillo, 2007). Detailed examination and measuring of albumin and globulin fractions is necessary to differentiate the causes of high protein levels. The liver is very important organ for protein sysnthesis. It is the only site of synthesis of albumin (Thrall et al., 2006). The decreased production and such low levels of albumin and thus total protein may occur in rabbits with significant and advanced liver disease (Thrall et al., 2006; Melillo, 2007), such as hepatic coccidiosis (*E stiedae*) or scarring and necrosis due to the migrations of *Cysticercus* (*Taenia*) *pisiformis* larvae (Melillo, 2007). Chronic malnutrition, poor diet and advanced dental diseases are common causes of hypoalbuminemia in rabbits. Cecotrophy (as mentioned in 2.3.3. Digestive tract) is a source of high-value proteins for rabbits. Therefore, all reasons which reduced cecotrophy (e.g., dental diseases, obesity, back pain) can cause a low protein, especially albumin (Melillo, 2007).

Urea (UREA)

Urea is the principal end product of protein catabolism in the liver of mammals. Urea is excreted by the kidneys (Thrall et al., 2006; Jenkins, 2008). It is filtered through the glomerulus, and then 25% to 40% is reabsorbed by the renal tubules. Thus blood urea nitrogen is commonly used to assess renal function (Thrall et al., 2006; Jenkins, 2008). Urea levels in rabbits depend on the circadian rhythm (Thrall et al., 2006; Melillo, 2007). It has a diurnal fluctuation, peaking in the late afternoon and early evening (Melillo, 2007). The urea nitrogen is influenced by diet (quantity and quality of proteins), liver function, gastrointestinal absorption, urease activity of the caecal flora, and hydratation (Thrall et al.,

2006; Melillo, 2007). It can be easily influenced by water intake, because only a few hours without drinking or losing fluids (due to ileus or diarrhoea) may cause an increase of urea level compatible with renal failure (Mellilo, 2007). Changes in urea levels are not easy to correctly interpret and slight elevations in blood urea is a common finding (Mellilo, 2007). A decrease in urea may be caused by anabolic steroids, diminished protein intake, and severe hepatic insufficiency (Jenkins, 2008). Increase may be indicative of a variety of renal perfusion or function problems, increased protein catabolism due to high protein diets, or vigorous exercise (Jenkins, 2008).

Oher blood biochemistry parameters

Cholephilic dyes have been used in past to assay liver function in rabbits (Jenkins, 2008). Now it is not commonly used.

Creatine kinase (also known as phosphocreatine kinase, creatine phosphokinase or creatinine kinase) is an enzyme present in muscle, brain, and other tissues of vertebrates. Creatine kinase causes the conversion of adenosine diphosphate and phosphocreatine into adenosine triphosphate and creatine (Jenkins, 2008). Creatine kinase is very sensitive indicator of muscle degeneration (Jenkins, 2008).

Lactate dehydrogenace (lac) activity is presenting in a wide variety of tissues and body cells (Thrall et al., 2006; Jenkins, 2008). Due to the high activity of lac in erythrocytes, high plasma lac activity may be caused by hemolysis (Jenkins, 2008). Because of the wide tissue disribution and the effect of hemolysis and handling, lac is not commonly used to detect liver disease in rabbits. But it can be used in this way (Thrall et al., 2006).

The gamma-glutamyl transferase (GGT) activity of normal rabbit is less than 8 IU/L (Thrall et al., 2006). The activity of GGT in rabbits is low. But it is a useful indicator of chronic liver disease in horses, cattle, and domestic carnivores (Mellilo, 2007). The highest activity is in kidney, but renal GGT is eliminated with the urine, so it does not reach the blood circulation (Mellilo, 2007). Higher GGT levels in the rabbit are most often linked to hepatobiliary obstuctions (Thrall et al., 2006; Melillo, 2007). Testing of GGT may also be used to determine the cause of elevated ALP. Both ALP and GGT are elevated in disease of the bile ducts and in some liver diseses, but only ALP is higher in bone disease.

Therefore, when the GGT level is normal and ALP is higher, it is most likely due to bone disease (Mellilo, 2007).

Bile acids measurement is not a common procedure in rabbits, but higher levels are usually associated with hepatic disease (Mellilo, 2007). It is used as an indicator of liver function in other animal species. In rabbits it is not used due to cecotrophy, which does not allow a comparison of preprandial and postprandial serum bile acid concentrations (Mellilo, 2007).

Triglicerides as cholesterol level peaks after a meal, and thus period of fasting is necessary for exact measurement (Mellilo, 2007). This limits triglicerides and cholesterol diagnostic value in rabbits as well especially because of cecotrophy. As in cholesterol, abnormal values can be caused by a diet rich on fats, obese patients, or hepatic disease (Mellilo, 2007).

