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The Effect of Different Concentrations and Processing Methods of Milk Thistle (*Silybum marianum*) on Fattening Performance in Broiler Rabbits

Dissertation thesis

Student: MSc. Akhir Pebriansyah

Supervisor: prof. MVDr. Daniela Lukešová, CSc.

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ABSTRACT

Food safety is one of the basic indicators of food quality. Since January 2006 legislative changes in European countries have limited the use of antibiotics (growth stimulants) and other chemical products in animal nutrition, which have been replaced by probiotics and other products, e.g. humic substances and plant extracts. The aim of this study was to investigate the effect of various methods of processing of milk thistle (Silybum marianum) on the growth and health status of broiler rabbits fed with different concentrations of milk thistle. Experiments were performed with a total number of 253 HYLA broiler rabbits. To rabbits were given a different concentration of 1% non-fermented milk thistle (experimental group E1) and 0.5% fermented milk thistle (experimental group E2) in the feed, and the results were compared with control group C, with standard diet, without the addition of Silybum marianum. Each experiment started at 42 days of rabbit age and was terminated at 85 days. The monitored parameters were: the average daily gain and daily feed consumption, total feed consumption, live slaughter weight and carcase weight. Selected biochemical parameters were examined by the VetTest analyzer (IDEXX Laboratories, Cymedica, USA). The molar profile of volatile fatty acid (VFA) was determined by gas chromatography. The best results were obtained in experimental group E2 (supplement of 0.5% of fermented milk thistle). Significant differences ($p<0.05$) were recorded in the average daily feed consumption, total feed consumption, slaughter live weight and carcase weight. No significant difference was found between males and females in groups C, E1, E2 in the monitored parameters, excluding the carcase weight, when the females of group C showed a significantly lower value compared to males.

In biochemical parameters, a significant difference ($p<0.05$) in higher cholesterol level was observed. The molar profile of VFA was a significant difference ($p<0.05$) in heptanoic and hexanoic acids. The results showed that 0.5% of the extract of the fermented milk thistle which was added to the feed dose had a positive effect on the fattening and health status of the broiler rabbits.

KEY WORDS

milk thistle, rabbit, fattening, growth performance, yield, health status

ABSTRAKT

Potravinová bezpečnost je jedním ze základních ukazatelů kvality potravin. Od ledna 2006 legislativní změny v evropských zemích omezily používání antibiotik (růstových stimulantů) a dalších chemických produktů ve výživě zvířat, které byly nahrazeny probiotiky a dalšími produkty, např. huminové složky a rostlinné výtažky. Cílem této studie bylo zjistit účinek různých metod zpracování ostropestřce mariánského (*Silybum marianum*) na růst a zdravotní stav brojlerových králíků, krmených rozdílnou koncentrací ostropestřce. Byly provedeny experimenty s celkovým počtem 253 of HYLA brojlerových králíků. Zvířatům byla podávána v krmivu rozdílná koncentrace 1% nefermentovaného ostropestřce (experimentální skupina E1) a 0.5% fermentovaného ostropestřce (experimentální skupina E2) a výsledky byly srovnávány s kontrolní skupinou C krmených standardní dietou, bez přídavku ostropestřce. Každý experiment začal ve věku 42 dnů ve věku králíka a byl ukončen ve věku 85 dnů. Sledovanými parametry byly: průměrný denní přírůstek a denní spotřeba krmiva, celková spotřeba krmiva, živá porážková hmotnost a hmotnost jatečně opracovaného trupu. Vybrané biochemické parametry byly vyšetřeny analyzátorem VetTest (IDEXX Laboratories, Cymedica, USA). Molární profil těkavých mastných kyselin (VFA) byl stanoven plynovou chromatografií. Nejlepších výsledků bylo dasaženo v experimentální skupině E2 (doplněk 0.5% fermentovaného ostropestřce). Významné rozdíly ($p<0.05$) byly zaznamenány v průměrné denní spotřebě krmiva, celkové spotřebě krmiva, živé porážkové hmotnosti a hmotnosti jatečně opracovaného trupu. Nebyl zjištěn významný rozdíl mezi samci a samicemi ve skupinách C, E1, E2 ve sledovaných parametrech, vyjma hmotnosti jatečně opracovaného trupu, kdy samice skupiny C vykázaly signifikantně nižší hodnotu v porovnání se samci. V biochemických parametrech byl významný rozdíl ($p <0.05$) ve vyšším obsahu cholesterolu. V molárním profilu VFA byl signifikantní rozdíl ($p <0.05$) v heptanové a hexanové kyselině. Výsledky ukázaly, že 0.5% extrakt fermentovaného ostropestřce, který byl doplněn do krmné dávky a měl pozitivní vliv na výkrmnost a zdravotní stav brojlerových králíků.

KLÍČOVÁ SLOVA

ostropestřec mariánský, králík, výkrm, růst, užitkovost, zdravotní stav

Declaration of integrity

I declare that I have developed and written the enclosed Ph.D. thesis completely presentation my own research work, any thoughts from others or literal quotations are clearly marked and all the sources have been quoted and acknowledged by means of complete references under the professional guidance of prof. MVDr. Daniela Lukešová, CSc. and Ing. Petra Silberová, Ph.D.

In Prague, 2018 Signature

MSc. Akhir Pebriansyah

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ADF	Acid detergent fiber
ALB	Albumin
ALKP	Alkaline phosphatase
ALT	Alanine transaminase
AMYL	Amylase
ANF	Antinutritional factors
ANOVA	Analysis of Variance
ASA	Acetylsalicylic acid
AV3	Herbal antioxidant
C	Control group
Ca	Calcium
Chol	Cholesterol
CR	Czech Republic
CZ	Czech
CULS	Czech University of Life Sciences Prague
CZK	Czech Krone (Czech currency)
DE	Digestible energy
DL	Dietary lignin
DM	Dry matter
Dr	Doctor
E1	Experimental rabbit groups 1% non-fermented milk thistle
E2	Experimental rabbit groups 0.5% fermented milk thistle
Et al	Et alia / and others
EU	European Union
FAO	Food and Agriculture Organization United Nations
g	Gram
GCA	Gas Chromatography Analysis
Hb	Hemoglobin

H1	Hypothesis 1
H2	Hypothesis 2
H_3PO_4	Phosphoric acid
Inc.	Incorporated (company)
GAP	Good Agricultural Practise
GMP	Good Manufacturing Practice
ha	Hectare
HIV	Human Immunodeficiency Virus,
INRA	Institut national de la recherche agronomique (French)
kg	Kilogram
kPa	kilopascal
ME	Metabolism energy
MJ	Mega joule
Mm	Milimeter
NDF	Neutral detergent fiber
Ltd	Limited (company)
OATP	Organic anion-transporting poly-peptide
OH	Hydroxyl group
P	Phosphorus
pH	Potential of hydrogen
SCFA	Short chain follatil fatty acids
StatSoft	Statistic software
TBIL	Total bilirubin
TDN	Total digestable energy
TP	Total Protein
USA	United Stated of America
VFA	Volatile fatty acids

1. INTRODUCTION

Milk thistle (*Silybum marianum*) has been used as a medicinal plant to remedy several diseases of animals. Animal studies reported that milk thistle can protect liver from poisoning and liver damage (Ibrahim et al. 2007), liver injury (Hikino et al. 1988), alcoholic hepatitis, protects kidney, improves blood sugar control in diabetes, prevents from viral hepatitis due to anti-viral activities properties. Milk thistle can encourage the immune function to against aflatoxin and improves the carcass and growth performance of chickens.

Furthermore, this annual plant contains several phytochemicals, commonly known as silybinin (Kroll et al. 2007) oil from 18 to 31% which is rich in unsaturated fatty acids, mainly linoleic acid from 42 to 54% and oleic acid from 21 to 36%, flavonoids that are used for coloration, producing yellow, blue or red color pigmentation in the animal products (Khan. et al. 2009).

Next, the rabbit is herbivorous species which thank to its morphological and functional parameters of the digestive tract can process fibrous feed. Broiler rabbits are well known because of their high-quality meat containing low fat and cholesterol and rich in unsaturated lipids. However, the experiments on rabbits concerning fattening performance and health status and are well documented, unfortunately, the current problem is that there is the shortage of information regarding the feed manipulation and processing concerning the effect of milk thistle (*Silybum marianum*) on rabbits.

Also, the question remains as what the effect of different concentrations and processing methods of milk thistle (*Silybum marianum*) on fattening performance and health status in broiler rabbits. Furthermore, some authors have studied the utilization of milk thistle as a feed for livestock and chickens and concluded that it was quite efficient as an immune function booster and growth performance enhancer. The processing of feed for rabbits is variable, depending on the purposes and period of breeding.

In general, feed can be divided into fresh feed and conserved feed. Fresh feed means grazed or fed as fresh cut materials and conserved feed as silage or dry feed or as hay as well as fermented feed. Dried feed (non-fermented) can be used for rabbit and it has benefit for farmer like ease and simple to feed the rabbits. The fermented feed can be used for rabbits as

well, this fermented feed needs to be processed using some basic technologies, e.g silage methods.

The mechanical process of feed from milk thistle such as fermented and non-fermented feed (dried feed) can influence the biological availability of milk thistle. Thus, it is assumed that the dried feed has low biological availability; somehow, however, the fermented feed has high biological availability which is important to utilize most protein present in feed.

The study was aimed to investigate the effect of milk thistle (*Sylibum marianum*) on feed consumption, growth performance, carcass evaluation of broiler rabbits fed by different concentration of milk thistle supplemented (0.5% fermented milk thistle and 1% non-fermented milk thistle).

2. LITERATURE REVIEW

2.1 The characteristics of milk thistle (*Silybum marianum*)

Milk thistle (*Silybum marianum*) (Figure 1) is an annual or biennial plant - shrub which belongs to the Compositae, family these plants are widely spread in arid and semi-arid areas of Mediterranean regions (Tagliapietra et al. 2014) for example in Europe, Egypt, China, and Argentina (Khan 2009). Milk thistle (*Silybum marianum*) is used as a medicinal plant (Křen et al. 2005; Ibrahim et al. 2007) and it has been used for animals due to the productive and reproductive performance increase and the health status improvement of livestock with the silymarin diet supplementation. The genus Silybum is divided into two species namely *S. eburneum* and *S. marianum*. Milk thistle can reach a height of around 0.6-1.8 metres depending on the soil moisture. The leaves of milk thistle are green–dark shiny and white and veined (Carrier et al. 2003; Gresta et al. 2006; Morazzoni and Bombardelli 1995).

These plants grow rapidly in early spring and the seed of milk thistle can be produced in high numbers. For example, each flower of milk thistle can have produced approximately 120 seeds. The size of the seed is about the size of wheat or grain (Gresta et al. 2006). Milk thistle has influences that are solitary large heads located at the apex of the stem, or at the primary and secondary branches. The florets are hermaphrodite, the shape is tubular, with a red-purple or white corolla (Gresta et al. 2006; Vaknin et al. 2008).

Milk thistle has fruits that are black or brown in color and the size is approximatelly 6-8 mm long, milk thistle is predominantly a self-pollinator (Hetz et al. 1995). The shape of the pollen grains in milk thistle is prolate in equatorial view and semi angular in polar view (Ahmad et al. 2008). The seeds are hard-skinned achenes, 6–8 mm long, shiny, generally brownish in colour and with a white pappus at the apex. *Silybum marianum* is a diploid species with 2n=2x=34 chromosomes. The karyotype consists of six pairs of meta-centric, ten pairs of submetacentric and one pairs of acro-centric chromosomes (Asghari-Zakaria et al. 2008).

The main constituent of milk thistle as we can seen in table 1 is flavolignans, in which are containsed silymarin about 70-80% and approximatelly 20-30% are chemical such as polymeric and oxidized polymeric whose fractions are not defined yet. The main content of the silymarin is silybinin and flavolignan mainly in silymarin seed e.g isosilybin,

dehydrosilibin, taxifolin, silychristin, silydianin (Křen et al. 2005; Radko et al. 2007; Usman et al. 2009).

Table 1 Milk thistle types and flavonolignan constituents

Variety	Components
Silybum marianum (milk thistle-purple flower):	3-OH-flavonolignans
1. Silybin type	high silybin: silydianin ratio
2. Silydianin type	low silybin: silydianin ratio
3. Silychristin/ dehydrosilybin type	high silychristin and dehydrosilybin, low silybin, no silydianin
4.*Silybum marianum* (milk thistle-white flower)	3 -deoxy and 3-OH-flavonolignans
5.*Silybum eburneum* (green milk thistle)	high isosilychristin

Source: Martin et al. (2006)

Other flavonolignans identified in milk thistle (*Silybum marianum*) are dehydrosilybin, dehydrosylchristine, deoxysilchristine, deoxysilydianine, silandrin, silybin, silyhermin anesylhermine. The flavonoids silybinol, apigenin and its 7-O-glucoside, 7-O-glucuronide, 4,7-diglucoside are also shed in the contents. In addition, kaemferol, its 7-O-glucoside and 3-sulfate, sitosterol and its glucoside, luteolin and its 7-O-glucoside, triterpen acetate, polyacetylenes and fumaric acid (Křen et al. 2005).

Other oils are also included in the oxygen droplet, including high-fat, unsaturated fatty acids, sugars (glucose, fructose, arabinose, xylose), amino acids, vitamin E (tocopherol), silica and carbohydrates. Oil accounts for about 20% of the fetus. With stearic, palmitic, arachidonic, linolenic and behenic acids. The presence of sitosterol, cholesterol, campesterol, stigmasterol and 24-lophenol has also been demonstrated. The drug is not occupied with saponins or alkaloids (Karkanis et al. 2011).

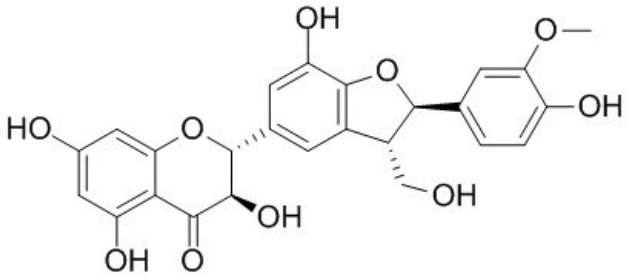
Figure 1 Milk thistle (*Silybum marianum)*

www.maghebati.com

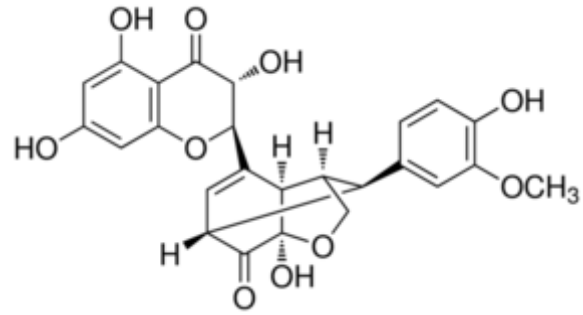
The most active complex of is silybin. It is a mixture of two diastereoisomers, silybin A and silybin B in a ratio of 1:1 and forms 50-70% silymarin (Bhattaram et al. 2002). The content and composition of milk thistle is changed depending on the variiety of milk thistle and the climate condition (Sholrpour et al. 2008). Hevia et al. (2007) reported a higher content of milk thistle in Chile than in Germany. Martin et al. (2006) also investigated that the milk thistle complex in New Zealand has much higher percentage of silybin compared to german milk thistle.

The percentage of milk thistle can be useful compounds can be increased by the so-called elicitor, biologically active compounds which induce stronger defence and secondary metabolite production in the plant. After application of jasmine acid elicitor, the content of milk thistle in cells and tissues increased to 1.36%, 0.68% and 0.007-0.0014%. Martin et al. (2006). Dvorakova (2006) also investigated the application of elicitor Acetylsalicylic acid (ASA) in the concentration (11H_2O+0,1 ml ASA) was increased about 22.5%, on the other hands there as a higconcentration of acetylsalicylic acid (11 H_2O+1 ml ASA). The content of

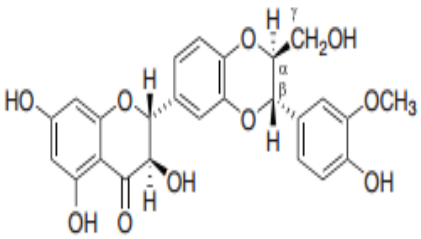
milk thistle is an effective compounds can be dropped about 43%. The biochemistry content of variety of milk thistle can be found in figure 2.



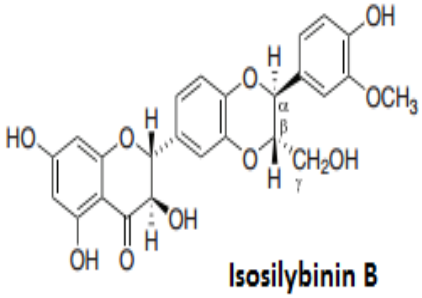
silychristin (SC)



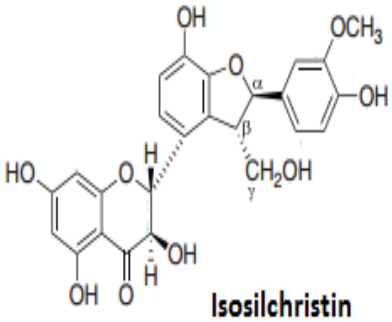
silydianin (SD)



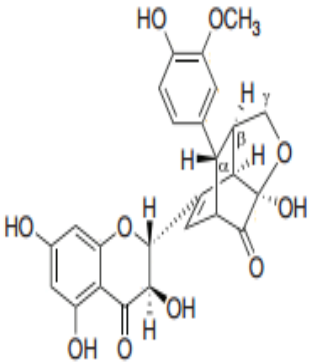
Silybinin B



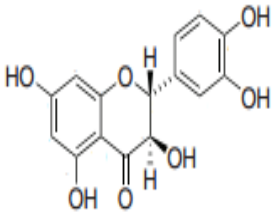
Isosilybinin B



Isosilchristin



Silydianin



Taxifolin

Figure 2 The biochemistry content of milk thistle

Source: Wen et al. (2008)

The yield of milk thistle is around 400-200 kg/ha, but its yield and the components of milk thistle are dependant on biotic and abiotic factors, such as on climate, temperature, humidity and cultivated genotype.

Different genera of *Silybum* spp. are widely cultivated in the agri-ecological conditions of Slovakia. Cultivar Silyb, originating from the Czech Republic, is the most cultivated one as well as the most used for the pharmaceutical processing. The taxonomy and distribution of milk thistle can be found in table 2.

Table 2 Milk thistle types and flavonolignan constituents

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Asterales
Family	Asteraceae / Compositae – Aster family
Genus	*Silybum* Adans. – Milk thistle
Species	*Silybum marianum* (L.) Gaertn. – Blessed milk thistle

Source: Martin et al. (2006)

The spreading of milk thistle is in arid and semi-arid areas of Mediterranean regions (Tagliapietra et al. 2014) for example in Europe -mediteranian regions, middle-east such as Egypt, Saudi Arabia, China, Mongolia, USA, Chile and Australia can be found in figure 3.

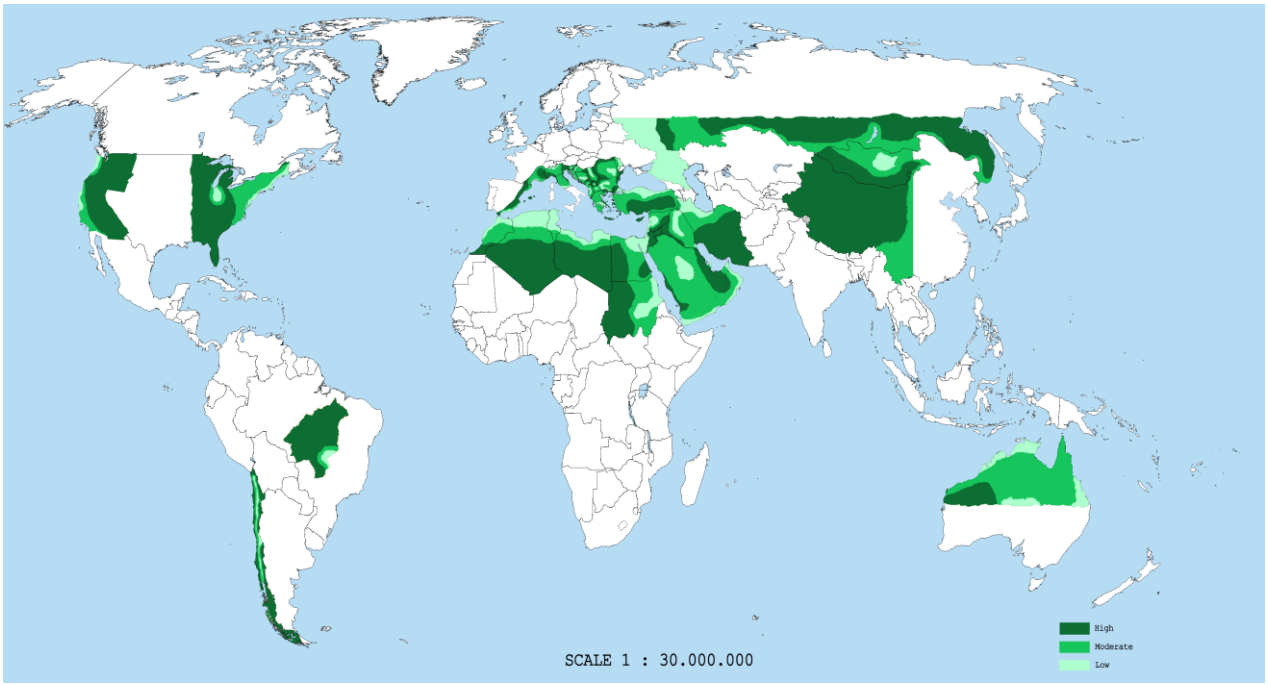


Figure 3 The spreading of milk thistle

Source: Khan (2009)

2.1.1 Production and distribution in the Czech Republic

In the Czech Republic, the company target is solely a processing of milk thistle run by Irel Inc., which was founded in 1994 and has grown considerably since then. Since its founding, Irel Inc. company has been a major manufacturing partner of international pharmaceutical companies. The company does not specialized in its own cultivation but cooperates with the plant breeders both in the Czech Republic and abroad. Focused on the pre-treatment of medicinal drugs, which is made possible by a much more effective final treatment of medical devices, which is also associated with their higher quality. Irel Inc. is the holder of the patent granted based on international patent protection law, pre-extraction technologies and procedures. Pressed seeds are used to produce food supplements, cosmetics and dietary

supplements. All production facilities, production organization, production operations and procedures, control and contracting obligations of Irel Inc. (Figure 4) are in full compliance with the principles of Good Manufacturing Practice (GMP) and Good Agricultural Practice (GAP). The benefit of Irel Inc. (2016) for the single year is 168.000.000 CZK.

Figure 4 Irel Inc., 671 72 Miroslavské Knínice 63, Czech Republic

2.1.2 The nutrition of broiler rabbits

Rabbit production and performance are highly dependent on feeding, which means the feeding must be adequate and proper in quality and quantity in terms of nutrition. External factor such as temperature influence the energy metabolism in rabbits. There was an experiment dealing with temperature and feed intake, Lebas et al. (1997) reported that in growing rabbits who facing the temperatures between 5°C and 30°C, the feed intake dropped down from 120 g to 180 g a day and the water intake increased between 330 g to 390 g a day.

Lebas et al. (1997) also said that rabbits without feed but with water available can survive about 4 weeks and reduce the high intake of water due to the Sodium chloride in water (0,45%), therefore rabbits are very adapted to hunger and lack of water. Moreover, rabbits feeding preference is always unpredictable. Cheeke et al. (1986) also reported that rabbits are prefer bitter taste compared to sweet one.

The type of broiler rabbits varies. The most common broiler feed is mixed feed. It's cheap and easy to maintain in intensive European farming. The pellets can be formed as granules or pellets. Feedstuff can be divided into four main categories. Firstly, standard feed which has protein and amino acids, protein is very important for maintaining body growth and performance. The content of fibre in feeding is also necessary to maintain the metabolism. The percentage of fibre can be estimated by the acid detergent fibre. The fibre can build energy which is important to maintain the body performance and regulate the body temperature (Lebas et al. 1997).