Creatinine is a breakdown product of creatine phosphate in muscle, and is produced at a fairly constant rate by the body muscle and it is excreted by glomerular filtration (Mellilo, 2007). Creatinine is one of the most trustworthy test of renal function in rabbits. Limited ability to concentrate urine in rabbits can cause dehydratation and thus prerenal azetomia. As urea, creatinine can be easily increased by few hours without drinking or by ileus and diarrhoea (Mellilo, 2007). This can return to normal when the water deficit is corrected.

Lipase There is little information on the function and diagnostic value of lipase in rabbits. Increased lipase values may indicate cellular damage to the pankreas as it does in other species. As with amylase, lipase is artifactually elevated by corticosteroids.(Melillo, 2007)

3. Aims of the thesis

The aim of this thesis is to evaluate the positive effect of the milk thistle (*Silybum marianum*) supplement in feed ration on the rabbit metabolism, blood chemistry and liver functions, which are the main indicators of a good health status and strong immunity. The second aim is to find out, which concentration of the milk thistle suplement is the most suitable for feeding of broiler rabbits.

4. Hypothesis

Based on the scientific articles and literature I supposed the following hypothesis of the research:

1) The group of HYLA broiler rabbits with the highest concentration of milk thistle will have better biochemical and liver parameters and thus better and stronger immunity.

2) The group with the highest concentration of milk thistle will show lower mortality, better growth and gain parameters.

5. Material and Methods

HYLA broiler rabbits from the farm of Mr. Kočár (Genetic Center HYLA in Ratibořice, CR), aged 35 days were used for the experiment. The animals were housed in metal fattening cages in the demonstration and experimental barn of CULS Prague (automatically air-conditioned). Mr. Karel Janda (FAFNR) was in charge of breeding, care, and handling of the animals.

The animals were divided into three experimental groups and one control group and fed complete mixtures for fattening rabbits and given water ad libitum. Feed mixtures for broiler rabbits with additives of Milk thistle (*Silybum marianum*) are produced and delivered ready for individual experimental groups by Biokron Ltd. company (Blučina), proceeds fruits of Milk thistle mechanically as well as by drying. The milk thistle is supplied by Irel L.T.D. company (Brno). Each group differs in the amount of milk thistle added to feed mixture. The control group was fed feed mixture without addition of Milk thistle.

Classification of the experiment:

Control group – white label: feed with feeding mixture KBO (feeding mixture for weaned broiler rabbits).

I. Experimental group – blue label: feed with feeding mixture KBO with 0.2 ml/kg of AV3 (antioxidant with 25,2 mg/l of silybinin).

II. Experimental group – red label feed with feeding mixture KBO with 0.2 % of untreated milk thistle pomace.

III. Experimental group – yellow label feed with feeding mixture KBO with 1 % of untreated milk thistle pomace.

Composition of BIOSTAN KBO feed mixture: 30 % alfalfa meal, *Avena sativa* 25 %, wheat bran 17 %, sunflower meal unscraped 10 %, 8 % malt sprouts, apple dried pomace 4 %, 2 % linseed, dicalcium phosphate 1.5 %, 1% calcium carbonate, sodium chloride 0.3 %, premix of amino acids, trace elements and vitamins 1 %, PROBIOSTAN E10 0.25 % (PROBIOSTAN is the yeasts and lactobacillus fermented feed of probiotic charakter, enriched of selected trace elements, which support growth of the desirable microflora in

the digestive tract of the animals). In all the feeding mixtures there was used EMANOX PMX as a coccidiostatics.

The exact composition and contents of each active substances of AV3 manufacturer does not specify. (Nečasová et al., 2015) made analysis of this preparation, and they determined the content of active component silybinin at 25,2 mg/l.

Whole research was realized in one year (2014). We realized 4 experiments and in one experiment 3 experimental groups and one control groups were compared there.
Blood samples were taken during slaughtering, while bleeding by cutting carotid artery. Rabbits were slaugtered by captive bolt into the head at first. Blood samples were taken into 3 millilitres VACUETTE® test tubes for serum with added clot activator.

The collecting of the blood samples was always performed in the morning (8:00 – 11:00). Samples were taken in 70, 77 and 84 days of rabbit age depending on the weight of the specific animal. The ideal slaughter weight of broiler rabbit is 2600 g of live weight. This weight should be achieved before 84 days of rabbit age.The stunted animals were not included in the research.

In total 420 rabbits were included in to the research. These rabbits were used for evaluation of fattening properties and mortality. Blood biochemistry was done for randomly selected 126 animals (30 animals in each experiment).

The collected blood samples were processed and investigated at the Department of animal science and food processing (FTA CULS Prague). Using plastic Pasteur´s pipets serum was separated from the blood clot and poured into 0.5 millilitres Eppendorf® tubes. In this tubes the samples were centrifuged there for three minutes at 11000 rpm. Eppendorf MiniSpin® was used for the centrifugation. Subsequent analysis was done on VetTest® chemistry analyzer (IDEXX Laboratories) using dry-slide technology.