Minerals and vitamins are also necessary in animal feeding to help as building blocks and serve as enzymes to build and rebuild the body proteins. As herbivores, rabbits have microbial fermentation in the intestine and utilize non-protein nitrogen (Arrington and Kelley 1976). Amino acids must be available for rabbits such as, histidine, arginine, isoleucine, tryptophane, methionine, lysine, phenylalanine, threonine, valine, leucine and for the rapid growth glycine (Arrington and Kelley 1976). Protein and amino acids are very important for rabbits, there is a recommendation for rabbits to get minumim dietary protein and amino acids see table 3.

Table 3 Minimum dietary protein and amino acids recommendations for rabbits

Dietary Level (for a DM of 89-90%)	Reproducing Does	Young at weaning	Fattening rabbits
Digestible energy (kcal/kg)	2500	2275	2400
Crude protein (%)	17.5	16.0	15.5
Digestible protein (%)	12.7	11.0	10.8
Arginine	0.85	0.90	0.90
Histidine	0.43	0.33	0.35
Isoleucine	0.70	0.65	0.60
Leucine	1.25 1.10	1.10	1.05
Lysine	0.85	0.75	0.70
Methionine + Cystine	0.62	0.65	0.65
Phenylalanine + Tyrosine	1.40	1.20	1.20
Threonine	0.65	0.60	0.60
Tryptophan	0.15	0.13	0.13
Valine	0.85	0.70	0.70

Source: Maertens (1992)

Crude protein

Protein is the nutrient most needed in rabbit diets primarily because the energy sources such as maize and other cereal grains and tuber crops are usually low in protein. The rabbit makes its own proteins from the proteins and amino acids, which it obtains from its food (Usman et al. 2009). This protein synthesis could build up the energy. Also, the ten essential amino acids which must be provided in the diet to support the growth and survival of animals such as: lysine; methionine; leucine; valine, phenylalanine; threonine; tryptophan; arginine isoleucine; histidine (Manning et al. 1994).

Essential amino acids need to be included in the ration for rabbits. Lysine and methionine are the amino acids that are found to be deficient in rabbit ration (Maertens 1992). While there is some bacterial protein synthesis in the caecum, it is not enough to meet the essential amino acid requirements of rabbits. For rabbits the recommended crude protein level in the dry matter of the ration is over 18% for newly weaned rabbits; 16-18% for rabbits from 12-24 weeks; 15-17% for breeding does; and 12-14% for all other stock (Manning et al. 1994). Several researchers have reported that the protein requirement of growing rabbits.

Usman et al. (2009) investigated that rabbits fed with decreasing crude protein levels of 18.08, 16.32, 14.22 and 12.50%, and they found that crude protein could be reduced to 16.32% with lysine and methionine supplementation without affecting weight gain and feed efficiency. Different results were obtained when Fang et al. (2002) used diets with increasing crude protein levels, 14.3, 17.2 and 21.4%, as they found no significant difference among groups of rabbits regarding the body weight, feed intake or feed conversion efficiency.

Kosina et al. (2017) established an experiment and investigated that there were no differences in live weight gain, final body weight, and feed intake when diets containing 16, 18 and 20% crude protein were fed to five-week-old rabbits. Manning et al. (1994) using mash diets containing crude protein ranging from 11.63 to 26.85% found that the diets containing 20.74% crude protein recorded the highest final live body weight and live weight gain. Maertens (1992) has shown that soya bean meal or fish meal promotes better growth rates

than other protein supplements when the alternative supplements do not have essential amino acids added.

Maertens (1992) further investigated that when essential amino acids were added to protein supplements such as rapeseed meal, cotton seed meal, horsebeans, and peas, growth rates like those achieved with soya bean and fish meals were attained. According to Suckow et al. (2011) dietary protein quality is particularly important for rapidly growing weanling rabbits, which may not have well developed caecal fermentation. Recent research has conducted that the amino acid requirements are age dependent and change during the reproduction cycle of the doe. In early growth stage (4-7 weeks of age), rabbits need a higher dietary amount of digestible crude protein and amino acids (Maertens et al. 1992). Also, during peak lactation the response to higher amino acids is more pronounced (Suckow et al. 2011).

Many research reports have shown that a reduction in the level of protein and essential amino acids in the diet, from an optimum level for growth in animals, is associated with a decreased growth rate and efficiency of feed utilization and a concomitant increase in body fat Suckow et al. (2011). Dietary protein level is one of the several non-genetic factors that influence the amount of body fat in animals Usman et al. (2009). Author Maertens (1992) reported that if the amino acid content in the feed of animals differed widely from the animal's requirement for amino acids, feed intake would be depressed and that if the deficient amino acid was supplemented, intake would be increased.

Energy

Energy is oxidized during the metabolism process such as carbohydrates, fats and protein. The energy also needed by rabbits for organic synthesizing is usually supplied by carbohydrates and fats. An excess of protein could help to supply energy after deamination. Rabbits also can adjust their feed intake as a function of their dietary energy concentration Percival (1998).

Percival (1998) reported that, the intake regulation is to achieve constant daily energy intake and is only possible at a dietary digestible energy (DE) concentration above 2250kcal/kg.

Several factors such as productive function (growth, lactation, maintenance, etc.), age, sex, body size and environment (temperature, humidity, air-movement) influence the energy requirements of rabbits Percival (1998). The rabbit requires more energy to maintain normal body temperature (Maertens 1992) as a result, the body temperature decreases and to increase energy, by the intake level of feed must be increased, or the energy content of the ration must be increased in feed ration.

The average maintenance requirement determined in growing rabbits is about 100kcal DE/ kg 0.75 Maertens (1992). Fed on energy-concentrated foods, rabbits can satisfy their requirements, but this is not possible with forage because forage is usually a dilute source of energy (Fielding 1991); hence when fed only on forage they cannot obtain as much energy as those fed on concentrated foods such as maize grains or cereal brans. Rabbits (Cheeke 1986) require a diet of 2200 kcal/kg of diet or 2.2 kcal/g of diet. For breeding rabbits (Fielding, 1991), a general recommendation is that the food should contain: 65-66% TDN; or 2600-2700 kcal DE/kg DM; or 2.4-3.5 MJ DE/kg DM; or 2.0-3.0 MJ ME/kg DM.

Products of microbial degradation of dietary fibre which contributes to the energy demand of the host animal, are the volatile fatty acids (VFA). An effective absorption of VFAs from the large intestine has been demonstrated in all non-ruminant herbivores which have been investigated Douglas et al. (1987). Authors Douglas et al. (1987) also investigated that in rabbits about 10-20% of maintenance energy expenditure comes from VFA.

Even though the apparently poorer utilization of fibre by rabbits than by horses or ruminants, Cheeke (1986) concluded from Carbon-14 studies that VFAs absorbed 30% of the maintenance energy requirement, a value similar to that attributed to those products from the caecum and colon of the horse (Percival, 1998). Douglas et al. (1987) investigated that digestible energy levels in typical rabbit diets are quite low, being in the range of 2400-2800kcal/kg weight diet. They further indicated that higher energy levels impair animal performance and result in reduced energy intake. Rabbits are efficient users of starch in cereal grains and prefer barley to corn. When given a choice of cereal grains. According to Douglas et al. (1987) diets that are based on corn only have produced poorer growth rates as compared to barley- or oat-based diets.

Pond et al. (1995) reported that approximately 3% fat is recommended in rabbit diets; dietary fat is well utilized by rabbits and improves diet palatability and increases energy level without causing carbohydrate overload of the hindgut. The rabbit, for instance the breeding doe, adjusts its feed intake according to the energy concentration of the feed as well as the protein and other dietary components present (Lebas et al. 1986) to around 220-240kcal of digestible energy (DE) per kg metabolic weight.

Crude fibre

Maertens (1992) reported that although fibre is not considered a real nutrient in rabbits because of its low digestibility, it means the average dietary digestibility is less than 20%, it is considered a nutrient to maintain the gut motility. Cell-wall constituents from feedstuffs having low lignin content or young plants have a considerable higher digestibility than highly lignified sources, 40-70% versus 5-20% respectively. It is not clear the minimum fibre intake for prevention of diarrhea in rabbits.

Research reports from Pond et al. (1995) examined the effect of low fibre diets to rabbits and observed that a sharp decrease in fibre level from 19-9% in the diet doubled the risk of digestive trouble. The population of cellulolytic bacteria decreased in the caecum, and the microbial ecology system in the caecum became unbalanced, which may cause death from diarrhoea.

Feeding rabbits with a diet low in fibre and high in energy or a finely ground concentrate diet; can result in high mortality due to intestinal disorders, such as enterotoxemia (Lukefahr and Cheeke 1991). The significant role of dietary lignin (DL) on the rate of passage and its protective effect against diarrhoea has been demonstrated by the French INRA (Institut National de la Recherche Agronomique) team (Gidenne and Perez 1994; Lebas et al. 1997). The mortality rate because of digestive disorders was closely related ($r = 0.99$) to the DL level in their experiments. The relationship was expressed as follows: Mortality rate (%) = 15.8-1.08 DL (%); n >2.000 rabbits.

Quite similar effects were observed by the same team of researchers with various cellulose (ADF-DL) levels (Gidenne and Perez 1994). They indicate that the recommendations in terms of dietary safety cannot be expressed as a single fibre fraction. Moreover, recommendations of dietary fibre are age dependent. Young rabbits require higher minimum levels than fattening or breeding does, probably because of their lower daily intake to reduce enteritis. An excess of dietary fibre is also not desirable because digestible energy (DE) content decreases and a too high protein- to- energy ratio is commonly the result. Such a situation is favorable for the proteolytic flora that produces ammonia with an increasing risk of digestive disorders (Lebas 1997).

Besides dietary fibre starch also plays an important role in the nutrition-enteritis interaction. Young rabbits have an immature pancreatic enzyme system that can lead to significant amount of starch reaching the caecum when using high-starch diets. Especially dietary starch with higher resistance (corn) against hydrolysis could lead to starch overload. The risk of destabilization of the caecal flora is higher if the increased ileal starch flow is not accompanied with a similar increase of fibre intake (Gidenne and Perez 1994).

Rabbits use crude fibre less efficiently due to a faster rate of passage of digesta and smaller holding capacity, compared to grazing ruminants. Rabbits are therefore more selective in their diets than ruminants (Gidenne and Perez 1994). Optimal fiber balance also includes a dietary recommendation for particle size. A sufficient number of large-size particles is required for optimal performance and to reduce the risk of digestive disorders.

Gidenne and Perez (1994) reported that chemical composition and form of fibre not only affect its susceptibility to digestion but can also influence feeding habits. in an experiment, compared oat husk with barley straw and pure cellulose in rabbit diets, and concluded that daily feed intake increased as the crude fibre content of the diet was increased from 3.9 to 27.0%. The optimum level of crude fibre for growing rabbits is 13-14% (Lebas et al. 1997).

Minerals and vitamins

Pond et al. (1995) concluded that the major mineral elements of concern in rabbit diet formulation are calcium and phosphorus (Ca and P), and that the other minerals are provided in adequate amounts by the ingredients used plus the addition of trace-mineralized salt. Studies on the calcium and phosphorus requirements of growing rabbits have shown that they need these minerals much less than lactating does. The amounts excreted through the milk are significant.

However, excesses of calcium (>40 g/kg) or phosphorus (>19 g/kg) induce significant alteration of fertility and prolificacy or higher proportions of stillbirths. Total dietary phosphorus intake ranging from 0.45 to 0.76% did not affect any of the does' reproduction performances (Lukefahr and Cheeke 1991). Pond et al. (1995) investigated that fed weaner rabbits peanut meal, sunflower meal and palm kernel meal diets containing 14.84, 23.24 and 38.89% crude fiber respectively and observed that the feed consumption was significantly ($p<0.05$) higher with the palm kernel and sunflower meal diets than with the peanut diet. This was however, attributed to the rabbits having to compensate for their energy requirement. In the same experiment they found feed to gain ratios to be significantly ($p<0.05$) poorer on the palm kernel and sunflower meal diets than on the peanut meal diet.

Differences in weight gain and final body weight for the three diets were however, not significant ($p>0.05$) though the diet with lower fibre content had higher weight gains than the one with higher crude fibre content. The performances of the progeny were independent of their mothers' diet. The lack of response to low-dietary phosphorus levels has been confirmed with fatteners (Lebas et al. 1997). The Ca:P ratio does not seem to be critical for rabbits (Lebas et al. 1997) and is usually 2:1, however, rabbits can tolerate much higher ratios.

Copper sulphate which is often used as a non-nutritive feed additive aids in preventing enteritis (Pond et al. 1995). Lebas et al. (1997) stated that rabbits are born with high levels of iron in their livers, sufficient for their preweaning growth. Rabbits require water-soluble (B group and C) as well as fat-soluble vitamins (A, D, E, and K). According to Lukefahr and Cheeke (1991) the major vitamins needed in rabbit diets are vitamins A, D and E and that

protein and carbohydrate dietary sources, fed in good variety, may largely meet the mineral and vitamin requirements.

Micro-organisms in the digestive flora synthesize sizeable amounts of water-soluble vitamins which are utilized by the rabbits through caecotrophy. Vitamin K and the B vitamins are not required in the diet, since they are synthesized through coprophagy and fermentation in the caecum or hindgut; likewise, vitamin C (Lukefahr and Cheeke 1991). Under practical conditions, the B-complex vitamins are not dietary essentials for rabbits; however, under stress situations and at high performance levels deficiencies can occur (Pond et al. 1992).

Gillespie (1998) has indicated that the use of iodized salt at the rate of 0.5% of the diet will supply the needed sodium, chlorine and iodine for rabbits. The vitamin A requirement of rabbits has not been adequately determined and a level of 10,000 IU/kg of diet is adequate while levels more than 40,000 IU/kg diet may adversely affect reproduction (Pond et al., 1995). They further stated that vitamin A-deficient rabbits exhibit poor growth, leg deformities, increased susceptibility and a high incidence of enteritis. Vitamin C supplementation is recommended for rabbits under stress (Lukefahr and Cheeke 1991).

Water as a nutrient for rabbits

Water is normally considered a nutrient, although its properties and functions are quite different from those of other nutrients found in feeds. Water is the major component of the rabbit body, making up 70% of the lean body mass (Maertens 1992). According to Maertens (1992) further indicated that rabbits will die more rapidly from water deprivation than from food deprivation.

Restricted drinking water or limited drinking time leads to reduced feed intake that is directly proportional to the amount of water being consumed (Pond et al. 1995). They further reported that water and feed consumption vary with changes in environmental temperature and humidity. As the temperature rises above 25^0C day and night, feed intake tends to drop while water consumption increases. At high temperatures, (35^0C and over) feed and water intakes decline, affecting the performance of growing and lactating animals (Maertens 1992).

According to Pond et al. (1995) water plays an important role in a number of functions vital to an animal such as digestion, nutrient transport, waste excretion and temperature regulation.

One of the most important properties of water in nutrition is its remarkable ability to dissolve substances. This property is due to its dielectric constant, which in turn is due to its hydrogen bonding (Lukefahr and Cheeke 1991).

2.2 Phytochemicals of milk thistle and their uses

Crozier et al. (2008) investigated that plants synthesize a huge number of organic material which are divided into two categories, primary and secondary metabolites. Primary metabolites are organic materials glutathione reductase, glutathione peroxidase, glutathione peroxidaroduced by the plant that have direct functions in growth and metabolism including solute transport, translocation, nutrient assimilation, respiration and photoshyntesis such as nucleotides, amino acids, phytosterol, and organic acids). However, secondary metabolites, on the other hand, are bioactive substances synthesized by plants that are not involved in 'primary' metabolic pathway. Secondary metabolites play great roles, especially in environmental adaptation. Secondary metabolites are very important to give protection against UV, microorganism such as viruses, bacteria and fungi, as they have antiviral, antibacterial and antifungal functions) and herbivores (arthropods and vertebrates), according to Wink (1988) and Mazid et al. (2011).

Pichersky and Gang (2000) in their experiments reported that the more secondary metabolites are synthesized, the more endurance the plants will have. Besides giving plant protection, the other functions are allelopathic agent, signaling molecules, and pollinators. It also contributes to odours, tastes and colours of plants, making each plant different among others. Secondary metabolites bring enormous benefits for human and have been used as traditional medicines for hundreds of years.

The use of secondary metabolites is also very promising for animal nutrition. They are assumed to be the bioactive compounds in phytogenic feed additives (Costa et al. 2013). Feed additives are animal feed supplements given by farmers that improve the dietary feed to get

the desired responses (better performances and health). The usage is completely different from veterinary drugs which are used only for prophylaxis and therapy in the short period of time. While phytogenic feed additives are feed additives obtained from plant and herbs sources (Windisch et al. 2008).

Wenk (2000) also have done an experiment and reported that the examples of feed additives which are commonly used are antioxidants, performance promoters, emulsifiers, stabilizers, flavours, and probiotics. The application of plant secondary metabolites as feed additives, however, is still recent compared to human-related usage so there are still relatively limited results of the trials (Nielsen 2008).

The demand for organic animal products have been also increasing, considering the perception that organically farmed animals are healthier. For these reasons, considerable attention to the benefit of natural additives like secondary metabolites in animal has been given (Nielsen 2008). A great diversity of secondary metabolites which is produced by plants not always give positive impact on animal health (Windisch et al. 2008). Use of phytogenic products as feed additives for swine and poultry. There are some compounds which are toxic and harmful to animals, such as linamarin in cassava (*Manihot esculenta;* Euphorbiaceae), and beans (Wenk 2000).

Most results showed promise of the efficacy of plant secondary compounds. Some secondary metabolites have a positive effect in intestinal digestions influencing positively nutrient digestibility while some others effect on change in colour, tissue metabolism, immunity, flavours, egg quality, and many others (Wenk 2000). Nielsen assumed the use of citrus, chestnut, grape, green tea extract, chestnut wood and white willow bar and oil as feed additives in piglets and finishers could improve daily gain and feed utilization (Nielsen 2008). In addition, tanins and saponins in the pods of *Acacia petanula* and *Enterolobium cyclocarpum* can assist in alleviating methane in rumen (Acton 2013).

Flavonoids

Costa et al. (2013) reported that the main category of plant according to their secondary metabolites is flavonoids. There are more than 7000 poliphenols were found (Bravo 1998).

The phenolic compounds are important to use as an antiallergic and anticarcinogenic. Antioxidant is the most important for health benefit (Wenk 2000).

Flavonoid as antioxidant (Peterson and Dwyer 1998) has an important health due to scavenge free radicals from their functional hydroxyl groups (Kumar and Pandey 2013). Milk thistle is an herb that is currently commonly used in herbal medicines or feed supplements because of the high of flavonoid content.

Human has antioxidants problem recently such as a high number of diseases, including cancer, neurological disorder, hypertension, asthma, inflammatory disease, acute respiratory, and pulmonary disease and other diseases. An oxidative stress may occur when cells cannot protect themselves against reactive oxygen species because of poor mechanisms defence, for example in under stress conditions (Krishnaiah et al. 2011). According to Grotewold (2006). The main problem of reactive oxygen species detoxification is lack of antioxidants which is therefore important for the survival of aerobic organisms.

2.3 The utilization of natural products and milk thistle

The antibiotic usage in animal feed has been prohibited since 2006 January^{1st} to overcome this problem the utilization of natural product is necessary to improve growth and medication for animals. Volek (2006) reported that a usage natural product to replace antibiotics for animal was effective. Furthermore, some experiments have shown that a positive effect of some natural products on digestion and therefore on the health and performance of rabbits; mainly chicory (*Cichorum intybus* L.), white lupin (*Lupinus albus*) and Jerusalem artichoke *(Helianthus tuberosus*).

The utilization of milk thistle could be a medicinal plant to remedy several diseases for animals (Awang 1993). Authors Křížová et al. (2011) investigated in cows was used milk thistle shows that influenced the rumen degradability and bioavailability in the rumen was low, even though milk thistle has high potential of natural hepatoprotective. Also, Tedesco et al. (2004) reported that the utilization of milk thistle as a feed can influence the dairy cow performance and raise the milk production.

Furthermore, milk thistle is also used as a feed for dogs and the performance of the dog which includes health status–against antimicrobial agents (e.g. *Trichomonas, Plasmodium*, *Leishmania* and *Toxoplasma* spp.) was better with treatment compared to those without milk thistle (Chon and Kim 2005).

This annual or biennial plant contains several chemicals, commonly known as silybinin (Kroll et al. 2007). This plant is rich or edible oil (18-31%) which is rich in unsaturated fatty acids, mainly linoleic acids (42-54%) and oleic acids (21-36%), flavonoids that are used for coloration, producing yellow, blue or red color pigmentation in the animal products (Bilia et al. 2001; Khan. et al. 2009). Seed oil content (%) and fatty acid profile (%) of oil extracted from Milk Thistle seed and several other commercially important oil crops.

The milk thistle obtained a fatty acid profile from the oil extract of corn, sunflower, and soybean. These plants were suitable to use in food processing to get a flavour and performances characteristic, however, in fact that the oil contains has ability in the sense of commercial products and these lower cost market compare to another product.

The effect milk thistle on feed ration

Schiavone et al. (2007) reported that the utilization of milk thistle (*Silybum marianum*) as a supplement in feed ration was influenced the on the meat quality and performance of broiler chicken and increases muscles resistance from oxidative stress. The positive effect of milk thistle for broiler chicken on feed ration have reported that milk thistle from its flavolignan was influenced to increasing the weight gain by 1.3%, decreased the cholesterol, increase a vitamin A content in the liver and economic benefit reason which is less cost feed by 2.7% (Fisinin et al. 2011).

Moreover, Kim et al. (2013) reported that the utilization of milk thistle *(Silybum marianum)* on steers (*Bos taurus*) was indicated by a positive influence on the characteristics of carcass, blood metabolite and liver function–better performance compared with control (without silymarin) during the fattening period, these studies also showed that costumers prefer to use it as an antibiotic or feed additive due to safety and economies. Kroll et al. (2007) investigated that milk thistle (*Silybum marianum*) contain flavonoid 80% and flavonolignans 65% and

influence palatability and the live weight of rabbits. Live weight of broiler chickens was also improved by (1078.3±21.3 g) with milk thistle supplementation 10g/kg in feed mixture compared to the control (804.3 ±8.7 g) Chand et al. (2011).

But, interestingly, Šťastnik et al. (2015) who evaluated milk thistle with 5% and 15% in feed mixture of broiler chicken reported negatively affected by dietary treatment, also the slaughter live weight of broiler chicken was lower ($P<0.05$) compared to the control group which was high (2169.24 ± 134.72 g). Furthermore, Křížová et al. (2011) investigated that milk thistle did not aaffect the daily feed intake as well as feeding consumption during the experiments the reason is because milk thistle contains substances with a bitter taste and influence the palatability for rabbits, according to Khan et al. (2009).

Daily gain was investigated by Cullere et al. (2016) shown that there was no difference among the groups. The control group was (52.3 ± 2.88 g) and treatment groups was (52.5 ± 2.86 g) and (51.5 ± 2.85 g). Moreover, Daneshvar (2017) shown his rabbit experiment and shown that the daily gain was not significant difference between the control group (conventional diet) was (41.15±1.24 g) and the treatment group (milk thistle extract) was (41.41±1.34 g).

It was interesting to notice regarding the carcass performance, Omid Fani Makki et al. (2013) observed that carcass of broiler chicken in treatment group 1% of milk thistle was 70.71g and 0.5% of milk thistle was 70.84g, compared with the control which was 65.77 g. Carcass weight as reported by Kosina et al. (2017) showed that experimental groups (0.2% and 1% milk thistle) were significantly higher at 1563.1 ± 9.1 g and 1580.1 ± 8.7 g compared to control group (basal diet) was 1553.2 ± 8.5 g.

Next, formation of volatil fatty acid (VFA) in the caecum is important because it reflects the concentration of fibre in a diet of broiler rabbits, by increasing concentrations of milk thistle in feed are needed and important to increase the level of propionate and butyrate in order to supply additional energy and health status in rabbits (Chrenkova et al. 2011), but heptanoic as well as hexanoic acids are not physiologically important to evaluate the health status of rabbits (Marounek et al. 2000).

2.4 Anatomy and general physiology of rabbits

The rabbit is an herbivore animal which has a high metabolic rate. Rabbits can consume a high amount food and large amount of plant, which later is easily fermented. Rabbits have an effective and fast digestive tract to digest the high level of fibre compared to cattle or other small ruminants (O'Malley 2005). In oral captivity, the feed either from plants or concentrate will be chopped and masticated in the mouth thanks to teeth and saliva glands such as parotid, mandibular, sublingual, and zygomatic during to ease movement food into next process. In the stomach which has a pH 1-2. The rabbits can feed frequently up to 30 times a day of 2-3 g of food over 4-6 minutes periods can be found in figure 5.

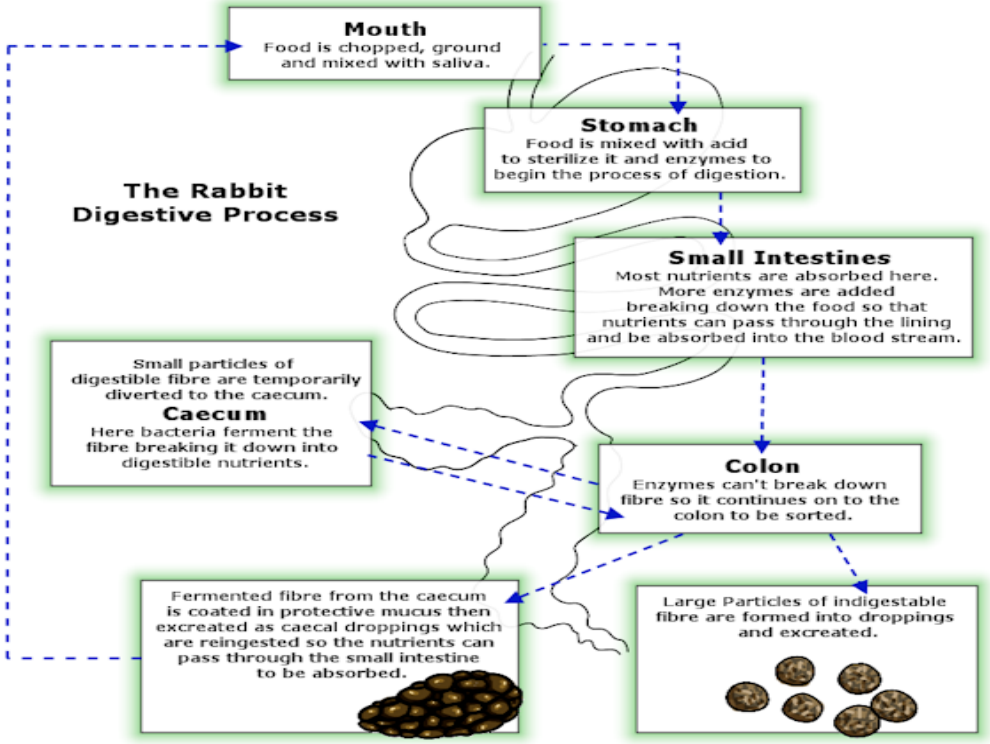


Figure 5 Anatomy and general physiology of rabbits

Source: O'Malley (2005)

The stomach normally contains a mixture of food, hair, and fluid for 24 hours fasting (O'Malley 2005). Rabbits are well known as an animal which are used for human utilization

such as meat and pets. Meat is one of their products to be used in many areas. And its products are in quite high demand in market sector. The customer considers the nutritional value as good quality at favorable prices. The meat from rabbit is very typical, rabbit has a high protein contain, low in fat and cholesterol which means these are very good for human nutrition (Kosina et al. 2017). To get good quality rabbit products, always we should establish the feeding for those rabbits. The experiment has been conducted regarding the utilization of feed for livestock and rabbit, particularly with milk thistle (*Silybum marianum*) that is used a supplement feed. In figure 6 showed that milk, water, dry feed intake of the young rabbit was influences. Water intake and dry feed intake are increasing according to age, otherwise milk intake is decreasing from 20 days.

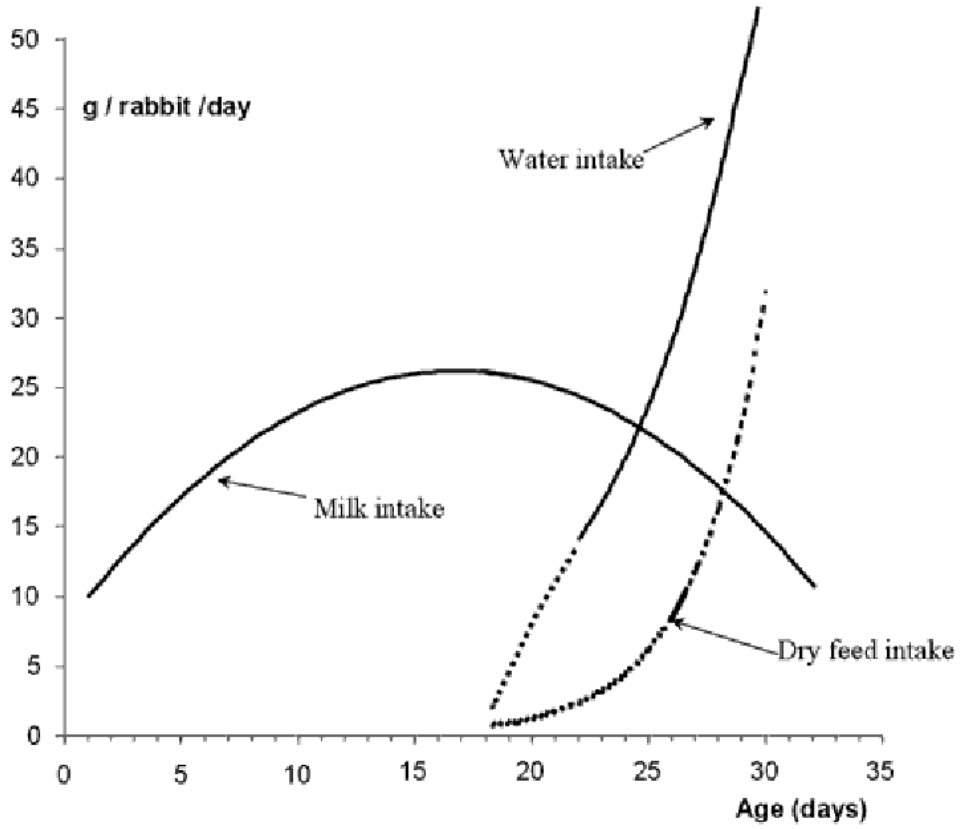


Figure 6 Milk, water, dry feed intake of the young rabbit. Values are means for litters of seven to nine kits with pelleted dry feed and one lactating doe available and weaned at 30 days.

Source: adapted from Szendro et al. (1999); Fortunlamothe and Gidenne (2000)

Tagliapietra et al. (2014) showed that in vitro fermentability and in vivo of milk thistle improved the efficiency of feeding for livestock and the profitability environmental system in arid and semi-arid areas. Silberova (2014) reported that the utilization of milk thistle in feed ration can improved health status of rabbits due to phytoadditive contain from milk thistle. The parameters of morbidity and mortality were lower in feed ration which are used 1% of milk thistle compared to control (without milk thistle). On the other hands, the parameters, such as growth and yield carcass of rabbit were not significant influences. Moreover, the utilization of milk thistle for rabbits could be feasible for improvement the performance of rabbits.

In fact, Banaee et al. (2011) observed that milk thistle is used as a supplementation feed for rabbits was useful to improve health status of fish, growth and physiological performances, followed by the biochemical blood parameters which influenced in the reduction of plasma glucose and cholesterol levels. However, the plasma total protein and globulin were increased. Grabowicz et al. (2004) reported that milk thistle as a silage for cows might influence the activity or blood serum and the level of triglyserides was lower compared to feeding without milk thistle, nevertheless the content of cholesterol and bilubirin were not influenced by milk thistle. However, Calani et al. (2010) observed in humans who consumed milk thistle as a supplement showed that milk thistle was influence a better on the absorption and metabolism in human body after digestion where might also reduce the hepatic problem.

2.4.1 The digestive system of rabbit

The caecum and colon are relatively important for rabbit digestion (Banaee et al. 2011). In fact, in the caecum there is a microbial activity which helps the digestion process to utilize the nutrition and to control digestive pathologies. Moreover, caecotrophy is the behavior of ingestion of soft faeces to make microbial digestion in the caecum more important for nutrition of rabbits. Furthermore, rabbits developed a strategy of high feed intake (65-80g/kg body weight (BW) and a fast transit of feed through the digestion system to meet nutritional requirements.

To reach its full functional capacity, the digestive system of the growing rabbit must go through a period of adaptation from milk base feeding to dependence on solid feed. This adaptation process not only affects the digestion process, but also microbiota colonization and the development of gut barrier mechanism that protects the animal against digestive pathologies.

2.4.2 The role of intestinal microbiom in the digestion and absorption of nutrients

The presence of microbial population in caecum, together with caecotrophy allows the rabbit to obtain additional energy, amino acids, and vitamins. The main genus of the microbial population in the caecum of rabbit is bacteroides Grabowicz et al. (2004), which comprises 109–1010 bacteria g-1. Other genera such as Bifidobacterium, Clostridium, Streptococcus and Enterobacter, this bacteria population is 1010-1012 bacteria g-1 (Calani et al. 2010) The role of the whole microbiom community in the digestive processes can be evaluated by its enzymatic activity or the end products of fermentation. The presence of cellulolytic bacteria in the caecum of rabbits indicated that the main activities were in decreasing order of ammonia use, ureolytic, proteolytic, and cellulolytic. The great importance of other activities such as xylanolytic, pectinoliytic, mucolytic has been indicated in studies conducted by (Marounek et al. 2003).

The composition of the microflora does not remain constant throughout the life of the rabbit and is strongly influenced by the time of weaning (Calani et al. 2010). During the first week of age, the digestive system of the rabbit is colonized by strict anaerobes, predominantly Bacteroides. At 15 days of age, the number of amylolytic bacteria seem to stabilize, whereas those of colibacilly decrease as the numbers of cellulolytic bacteria increase Grabowicz et al. (2004). Milk intake may delay the colonization by cellulolytic flora but does not seem to affect the evolution of the colibacilli population (Banaee et al. 2011). Because of age related changes in the microbial population, the procuction of volatile fatty acids (VFA) increases with age (Marounek 2000).

Moreover, as caecotrophy is initiated, the presence of bacteria of caecal origin can be detected (Banaee et al. 2011). We can detect precaecal microbial flora after only 16-17 days of age,

respectively. The presence of these precaecal microbes is dependent on caecotrophy and no viable cells after 5-6 hours (Calani et al. 2010). The composition of the microflora does not remain constant during the life of the rabbit.

Because of fermentative activity of the microflora, VFAs are produced in the proportion of 60-80 mol acetate, 8-20 mol of butyrate and 3-10 mol of propionate 100 mol-1 of VFAs. However, the proportions change with the time of the day, and with the developmental stage of the rabbit with increases in acetate concentration from 15-25 days of age and for butyrate 15-29 days of age Grabowicz et al. (2004).

According to (Calani et al. 2010), VFAs can be metabolized in the hindgut tissues, with butyrate being the preferred substance for colonocytes. The liver is the main organ metabolizing absorbed propionate and butyrate. However, acetate is available for extrahepatic tissue metabolism. It is estimated that the rabbit obtains up to 0.40 of its maintenance energy requirement from VFAs produced by fermentation in the hindgut (Parker 1976).

2.4.3 Caecotrophy

Soft faeces are excreted according to a circadian rhytm, which is opposite to the feed intake and hard faeces excretion. Caecotrophy occurs mainly during the light period, whereas feed intake and hard faeces excretion occur during darkness (El-Adawy 1996). Most of the rabbit show monophastic patterns of soft faeces excretion from 08.00 to 17.00 h, with a maximum at 12.00 h. In figure 7 showed about the process of caecotrophy in the rabbit.

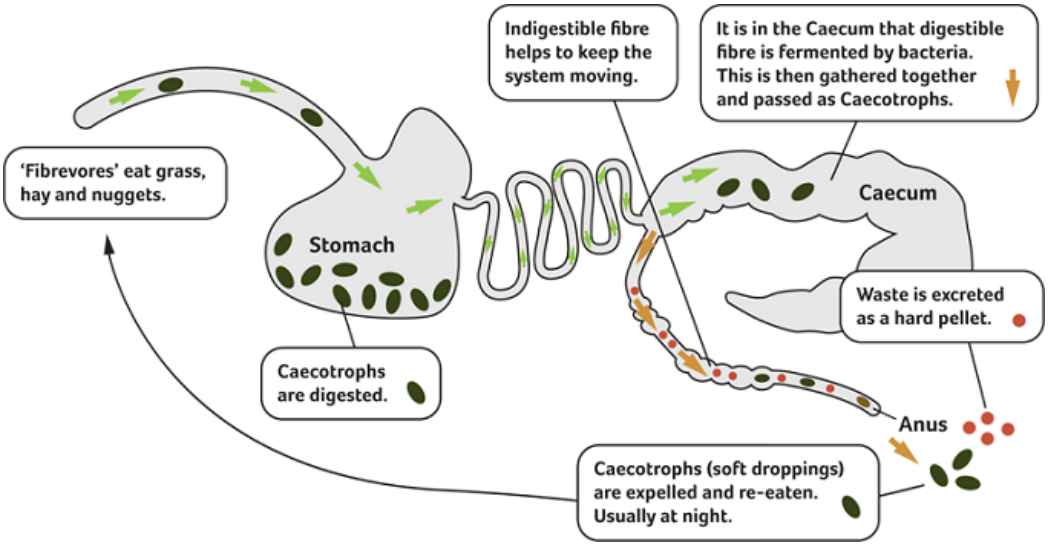


Figure 7 Caecotrophy

Source: El-Adawy (1996)

The caecotrophy plays an important role in rabbit nutrition, because providing B vitamins and proteins from bacterial sourches. Caecotrophy probably starts around 25 days of age, when a significant dry feed intake occurs that leads to both caecal and colon filling (El-Adawy, 1996). The nutrition and composition and hard feces nutrient can be found in table 4.

Table 4 Nutrient composition of caecotropes and hard feces

	Cecotropes	Feces
Dry Matter, %	34	47
Protein, %	30	17
Crude Fiber, %	18	30
Potassium, mmol/kg DM	260	84
Bacteria 10 ^{9}/g DM	142	31
Nicotinic acid, mg/kg	139	40

Source: El-Adawy (1996)

2.5 Rabbit breeding

Today's rabbit belongs to the family Leporidae; it was domesticated from the European wild rabbit (*Oryctolagus cuniculus*), according to Lebas et al. (1997). It is an herbivore that has a high reproduction capability with multiple gestations throughout the year (Banaee et al. 2011) and who can efficiently convert fodder into food having the capacity of turning 20 % of the protein they eat into meat (Lebas et al. 1997). Rabbit meat consumption depends on cultural, traditional and religious beliefs (Dalle Zotte 2010). It is important to say that in some countries, rabbits represent a pest and in others it is a key prey species as a source of food (Tablado et al. 2009).

Lebas et al. (1997) explained that the first controlled breeding records, date back to the sixteenth century when rabbits were kept with chickens in the garden of houses and their diet was based on green forage, hay, and some grains. The same authors said that in the late 19th and early 20th centuries the breeding of these herbivores reached its peak and during that time, breeding, selection and hygiene techniques were improved and breeder's associations formed, this all meant that rabbits were not raised with other animals anymore but were rather divided into groups. Later, rabbits started being bred for meat, skin, fur, and as laboratory animals for the testation of medicine and production of hormonal prepares (Banaee et al. 2011).

Banaee et al. (2011) described that the normal weight of an adult rabbit is between 4-4.5 kg, their skeleton is formed from 212 bones representing 9–10% of the total body weight, their digestive tract is around 4.5 to 5 m and the intestines' length represents 8 to 12 times the length of the rabbit's body. This author also stated that adult animals have a total of 28 teeth, while young animals have 16. According to Lebas et al. (1997), the small intestine is about 3 m long and the colon, which is divided into proximal and distal colon, measures 1.5 m.

Rabbits have a distinguishing feature in the function of the proximal colon, they produce two types of manure hard and soft (Tablado 2009). Both types are also known as pellets; the latter ones also as night pellets or cecotrophs (Manning et al. 1994). Authors Banaee (2011) and Manning et al. (1994) explained that the hard pellets are expelled, but soft pellets are recovered directly from the anus and swallowed without chewing; they also remarked that

cecotrophs are rich in water, vitamins, minerals and nitrogen and its consumption highly depends on the composition and productive state of the animal. Soft pellets can be recycled once, twice or even 3 to 4 times. This behavior, known as cecotrophy, starts at an age of 3 weeks, when young kids start to consume solid feed (Calani et al. 2010).

2.6 Health problems of broiler rabbits

The health problem can be very complicated and mostly the health problem of rabbits shows very confusing signs, and this can be a problem in analising the treatment and prevention as well as the right diagnosis of broiler rabbits (Melillo 2007). Common and chronic infection of the rabbits is hepatic coccidiosis caused by *Eimeria stiedae*. This chronic infection found in both domestic and wild rabbits. Clinical signs are not visible due to the young rabbits can be immune from non-lethal infections from a young age. Otherwise, the young rabbits (5-12 weeks of age) are the most severely affected (Thompson 1976). The signs can be described as constipation, diarrhea, debilitation, enlargement of the liver, anorexia, and death (Manning 1994). Sulfanomides is one of the best treatment for the chronic infections.

Thompson (1976) found pasteurellosis, this is the persistent infection diseases of domestic rabbits world-wide and caused by by the bacterium *Pasteurella multocida*. The clinical sign can be seen such as sneezing, coughing, nasal discharge, shaking of the head and abnormal respiration (Jenkins 2008). Arrington and Kelley (1976) also conducted the experiment that conjuctioval of rabbit is reddening and wet fur under the eyes because of epiphora. Pasteurellosis can also be linked to reproduction problems for example septicemia or infection via copulation. The treatment is usually by penicillin and streptomycin for 7-10 days (Manning et al. 1994). The best preventive choice is to watch the feeding management and housing system. Isolation for 30 days of all infected rabbits is needed from healthy colony rabbits (Thompson 1976).

2.6.1 The effect of milk thistle on the health status of rabbits

Kosina (2017) reported that the utilization 1% of milk thistle in feed was influenced the health status of rabbit due to this milk thistles are containing phytoadditive. The digestion of rabbits,

which includes recycling the microbial protein, is affected by bacterial colonization of the cecum (Leser et al. 2002). The composition of bacterial colonization in the intestinal ecosystem of rabbits has not yet been fully clarified (Abecia et al. 2007). Rabbits aged between 17 and 42 days are most prone to digestive disorders. This is 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. Furthermore, young rabbits, 22–28 days old, begin to show cecotrophy.

Milk thistle (*Silybum marianum*) is used as a medicinal plant to recover several diseases of animals. Several research regarding the animal reported that milk thistle can protect liver from dangerous poisoning and liver damage (Muriel et al. 1992; Brinker et al. 1998; Ibrahim et al. 2007), liver injury and liver problem (Hikino et al. 1988; Paulova et al. 1990; Allain et al. 1999; Shaker et al. 2010), alcoholic hepatitis in the body (La Grange et al. 1999), protects kidney from any diseases (Sonnenbichler et al. 1999), improves blood sugar control in diabetes (Huseini et al. 2006), prevents from viral hepatitis due to anti-viral activities properties (Berenguer et al. 1977), and milk thistle boosts immune function against aflatoxin. By receiving breast milk, solid food, water and faeces, suckling rabbits gradually develop microflora in the cecum (Volek et al. 2006). Such animals have not yet fully developed the digestive enzyme complex, especially the amylase, which enables starch breakdown (Abecia et al. 2007). Viability of weaned rabbits is affected by nutrition before weaning (Volek 2006).

Antibiotics were used in rabbit nutrition both for growth and as a medication. Potential problems with diseases of the gastrointestinal tract of rabbits in intensive farming led to the frequent use of antibiotics (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 ruminants and rabbits (O'Malley 2005). Nevertheless, since 1 January 2006 antibiotics usage in fodder mixtures has been prohibited, except for coccidiostatics and histomoniostatics (Directive no. 1831/2003). At present, there are efforts to replace the fodder antibiotics and other chemical medicines by natural products (Volek 2006).

2.6.2 The effect of milk thistle in human medicine

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

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

Silymarin has also been used in the treatment against liver poisoning with “green death cap mushrooms” *Amanita phalloides* (Capasso et al. 2009). This mushroom is characterized for having various types of toxins, but alpha–amanitin is the main hepatotoxin (Ward et al. 2013). Per oral application of a derivate silibinin-bis-succinate (SIL-BS) is used as a treatment in non-acute poisoning cases, but intravenous administration of the compound is applied in case of acute poisonings (Balszuweit et al. 2013) besides the previous mentioned derivate of silybin is indicated in many countries for the treatment of death cup intoxication (Jacobs et al. 2002). Silybin inhibits the action of OATP (Organic anion-transporting poly-peptide)

transporters, thus preventing the uptake of the toxin by the liver cells (Balszuweit et al. 2013). Besides this, Stickel and Schuppan (2007) explained that Silymarin interrupts the entero-hepatic recirculation of the toxin, inhibits of toxins to hepatocyte membranes and competes with the toxin for transmembrane transporters. Furthermore, *Silybum marianum* has a potential as an antidote to sulfur mustard poisoning. This chemical warfare causes skin blistering, ulceration, impaired wound healing and permanent lesions.

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

2.6.3 The effect of milk thistle in animals

There have been some studies focused on the potential of milk thistle as an additive to animal feeds. Tedesco (2004) reported that the whole milk thistle's fruit can be used only in ruminants, due to its high content of cellulose-lignin fraction. Schiavone et al. (2007) studied the effects of different doses of Silymarin in diet on the performances of broilers and their quality and concluded that Silymarin did not affect the growth performance, but to some extent affected the slaughter yields and that the treatment reduced the lipid content in the breast and thigh. Chand (2011) also analyzed the possibilities of milk thistle as a hepatoprotectant in chicken fed with aflatoxin B1. They reported that 10 g/kg of milk thistle in feed helped with body weight gain, feed conversion ratio and minimized the harmful effects of toxin-contaminated feed. They concluded that milk thistle has a good potential as a mycotoxin binder. In other research focused on the possible hepatoprotective effect of milk thistle in dairy cows, Tedesco et al. (2004) reported that *Silybum marianum* has neither a harmful nor protective effect on the liver of lactating cows.

Milk thistle has been also tested in reproduction and lactation of some farm animals having in some cases positive results. Tedesco et al. (2004) reported that a concentration of 4.1% of Silymarin should not be fed more than 4 to 5 weeks after calving, because it can cause some adverse effect on the cow's fertility. They also stated that this concentration has a positive effect on the involution of the uterus and is preferably to be given to cows with low levels of estrogens. Capasso et al. (2009) reported some studies demonstrated that milk thistle increases lactation in cows. The same authors analyzed the effects of the silymarin extract on the prolactin levels on female rats. It was found out that 14 days after the treatment, rats had increased their total body weight as well as the circulating levels of prolactin. Moreover, they observed that this increase was at the silymarin doses of 50, 100 and 200 mg/kg and noted that the stimulatory action of the extract on prolactin levels remained for up to 66 days after the discontinuation of the treatment.

There have been researches focused on the protection of milk thistle against *Amanita phalloides* toxin. Stickel and Schuppan (2007) demonstrated that dogs which were treated with milk thistle against poisoning with amanita toxin reported no cases of fatalities compared to those which were not given the preparation. In other study Desplaces et al. (1975) reported the hepatoprotective effect of silymarin in rats and explained that silymarin inhibits the toxin when given in a dose of 15 mg/kg 60 minutes before or 100 mg/kg 10 minutes after the poisoning with phalloidine. They also remarked that if silymarin is given 30 minutes after the administration of the toxin, it has no curative effect. *Silybum marianum* has a potential as a new non-edible feedstock for biodiesel, due to its content of oil, from which could be obtained around 25% of oil (Karkanis et al. 2011).