We focused mainly on thirteen blood parameters: alanine aminotransferase, albumin, alkaline phophatase, amylase, asparate aminotransferase, calcium, cholesterol, globulin, glucose, phophorus, total bilirubin, total protein and urea.

Feed conversion was calculated from rabbit´s weights and feed consumption. All rabbits were weighted once a week and feed consumption was recorded daily. Deaths (mortality, morbidity) and other parameters (stunted animals, weighing of carcasses, parts of the body and liver) were recorded during each slaughter.

Statistical evaluation was done in program SAS System V 9.3 by General linear model procedure (GLM). The mortality and morbidity were evaluated by χ^2 in the programm Microsoft Excel.

6. Results

In total, 420 rabbits were examined and their values then compared. For the blood biochemistry we examined 126 rabbits. For each blood parameter we compared sexes, ages of rabbits (70, 77 and 84 days old), groups (1controll + 3 with milk thistle supplement) and repetitions of the experiment (January, February + March, May, September + October and December 2014).

All parameters do not significantly differ between males and females, except cholesterol. Cholesterol was significantly higher in females compared to males. The mean of cholesterol level in males is 1.196 mmol/L and the mean in females is 1.447 mmol/L ($p=0.0119$). The level of probability was estimated at $p < 0.05$. 32.86 % of all males fitted to the normal range values for cholesterol, 28.57 % of all males have higher and 24.29 % lower value compared to the normal range. In females, 31.43 % fitted to the range value, 58.57 % were above and 10 % were below the range.

All examined blood parameters do not significantly differs among ages of slaughtering. Alarming is conclusion, that every parameter significantly differs among repetitions of sampling, except total bilirubin. It is the only parameter, which values were similar among repetitions.

Comparing groups, we concluded that significant differences occur in total protein, globulins, cholesterol and phosphorus. The signifficant difference in globulins occurs between the first experimental group (AV3) and the third experimental group (1 %) ($p = 0.0121$) and between the second experimental group (0.2 %) and the third experimental group (1 %) ($p = 0.0386$). When, the third experimental group has the highest mean value (23.048 g/L) and the third experimental group has the lowest mean value (21.512 g /L) (see Figure 2).

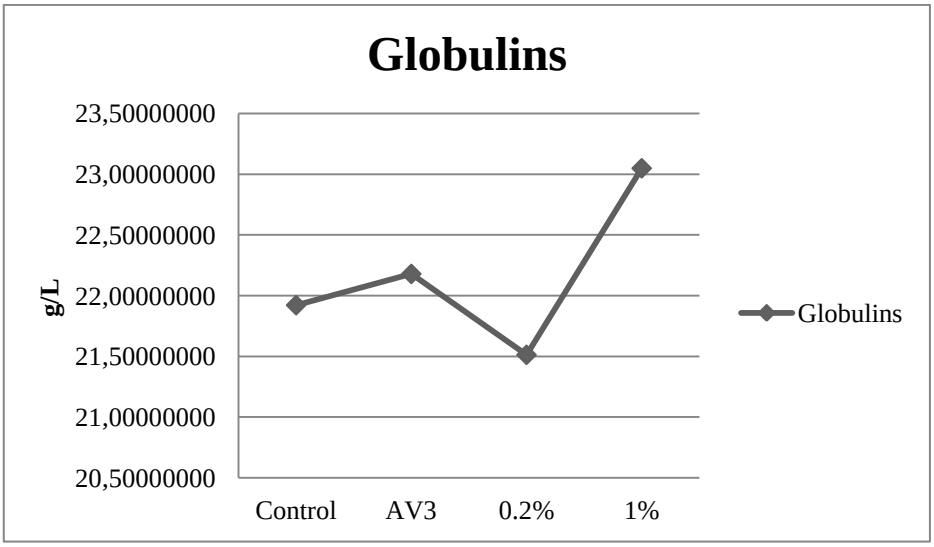

Figure 2: Graphical representation of globulins levels in different experimental groups of rabbits.

Signifficant difference in total protein occurs between the first and the third (AV3 and 1 %) experimental group (p = 0.012) and between second and the third experimental groups (0.2 % and 1%) (p = 0.0386) (see Figure 3).