2.7 Antinutritional factors

Anti-nutritional factors are compounds mainly in organic matter, which can present in a diet, may affect the health of the animal or interfere with normal feed utilization. Barnes and Amega (1984) reported that anti-nutritional factors may occur as natural constituents of plant and animal feeds, as artificial factors added during processing or as contaminants of the ecosystem. Ingestion of feed containing such substances induces, in some cases, chronic intoxication and in others interferes with the digestion and utilization of dietary protein and

carbohydrate and interferes with the availability of some minerals, thus feed efficiency and growth rate and, consequently, the production of the edible products. Although anti-nutritional factors are present in many conventional feeds, they are more common in most of the non-conventional feeds.

According to Nityanand (1997) classified the various antinutritional factors (ANF) in feedstuffs to their chemical nature and their activity in animals as:

1. Chemical nature, in this category are acids, tannins, enzymes, nitrogenous compounds, saponins, glucosinolates and phenolic compounds.

2. Factors interfering with the digestion and utilization of dietary proteins and carbohydrates, for example, tannins, trypsin or protease inhibitors, and saponins.

3. Factors interfering with the availability of minerals are for example, phytates or phytic acid, oxalates or oxalic acid, glucosinolates and gossypol.

Tannins which are complex polymeric phenols having molecular weight greater than 500 are natural constituents of many plants and grouped into two forms-hydrolysable and condensed tannins (Nityanand 1997). Hydrolysable tannins are potentially toxic and cause poisoning if large amounts of tannin-containing plant material such as leaves of oak (*Quercus* spp.) and yellow wood (*Terminalia oblongata*) are consumed Barnes and Amega (1984) reported that tannins can inhibit the activities of rumen microbes.

The tannins form complexes with protein, cellulose, hemicelluloses, lignin and starch and interfere with their optimum utilization in the digestive tract and systems. Protein sources of plant origin containing high amounts of tannins and hydrolysable tannins should be used with caution Barnes and Amega (1984) reported that soaking and washing removes substantial amount of tannins and this is usually accompanied by some loss of dry matter. Tannins have been found to affect digestibility and therefore rate of utilization of dietary nutrients in both ruminants (Tedesco 2004) and non-ruminants (Schiavone 2007).

Saponins are bitter in taste and hence reduce palatability; they are also haemolytic and alter the permeability of cell membranes and produce toxic effects on organized tissues when ingested. Lucerne, white and red clovers, mahua seed cake and soyabean are rich sources of saponins. Soaking and washing in water is quite effective in removing a greater proportion of saponins (Nityanand 1997). Saponins have been reported to cause depressions in feed intake (Cheeke 1986).

Phytates (salts of phytic acid) are found in almost all feeds of plant origin. The phytates are present in association with protein and generally high in protein feeds e.g. groundnut cake, soyabean cake and sesame cake. Phytic acid possesses high chelating ability and in plants, it is found as phytates of many minerals which are mostly not available to monogastrics as they lack the enzyme phytase. The use of the enzyme phytase can make minerals such as phosphorus available to monogastrics (Nityanand 1997).

Nityanand (1997) reported that anti-vitamin activities against vitamins A and D have been observed in soyabean, against vitamin E in kidney bean (*Phaseolus vulgaris*), against vitamin K in sweet clover and against pyridoxine in linseed cake (Cheeke 1986) had observed that most processing methods employed in improving the food value of non-conventional or alternative feedstuffs do not eliminate anti-nutritional factor substances, but only reduce their concentrations to tolerable levels in feedstuffs. It is a common practice in feeding trials to use the weights of some internal organs like liver and kidney as indicators of toxicity. Bone (1979) reported that if there are toxic elements in the feed, abnormalities in weights of liver and kidney would be observed. The abnormalities will arise because of increased metabolic rate of the organs to reduce these toxic elements or anti-nutritional factors to non-toxic metabolites.

2.8 Biochemical compounds of rabbits

The internal organ such as kidney, liver, lung, and other vital organ can be evaluated by biochemistry evaluation in blood chemistry to view the health status of rabbits and to diagnosis certain diseases (Melillo 2007; Jenkins 2008). Blood is a complex fluid containing a large variety of dissolved suspended inorganic and organic substances or specialized

circulating tissues and cells suspended in the intercellular fluid substance (Jenkins 2008). Blood circulates in the arteries, veins and capillaries of man and animals. Its primary function is to transport oxygen from respiratory organs to body cells, distributing nutrients and enzymes to cells and carrying away waste products, thereby maintaining homeostasis of the internal environment. The various functions of blood are made possible by the individual and collective actions of its constituents–biochemical and haematological components (Melillo 2007).

Generally, both the biochemical and haematological blood components are influenced by the quantity and quality of feed and the level of anti-nutritional elements or factors present in the feed (Akinmutimi 2004). Biochemical components are sensitive to elements or factors present in the feed (Akinmutimi 2004), including elements of toxicity. They can also be used to monitor protein quality of feeds. Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle 1998).

Abuet et al. (1988) reported that low level haemoglobin (Hb) of treatment diets could imply that dietary proteins were not of high quality. Diets containing poor protein would usually result in poor transportation of oxygen from the respiratory organs to the peripheral tissues (Melillo 2007).

High white blood cells count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system. High blood urea levels are associated with poor protein quality or excess tissue catabolism associated with protein deficiency (Oduye and Adadevoh 1976). There is evidence in literature that haematological characteristics of livestock suggest their physiological disposition to the plane of nutrition (Madubuike and Ekenyem 2006).

Reduction in packed cell volume and red blood cell values are indicative of low protein intake or mild anaemia. Blood chemistry constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding (Oyawoye and Ogunkunle 1998).

Blood chemistry studies are usually undertaken to establish the diagnostic baselines of blood characteristics for routine management practices of farm animals (Akinmutimi 2004). The haematopoietic system is an important index of physiological and pathological status in animals and human. Normal range of blood sugar level indicates that animals are not surviving at the expense of body tissues (Oyawoye and Ogunkunle 1998). They reported that the number of neutrophils in the blood increases rapidly when acute infection is present, hence a blood count showing this increase is useful in diagnosis of infections. He reported further that eosinophils which normally are scarce increase in numbers in certain chronic diseases, such as infection with parasites and in allergic reactions.

In the last decades the investigation of blood chemistry value in rabbits has been evaluated. Each species can be differed and have typical blood value profiles. The parameters of breed, gender, age, pregnancy status and type of animals is also considered to provide reference range, this is time-consuming and expensive task. Otherwise, by following the single references range of animal species should be taken (Thrall et al. 2006). Author Meillo (2007) reported that most reference ranges are from homogenous rabbit laboratory research which belonged the same breed, age, strain, and environmental conditions and Thrall et al. (2006) also reported that the abnormal value and animal behavior should be taken separately.

Biochemical parameters can be evaluated from serum, whole blood, plasma or urine. We evaluated rabbit blood serum. Serum is a fluid that separates from clotted whole blood plasma which allowed to stand. In room temperature the blood easy to clots and coagulate very quickly, so the best way is to mix the blood with anticoagulant such as heparin during the experiment collection (Meillo 2007). The range of blood parameters and physiological ranges of haematological is varies according to type of bloods, see table 5.

Table 5 Normal physiological ranges of haematological and biochemical components for rabbits.

Parameter	Range
Haematological components	
Haemoglobin (g/dl)	8.0-17.5
Packed cell volume (%)	30.0-50.0
Red blood cell (x 106/µl)	4.0-8.0
White blood cell (x 103/dl)	5.0-12.0
Neutrophils (%)	35.0-55.0
Lymphocytes (%)	25.0-50.0
Eosinophils (%)	0-5.0
Biochemical components	
Cholesterol (mg/dl)	35.0-60.0
Total protein (g/dl)	5.4-7.5
Albumin (g/dl)	2.5-4.5
Globulin (g/dl)	1.9-3.5

Sources: Jenkins (1998)

Biochemical parameters in research

Thrall et al. (2006) conducted the experiments and shown that approximately 40% to 60% of total protein is **albumin (ALB).** The higher albumin concentrations are female compared to male rabbits. Milk thistle could increase the albumin and protect it from declining due to toxicity, but the level of albumin would when it was combined with grape seed in intoxicated fumonisin rats (Cork 2002). Another significant increase of total protein was shown in the research on hepatoprotective of silymarin towards acethylsalysilic acid but there was no effect on alanine aminotransferase and bilirubin. Similarly, the increase of protein after being exposed to the hepatotoxic agent shown by Jeklova et al. (2009).

Total protein (TP) is an important parameter in any animal species and it is the sum of albumin and globulin (Melillo 2007). Total protein levels vary in rabbits depends on age, breed, and reproductive status (Meredith and Rayment 2000. Protein sysnthesis can be investigated by the evaluation of the liver. Thrall et al. (2006) and Melillo (2007) reported that the level of total protein may be influence by the liver problem cause by hepatic coccidiosis (*Eimeria stiedae*). According to Melillo (2007) reported the high value of protein is from cecotrophy and hypoalbuminemia cause by malnutrition, poor diet and dental diseases problem.

Melillo (2007) reported that **cholesterol (CHOL)** is biosynthetised cells and it is a lipid molecule obtained from the diet as a precursor of steroids. The liver can metabolize the lipid and excreted in bile. Fasting is very important to measure the cholesterol level, because the meal the cholesterol level can increase very high (Melillo 2007). Age, breed, strain, and sex are important consideration to evaluate the cholesterol concentration in rabbits (Thrall et al. 2006). Usually the cholesterol concentration in adult male rabbits have higher compared to adult female rabbits (Thrall et al. 2006).

Glucose (GLU) was investigated by Jenkins (2008) and was observed that carbohydrate metabolism can influence the blood glucose value. Volatile fatty acids are used for rabbits as an energy (Melillo 2007). The experiment shown that after fasting within 5 days the blood glucose levels in rabbits was reduce (Melillo 2007). Heat stress, transportation, fear, and pain

are common causes of the hyperglycemic rabbit which is can influence the glucose level (Melillo 2007; Jenkins 2008).

Globulins (GLOB) are act as an enzimes which belong to family of globular proteins and globulin is water solubility than the albumins. Liver can produce the globulin to protect the body from any dangerous bacteria or parasites, they created the enzimes as the immune system. (Porter 1959).

The absorption of **calcium (Ca)** in rabbits can be found in the gut and the kidney (Melillo 2007). Calcium usually able to be bounded to serum. The calcium content of the diet of course is influenced by blood calcium level. In rabbits, the blood level of calcium at which it is moved to the bones is very high (Melillo 2007). Youngs rabbits and pregnant does need more calcium, which is results in lower blood calcium levels. In fact, that rabbits excrete about 45% to 65% of calcium through the urine, whereas most mammals do not excrete more than 2% of Ca in urine. The most common problem of low blood calcium levels is caused by lack and poor nutrition (Melillo 2007).

In rabbit's **phosphorus (PHOS)** play an important role to maintain the proper formation of bones and teeth (Melillo 2007). The amount of phosphorus in the blood of rabbits is influence by environmental conditions such as feed and climate (Brown 1928). There is a relation between the calcium and phosphorus and it could be influence the disease. By determination of kidney we can evaluate the phosphate levels because kidney is important for regulation, filtration and reabsorption phosphorus (Melillo 2007).

The highest levels of **alkaline phophatase (ALKP),** sometimes called as AP or ALP, the 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). 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). Young animals have higher plasma alkaline phophatase 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 three 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 because of liver necrosis are minimal. Increases because 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). In this research is focused on blood parameters related to health status of rabbits. The problem is lack of experiments which are focused on effects of blood test of rabbits available.

2.9 Short chain fatty acids

Short chain fatty acids (SCFA) are the products of colonic bacterial degradation of unabsorbed starch and non-starch polysaccharide (fibre). They are important anions in the colonic lumen, affecting both colonocyte morphology and function. The three main acids (acetate, propionate, and butyrate) stimulate colonic sodium and fluid absorption and exert proliferative effects on the colonocyte (Wong et al. 2006).

Experimental animal studies have shown that they promote adaptive responses to small intestinal resection and colonic anastomosis. Acetate increases colonic blood flow and enhances ileal motility. Butyrate has been shown to be the preferred energy substrate for the colonocyte and to be a potent differentiating agent in cell culture. Butyrate may also have a role in preventing certain types of colitis. A diet low in resistant starch and fibre, which will result in a low production of SCFA in the colon (Wong et al. 2006).

In most ruminants as well as rabbits occurred extensive anaerobic fermentation in forestomach. Short chain fatty acids and (SCFA) and non-carbohydrates are the most important substrates for energy metabolism. Studies of SCFA production to energy

metabolism have been obtained. Estimates contributions of SCFA to DE have been found in the range from 20 to 65% of DE (Bergman 1990). We can say that in the ruminal epithelium had a unique role in ruminal energy metabolism by its huge SCFA metabolism (Bergman 1990).

Colonocyte metabolism of SCFA

The intact walls of the guinea pig caecum and upper colon were shown in vitro to metabolise 14C-butyrate to ketone bodies; the presence of acetate and propionate did not affect ketogenesis from butyrate. In suspensions of isolated epithelial cells from the human colon butyrate had a sparing effect on glucose and glutamine oxidation. On the other hand, labelling of butyrate showed that glucose or glutamine did not diminish the entry of fatty acid carbon into the citric acid cycle (Bergman 1990).

Wong (2006) reported that the preference of colonocytes for butyrate as a fuel was more pronounced in the distal than the proximal colon.3 Isolated rat colonocytes responded to nutrient deprivation by a general reduction of oxidative metabolism (butyrate, 3-hydroxybutyrate, glutamine, glucose). Generally, the effect of acute fasting on substrate oxidation was greater than that of chronic malnutrition. These and other findings show that butyrate is the preferred fuel for colonic epithelial cells, accounting for about 70% of total energy consumption. The colonic mucosa may be especially vulnerable during starvation and malnutrition, as luminal fuels make such a great contribution to energy metabolism. The hypothesis has been put forward that a lack of luminal nutrients may impair the morphology (atrophy, colitis) and function (sodium absorption, gut barrier) of colonic epithelial cells.

The experiment conducted by Melillo (2007) evaluated the effects of short chain fatty acids (SCFA) on colonic morphology and function (facts and hypotheses). Their production during bacterial carbohydrate (starch, fibre) fermentation is well established. SCFAs are the preferred energy substrates of colonocytes, especially in the distal large bowel. Probably linked to this property, they affect a range of mucosal events (absorptive processes, blood flow, mucus release, cellular differentiation and proliferation). These effects of SCFAs are possibly

clinically important (adaptation to postoperative conditions, prevention of colitis). The metabolism of SCFA can be found in figure 8.

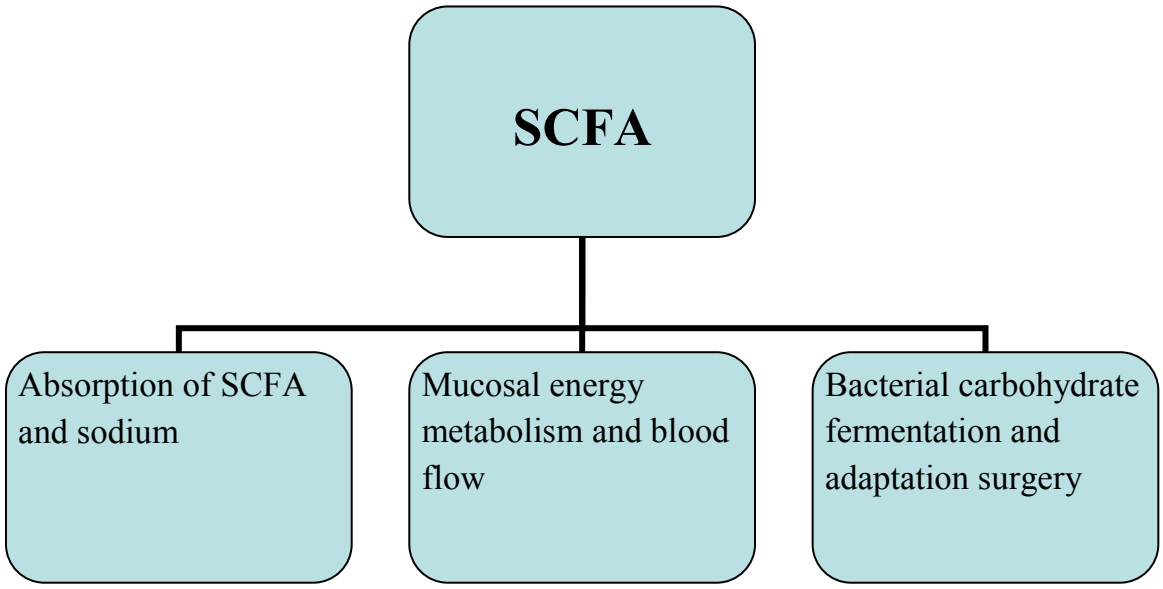


Figure 8 Metabolism of SCFA

Source: Wong (2006)

3. AIM

The aim of this study is to evaluate the effect of different concentrations and processing methods of milk thistle (*Silybum marianum*) on fattening performance and health status.

4. HYPOTHESES

Our hypotheses were:
H1: The feed mixture with milk thistle supplement will improve the fattening performance of broiler rabbits.

H2: The fermentation of milk thistle will increase the biological availability of silymarin complex with effect on fattening performance and health status of broiler rabbits.

5. MATERIALS AND METHODS

Experiments were carried out with 253 HYLA rabbit broilers based on all permissions to experiment (decision of the Animal Protection Commission, CR). The animals were housed in all-metal fattening cages into automatically air-conditioned hall of the demonstration and experimental barn at the CULS Prague, Czech Republic. Feed and water were available ad libitum. The anticoccidial solution Emanox from Biokron Inc. (Blučina, Czech Republic) was added to all groups of rabbits during the experiment. The rabbits were fed with a feed mixture containing 0.5% fermented milk thistle (*Silybum marianum*) and 1% nonfermented milk thistle (*Silybum marianum*) and these two groups will be compared to the control group without the addition of milk thistle (Silybum marianum). Feed mixtures for broiler rabbits with milk thistle (*Silybum marianum*) were produced and delivered ready for individual group of broilers rabbits by Biokron Inc. (Blučina, Czech Republic).

Each experiment begun when the rabbits reach the age of 42 days; the daily feed consumption were monitored. Weight gain was recorded once a week, followed by conversion of feed and these experiments were terminated at a slaughterhouse with the live weight of 2.6 kg of rabbits. The slaughter live weight and carcase yield, and total gains and metabolic parameters were monitored.

5.1 Experimental design

The total of 253 HYLA broiler rabbits, 42 days old, were obtained from genetic center HYLA (Ratibořice, Czech Republic) and four experiments were conducted. There were 32 cages with two rabbits into one. Experiments were conducted during 2013 till 2015, see table 6.
During the experiments both male and female rabbits were together, which means every single group had male and female, in groups C (control), E1 (1%) and E2 (0.5%). The average initial weight both males and females, see table 7.

Table 6 The fattening experiment of 42-day repeated

Experimental time	Number of rabbits (two in one cage)	Age of rabbits
2013 to 2014 Summer	63 HYLA	42 days
2013 to 2014 Winter	63 HYLA	42 days
2014 to 2015 Summer	63 HYLA	42 days
2014 to 2015 Winter	64 HYLA	42 days
Total (n)	253 HYLA	

Note: From 2013 to 2015 three rabbits died (one rabbit in each experimental time), there were four independent repetitions without overlapping time

Table 7 Average initial weight of 42 – day in fattening rabbits

Parameters	**Groups**					
	C		**E1**		**E2**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Number of rabbits	127	126	126	126	126	126
Average initial weight (kg)	1.39±165.9	1.35±147.7	1.34±150.7	1.39±182.3	1.42±145.9	1.41±149.6

In each group were males and females, randomly allocated to three groups according to the diets: such as standard diet as a control (C), diet with 1% unfermented milk thistle (E1) and diet with 0.5% fermented milk thistle (E2). The supplemented diets were prepared in the same way as standard diet by Biokron Inc., Blučina, The Czech Republic, up to one week before start of the experiments and the same batch of feed was used during all individual experiments, see table 8.

Table 8 Experimental groups of fattening rabbits each time

Group C	Group E1	Group E2
standard diet as a control (n=21), two animals in cage	diet with 1% unfermented milk thistle–mechanically processed seeds of *S. marianum* (n=21), two animals in cage	diet with 0.5% fermented milk thistle-mechanically processed seeds of *S. marianum* (n=21), two animals in cage

Note: The independent repetitions without overlapping time

The animals were housed in all-metal fattening cages from Velaz Inc. (Prague, Czech Republic) into automatically air-conditioned hall of the demonstration and experimental barn at the CULS Prague, Czech Republic. The rabbit fattening housed contains 32 cages and each cage were two rabbits.

Feed and water were provided *ad libitum*. Emanox as anticoccidial solution (Biokron Inc., Blučina, Czech Republic) was added to the water for all groups of rabbits in the experiments.

The experiments started on the 42 days of rabbit age (after 7 days of adaptation). During the experiments, the feed consumption of rabbits was monitored every day; their weight gain was calculated weekly. The experiment was finished by slaughter when rabbit reached 2600 g of live weight or when the rabbits were 84 days old. The rabbits were slaughter by captive bolt into the head.

- The weight of the body parts was recorded weekly for each animal, and samples of blood were taken from individual animals.
- The health status of individual rabbits was monitored daily.
- Feed consumption was checked daily per cage, the half of feed consumption per cage was used for calculation of feed consumption for individual rabbit in the cage.
- The experiment was finished by slaughtering when the ideal slaughter weight of a broiler rabbit was reached 2600 g or when rabbits were 84 days of age.
- One experiment was prepared for collecting of metabolic parameters from 40 bloods serum.

This study was conducted in accordance with Good Agricultural Practices (GAP), published by FAO (2003) and is not classified as an experiment in accordance with Act No 246/1992 Coll., on the protection of animals against cruelty.

5.2 Feed mixture

Feed mixtures for broiler rabbits with milk thistle (*Silybum marianum*) were produced and delivered ready for individual group of broilers rabbits (E1, E2 and C) by Biokron Inc. (Blučina, Czech Republic). Diet formulas of feed mixture in table 9.

By the Biokron Inc. (Blučina, Czech Republic) prepared: complete mixtures for fattening of rabbits with 10% Probiostant E10 and 0.25% Emanox and supplemented with milk thistle (*Sylibum marianum*). Milk thistle was supplied by Irel Inc. (Brno, Czech Republic) to Biokron Inc. (Blučina, Czech Republic), where the seeds of milk thistle were mechanically processed and fermented and dried. Finally, a feed mixture for broiler rabbits with the milk thistle were prepared ready for individual experimental groups with milk thistle (E1 and E2) and standard diet as a control group (C), without milk thistle supplementation.

Table 9 Composition of the control diet and diets containing non-fermented milk thistle (1%), and fermented milk thistle (0.5%)

Main Compounds	Amount (%)
Crude protein	16.5%
Crude fat	2.5%
Crude ash	8.0%
Crude fiber	14.5%
Calcium	1.10%
Natrium	0.35%
Phosphorus	0.6%
Vitamin A	11000 m.j./kg
Vitamin D_3	1200 m.j./kg
$FeSO_4.7\ H_2O$	55 mg/kg
KI	1.1 mg/kg
$CuSO_4.5H_2O$	12.1 mg/kg
MnO	33 mg/kg
ZnO	33 mg/kg
Na_2SeO_3	0.05 mg/kg
Emanox E10	0.25 g/kg
Probiostan E10 (probiotic)	2.5 g/kg

Compound: alfalfa flour, oat, wheat bran, malt sprouts, sunflower meal, barley, limestone, monocalcium phosphate, salt, dried slops, fruit pulp

5.3 Analyses of short chain volatile fatty acids (SCFA)

The total SCFA concentration was estimated by titration after steam distillation. The molar profile of volatile fatty acids (VFA) was estimated by gas chromatography at 140^{o}C using a Chromosorb glass column (2mX3mm i.d.) with 15% SP 1220 and 1% H_3PO_4 (Supelco, Bellefonte, USA), as was described by Ottenstein and Bartley (1971). Nitrogen at an inlet pressure of 80 kPa was employed as the carrier gas.

Apparatus

The apparatus used was a model 2500 gas chromatograph (Bendix Process Instrument, Ronceverte) equipped with a Honeywell 1-mV span recorder, Model electronic 194. Both

glass and stainless steel coloumns were used. The glass coloumn were 6ft X 1/8-in. o.d., 0.085-in.id., also in a U-configuration. Nitrogen was employed as the carrier gas.

The acids standards were the Supelco with two standard containing 0.10% of each acid: acetic, propionic, iso- and n-butyric, iso- and n-valeric acids in a water. The SP-1200 was synthesized in the laboratory of Institute of animal sciences Uhrineves Prague. Packings were prepared by dissolving 1.0 gram of SP-1200, 0.1 gram H_3PO_4
In 40 ml of acetone and slurrying them with 8.9 gram of acids washed Chromosorb W 80/100 mesh. The acetone was removed with use of a heat lamp while the packing was continuously stirred till dry. Coloumn were packed with aid of a vibrator.
Glass columns were packed leaving two inches on the inlet side empty to allow for on-coloumn injection. Stainless steel coloumn were packed almost completely full at both ends leaving a minimum amount of space for glass wool plugs. Silanized glass wool was used for each type of coloumn. Coloumn was conditioned overnight at 200^0C with a flow of carrier gas 40 ml/min nitrogen. Before the initial use and after the coloumn has been left unused for some time, several microliters of water were injected to clear it of extraneous material. The injection port inlet and detector were operated at 25^0C above the coloumn temperature.

Analyzed samples

The rabbit feaces liquid (n=10) were sampled by a stomach tube and filtered through an ordinary sieve in the standard way. Approximately 10 ml of the sample was preserved with 2-3 drops of toluene to prevent fermentation. The samples were preserved by this way immediately and analyzed or stored at -20°C temperature pending analysis.

For the analyses, the samples were thoroughly centrifuged (or filtered) and 200x diluted in distilled water. Prepared by this way, the samples were used for the analysis without any further treatment.

For the gas chromatography (GC) analysis, the samples were prepared in the following way: 200 µl of metaphosphoric acid (25%) and formic acid (3:1) mixture was added to 1 ml of rumen liquid (Cottyn and Boucque 1968). After 30 min of centrifugation, the clear supernantant was 10x diluted in water and injected in the chromatograph. In trial were used chemicals: metaphosphoric acid, ε-aminocaproic acid (EACA), hydroxyethylcellulose (HEC),

caproic acid (Sigma Aldrich, Czech Republic), HCl–refined by isothermic distillation, formic acid (Lachema Brno, Czech Republic). Standard solutions of appropriate concentration were prepared from the individual substances of analytical purity (Sigma-Aldrich, Czech Republic). The analyses were carried out in a two-capillary isotachophoretic analyser IONOSEP 2002 (Recman Laboratory technology, Czech Republic). The internal diameter of the pre-separation capillary was 0.60 mm and that of the analytical capillary 0.25 mm. Detection was carried out with contactless high-frequency conduction detectors. The leading electrolytes were: 10 mM HCl + 22 mM EACA + 0.05% HEC (pH 4.5) and the terminal electrolytes: 5 mM caproic acid. The driving power in the pre-separation capillary was: 120 μA; in the analytical capillary: 40 μA, during the detector passage: 20 μA. Thirty μl of sample were applied per column using an autosampler. The analysis time was lasted approximately 20 min.

Gas chromatography (GC)

The analyses were conducted on a 6820 GC System gas chromatograph, Agilent Technologies. A capillary column was used, 30 m×250 μm×0.25 μm (Quadrex Corporation). Carrier gas–nitrogen, flow 1.0 ml/per min, detector and temperature programme used: 60–200°C (20°C/min, 10 min), injector: 250°C, detector: 300°C. The injector was equipped with a glass liner of glass wool to separate particles of dirt from the sample. The samples were dosed by a HT 300A automatic dosing device at an injection size of 1 μl using the split method and a 30:1 splitting ratio. The analysis time was lasted approximately 15 minutes.

5.4 Biochemical parameters and analyses

The rabbits were slaughter by captive bolt into the head and we randomly collected the blood samples by bleeding the carotid artery from 120 rabbits (30 animals in each experiment). The collecting of blood samples performed in the morning from 8:00 to 11:00. Sample of bloods were taken in 70, 77 and 84 days of age depending on the weight of the specific of rabbit. The blood samples were collected into 3 ml vacuette test tube for the serum with additional clot activator. Samples were left for 15 minutes at room temperature and then keep and stored on ice.

For biochemical analyses were collected 40 samples of rabbit's serum and than were processed for a subsequent laboratory analysis at the Department of Animal Science and Food Processing (AFS) at CULS Prague. Blood serum (centrifuged for 3 minutes at 3000 rpm) was examined employing the VetTest analyzer (IDEXX Laboratories, Cymedica, USA) by using dry slides technology for 12 biochemical parameters: albumin (ALB), total protein (TP), cholesterol (CHOL), urea (UREA), glucose (GLU), total bilirubin (TBIL), globulin (GLOB), amylase (AMYL), alanine transaminase (ALT), calcium (Ca), phosphorus (P), and alkaline phosphatase (ALKP). All experimental data of biochemical parameters was carried out in four repetitions from 2013 to 2015. Serum chemistry reference range data for rabbits, in all ages, are shown in table 10 (Suckow et al. 2011).

Table 10 Ranges of normal level of some blood biochemistry in rabbit blood

Biochemistry	Range	Biochemistry2	Range
Glucose	75-155 mg/dl	Total Protein	5.4-8.3 g/dl
Total Cholesterol	10-80 mg/dl	Globulin	2.4-4.6 g/dl
ALP	10-140 IU/l	Albumin	1.5-2.8 g/dl
ALT	14-80 IU/l	Calcium	5.6-17 mg/dl
AST	14-113 IU/l	Phosphorus	2.3-6.7 mg/dl
Amylase	200-500 IU/l	Total bilirubin	0-0.8 mg/dl

Source: Suckow et al. (2011)

VetTest analyser

Blood samples from 40 clinical cases were collected, submitted to laboratory analysis at the Department of Animal Science and Food Processing (AFS) at CULS Prague over an experimental period. The rabbit showed a variety of clinical signs and had been presented to State Veterinary Institute (Prague, Czech Republic) for further investigation. Venous blood samples were collected into plain tubes and serum samples were analysed for 12 analytes including albumin (ALB, g/l), total protein (TP, g/l), cholesterol (CHOL, mg/dl), urea (UREA, mmol/l), glucose (GLU, mg/dl), total bilirubin (TBIL, µmol/l), globulin (GLOB, g/dl), amylase (AMYL, iu/l), alanine transaminase (ALT, iu/l), calcium (Ca, mmol/l),

phosphorus (P, mg/dl), and alkaline phosphatase (ALKP, iu/l) by using VetTest analyser (IDEXX Laboratories, Cymedica, USA) by using dry slides technology.

Preparation instruments. The samples were used appropriate tubes to draw and clot over 30 minutes. The sample was centrifuge in 3000 rpm within 3 minutes and high-speed centrifuge (StatSpin≥8000 rpm) within 60 seconds and the serum sample were transfered to sample cup.

Running test were run by enter 'new sample' on the VetTest and follow the screen prompts devices to enter sample informations: insert dry slides in prompted devices, place the tip securely on the pipettor. Press the pipettor button to one beep, at two beeps, remove the pipettor from the sample cup. The sample tip was assessed and replaced the pipettor in the analyser holder. The rest of the tested process occurs automatically.

5.5 Prevalence of coccidiosis and mortality

Rabbit infections, possibly suspected of coccidiosis, were evaluated by coprological examination of faecal samples taken at weekly intervals. The clinical features were manifested by loss of appetite, diarrhea, weight loss and death. Numbers of developmental stages of coccidiosis oocysts were morphologically detected and semiquantitative evaluated at 10x magnification by microscope (Nikon, Japan). During experiments, the deaths of three rabbits were recorded with confirming of coccidiosis. Their necropsy was performed at the State Veterinary Institute (Prague, Czech Republic).

5.6 Data analysis

The program Statistica CZ v. 9 (StatSoft, Inc., Tulsa, USA) was used for statistical evaluation of the fattening performances. Data were expressed as means ± SD (standard deviation). One-way analyses of variance, two-way analysis by ANOVA and by following select POST-HOC test (Tukey HSD test) were utilized. And 95% confidence interval was selected. The graphs were created by the statistical program Statistica.cz (v.9) as well. Others parameters (blood parameters, VFA) were evaluated by using SPSS or Statistical package for social sciences

with Analyses of variance (ANOVA) and descriptive statistics and a one-way analysis of variance (ANOVA), or the t-test (where appropriate), which was calculated using Statistica10 software (StatSoft, Inc.,Tulsa,USA).Significant differences were identified with Tukey HSD test.

6. RESULTS

The efficacy of different concentrations of milk thistle (*Silybum marianum*) were evaluated during fattening experiments and average daily gain and total gain; daily feed consumption, total feed consumption and feed conversion; values of slaughter live weight and finally carcasses weight were obtained.

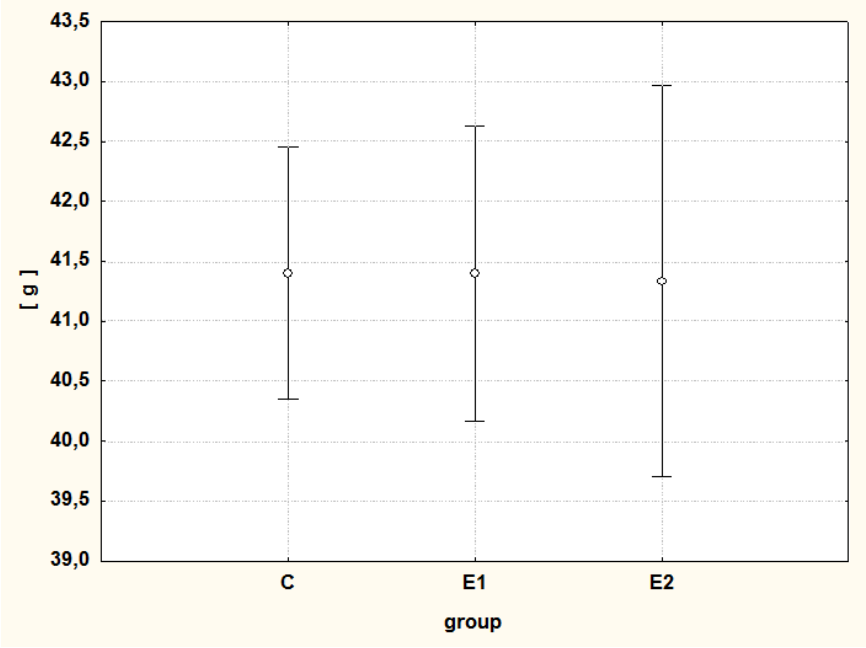
6.1 Daily gain and total gain

The average **daily gain** was 41.39 ± 5.8 g in group E1, 41.33 ± 5.6 g in group E2 and 41.40 ± 5.9 g in group C. The significant differences between experimental groups E1, E2 and control group C were not found out ($p>0.05$). The results are documented in figure 9.

The significant differences between males and females in experimental groups E1, E2 and control group C were not found out ($p>0.05$). The average daily gain in group E1 were observed in males 41.51 ± 5.3 g and 41.30 ± 6.2 g in females. In group E2 were detected 40.0 ± 4.9 g in males and 42.83 ± 6.0 g in females. In control group C were documented average daily gain 40.99 ± 6.4 g in males and 42.00 ± 5.2 in females.

Similarly, the results of average **total gain** did not show significant differences between observed groups ($p>0.05$). Average total gain in group C were found out 1380.1± 159.3 g, in group E1 were documented 1374.2 ± 169.5 g and in group E2 were reported 1410.8 ± 146.2 g.

No significant differences between males and females were found in experimental groups E1, E2 and control group C ($p>0.05$). The average total gain in group E1 were reported in males 1345.7 ± 150.7 g and 1398.9 ± 182.3 g and in group E2 were documented in males 1415.4 ± 145.9 g and 1405.6 ± 149.6 g in females. The average total gain in group C were detected in males 1396.0 ± 165.9 g and 1356.9 ± 147.7 g in fameles.



Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle

Figure 9 Average daily gain of rabbits in groups

6.2 Daily feed consumption, total feed consumption and feed conversion

On the other hand, the significant differences were recorded in average **daily feed consumption.** Experimental group E2 showed higher value (162.05 ± 10.2 g) in this parameter than group C (155.71 ± 10.4 g) (p=0.0023) and group E1 (154.65 ± 12.6 g) (p=0.0006). Average daily feed consumption did not differ significantly between group C and group E1. The results are showed in figure 10.

No significant differences were recorded in average daily feed consumption according gender. Experimental group E2 showed higher value both in males and females (in males 163.21 ± 10.0 g, in females 160.74 ± 10.4 g). Average daily feed consumption in control group C were showed 155.79 ± 10.9 g in males and 155.58 ± 9.8 g in females and in group E1 were detected the values 152.30 ± 11.9 g in males and 156.70 ± 12.8 g in females.

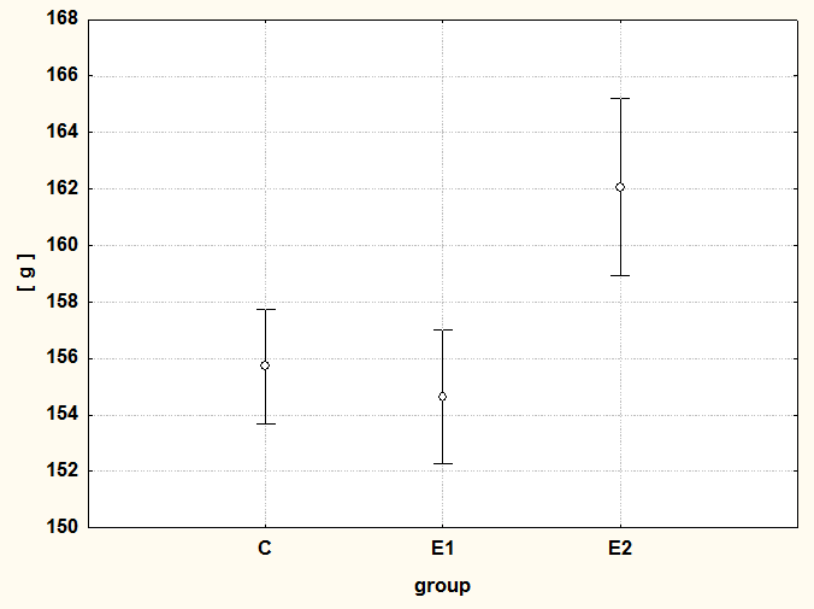
Average **total feed consumption** was significant between groups E2 a E1 (p= 0.02276), however in group C and E1, as well as in group C and E2, were not significant differences

(p>0.05) in figure 11. Average total feed consumptions were found out 5201.51 ± 876.7 g in group E1 and 5618.16 ± 915.6 g in group E2 and finally in group C 5279.57 ± 923.6 g

In average total feed consumption were not significant results between males and females in groups C, E1 and E2 (p>0.05). Theses results were found: in group C in males 5399.93 ± 926.3 g and in females 5104.06 ± 900.4 g. In group E1 in males were found 4987.38 ± 753.9 g and 5387.72 ± 939.7 g in females and in group E2 in males 5852.31 ± 923.8 g and 5353. 48 ± 849.3 g in females.

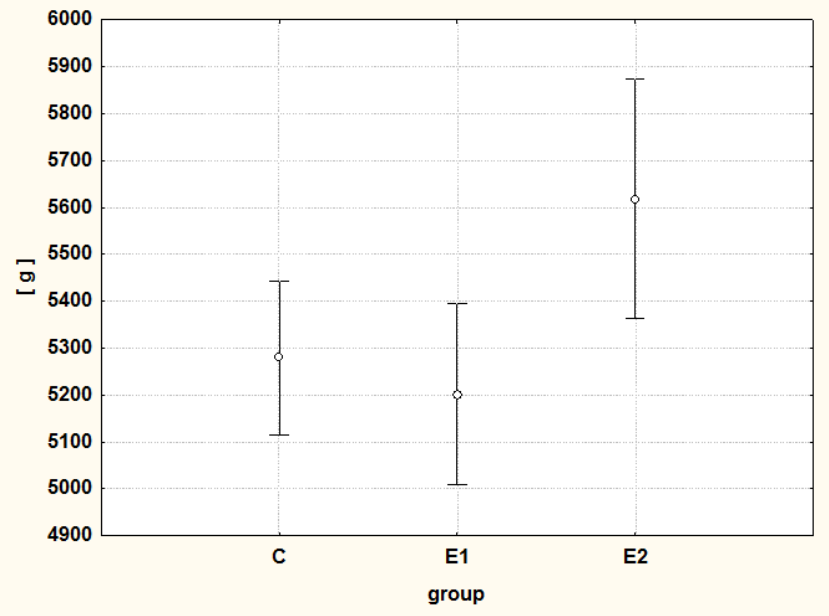
However, average **feed conversion ratio** was nonsignificant amongst the groups. Group C showed the value 3.8 ± 0.59 g, group E1 3.78 ± 0.45 g and group E2 3.98 ± 0.55 g.

Average feed conversion were not significant between males and females in groups **C,** E1 and E2. Control group C showed the value in males 3.89 ± 0.6 g and in females 3.76 ± 0.5 g. In group E1 in males were found feed conversion 3.71 ± 0.4 g and in females 3.85 ± 0.5 g. In the last rabbit group E2 in males were detected the value 4.13 ± 0.5 g and 3.82 ± 0.5 g in females.



Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle.

Figure 10 Average values of daily feed consumption of rabbits in groups



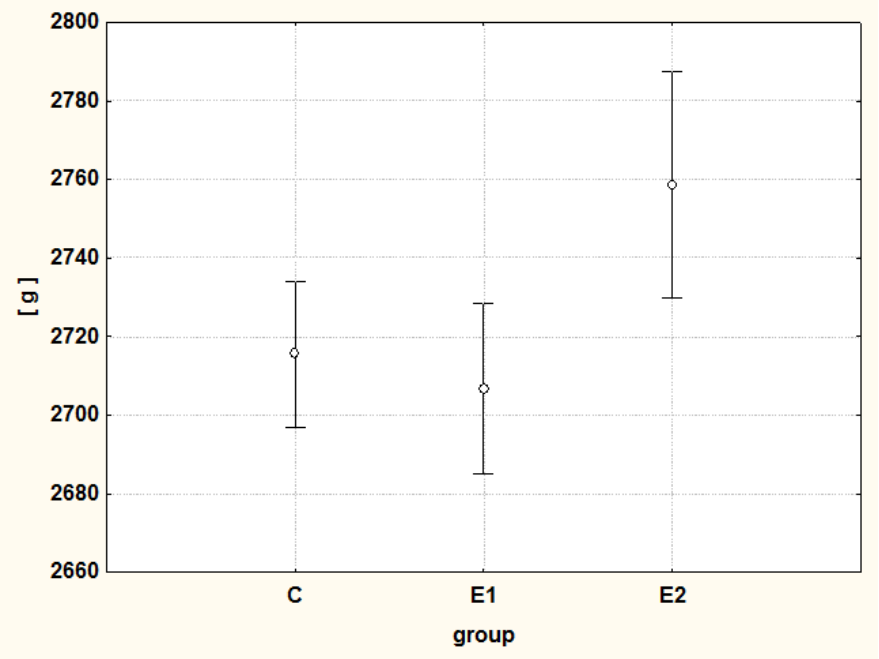
Legend C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle.

Figure 11 Average values of total feed consumption of rabbits in groups

6.3 Slaughter live weight

The highest average **slaughter live weight** was recorded in group E2 (2758.57 ± 113.7 97 g), in compared to group E1 (2706.86 ± 98.8 g) and group C (2715.43 ± 99.5 g). The differences in average slaughter live weight were significant between the groups E2 and E1 ($p=0.0130$) and E2 and C ($p=0.0347$). Nonsignificant difference ($p>0.05$) was between groups E1 and C. These results are documented in figure 12.

Nonsignificant difference ($p>0.05$) were found between males and females in group C, E1, and E2. In group E1 average slaughter live weight were achieved 2692.75 ± 106.4 g in males and 2719.13±91.1 g in females, in group E2 were detected the value 2756.15± 89.2 g in males and 2761.30 ± 138.4 g in females and finally in group C were reported the values 2730.57 ± 111.2 g in males and 2693.33 ± 75.4 g in females.



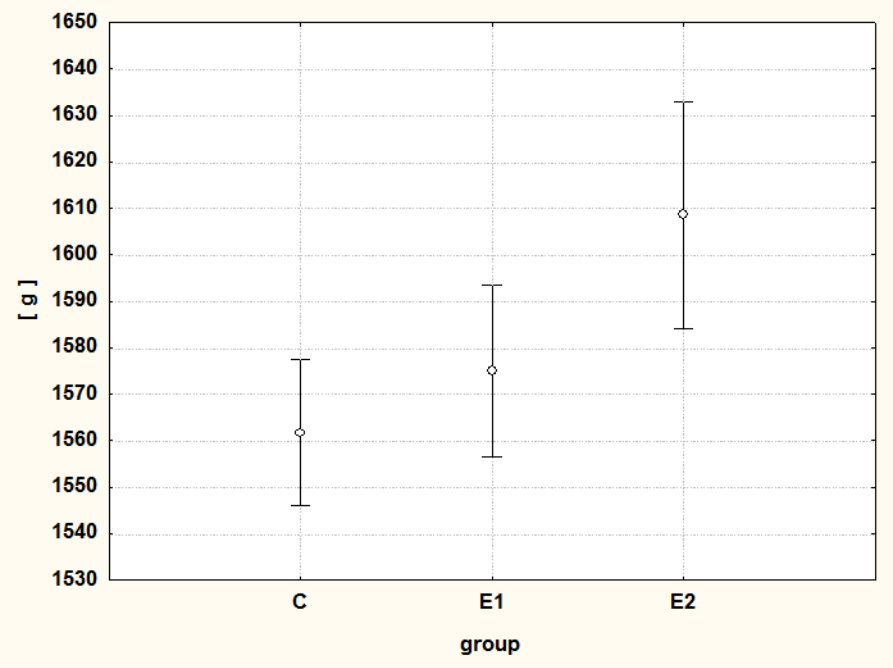
Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle.

Figure 12 Average slaughter live weight of rabbits in three groups

6.4 Carcass weight

In the next figure 13 the results in average **carcass weight** of broiler rabbits were showed. The value of this observed parameter was 1574.94 ± 99.3 g in group E1, than 1608.57 ± 83.9 g in group E2 and finally 1561.69 ± 77.8 g in group C. In the group E2 was achieved the highest average carcass weight, compared to other groups, but significant difference was only found between groups E2 and C ($p=0.0043$). The other differences were insignificant ($p>0.05$).

The value of average carcass weight of HYLA males rabbits were in control group C 1585.43 ± 78.8 g and 1527.08 ± 62.4 g in females. In other group E1 was found 1584.00 ± 125.0 g in males and 1567.07 ± 70.4 g in females. The last group E2 achieved the value 1616.15 ± 71.2 g in males compared to females with the value 1600.00 ± 97.3 g. In the group E2 in males were achieved the highest average carcass weight, compared to other groups, but significant difference was only found in groups C ($p=0.003$) between males and females. Nonsignificant difference ($p>0.05$) were found between males and females in group E1 and E2.



Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle

Figure 13 Average values of carcass weight of rabbits in groups

6.5 Profile of caecal VFA

The main molar profile of volatile fatty acids in acetat achieved not significant difference, the experimental results were (81.79 ± 2.39) compared to control standard diet group was (80.75 ± 1.90). Propionate was not significant difference in experimental group (5.14 ± 2.18) compared to control standard diet group (5.02 ± 1.36). In butyrate was not significant difference in experimental group (8.96 ± 2.65) compared to control standard diet group (11.10 ± 1.90). However, hexanoate and heptanoate were significant difference ($p<0.05$) in experimental groups (0.30 ± 0.23) and (1.75 ± 0.67) compared to control groups (0.56 ± 0.27; 0.90 ± 0.24). These results are documented in table 11 and figure 14.