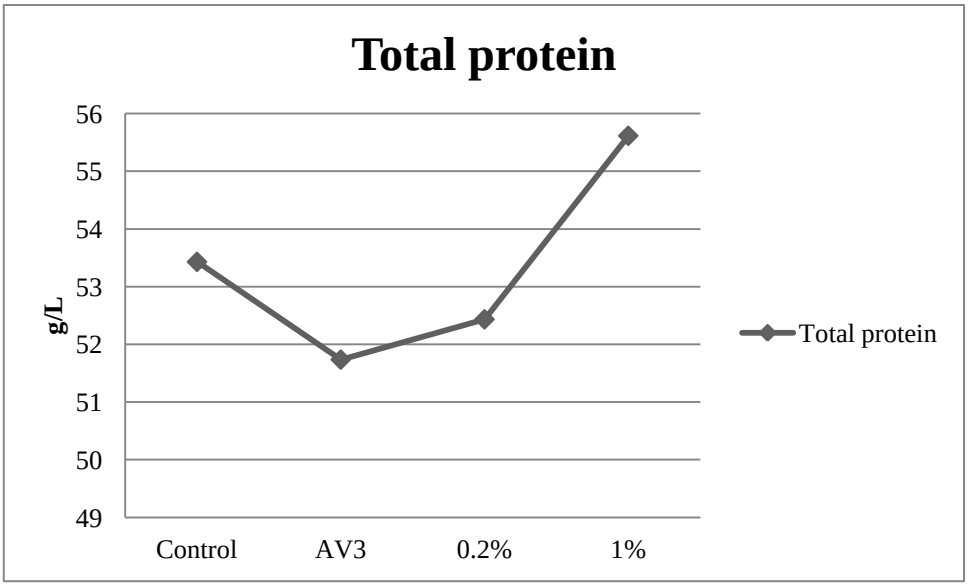

Figure 3: The comparison of values of total protein in experimental groups.

Cholesterol significantly differs between control group and the first experimental group (AV3) (see Figure 4) (p = 0.0134) and also control group and the second experimental group (0.2 %) (p = 0.0166). The mean of cholesterol for control group is 1.084 mmol/L, for the first experimental group = 1.449 mmol/L, for the second experimental group = 1.434 mmol/L and for the third experimental group = 1.320 mmol/L.

Significant diffence of phosphorus occurs between the second (0.2 %) and the third (1 %) experimental groups (p = 0.0257). The highest mean value occurs in the third experimental group (2.242 mmol/L) and the lowest in the second experimental group (2.107 mmol/L).

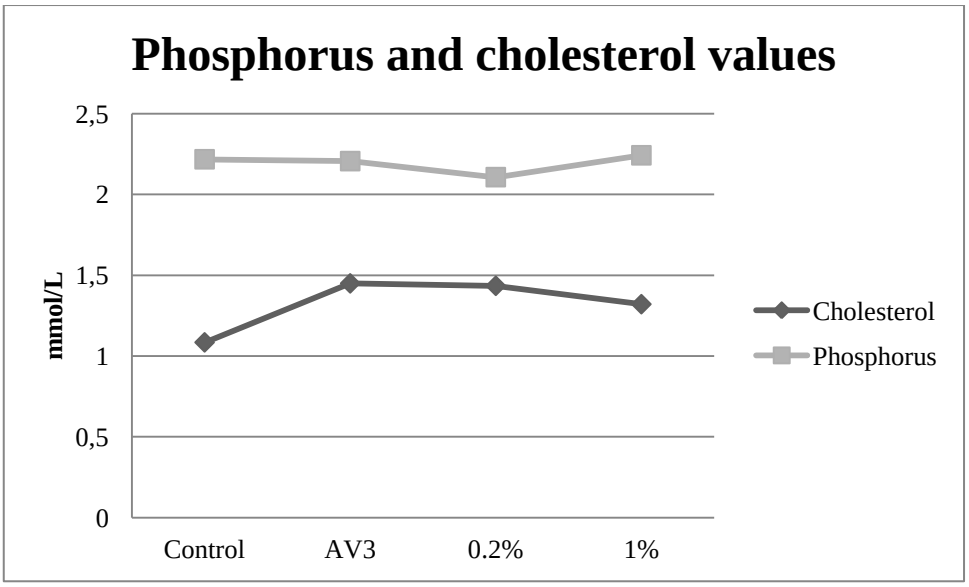

Figure 4: Average vaules of phosphorus and cholesterol for each group of rabbits.

There were no significant differences among feeding groups in slaughter weight, daily gain, feed conversion, total weight gain, liver weight and carcass weight (see Annexe 2). However the highest slaughter weight (2722.57g) occurs in the first experimental group (AV3) and the lowest in the third experimental group (1% = 2699.78g). The daily gain was the highest in the second experimental group (0.2% = 41.21g) and lowest in the first experimental group (40.64g). The highest value of feed conversion was in the second experimental group (3.88 $kg.kg^{-1}$) and the lowest in the third experimental group (1%= 3.81 $kg.kg^{-1}$). Total weight gain was highest in the second experimental group (1419.32g) and the lowest in control group (1390.58g). The heaviest liver occurs in the first experimental group (98.62g) and the lowest in the control group (94.54g) (see Figure 5).

Carcass weight was also the highest in the first experimental group (1590.55g) and the lowest in control group (1562.11g).

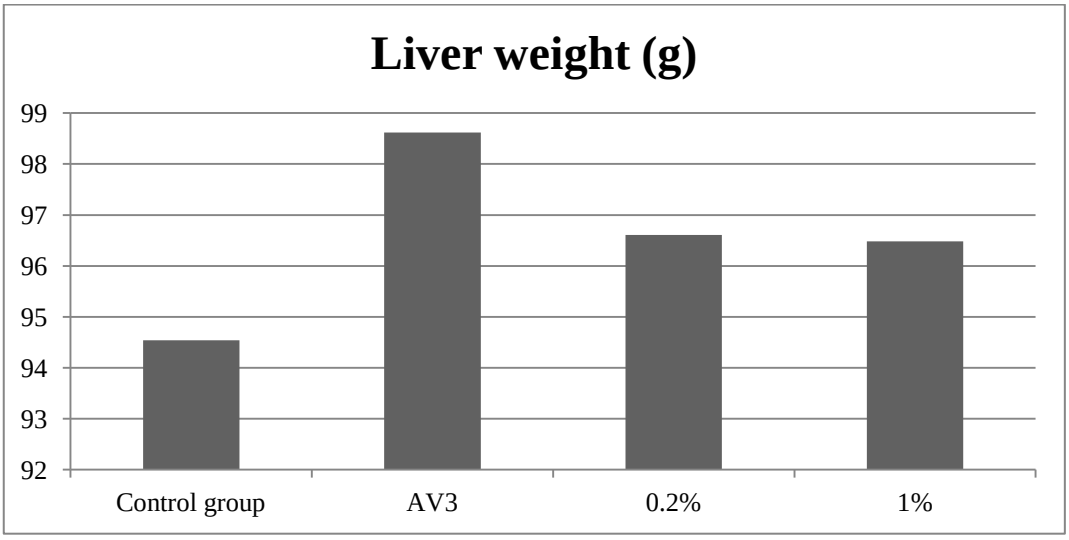

Figure 5: Comparison of the mean of rabbit liver weight in experimental groups.

In carcass yield, kidney fat weight, total feed intake and daily feed intake occur significant differences among feeding groups (see Annexe 2). The significantly different carcass yield occurs in the control group, where the value was the lowest (57.22g). Highest value occurs in the first experimental group (AV3 = 58.31g) (see Figure 6). The weight of kidney fat was significantly different in the control (43.1g) and the first experimental group (AV3 = 46.59g). Total feed intake significantly differed in the second experimental group (0.2 % = 5500.81g) from the third experimental group (1% = 5323.46g) and the control group (5368.11g). The difference from the first experimental group (AV3) was not significant. Daily feed intake was significantly different in the second experimental group (0.2% = 157.72g), where the value was the highest, from other groups. The lowest value was in the first experimental group (AV3 = 153.46g).

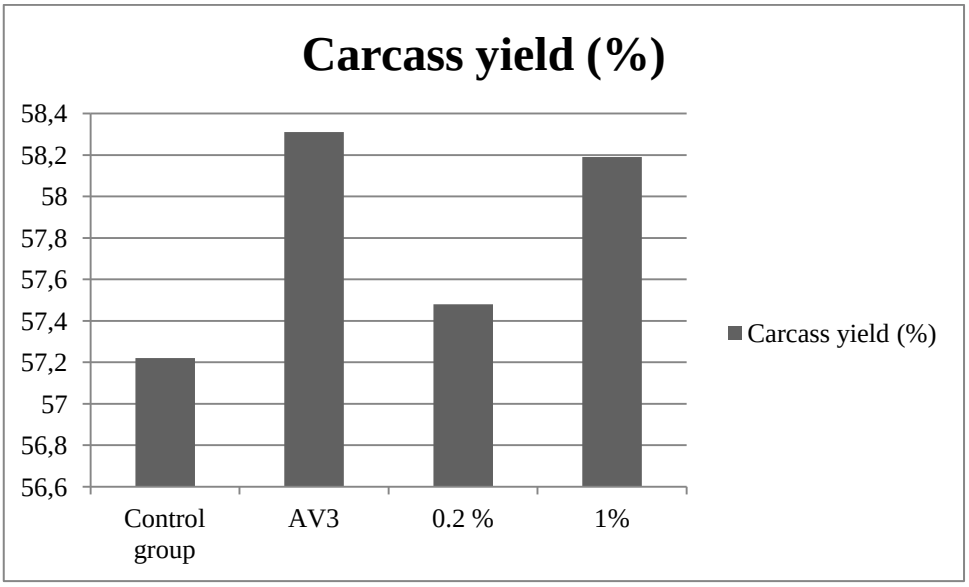

Figure 6: Representation of the differences in means of carcass yield in the experimental groups.