Table 11 Profile of volatile fatty acids (VFA) in the caecal contents of 10 control rabbits and 10 rabbits fed milk thistle at 1%

VFA (mol. %)	Control rabbits	Rabbits fed milk thistle
Acetate	80.75 ± 1.90	81.79 ± 2.39
Propionate	5.02 ± 1.36	5.14 ± 2.18
Isobutyrate	0.79 ± 0.35	1.24 ± 0.60
Butyrate	11.10 ± 1.90	8.96 ± 2.65
Isovalerate	0.23 ± 0.07	0.24 ± 0.12
Valerate	0.65 ± 0.14	0.53 ± 0.13
Isohexanoate	0	0.05 ± 0.05
Hexanoate	0.56 ± 0.27	**0.30 ± 0.23***
Heptanoate	0.90 ± 0.24	**1.75 ± 0.67***

*Significantly different from the control at $p < 0.05$

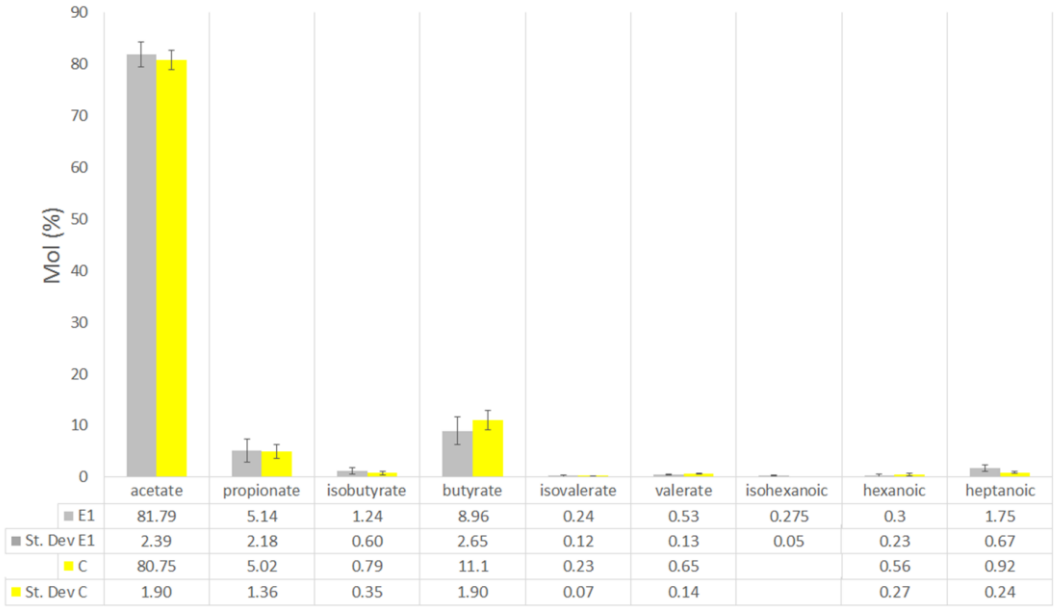
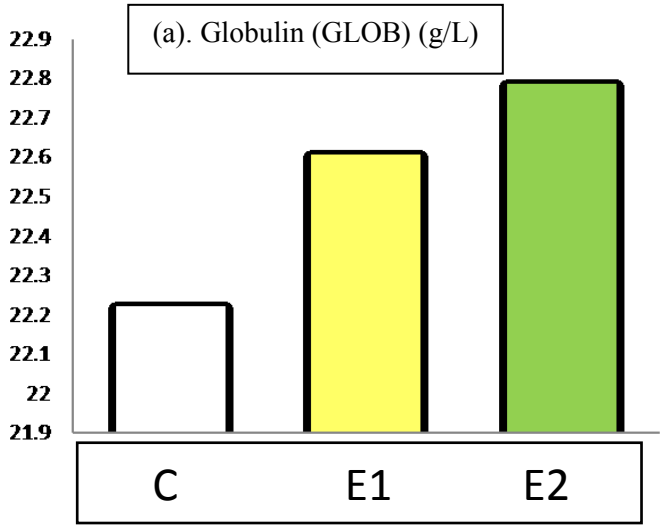


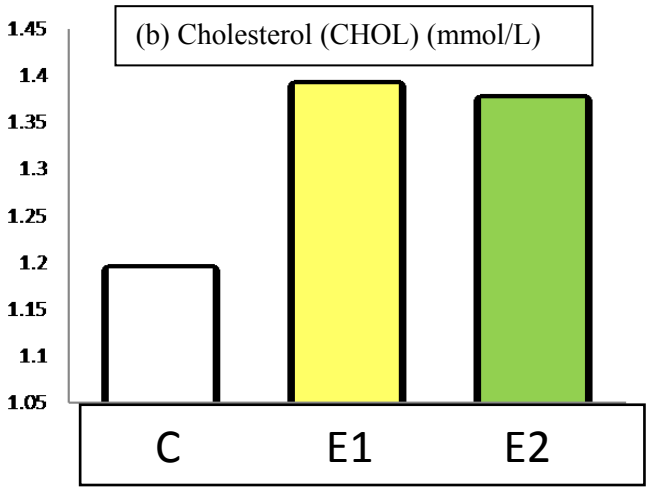
Figure 14 Profile of volatile fatty acids (VFA) in the caecal contents of 10 control rabbits and 10 rabbits fed milk thistle at 1%

6.6 Blood biochemistry

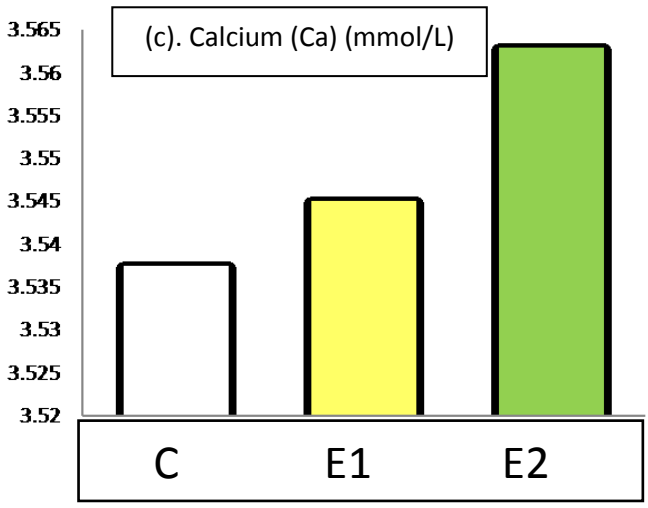
The effects of feeding on blood profiles showed that there was significant difference $p<0.05$ to globulin (GLOB), cholesterol (CHOL), calcium (Ca), and phosporous (P) in group E2 compared to group standard diet (control). In the presented figure 15 the significant differences in blood parameters of rabbits in group E2 compared to group C were observed. The results are documented in figure 15 a, b, c, d and table 12.



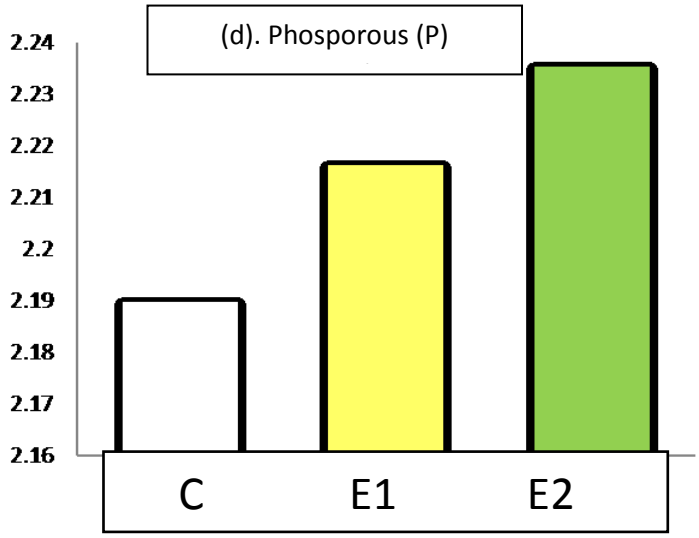
Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle



Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle



Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle



Legend: C: control group (standard diet group); E1: experimental rabbit groups 1% non-fermented milk thistle; E2: experimental rabbit groups 0.5% fermented milk thistle

Figure 15 Blood biochemistry with feeding C control rabbits, rabbits fed E1 with milk thistle (1%), and rabbits fed E2 with fermented milk thistle (0,5%)
(a) globulin, (b) cholestrol, (c) calcium, (d) phosporous

Table 12 Blood profiles with feeding C control rabbits, rabbits fed E1 with milk thistle (1%), and rabbits fed E2 with fermented milk thistle (0.5%)

Blood profile International Unit (IU)	Feeding P-value
Albumin (g/L)	0.849
Total proteins (g/L)	0.553
Globulin (g/L)	0.485
Alkaline phosphatase (µ kat/L)	0.266
Alanine aminotransferase (µ kat/L)	0.453
Amylase (µ kat/L)	0.330
Calcium (mmol/L)	0.769
Cholesterol (mmol/L)	0.031
Glucose (mmol/L)	0.064
Phosporus (mmol/L)	0.775
Urea (mmol/L)	0.169
Total bilirubin (µmol/L)	0.638

The statistical analyses can be found in annexes

6.7 Health status

Table 13 showed that clinical signs of diarrhea were detected in seven rabbits in control group C, unlike experimental groups. In group E1 had only disorders four animals and two in group number E2. Clinical examinations revealed watery, mucoid and abnormally soft feces. Oocysts of coccidia Eimeria spp. were detected microscopically under the light microscope after using the flotation method, but some of the affected rabbits were gradually survived until the experiments were finished.

During the experiment, eight rabbits died in the control group C, unlike experimental groups (E1=3 deaths, E2=2 deaths). Infectious intestinal coccidiosis and bacterial pasteurellosis in lungs were confirmed by necropsy at the State Veterinary Institute (Prague, Czech Republic).

Table 13 Health status of rabbits and report of mortality and morbidity

Group	**Number of slaughtered rabbits**	**Mortality** (in animal numbers)	**Diarrhea** (in animal numbers)
Control C	85	8	7
Milk thistle (1%) E1	85	3	4
Fermented milk thistle 0.5%) E2	85	1	2

7. DISCUSSION

Daily gain and total gain

The daily gain and total gain are important because it shows how efficiently the rabbit is performs and growth. In our experiments, the daily gain and total gain were not significant difference. Indeed, according to Tagliapietra et al. (2014) and Stastnik et al. (2015) were investigated that milk thistle was low-quality forages and high of NDF and ADF level which may influence the daily gain and rabbit performance. Cullere et al. (2016) also investigated that daily gain was not difference among the groups, control group was (52.32 ± 2.8 g) and treatment groups were (52.54 ± 2.8 g) and (51.55 ± 2.8 g).

Furthermore, Daneshvar (2017) documented that the daily gain was not significant difference between control group (conventional diet) was (41.15±1.24 g) and treatment group (milk thistle extract) was (41.41±1.34 g). However, lower of daily gain was corresponding with Omid Fani Makki et al. (2013) who investigated daily gain in growing broilers, found the average of daily gain was not difference among the treatments (94.4 ± 6.1 g) compared to control group (94.11 ± 6.1 g). The higher weight gain was reported by Tedesco et al. (2004) in treatment groups on his beef catle trials.

Based on these results, the effects of milk thistle 1% and 0.5% in experimental diets to improve the gain of the rabbits were not affected, we could say that the content of crude fiber was higher 14.5% in experimental groups and crude fiber can be linked to NDF level (cellulose, hemicellulose, and lignin). High of lignin content in milk thistle according to Gidenne et al. (2001) can influence the rabbits gain, the optimum supply in a diet at least 6 g/day.

The dietary lignocellulose also must be minimum supply in a diet to ensure the growth performance of the rabbit (Gidenne et al. 2000). Stastnik et al. (2015) observed that the diets with higher of fiber contents and bitter in taste could influence the palatability and the gain performance. High amount of fiber with high of lignin content can increase the digestive disorder and reduce the performance for the rabbits (Maertens 1992). Nicodemus et al. (1999) also reported that excessive of fiber intake can reduced the gain performance and feed efficiency.

Pond (1995) observed how to improve the weight gain of rabbit by lowering the crude fiber content from 9 to 10% in a diet. The fiber in a diet is very important for population of cellulolytic bacteria in the caecum. Unbalance of total bacteria can cause problem of gain performance and diseases (Maertens 1992). An appropriate balance of nutritients between dietary crude fibre and energy is therefore, crucial to optimal growth performance of rabbits (Nicodemus et al. 1999).

Daily feed consumption, total feed consumption and feed conversion

Feed utilization and feed conversion play a key role to get the energy intake and improve the rabbit performance for maintenance and growth (Cullere et al. 2016). In our experiment, the treatment groups E1 and E2 were not significant difference compared group C in the daily feed consumption, total feed consumption and feed conversion. Indeed, this experiment was related to Šťastnik et al. (2015) who observed milk thistle 5% and 15% in feed mixture and found that the daily feed consumption was higher in control group (without additional milk thistle) was 3.3 kg the reason is because milk thistle contain substances a bitter taste and may influence the palatability, according to Khan et al. (2009) found a source of edible oil as the mature seeds contain 25–30% high-quality oil (42% linoleic acid and 36% oleic acid) which may influence the palatability of feed. Furthermore, Křížová et al. (2011) reported that milk thistle was not affected the daily feed intake or feeding consumption among the groups.

In recent study daily feeding consumption was improved by 12.5% milk thistle compared to control group – without additional milk thistle. The improvement shown shows very sufficient in the rabbit's performance especially in feeding consumption may be due to the pure form of silymarine phytosome while in the present study milk thistle seed were used in experiments may contain anti-nutritional factor which can leading to depress the appetite (Tedesco et al. 2004).

Total feed consumption was higher in group E2 compared to group C and group E1, this experiment was not corresponding with Šťastník et al. (2015) who found the highest average total feed consumption during the experiment was observed in group C, the treatment group 5% and 15% of milk thistle were low. This was the fact that the treatment group which contained milk thistle has a bitter tasted, so it is influence the palatability of feeding consumption.

However, Cullere et al. (2016) reported that the addition 5 g/kg and 10 g/kg of milk thistle supplementation total feed consumption with was not affected among the groups, group control 145 g/day, and treatments group were 145 g/day and 142 g/day. Other authors investigated that diets supplementation of milk thistle was not significant difference which feeding control was (28.79±1.27 g) and the treatment group was (30.01±0.31 g).

Moreover, feed conversion ratio was not influenced by treatments (Tedesco et al. 2004), it was interesting to notice that, these results was contrast different with Chand et al. (2011) who were reported that daily gain and feed conversion were increased in productive and reproductive performance, the addition of *Silymarin phytosome* in the feed at 600 mg/kg of body weight resulted in an increase of 14.83% in daily gain.

Similarly, Gowda Sastry (2007) also confirmed significant ($p<0.05$) improvement of milk thistle on daily weight gain and attributed the effect to antioxidant activity that stimulated protein synthesis by bird's enzymatic system. Higher gain was also reported by Chakarverty and Parsad (1991), in milk thistle supplemented group. The exact mechanism of improving weight gain was not well established; however, this effect might be due to the improved immune function of the birds receiving milk thistle.

In our results, in experimental treatments were not significant difference between males and females compared to control group, because the chemical composition of the diets and fiber content and NDF level can influence the feeding habbits, Lebas et al. (1986) observed that the optimum of crude fiber for rabbits is 13 to 14% and NDF level optimum is 33% in a diet, while in our diets total amount of crude fiber was 14.5% and NDF level was 50.8% which were higher than optimum recommendation. The dietary fiber play an important role to provide the energy and to improve the performance of rabbits (Gidenne and Perez 1994).

An excess of dietary fiber and NDF level in our results were not desirable because can influence the digestable energy (DE) and increasing the digestive disorder (Lebas 1997). The standard digestable energy for fattening rabbit was 2400 kcal/kg and DE can influence the feeding habbit of rabbits (Xiccato et al. 2010). So, it's also obvious in our results because the parameters of daily feed consumption, total feed consumption and feed conversion did not differ compared to control groups because of the gain performance of rabbit did not differ compared to group as well.

Slaughter live weight

The average slaughter live weight was significant differences between group C compared group E2, furthermore group E1 and group E2 were significant difference compared group C, these results was related to Kroll et al. (2007) who reported that milk thistle contains flavonolignans 65% and flavonoid 80% that may influence its palatability which was influence the live weight.

Chand et al. (2011) reported live weight of broiler chicken was improved by (107.3±21.3 g) with milk thistle supplementation 10 gram/kg in feed mixture compared to control (80.3 ±8.7 g), interestingly, according to Šťastnik et al. (2015) who evaluated milk thistle with 5% and 15% in feed mixture of broiler chicken shown negatively affected by dietary treatment, the slaughter live weight was lower ($p<0.05$) compared to the control group which was high (2169.24 ± 134.72 g). But, Sutanto (2015) investigated feeding with additional of AV3 which milk thistle and ginkgo biloba extracts was not improved the average slaughter live weight (2722.57 g±13.68) compared to control group (standard diet) (2717.89 g±8.96), the reason was because the diet is high content of antioxidant which is influence for health benefit for the rabbits.

The parameter of slaughter live weight between males and females in our experiment were not significant differences in group C, E1, and E2, the slaughter live weight related to low degree of crude fat and lipid unsaturated reported by Chand et al. (2011), in our diets we have got 2.5% of crude fat which was very low lipid unsaturated, lack amount of lipid unsaturated and crude fat in a diet can not influence the slaughter live weight of the rabbits.

Crude lipid should provide minimum about 3% in a diet, lipid can improve the palatability and energy level of rabbits (Pond et al. 1995). Most researchers assumed and frequently mentioned that the parameters of gain performance related to slaughter of live weight, which were corrected if we have got ideal nutrition composition in a diet during our trials. Lack or excess of nutrient component in a diet can influences the performance and health status of animals (Cullere 2016).

Carcass weight

Carcass weight was significant difference ($p<0.05$) between group C compared to group E2, this result is corresponding to Omid Fani Makki et al. (2013) who observed that carcass of broiler chicken in treatment group 1% of milk thistle was 70.71 g and 0.5% of milk thistle was 70.84 g, compared with control was 65.77 g. Carcass weight was investigated by Kosina et al. (2017) shown that experimental groups (0.2% and 1% milk thistle) were significantly higher were 1563.1 ± 9.1^{a} g and 1580.1 ± 8.7^{b} g compared to control group (basal diet) was 1553.2 ± 8.5^{a} g.

According to Chand et al (2011) who performed the experiment found that carcass weight and carcass yield in rabbits and chicken fed with fermented milk thistle were significantly higher than in rabbits of other groups, interestingly, Šťastník et al. (2015) reported that in carcass weight of broiler chicken was found the significant higher ($p<0.05$) differences in control group (73.50 ± 4.14 g) compared treatment group 5% milk thistle (69.28 ± 0.85 g), according to Cullere (2016) observed that carcass attributes with *Silybum marianum* dietary supplementation at 5 and 10 g/kg levels were not statistically different among dietary groups of the present experiment (1517 ± 46.1 g), (1546 ± 46.8 g), (1490 ± 46.3 g), the dietary inclusion of milk thistle did not affect carcass traits and did not change neither colour nor oxidative status.

Moreover, Schiavone et al. (2007) reported that additional milk thistle into feed mixture compared to control group (without silymarin) achieved significantly highest of carcass yield 75%. Šťastník et al. (2015) observed that 5% and 15% milk thistle into feed mixture do not worsened the performance characteristics and improved the sensory quality traits such as odour and flavour, these results also related to Sutanto (2015) reported that additional milk thistle and AV3 extract in feed was not significant difference in the experimental group (1590.55 g ± 12.4) compared to the control group (1562.11 g± 8.12).

Carcass weight was higher in experimental group E2 because the best treatment with a standard cage during the experiment. Cullure (2006) observed that the experimental trials and the diets can improver the carcass weight, especially high content of crude protein. In our diets the crude protein was 16.5% in a diet, this amount was suffecient, while the standard requirement of crude protein for fattening rabbit 15-17% (Schiavone et al. 2007).

Usman et al. (2009) mentioned that rabbits fed with crude protein from 14% to 17% in a diet could reduce 16.3% of lysine and methionine content without affecting the weight gain and feed consumption, these were the reason why the parameters of weight gain and feed consumption did not differ during the trials. Manning et al. (1994) observed that crude protein in a diet is very essential to build up the energy and essential amino acids.

In our results the parameter of slaughter live weight was related to carcass weight. Andersen et al. (1975), Jenkin (2008) and Panea et al. (2012) observed that slaughter live weight depending by fat composition, the amount of crude protein and genetics which can influence the carcass weight. So, the parameters of slaughter live weight were related to carcass weight. During our trials, we had no problem with the external influences such as light regime and the transportation.

Effect of gender

No significant difference was found between males and females in groups C, E1, E2 in the monitored parameters, excluding the carcase weight, when the females of group C showed a significantly lower value compared to males, these results are conected to Cullere et al. (2016) also investigated that carcass weight in female rabbit were significant difference among the groups. These results also related to Štastnik et al. (2015) who explained that crude protein which present in a diet can influence the performance of rabbits between males and females. Moreover, Kosina et al. (2017) reported that control group in females of group C achieved lower value compared to males due to males are likely to be feeding to support high activity.

In our results were not found significant differences in the performance parameters, such as daily gain, total gain, feed consumption, total feed consumption, feed conversion, slaughter live. This results related to Tagliapietra et al. (2014) and Stastnik et al. (2015) who reported that milk thistle was low-quality forages and high of NDF and ADF level which may influence the daily gain, feed consumption, and slaughter live weight and health status amongst males and females rabbits.

Profile of caecal VFA (Volatile fatty acids)

Nicodemus et al. (1999) reported that nutrients provide an important source of energy from caecal fermentation of digestable fiber or undigested nutrients. High amount of VFA and acetic acids concentration because of high content of fiber in the caecum with high number of fiber fermentation (Chao and Li 2008). Tao and Li (2006) mentioned fibrolytic activities in the caecum of rabbits enhanced the NDF (neutral digestable fiber) to improves the production of energy.

The performance of molar profile of caecal VFA in our experiment was very similar in control rabbits and rabbits fed the milk thistle. The main profile of caecal VFA is acetate, propionate and butyrate were not significant differences, this is because lower number of additional milk thistle in the feed during the experiment (Chrenkova et al. 2011). Bannink (1997) used six models of VFA in dairy cattle and compared with Sveinbjörnsson et al. (2006) which showed that the performance of VFA (acetate, propionate and butyrate) reduced compared to controls. Sveinbjörnsson et al. (2006) assumed that by supplemented milk thistle produced high impact of VFA profile, sugar and pH during the metabolism.

Friggens et al. (1998) investigated that VFA molar profile is influenced by feed composition so the physical and chemical processing could influence the fermentation patern and can be reflected on the molar profile of VFA. Bannik (1997) also reported that the molar profile of caecal VFA could be different and varies according to the quality of feed and the fermentation patern of feed. Additionally, Krause et al. (2003) investigated that acetate, propionate and butyrate has a significant effect on health status of animals. Sveinbjörnsson et al. (2006) also documented that protozoa in the caecum are useful to improve the butyrate production and VFA prediction.

In our results, the significant differences ($p<0.05$) only were in hexanoic and heptanoic acids which are not physiologically important to evaluate the health status of rabbits (Marounek et al. 2000). Hexanoic acids is important as a fats and oils components especially for food manufacture (Hanigan et al. 2002). However, heptanoic acids or called enanthic acid is important for odor properties which well soluble in ethanol and ether.