No significant differences occur in the total mortality. However the lowest mortality was in the third experimental group (1 % = 10 % of dead rabbits). The highest mortality occurred in the second experimental group (0.2 % = 13.63% of dead animals). The lowest number of stunted rabbits occurred in the control group (9.33 %) and the highest in the first experimental group (AV3 = 22.22 %) (see Figure 7).

		Control	**AV3**	**0.2 %**	**1 %**
Total rabbits	animals	150	90	110	120
Total mortality	animals	18	12	15	12
	%	12	13.33	13.63	10
Stunted rabbits	animals	14	20	16	24
	%	9.33	22.22	14.54	20

Figure 7: Table representing the mortality of rabbits.

From a descriptive statistics (see Annexe 1), we can estimate the lowest and the highest values of the biochemical examination. The highest maximal values occurred most often (six times) in the second experimental group (0.2 %). The highest numbers of mean occurred most often (seven times) in the third experimental group (1 %).

7. Discussion

The significant difference in cholesterol between males and females differs from other authors results (Thrall et al., 2006; Melillo, 2007). Thrall et al. (2006) writes, that normal adult male rabbits have twice higher the cholesterol concentration of adult female rabbits. Fact, that all or most of females suffered from hypercholesterolemia is highly unlikely. The narrowest range for cholesterol states IDEXX Labratories (2015) (0.9-1.37 mmol/L). The range of our values is 0.18 – 2.32 mmol/L with the average value 1.01 ± 0.15 mmol/L. The average value for females is out of this range, but this value still fits in all other authors ranges. The highet acceptable value states Inlab medical s.r.o. (2015): 2.1 mmol/L, which seems suitable and adequate also for our animals.

Thrall et al. (2006) states, that cholesterol concentration varies with age. Although there were no significant differences among ages of the rabbits, the second group (77 days old rabbits) had the lowest mean of cholesterol (1.1927 mmol/L). Thrall et al. (2006) states, that higher cholesterol is often associated with fatty infiltration of many tissues and even in our experiment first experimental group (AV3) has the highest mean of cholesterol concentration and the kindey fat was the heaviest in this group.

Cholesterol significantly differs among control group and the first (AV3) and the second (0.2 %) experimental group. This result shows opposite effect of milk thistle, than was expected. The fact, that cholesterol is the lowest in the control group do not matches in the first hypothesis. We expected the lowest value in the third experimental group (1 %) and the highest in the control group. That is the reason, why we can not evaluate, that the milk thistle supplement has a positive influence on the cholesterol levels in broiler rabbits.

The fact, that cholesterol levels are the highest after a meal and so fasting is needed for accurate measurement (Mellilo, 2007) is very important. Our rabbits were not fastened and it can be the reason, which probably influenced our cholesterol values. It probalby moves or values slightly higher. We did not restrict the feed, because when rabbits fastened before the slaughtering, they drink a lot and full urinary bladders are not ideal. Cholesterol should be tested not togehter with the slaughtering, but in really fastened rabbits.

The signifficant difference in globulins occurs between the first experimental group (AV3) and the third experimental group (1 %) ($p = 0.0121$) and between the second experimental group (0.2 %) and the third experimental group (1 %) ($p = 0.0386$).

According (Porter, 1959) we can suppose, that the higher level of globulins, the better for the rabbit, because it indicates healthier livers. Of course the levels should be in the reference range, because too high levels can indicate some disorders. The highest normal value states Inlab medical s.r.o. (2015) 46 g/L and the lowest is the same in IDEXX Laboratories (2015), Laboklin (2015) and Mellilo (2007) = 15 g /L. Our highest measured value is 31 g/L and the lowest value is 17 g/L. And our mean value is 22.25 ± 1.04 g/L. So we can valorize the observed animals as healthy, with respect to globulins.

The value of total protein was significantly different in the first experimental group (AV3) compared to the third experimental group (1 %) ($p = 0.0121$) and also in the second experimental group (0.2 %) compared to the third experimental group (1 %) ($p = 0.0386$). The mean of this parameter is 53.24 ± 2.7 g/L and thus we can evaluate that our rabbits had lower values of total protein than states other authors. Because Thrall et al. (2006) states the rdest range of normal values for total protein and it is 50-85 g/L. Our values are in the lower limit of normal range. The low protein can be caused by the reduced cecotrophy, which is a great source of high-value proteins (Melillo, 2007). Other causes of lower total protein values can be some liver diseases, malnutrition, poor diet or advanced dental diseases (Thrall et al., 2006; Melillo, 2007). However the presence of these diseases is highly unlikely and the decrease of our values is not such significant or alarming.

Phosphorus was significantly different in the second experimental group (0.2 %) and the third experimental group (1 %), when in the second experimental group it was the lowest (2.107 mmol/L) and in the third, the higest (2.242 mmol/L). Both of these values are almost over the normal range. Thrall et al. (2006) states the highest normal value 2.9 mmol/L. The lowest normal value states IDEXX Laboratories (2015): 0.39 mmol/L. In the table representing descriptive statistics (see Annexe 1) we can see that the highest value (2.76 mmol/L) was recorded in the second experimental group (0.2 %) and the lowest value (1.74 mmol/L) was measured in the control group. For comparing and evaluation of our samples is the best to follow the normal range which states Thrall et al. (2006) 1.8 – 2.9 mmol/L. Blood phosphate concentrations can easily increased by hemolysis, because

phosphorus is present inside the blood cells (Melillo, 2007). Hemolysis can be caused by sampling difficulties or it can spontaneous. The sampling difficulties can little bit rise our levels of phosphorus.

Alarming is conclusion, that every parameter significantly differs among repetitions of sampling, except total bilirubin. It is the only parameter, which values were similar among repetitions. All samples were taken in the morning, circa from 8 AM till 11 hours AM, so it can not be influenced by the time of sampling. Also the presence of automatical air-condition in the experimental stable (where samples were collected) decreases the effect of the season and the environmental changes.

These differences can probably be caused by the transport of weaned animals from Ratibořice to Prague. Other reason can be higher occurrence of the diarrhea or other diseases like Pasteurellosis in the breeding of rabbit´s dams.

Brown (1928) states, that the amounts of phosphorus in the blood of rabbits are subject to spontaneous (seasonal) variation, and so it may be affected by environmental conditions. It can bet the reason, why we recorded statisticaly significant difference in this parameter comparing repetitions of the experiment.

Interesting is also the result, that all examined blood parameters do not significantly differs among ages of slaughtering. We expected, that some parameters (especially AST, cholesterol, glucosis or creatinine (Thrall et al., 2006; Jenkins, 2008)) will be higher in older animals. The reason could be, that the youngest rabbits were only about 14 days younger. And 14 days is not such a big difference.

All fattening parameters were the higest in the first experimental group (AV3 – slaughter weight, liver weight, kidney fat weight, carcass weight and carcass yield) or in the second experimental group (0.2 % - daily feed intake, daily gain, feed conversion, total weight gain and total feed intake). Total weight gain, total feed intake, liver weight, kidney fat weight, carcass weight and carcass yield were the lowest in the control group. From these results, the using of AV3 and 0.2 % of milk thistle supplement can be recommended.

According to the moratlity, which was the lowest in the third experimental group (1 %) we can recommend using of milk thistle supplement. On the other hand, the differences in

mortality were not statistically significant. Thus it is the question of the economical view, if the milk thistle supplement is really worhtwile.

The percentage expression of the stunted rabbits is the lowest in the control group and highest in the first experimental group (AV3). Therefore the using of milk thistle supplement can be evaluated as counterproductive in this parameter.

According the results of the descriptive statistics, that the highest numbers of the mean occurred most often (seven times) in the third experimental group (1 %), we can suggest that such a high concentration of the milk thistle supplement slightly rises the biochemical values. But on the other hand, the lowest means of blood parameters occurred most often (five times) in the second experimental group (0.2 %). And this result is disproving the first suggestion.

From the above mentioned results, we can say, that setted hypothesis were not confirmed.

8. Conclusion

We can not conclusively say which one of the feeding groups evinced the best results. Conclusively positive effect of the milk thistle (*Silybum marianum*) supplement in feed ration on the rabbit metabolism, blood chemistry and liver functions was not confirmed. Because in most of the parameters were no significant differences. Therefore one of the feeding groups can not be evaluated as the best and thus we can not say which concentration of the milk thistle supplement is the most suitable for feeding of broiler rabbits.

The first hypothesis was not confirmed, because the group with the highest concentration of milk thistle supplement did not have better biochemical parameters. The second one can be partly accepted, because the mortality was really the lowest in the third experimental group with the highest concentration of milk thistle supplement (1 %). Positive effect on the growth and gain parameters was also demonstrated, but not in the group with the highest concentration of the milk thistle supplement. It was demonstrated in the first (AV3) and the second (0.2 %) experimental groups. But the usage of the milk thistle supplement was counterproductive in the number of stunted rabbits.

It is evident, that the positive effect on broiler rabbits was not demonstrated, but neither any negative effect, like deterioration of liver parameters, mortality or yields. Therefore according my research, the milk thistle supplement can not be strongly recommended for fattening of broiler rabbits.

Feeding of milk thistle supplement can be meaningful in sick animals, but in healthy young animals, there is almost no reason. It can bring also possitive effect on older animals. Our samples were taken from quite young animals, which did not show definite improvement when they were fed by milk thistle supplement. Further, I see the utilization of milk thistle extract in pet rabbits, but no in broiler rabbits. Pet rabbits live much longer and their breeders are usually willing to pay for healthier diet or prevention. Because using of the milk thistle supplement in fattening of broiler rabbits is not probably economically effective.