We assumed that the formation of VFA in the caecum reflected increased concentrations of fiber and NDF in a diet of broiler rabbits but increasing concentrations of milk thistle in feed are needed to improve VFA production patterns and increase the level of acetate, propionate

and butyrate to supply additional energy during the metabolism process (Chrenkova et al. 2011). Friggens et al. (1998) conclude that the fermented of feed could be very benefical to improve the VFA performance such as acetate, propionate and butyrate. Inadequate prediction of molar caecal VFA production rates remains a weakness of animals.

Moreover, Winsen et al. (2001) observed that fermented feed can influences the gastrointestinal tract in the ecology of bacteria and fermented feed can decreasing the Enterobacteriaceae level of rabbits. According to Ørskov et al. (2007) there were no influences significance in the efficiency of performance parameters on VFA to improves the gains, live weight and carcass weight, but VFA can supply the energy during the metabolism process (Chrenkova et al. 2011).

Moreover, Andersen et al. (1975), Jenkin (2008) and Panea et al. (2012) observed and explained that carcass weight slaughter was depending by fat composition, the amount of crude protein and genetics which can influence the carcass weight after the slaughter of animals and metabolism of VFA.

Biochemical parameters

The biochemical parameters from blood were investigated to show the health status of rabbits, such as kidney, liver and other important organ function (Awang 1993; Chand et al. 2011; Schulz et al. 1998; Melillo 2007). In parameters of groups C and E1 was not significant different ($p>0.05$) correlated with suggestions by Chand et al. (2011) who reported that biochemical parameters in rabbits were not affected by sex and genotype without any supplementation feed.

Moreover, the effect of feeding on blood profiles showed that cholesterol (CHOL) was significantly different ($p<0.05$) which is corresponding to Thrall et al. (2006), who reported that the additional milk thistle in a diet might increase the concentration of cholesterol, creatine kinase, magnesium and alkaline phosphatase which play an important role in order to maintaining homeostasis within the body and source of energy from adenosine diphosphate (ADP), deposit of energy and make up the health status of body.

Thrall et al. (2006) also reported that the consumption of feed with additional of fermented milk thistle could influence the cholesterol contents in the blood profile. In our results the

cholesterol content was very low (1.2-1.4 mg/dl) compared with the normal cholesterol range (10-80 mg/dl) was investigated by Suckow et al. (2011), the studied was because of the rabbit meat has very low of cholesterol (Melillo 2007) and the fermented milk thistle can influence the cholesterol content in rabbit meat (Suchý et al. 2008).

However, alanine transaminase (ALT) was not significantly different ($p>0.05$) which was related to Suchý et al. (2008) who reported that alanine transaminase (ALT) activity in blood parameters was lower in treatment with 2.95 % milk thistle compared to control with standard diet. In this studies of alanine transaminase was lower compared to normal range of ALT, which was 14-80 IU/80 IU/l. these results obtained because of the ALT was attributed to be easy to decrease the concentration due to the content of nutrition (Jenkins 2008).

In our experiment the blood parameters was not significantly different among the gender (male and female). However, parameters of cholesterol was significant different in male ($p<0.05$) compared to female. This results is related to Mellilo (2007) who evaluated that male has higher in cholesterol content compared to female because of feed behavior influences. Procházková (2015) reported that cholesterol was reduce in experimental groups compared to control groups, globulin and phosporous were significant different in experimental groups compared to control standard diet groups, these results because of the diet feed which contains AV3 has high of antioxidant and flavonoids, so those antioxidant and flavonoids could improve the blood parameters.

Interestingly, Sutanto (2015) investigated that the diet which contains of AV3 was not influences difference in blood parameters but they can improve the performance of rabbits. Furthermore, the diet containing AV3 is important because they have a positive effect in the performance paramters such as improve the carcass weight and daily gain. Jones (1975) mentioned that the normal value of cholesterol in New Zealand rabbits showed in the range of 1.30 to 2.84 mmol/l with a mean of 1.96 mmol/l.

The protein was present in blood parameters as a globulin, globulin play‘s an important role to protect the body, most globulin work together with lipid and hormones to protect from any bacteria, viruses and parasites (Porter 1959). In our results the feed with fermented milk thistle 0.5% (E2) was significant different compared to other groups E1 and C. Fermented milk thistle can increase the globulin content in the blood which is important for rabbit health

(Jenkin 2008). Normal range of globulin from 1.5 g/dl to 2.8 g/dl so our results for total amount of globulin in blood was very high.

Calcium and phosporous in our results was higher in E2 groups compared to group E1 and C. Calcium and phosphorus are very important for bone improvement, growth and greatly essential during rabbit pregnant (Mellilo 2007). According to our experiment E2 was the highest amount than groups E1 and C. normal range for calcium from 5.6 to 1.7 mg/dl and phosphorus 2.3 to 6.7 mg/dl.

In our results E2 for calcium were 3.56 mg/dl which was very low compared to normal range of calcium and phosphorus was 2.23 mg/dl. These results showed that the calcium and phosphorus in our results were low, so the supplementation of milk thistle in experimental group needs additional vitamins and mineral resources. The effects of feeding on blood profiles showed that there was significant difference $p<0.05$ to globulin (GLOB), cholesterol (CHOL), calcium (Ca), and phosporous (P) in group E2 compared to group standard diet (control) which is corresponding to Martinec et al. (2012) and Dalle Zotte (2014), who reported that feeding parameters of rabbit might influence the serum globulin (GLOB), cholesterol (CHOL), calcium (Ca), and phosporous (P). Milk thistle was mentioned as a promosing effect on hypocholesterol agent when we supplied a high fat diet (Bialecka 1997; Kilic et al. 2008).

Health status

The health status was investigated during the experiments and found that the diet with fermented milk thistle (E2) was the best choice because according to the experiments they have the lowest number of mortality and morbidity, compared to groups C and E1, with higher numbers. Coccidiosis and pasteurellosis were confirmed by necropsy, in the histological sections of a rabbit intestine were coccidian developing stages (meronts, gamonts and oocysts). Chrenkova et al. (2011) also described a case of intestinal coccidiosis, with the intestinal epithelium and lymphocytic infiltration into the lamina of the villi, in the groups with standard diets, that was happend because of the type of cage and management of rabbits in control group was used.

The diarrhea can cause problem, this was because of poor management during the experiment (Chand et al. 2011). Kosina et al. (2017) reported that the different concentration of feed in rabbits has an effect on the problém with coccidiosis. There is influence of the microbial contents from the small intestine, large intestine or caecum, so the microorganisms play an important role in level of metabolism of organism. This situation of course can be affected by absorption and metabolism status of rabbits (Cullere et al. 2016).

Since the use of antibiotic banned in European union law, the disease problem of the rabbits during the experiments was solved. Animals were treated with growth-promoting effect from natural herbs or extract plant compounds as probiotics, as in the scientific publication of Kosina et al. (2017). These preventions of rabbits can protect them from any dangerous protozoans and can improve the rabbit performances (Chrenkova et al. 2011).

Since the deaths in the experimental groups were low, compared to the control group, we believe that both probiotics, Emanox and milk thistle allowed the growth and maintenance of rabbit health. If rabbit mortality has occurred, we believe that coinfection should be avoided and effective vaccination of rabbits against pasteurellosis that affects their lungs can minimize losses in rabbit breeds.

The light regime did not differ for all of our trials on performance parameters. Same conditions on the transportation of rabbits were not differ during the trials. The rabbits were healthy and not stressed, when compared with Sutherland et al. (2014), who described that transportation could had the potential negative impact on the performance and health status of animals. But in our experiments the transported rabbits were withouth feed and water for no more than three hours and welfare conditions were established to reduce stress.

We also believe that fermented milk thistle is economical benefit for farmer in small and large farm scale because fermented milk thistle could reduce the cost of expenses about half percentage of total cost and the animals were more healthy with fermented trials compared to others (Dr. Ondráček personal communication, Biokron Inc. Blučina, Czech Republic).

8. CONCLUSION

Feeding is the most important parameters in animal farming as a source of energy and to improve the performance, especially for the rabbits, in fact rabbits are very nutritious for human consumption. In our experiments, the results of the study showed that 0.5% fermented milk thistle extract, enriched to standard diets, was safe after application of broiler rabbits HYLA and had a positive effect on fattening and rabbit's health.

HYLA rabbit broilers, fed with milk thistle fermented milk in a standard diet, reached significantly higher values: average daily feed consumption, total feed consumption, live weight and carcase weight. Fermentation has allowed an increase in the bioavailability of silymarin complex and has influenced health and fattening performance.

Non-significant differences were found between males and females into groups C, E1, E2 in the parameters of the average daily gain, total gain, daily feed consumption, total feed consumption, feed conversion and live slaughter weight. But significant results were found in carcass weight between males and females in control group C into the groups E1 and E2 were found no significant.

In case of increased mortality in broiler rabbit breeds, it is recommended to feed a standard diet with anticoccidics and milk thistle as a suitable substitute for the use of feed antibiotics, the ban of which is valid from 1.1.2006 in EU countries.

In order to demonstrate other desirable effects of milk thistle in standard diet, further experimental studies on nutrient digestibility and the effect of milk thistle on the intestinal microbe of rabbits will have to be carried out. Hence, for further studies need to implement the present results considering digestibility of nutrients as well as the effect of this herb on the intestinal intestinal microbiom of rabbits.

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10. ANNEXES

Annexe 1 Statistical data for performance parameters

1. Average daily gain

Efekt	Úroveň Faktor	N	prumpri Průměr	prumpri Sm.odch.
Celkem		253	41,38773	5,778813
group	C	118	41,40517	5,906829
group	E1	86	41,39563	5,778437
group	E2	49	41,33188	5,580630

Average daily gain according to the gender

Efekt	Úroveň Faktor	Úroveň Faktor	N	prumpri Průměr	prumpri Sm.odch.
Celkem			253	41,38773	5,778813
group*sex	C	M	70	40,99422	6,364981
group*sex	C	F	48	42,00446	5,17306(
group*sex	E1	M	40	41,50595	5,27458
group*sex	E1	F	46	41,29969	6,240081
group*sex	E2	M	26	40,00092	4,935904
group*sex	E2	F	23	42,83644	5,983625

Č. buňky	group	sex	{1} 40,994	{2} 42,004	{3} 41,506	{4} 41,300	{5} 40,001	{6} 42,836
1	C	M		0,93869	0,99779	0,999778	0,976018	0,772188
2	C	F	0,93869		0,99865	0,99175	0,71458(	0,99315
3	E1	M	0,99779	0,99865		0,99998	0,90749	0,95189
4	E1	F	0,99977	0,99175	0,99998		0,94312(	0,90486(
5	E2	M	0,97601	0,71458	0,90749	0,94312(		0,52497
6	E2	F	0,77218	0,99315	0,95189	0,90486(	0,52497	

2. Average total gain

Popisné statistiky (Kopie - RESEARCH DATAS RABBITS AKHIR S3)				
Efekt	Úroveň Faktor	N	konv Průměr	konv Sm.odch.
Celkem		253	3,847394	0,543736
group	C	118	3,834269	0,592949
group	E1	86	3,785960	0,450807
group	E2	49	3,986824	0,555202

Č. buňky	group	{1} 41,405	{2} 41,396	{3} 41,332
1	C		0,999926	0,996961
2	E1	0,999926		0,997926
3	E2	0,996961	0,997926	

Efekt	Úroveň Faktor	N	celpri Průměr	celpri Sm.odch.
Celkem		253	1384,032	160,385(
group	C	118	1380,085	159,3013
group	E1	86	1374,186	169,4869
group	E2	49	1410,816	146,2138

Average total gain according to the gender

Efekt	Úroveň Faktor	Úroveň Faktor	N	celpri Průměr	celpri Sm.odch.
Celkem			253	1384,032	160,385(
group*sex	C	M	70	1396,000	165,9265
group*sex	C	F	48	1356,875	147,7365
group*sex	E1	M	40	1345,750	150,6718
group*sex	E1	F	46	1398,913	182,3212
group*sex	E2	M	26	1415,385	145,9378
group*sex	E2	F	23	1405,652	149,6306

Č. buňky	group	sex	{1} 1396,0	{2} 1356,9	{3} 1345,8	{4} 1398,9	{5} 1415,4	{6} 1405,7
1	C	M		0,783018	0,609549	0,999999	0,995108	0,999866
2	C	F	0,783018		0,999524	0,800146	0,663824	0,836437
3	E1	M	0,609549	0,999524		0,640951	0,514109	0,708862
4	E1	F	0,999999	0,800146	0,640951		0,998354	0,999983
5	E2	M	0,995108	0,663824	0,514109	0,998354		0,999941
6	E2	F	0,999866	0,836437	0,708862	0,999983	0,999941	

3. Average daily feed consumption

Efekt	Úroveň Faktor	N	prumspo Průměr	prumspo Sm.odch.
Celkem		253	156,5772	11,43502
group	C	118	155,7062	10,42085
group	E1	86	154,6527	12,55615
group	E2	49	162,0522	10,17373

Č. buňky	group	{1} 155,71	{2} 154,65	{3} 162,05
1	C		0,78296	0,00234
2	E1	0,78296		0,00062
3	E2	0,00234	0,00062	

Average daily feed consumption according to gender

Efekt	Úroveň Faktor	Úroveň Faktor	N	prumspo Průměr	prumspo Sm.odch.
Celkem			253	156,577	11,43502
group*sex	C	M	70	155,792	10,86827
group*sex	C	F	48	155,580	9,84345
group*sex	E1	M	40	152,301	11,94475
group*sex	E1	F	46	156,696	12,84362
group*sex	E2	M	26	163,212	10,02347
group*sex	E2	F	23	160,740	10,40510

Č. buňky	group	sex	{1} 155,79	{2} 155,58	{3} 152,30	{4} 156,70	{5} 163,21	{6} 160,74
1	C	M		0,99999	0,61006	0,99817	0,04290	0,43349
2	C	F	0,99999		0,74143	0,99665	0,05483	0,44726
3	E1	M	0,61006	0,74143		0,44822	0,00139	0,04360
4	E1	F	0,99817	0,99665	0,44822		0,16084	0,71316
5	E2	M	0,04290	0,05483	0,00139	0,16084		0,97156
6	E2	F	0,43349	0,44726	0,04360	0,71316	0,97156	

4. Average total feed consumption

Efekt	Úroveň Faktor	N	celspo Průměr	celspo Sm.odch.
Celkem		253	5318,617	915,3366
group	C	118	5279,576	923,6037
group	E1	86	5201,512	876,7121
group	E2	49	5618,163	915,6155

Č. buňky	group	{1} 5279,6	{2} 5201,5	{3} 5618,2
1	C		0,816045	0,071492
2	E1	0,816045		0,027602
3	E2	0,071492	0,027602	

Average total feed consumption according to gender

Efekt	Úroveň Faktor	Úroveň Faktor	N	celspo Průměr	celspo Sm.odch.
Celkem			253	5318,617	915,3366
group*sex	C	M	70	5399,929	926,3347
group*sex	C	F	48	5104,062	900,4128
group*sex	E1	M	40	4987,375	753,9282
group*sex	E1	F	46	5387,717	939,6930
group*sex	E2	M	26	5852,308	923,8217
group*sex	E2	F	23	5353,478	849,2770

Č. buňky	group	sex	{1} 5399,9	{2} 5104,1	{3} 4987,4	{4} 5387,7	{5} 5852,3	{6} 5353,5
1	C	M		0,485028	0,180362	1,000000	0,233519	0,999935
2	C	F	0,485028		0,990270	0,637178	0,007528	0,880396
3	E1	M	0,180362	0,990270		0,299576	0,001654	0,619231
4	E1	F	1,000000	0,637178	0,299576		0,275061	0,999989
5	E2	M	0,233519	0,007528	0,001654	0,275061		0,369084
6	E2	F	0,999935	0,880396	0,619231	0,999989	0,369084	

5. Average feed conversation

Č. buňky	group	{1} 3,8343	{2} 3,7860	{3} 3,9868
1	C		0,803805	0,221223
2	E1	0,803805		0,095291
3	E2	0,221223	0,095291	

	Popisné statistiky (Kopie - RESEARCH DATAS RABBITS AKHIR S3)			
Efekt	Úroveň Faktor	N	konv Průměr	konv Sm.odch.
Celkem		253	3,847394	0,543736
group	C	118	3,834269	0,592949
group	E1	86	3,785960	0,450807
group	E2	49	3,986824	0,555202

Average feed conversation according to gender

Efekt	Úroveň Faktor	Úroveň Faktor	N	konv Průměr	konv Sm.odch.
Celkem			253	3,847394	0,543736
group*sex	C	M	70	3,886832	0,638877
group*sex	C	F	48	3,757614	0,515651
group*sex	E1	M	40	3,709493	0,417106
group*sex	E1	F	46	3,852452	0,472626
group*sex	E2	M	26	4,137359	0,529862
group*sex	E2	F	23	3,816655	0,544474

Č. buňky	group	sex	{1} 3,8868	{2} 3,7576	{3} 3,7095	{4} 3,8525	{5} 4,1374	{6} 3,8167
1	C	M		0,793021	0,552870	0,999422	0,322878	0,994309
2	C	F	0,793021		0,998357	0,956493	0,042352	0,998055
3	E1	M	0,552870	0,998357		0,820717	0,019220	0,973535
4	E1	F	0,999422	0,956493	0,820717		0,254172	0,999836
5	E2	M	0,322878	0,042352	0,019220	0,254172		0,292884
6	E2	F	0,994309	0,998055	0,973535	0,999836	0,292884	

6. Average slaughtery live weight

Č. buňky	group	{1} 2715,4	{2} 2706,9	{3} 2758,6
1	C		0,824924	0,034653
2	E1	0,824924		0,013034
3	E2	0,034653	0,013034	

Efekt	Úroveň Faktor	N	hmpor Průměr	hmpor Sm.odch.
Celkem		253	2720,870	103,5177
group	C	118	2715,424	99,5343
group	E1	86	2706,860	98,8434
group	E2	49	2758,571	113,7248

Average slaughtery live weight according to gender

Efekt	Úroveň Faktor	Úroveň Faktor	N	hmpor Průměr	hmpor Sm.odch.
Celkem			253	2720,870	103,5177
group*sex	C	M	70	2730,571	111,1584
group*sex	C	F	48	2693,333	75,3808
group*sex	E1	M	40	2692,750	106,4337
group*sex	E1	F	46	2719,130	91,1367
group*sex	E2	M	26	2756,154	89,1774
group*sex	E2	F	23	2761,304	138,4428

Č. buňky	group	sex	{1} 2730,6	{2} 2693,3	{3} 2692,8	{4} 2719,1	{5} 2756,2	{6} 2761,3
1	C	M		0,369480	0,417061	0,991562	0,883568	0,808304
2	C	F	0,369480		1,000000	0,822683	0,113684	0,088976
3	E1	M	0,417061	1,000000		0,837268	0,131713	0,103294
4	E1	F	0,991562	0,822683	0,837268		0,674956	0,582857
5	E2	M	0,883568	0,113684	0,131713	0,674956		0,999976
6	E2	F	0,808304	0,088976	0,103294	0,582857	0,999976	

7. Average carcass weight

Č. buňky	group	{1} 1561,7	{2} 1574,9	{3} 1608,6
1	C		0,52903	0,00426
2	E1	0,52903		0,07753
3	E2	0,00426	0,07753	

Efekt	Úroveň Faktor	N	jatel Průměr	jatel Sm.odch.
Celkem		253	1575,27	88,2193
group	C	118	1561,69	77,8043
group	E1	86	1574,94	99,3315
group	E2	49	1608,57	83,9394

Average carcass weight according to gender

Efekt	Úroveň Faktor	Úroveň Faktor	N	jatel Průměr	jatel Sm.odch.
Celkem			253	1575,277	88,2194
group*sex	C	M	70	1585,429	78,7895
group*sex	C	F	48	1527,083	62,3974
group*sex	E1	M	40	1584,000	125,0344
group*sex	E1	F	46	1567,065	70,3687
group*sex	E2	M	26	1616,154	71,1661
group*sex	E2	F	23	1600,000	97,3275

Č. buňky	group	sex	{1} 1585,4	{2} 1527,1	{3} 1584,0	{4} 1567,1	{5} 1616,2	{6} 1600,0
1	C	M		0,003334	0,999999	0,864684	0,614219	0,980278
2	C	F	0,003334		0,021438	0,200767	0,000250	0,009205
3	E1	M	0,999999	0,021438		0,940739	0,661705	0,979523
4	E1	F	0,864684	0,200767	0,940739		0,171448	0,651544
5	E2	M	0,614219	0,000250	0,661705	0,171448		0,985707
6	E2	F	0,980278	0,009205	0,979523	0,651544	0,985707	

Annaxe 2. Blood biochemistry with feeding C control rabbits, rabbits fed E1 with milk thistle (1%), and rabbits fed E2 with fermented milk thistle (0.5%)

Note: **White = C**

Yellow = E1

Green = E2

First step is Descriptive Statistics

Descriptive Statistics

	Sex	Feeding	Mean	Std. Deviation	N
ALB	Male	White	31.4000	3.71019	30
		Yellow	31.1176	3.38900	17
		Green	31.0000	1.48324	11
	Female	White	30.6471	2.08989	17
		Yellow	31.8571	2.63222	21
		Green	31.2857	5.55294	14
TP	Male	White	53.2000	4.55919	30
		Yellow	53.9412	5.57304	17
		Green	53.8182	2.63887	11
	Female	White	53.1765	3.86062	17
		Yellow	54.3810	4.15303	21
		Green	54.5000	7.11175	14
GLOB	Male	White	21.8000	2.35475	30
		Yellow	22.6471	3.63904	17
		Green	22.3636	1.85864	11
	Female	White	22.6471	2.62062	17
		Yellow	22.5714	2.58014	21
		Green	23.2143	2.63639	14
ALKP	Male	White	145.4333	47.69059	30
		Yellow	125.0588	33.26122	17
		Green	145.0909	38.91389	11
	Female	White	121.6471	31.32280	17
		Yellow	121.3333	36.45179	21
		Green	124.9286	37.45041	14
ALT	Male	White	59.6000	16.56044	30
		Yellow	61.4118	19.57377	17
		Green	62.2727	8.64975	11
	Female	White	67.2941	15.32467	17
		Yellow	57.7619	16.57982	21
		Green	66.7857	21.19208	14

AMYL	Male	White	204.9667	105.27189	30
		Yellow	238.9412	133.90784	17
		Green	342.9091	511.99677	11
	Female	White	195.0588	68.73815	17
		Yellow	184.9524	92.32739	21
		Green	209.3571	105.72359	14
Ca	Male	White	3.5563	.11171	30
		Yellow	3.5318	.11271	17
		Green	3.5655	.08104	11
	Female	White	3.5188	.07407	17
		Yellow	3.5586	.12531	21
		Green	3.5607	.15877	14
CHOL	Male	White	.9343	.48181	30
		Yellow	1.2835	.66782	17
		Green	1.1482	.31080	11
	Female	White	1.4565	.56168	17
		Yellow	1.4995	.50346	21
		Green	1.6050	.37108	14
GLU	Male	White	88.5577	246.76135	30
		Yellow	7.9853	.77443	17
		Green	8.2273	.57910	11
	Female	White	55.1771	193.99845	17
		Yellow	8.3000	1.05441	21
		Green	8.2436	.40174	14
PHOS	Male	White	2.2193	.23163	30
		Yellow	2.2512	.21313	17
		Green	2.2264	.23754	11
	Female	White	2.1606	.21153	17
		Yellow	2.1819	.20985	21
		Green	2.2450	.21611	14
UREA	Male	White	6.0433	1.64478	30
		Yellow	6.6588	1.18536	17
		Green	5.9909	1.90864	11
	Female	White	6.5118	1.19472	17
		Yellow	6.5476	.70044	21
		Green	5.9286	1.37417	14
CREA	Male	White	16.1667	27.84821	30
		Yellow	7.1765	16.19890	17
		Green	.0000	.00000	11
	Female	White	25.5882	28.66413	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14
TBIL	Male	White	2.8667	1.85199	30
		Yellow	4.0588	7.74976	17
		Green	2.0909	.30151	11