I would like to recommend more extensive researches dealing with this topic. Especially on the breeding rabbits and the dams of the broiler rabbits, where the utilization of the milk thistle supplement promises more significant results.

This work should prompt more scientists to fill the gaps in contemporary science, dealing with this topic. And it should raise the awareness and interest in the given topic and stimulate further research, which is necessary.

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Annexes

Annexe 1: Table representing mean and standard deviation, minimal and maximal value of each parameter for each feeding group.

Parameter	Control group			AV3			0.2%			1%		
	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.
ALB (g/L)	31.46 ± 1.88	18	37	31 ± 1.54	28	34	31 ± 3.73	10	39	31.85 ± 2.22	26	38
TP (g/L)	53 ± 3.47	39	59	52 ± 1.35	20	59	53 ± 3.64	28	61	54.96 ± 2.33	47	69
GLOB (g/L)	22 ± 1.57	18	28	22 ± 1.57	17	28	22 ± 0.50	18	26	23 ± 0.53	19	31
ALKP (U/L)	138 ± 5.59	71	270	120 ± 17.43	75	264	119 ± 8.98	75	186	124.34 ± 8.49	58	226
ALT (U/L)	61 ± 2.44	14	108	57 ± 5.43	36	91	58 ± 1.01	38	77	59.26 ± 6.44	26	102
AMYL (U/L)	230 ± 31.25	90	440	189 ± 34.6	95	274	180 ± 9.08	78	352	228.16 ± 56.10	59	424
Ca (mmol/L)	3.56 ± 0.04	3.44	3.77	3.54 ± 0.1	3.41	3.71	3.56 ± 0.05	3.21	3.73	3.56 ± 0.03	3.32	3.72
CHOL (mmol/L)	1.01 ± 0.15	0.18	2.32	1.4 ± 0.16	0.69	2.09	1.38 ± 0.25	0.56	4.00	1.42 ± 0.25	0.55	2.51
GLU (mmol/L)	7.94 ± 0.13	6.85	9.32	7.97 ± 0.29	7.29	8.72	7.80 ± 0.38	6.79	9.50	8.16 ± 0.26	7.25	9.27
PHOS (mmol/L)	2.19 ± 0.03	1.74	2.47	2.18 ± 0.08	1.87	2.50	2.12 ± 0.13	1.78	2.76	2.23 ± 0.03	1.98	2.54
UREA (mmol/L)	6.9 ± 0.59	5.4	8.9	7 ± 0.08	5.0	10.1	7.4 ± 0.78	5.3	17.2	6.7 ± 0.44	5.2	9.3
AST (U/L)	19.46 ± 5.88	2	78	26.39 ± 4.58	0	55	31.18 ± 6.18	0	131	33.09 ± 8.73	1	83

Annexe 2: Table representing the means of fattening parameters with standard errors of the mean.

Parameter	**Feeding group**							
	Control		AV3		0.2%		1%	
	mean	SE	mean	SE	mean	SE	mean	SE
Slaughter weight (g)	2717.89	8.96	2722.57	13.68	2714.32	11.2	2699.78	10.82
Daily feed intake (g)	154.64 B	0.89	153.46 B	1.36	157.72 A	1.11	154.36 B	1.7
Daily gain (g)	40.84	0.35	40.64	0.54	41.21	0.44	41.08	0.61
Feed conversion ($kg.kg^{-1}$)	3.87	0.04	3.85	0.06	3.88	0.05	3.81	0.07
Total weight gain (g)	1390.58	12.36	1404.84	18.87	1419.32	15.45	1393.59	14.93
Total feed intake (g)	5368.11 AB	33.2	5376.88 ABC	50.69	5500.81 C	41.5	5323.46 AB	57.54
Liver weight (g)	94.54	1.7	98.62	2.6	96.61	2.13	96.48	2,05
Kidney fat weight (g)	43.1 B	0.92	46.59 A	1.4	44.48 AB	1.15	44.49 AB	1.11
Carcass weight (g)	1562.11	8.12	1590.55	12.4	1563.47	10.15	1569.21	9.8
Carcass yield (%)	57.22 B	0.28	58.31 A	0.43	57.48 AB	0.35	58.19 A	0.34

(The letters A,B,C represents the statically significant differences. Number with a certain letter significantly differs from numbers in the same line, which do not include this letter.)

Annexe 4: Vacuette test tubes marked with the number of each animal (Procházková, 2014).

Annexe 5: VetTest® chemistry analyzer from IDEXX Laboratories (Procházková, 2014).

Annexe 6: The dry- slides for the detection of blood biochemistry values (Procházková, 2014).

Annexe 7: Me in the experimental stable of CULS Prague (Pebriansyah, 2015).