	Female	White	3.5294	4.83629	17
		Yellow	2.8095	2.11232	21
		Green	2.7143	1.48989	14
AST	Male	White	25.9333	23.44610	30
		Yellow	31.8235	32.67881	17
		Green	42.3636	42.37752	11
	Female	White	20.9412	20.74389	17
		Yellow	27.2381	18.19314	21
		Green	35.5714	26.91215	14
LIPA	Male	White	234.6667	743.00148	30
		Yellow	.0000	.00000	17
		Green	.0000	.00000	11
	Female	White	152.3529	628.16727	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14
GGT	Male	White	.0667	.36515	30
		Yellow	.3529	1.45521	17
		Green	.0000	.00000	11
	Female	White	.0000	.00000	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14
CK	Male	White	56.9667	231.52902	30
		Yellow	.0000	.00000	17
		Green	.0000	.00000	11
	Female	White	39.2941	162.01380	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14
TRIG	Male	White	22.8333	71.45825	30
		Yellow	.0000	.00000	17
		Green	.0000	.00000	11
	Female	White	7.1176	29.34681	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14
Mg	Male	White	19.7667	45.07441	30
		Yellow	.0000	.00000	17
		Green	.0000	.00000	11
	Female	White	18.8235	42.02564	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14
LAC	Male	White	30.4543	164.80317	30
		Yellow	.0000	.00000	17
		Green	.0000	.00000	11
	Female	White	.2088	.86100	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14

NH3	Male	White	11.8000	36.74835	30
		Yellow	.0000	.00000	17
		Green	.0000	.00000	11
	Female	White	5.8824	24.25356	17
		Yellow	.0000	.00000	21
		Green	.0000	.00000	14

Second step is Tests of Between-Subjects Effects

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	ALB	15.543	5	3.109	.266	.931
	TP	31.554	5	6.311	.272	.927
	GLOB	22.563	5	4.513	.634	.674
	ALKP	12984.760	5	2596.952	1.695	.142
	ALT	1352.704	5	270.541	.940	.458
	AMYL	216940.839	5	43388.168	1.246	.293
	Ca	.029	5	.006	.443	.818
	CHOL	6.795	5	1.359	5.285	.000
	GLU	137640.236	5	27528.047	1.209	.310
	PHOS	.109	5	.022	.450	.813
	UREA	9.180	5	1.836	.981	.433
	CREA	9976.663	5	1995.333	5.209	.000
	TBIL	35.125	5	7.025	.470	.798
	AST	4151.929	5	830.386	1.168	.330
	LIPA	1203584.724	5	240716.945	1.121	.354
	GGT	1.669	5	.334	.920	.471
	CK	72239.695	5	14447.939	.761	.580
	TRIG	10596.287	5	2119.257	1.362	.245
	Mg	10167.263	5	2033.453	2.426	.040
	LAC	20177.287	5	4035.457	.533	.751
	NH3	2891.653	5	578.331	1.238	.297
Intercept	ALB	97202.873	1	97202.873	8325.209	.000
	TP	289080.398	1	289080.398	12473.283	.000
	GLOB	50675.854	1	50675.854	7116.144	.000
	ALKP	1700738.873	1	1700738.873	1110.291	.000
	ALT	389873.157	1	389873.157	1354.939	.000
	AMYL	5247135.630	1	5247135.630	150.701	.000
	Ca	1255.994	1	1255.994	96022.394	.000
	CHOL	174.097	1	174.097	677.081	.000
	GLU	86300.424	1	86300.424	3.790	.054
	PHOS	488.934	1	488.934	10067.568	.000
	UREA	3933.814	1	3933.814	2102.751	.000
	CREA	6633.508	1	6633.508	17.319	.000
	TBIL	904.621	1	904.621	60.555	.000

	AST	93669.002	1	93669.002	131.808	.000
	LIPA	414986.921	1	414986.921	1.933	.167
	GGT	.488	1	.488	1.344	.249
	CK	25672.447	1	25672.447	1.352	.248
	TRIG	2485.367	1	2485.367	1.597	.209
	Mg	4125.936	1	4125.936	4.922	.029
	LAC	2604.967	1	2604.967	.344	.559
	NH3	866.261	1	866.261	1.855	.176
Sex	ALB	.205	1	.205	.018	.895
	TP	3.341	1	3.341	.144	.705
	GLOB	7.290	1	7.290	1.024	.314
	ALKP	6296.998	1	6296.998	4.111	.045
	ALT	202.879	1	202.879	.705	.403
	AMYL	108013.125	1	108013.125	3.102	.081
	Ca	.001	1	.001	.051	.823
	CHOL	3.956	1	3.956	15.386	.000
	GLU	3026.223	1	3026.223	.133	.716
	PHOS	.033	1	.033	.683	.411
	UREA	.241	1	.241	.129	.720
	CREA	13.965	1	13.965	.036	.849
	TBIL	.004	1	.004	.000	.987
	AST	742.429	1	742.429	1.045	.309
	LIPA	18772.106	1	18772.106	.087	.768
	GGT	.488	1	.488	1.344	.249
	CK	865.300	1	865.300	.046	.831
	TRIG	684.282	1	684.282	.440	.509
	Mg	2.464	1	2.464	.003	.957
	LAC	2534.488	1	2534.488	.335	.564
	NH3	97.021	1	97.021	.208	.650
Feeding	ALB	4.508	2	2.254	.193	.825
	TP	24.159	2	12.079	.521	.595
	GLOB	5.818	2	2.909	.408	.666
	ALKP	2897.701	2	1448.850	.946	.392
	ALT	456.112	2	228.056	.793	.455
	AMYL	96986.216	2	48493.108	1.393	.253
	Ca	.010	2	.005	.393	.676
	CHOL	.928	2	.464	1.805	.170
	GLU	103707.712	2	51853.856	2.277	.108
	PHOS	.035	2	.018	.363	.696
	UREA	6.290	2	3.145	1.681	.191
	CREA	9142.250	2	4571.125	11.934	.000
	TBIL	16.597	2	8.298	.555	.575
	AST	3796.222	2	1898.111	2.671	.074
	LIPA	957417.988	2	478708.994	2.230	.113
	GGT	.601	2	.300	.827	.440

	CK	59229.005	2	29614.503	1.560	.215
	TRIG	5734.001	2	2867.000	1.842	.164
	Mg	9518.963	2	4759.481	5.678	.005
	LAC	6009.929	2	3004.965	.397	.674
	NH3	1998.553	2	999.277	2.139	.123
Sex * Feeding	ALB	11.781	2	5.891	.505	.605
	TP	2.216	2	1.108	.048	.953
	GLOB	5.167	2	2.584	.363	.697
	ALKP	2179.006	2	1089.503	.711	.493
	ALT	668.456	2	334.228	1.162	.317
	AMYL	60074.392	2	30037.196	.863	.425
	Ca	.021	2	.010	.798	.453
	CHOL	.500	2	.250	.972	.382
	GLU	7206.808	2	3603.404	.158	.854
	PHOS	.033	2	.016	.335	.716
	UREA	2.015	2	1.008	.539	.585
	CREA	1401.153	2	700.577	1.829	.166
	TBIL	21.804	2	10.902	.730	.484
	AST	19.514	2	9.757	.014	.986
	LIPA	43309.200	2	21654.600	.101	.904
	GGT	.601	2	.300	.827	.440
	CK	1996.338	2	998.169	.053	.949
	TRIG	1578.710	2	789.355	.507	.604
	Mg	5.686	2	2.843	.003	.997
	LAC	5847.328	2	2923.664	.386	.681
	NH3	223.838	2	111.919	.240	.787
Error	ALB	1214.276	104	11.676		
	TP	2410.301	104	23.176		
	GLOB	740.610	104	7.121		
	ALKP	159306.695	104	1531.795		
	ALT	29925.196	104	287.742		
	AMYL	3621083.925	104	34818.115		
	Ca	1.360	104	.013		
	CHOL	26.741	104	.257		
	GLU	2368047.388	104	22769.686		
	PHOS	5.051	104	.049		
	UREA	194.563	104	1.871		
	CREA	39834.755	104	383.026		
	TBIL	1553.647	104	14.939		
	AST	73907.062	104	710.645		
	LIPA	##########	104	214644.140		
	GGT	37.749	104	.363		
	CK	1974540.496	104	18985.966		
	TRIG	161861.931	104	1556.365		
	Mg	87177.837	104	838.248		

	LAC	787654.306	104	7573.599		
	NH3	48574.565	104	467.063		
Total	ALB	108808.000	110			
	TP	320400.000	110			
	GLOB	56181.000	110			
	ALKP	2070496.000	110			
	ALT	452755.000	110			
	AMYL	9124252.000	110			
	Ca	1386.600	110			
	CHOL	214.785	110			
	GLU	2659304.483	110			
	PHOS	543.384	110			
	UREA	4552.020	110			
	CREA	59682.000	110			
	TBIL	2609.000	110			
	AST	171791.000	110			
	LIPA	##########	110			
	GGT	40.000	110			
	CK	2098145.000	110			
	TRIG	178364.000	110			
	Mg	104923.000	110			
	LAC	815479.039	110			
	NH3	53340.000	110			
Corrected Total	ALB	1229.818	109			
	TP	2441.855	109			
	GLOB	763.173	109			
	ALKP	172291.455	109			
	ALT	31277.900	109			
	AMYL	3838024.764	109			
	Ca	1.389	109			
	CHOL	33.536	109			
	GLU	2505687.624	109			
	PHOS	5.160	109			
	UREA	203.742	109			
	CREA	49811.418	109			
	TBIL	1588.773	109			
	AST	78058.991	109			
	LIPA	##########	109			
	GGT	39.418	109			
	CK	2046780.191	109			
	TRIG	172458.218	109			
	Mg	97345.100	109			
	LAC	807831.593	109			
	NH3	51466.218	109			

Third step is mean of blood biochemistry

Mean of Blood profile

Sex	MALE			FEMALE		
Feeding	White	Yellow	Green	White	Yellow	Green
ALB	31.40	31.12	31.00	30.65	31.86	31.29
TP	53.20	53.94	53.82	53.18	54.38	54.50
GLOB	21.80	22.65	22.36	22.65	22.57	23.21
ALKP	145.43	125.06	145.09	121.65	121.33	124.93
ALT	59.60	61.41	62.27	67.29	57.76	66.79
AMYL	204.97	238.94	342.91	195.06	184.95	209.36
Ca	3.56	3.53	3.57	3.52	3.56	3.56
CHOL	.93	1.28	1.15	1.46	1.50	1.61
GLU	88.56	7.99	8.23	55.18	8.30	8.24
PHOS	2.22	2.25	2.23	2.16	2.18	2.25
UREA	6.04	6.66	5.99	6.51	6.55	5.93
CREA	16.17	7.18	.00	25.59	.00	.00
TBIL	2.87	4.06	2.09	3.53	2.81	2.71
AST	25.93	31.82	42.36	20.94	27.24	35.57
LIPA	234.67	.00	.00	152.35	.00	.00
GGT	.07	.35	.00	.00	.00	.00
CK	56.97	.00	.00	39.29	.00	.00
TRIG	22.83	.00	.00	7.12	.00	.00
Mg	19.77	.00	.00	18.82	.00	.00
LAC	30.45	.00	.00	.21	.00	.00
NH3	11.80	.00	.00	5.88	.00	.00

Fourth step is tests of between-subjects effect

Tests of Between-Subjects Effects

Blood profile	Significant		
	Sex	Feeding	Sex*Feeding
ALB	.895	.825	.605
TP	.705	.595	.953
GLOB	.314	.666	.697
ALKP	.045	.392	.493
ALT	.403	.455	.317
AMYL	.081	.253	.425
Ca	.823	.676	.453
CHOL	.000	.170	.382
GLU	.716	.108	.854
PHOS	.411	.696	.716
UREA	.720	.191	.585
CREA	.849	.000	.166
TBIL	.987	.575	.484
AST	.309	.074	.986
LIPA	.768	.113	.904
GGT	.249	.440	.440
CK	.831	.215	.949
TRIG	.509	.164	.604
MG	.957	.005	.997
LAC	.564	.674	.681
NH3	.650	.123	.787

Last step is blood biochemistry with the sex signifikan to alpha 0.05 signifikan to alpha 0.1

Blood profile	Sex
ALB	.918
TP	.594
GLOB	.226
ALKP	**.025**
ALT	.412
AMYL	.198
Ca	.828
CHOL	**.000**
GLU	.370
PHOS	.362
UREA	.554
CREA	.609
TBIL	.946
AST	.514
LIPA	.422
GGT	.231
CK	.528
TRIG	.213
MG	.478
LAC	.342
NH3	.316

Blood biochemistry with the sex signifikan to alpha 0.05signifikan to alpha 0.1

Blood profile	Feeding
ALB	.849
TP	.553
GLOB	.485
ALKP	.266
ALT	.453
AMYL	.330
Ca	.769
CHOL	.031
GLU	.064
PHOS	.775
UREA	.169
CREA	.000
TBIL	.638
AST	.092
LIPA	.072
GGT	.541
CK	.160
TRIG	.081
MG	.003
LAC	.505
NH3	.069

signifikan to alpha 0.05
signifikan to alpha 0.1

Annexe 3: Analyses of Short chain fatty acids by T-test evaluation

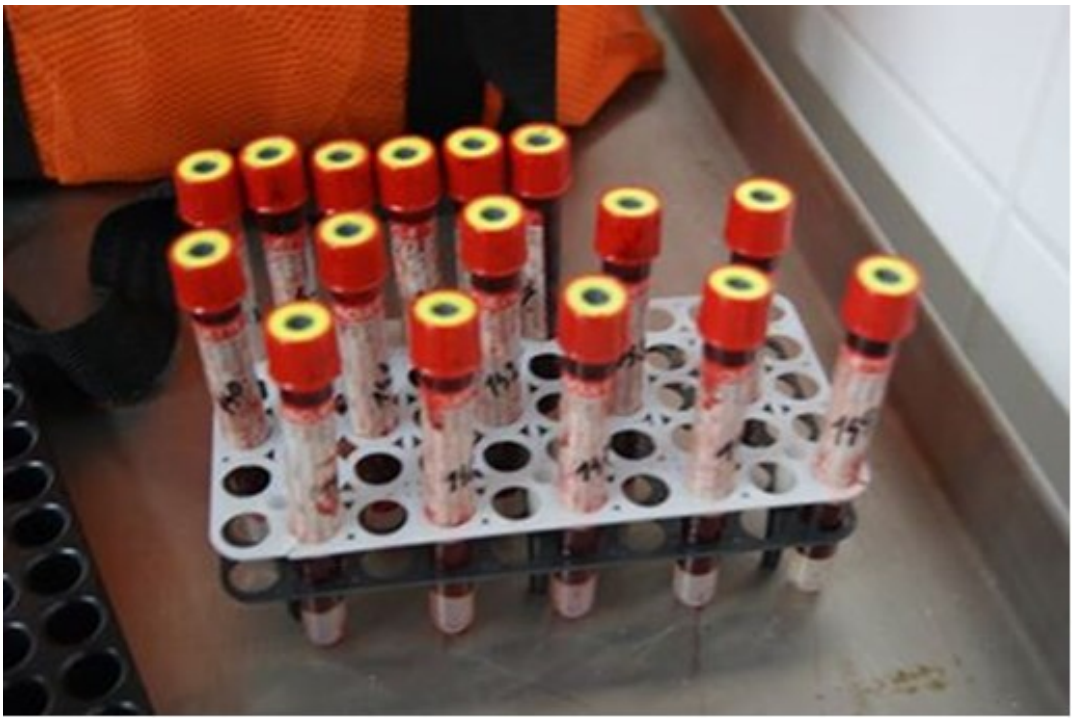
sl.stř.králík	acetát	propionát	isobutyrát	butyrát	isovalerát	valerát	isohexanoic	hexanoic	heptanoic	suma	žlutá
	mol%										E1
P1	83.9	4.2	1.4	8.4	0.1	0.5	0.3		1.2	100	
P2	77.5	5.3	1.2	11.7	0.3	0.7		0.3	3	100	
P3	79.8	4.1	1.9	9.8	0.1	0.7	0.3	0.6	2.7	100	
P4	81.4	5.3	1.7	8	0.2	0.7	0.2	0.3	2.2	100	
P5	83.8	4.2	1.2	8.5	0.2	0.4		0.1	1.6	100	
P6	84.2	5.5	0.6	7.8	0.2	0.5		0.1	1.1	100	
P7	83.5	4.6	0.5	9.3	0.3	0.4		0.2	1.2	100	
P8	80.4	11	2.3	3.7	0.5	0.4			1.7	100	
P9	83.9	3.9	0.6	8.5	0.3	0.5		0.7	1.6	100	
P10	79.5	3.3	1	13.9	0.2	0.5	0.3	0.1	1.2	100	
	81.79	**5.14**	**1.24**	**8.96**	**0.24**	**0.53**	**0.275**	**0.3**	**1.75**	**100**	
	2.39	**2.18**	**0.60**	**2.65**	**0.12**	**0.13**	**0.05**	**0.23**	**0.67**	**0.00**	
sl.stř.králík	acetát	propionát	isobutyrát	butyrát	isovalerát	valerát	isohexanoic	hexanoic	heptanoic	suma	kontrola
	mol%										C
K1	82.4	4.4	1.1	9.9	0.3	0.5		0.4	1	100	
K2	78.5	5.2	1	12	0.2	0.9		1	1.2	100	
K3	78.1	4.1	1	13.9		0.7		0.9	1.3	100	
K4	78.9	5.2	0.9	12.4	0.3	0.7		0.6	1	100	
K5	82.1	4	0.4	12.1	0.2	0.5		0.1	0.6	100	
K6	82.1	4.5	0.3	11.2	0.2	0.6		0.5	0.6	100	
K7	79.4	5	1.4	11.6	0.3	0.8		0.5	1	100	
K8	81.9	3.4	0.4	11.8	0.1	0.7		0.7	1	100	
K9	80.6	8.2	0.7	8.4	0.2	0.6		0.6	0.7	100	
K10	83.5	6.2	0.7	7.7	0.3	0.5		0.3	0.8	100	
	80.75	**5.02**	**0.79**	**11.1**	**0.23**	**0.65**		**0.56**	**0.92**	**100**	
	1.90	**1.36**	**0.35**	**1.90**	**0.07**	**0.14**		**0.27**	**0.24**	**0.00**	
P	0.296	0.884	0.055	0.053	0.822	0.062		0.032	0.002		

Annexe 4: HYLA Rabbits, me (akhir pebriansyah), slaughter place in Experimental Stable at Czech University of Life Sciences Prague (Pebriansyah, 2015).

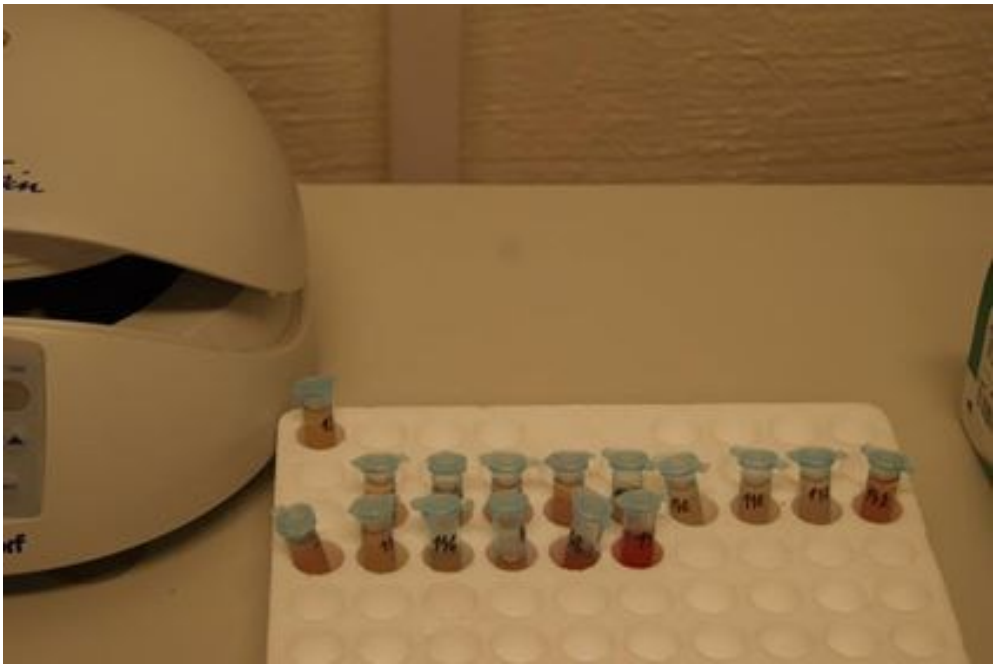

ECA 400 QC

Annexes 5: Carcass evaluation during the experiment (Pebriansyah, 2015).

Annexe 7: Vacuette test tubes marked with the number of each animal (Pebriansyah, 2015).

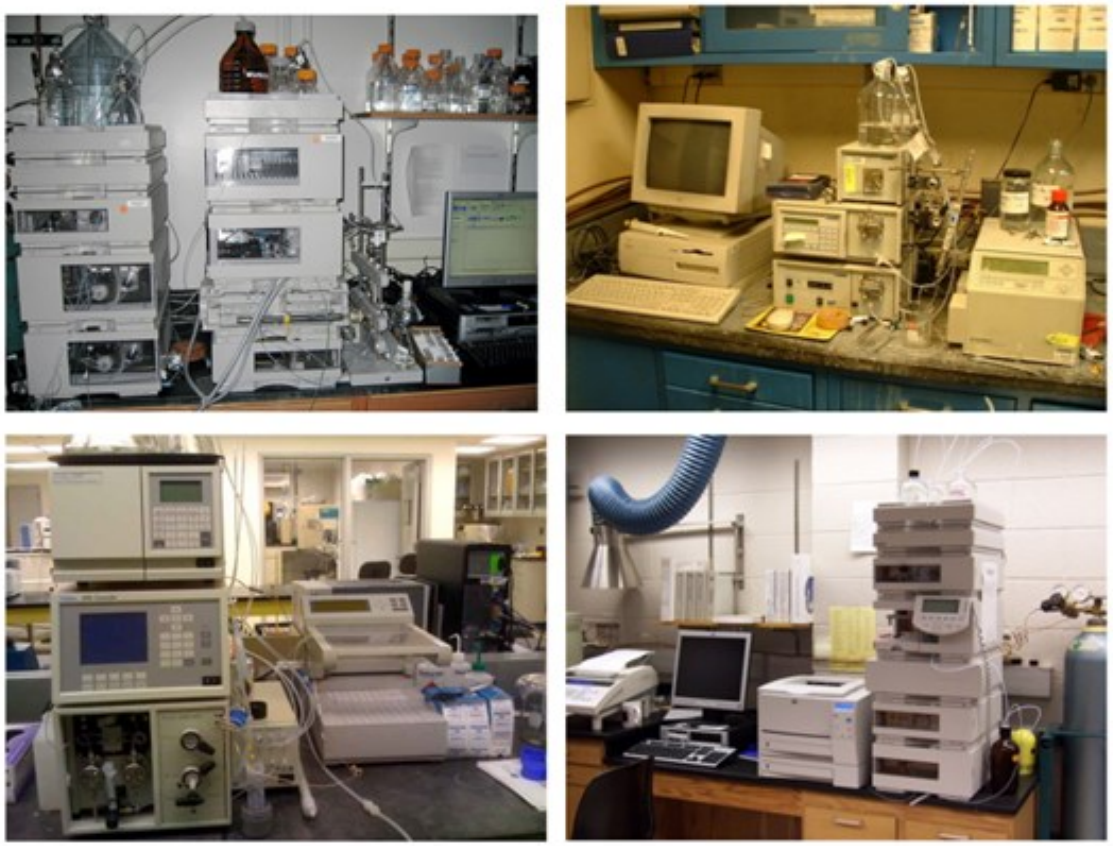


Annexe 6: VetTest® chemistry analyzer from IDEXX Laboratories (Pebriansyah, 2015).



Annexe 7: The dry- slides for the detection of biochemical parameters values (Pebriansyah, 2015)

Annexe 8: High-performance liquid chromatography – VFA Analyses of Rabbits (Pebriansyah, 2015).



Annexe 9: *Silybum marianum* in flower (Pebriansyah, 2007)