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The Impact of the Seabuckthorn (*Hippophae rhamnoides*) Supplement in the Feed Ration on the Quality of Poultry Products

Master thesis

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Declaration of Integrity

I, Akhir Pebriansyah declare that I have worked my thesis titled: “The impact of the Seabuckthorn (*Hippophae rhamnoides*) supplements in feed ration on the quality of poultry products” by myself, I used only sources that are listed the part of references.

In Prague, 18 April 2014

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Akhir Pebriansyah, BSc

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„In the Name of God, the Most Beneficial, the Most Merciful”

I do enter into His gates with thanksgiving, and into His courts with praise: I am thankful unto Him, the Almighty, and bless His name, for health and vigor, bestowed upon me, all through the life span of these crucial days of my study.

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Abstract

Seabuckthorn *(Hippophae rhamnoides*) (SB) is a shrub which belongs to the family Elaeagnaceae, it have been used in Asia, Europe, and North America for human consumption, cosmetics, and also animal feeding as a dried by-product after fruit processing for juices. This study was aimed to find any positive effect of the SB supplement in feed ration on the laying hens´ productivity and quality of eggs. Two experiments were conducted at the ITP (International Testing of Poultry) in Ústrašice, Czech Republic. Total number of laying hens was 2160. In the first experiment there were used 1440 hens, divided into 48 pens - 7 periods with a diet containing 5 % of SB - Treatment (T) vs. diet without SB – Control. During the second experiment 720 hens were divided into 12 pens - 2 periods with a diet containing 13.5 % of SB - T vs. control group without SB. The data collected was analyzed by statistical software SAS System 9.3. Parameters of the production of eggs, quality of eggs, live weight of hens and also feed consumption were measured. There were not found any statistical significant differences between groups of hens fed by 5 % of SB in a diet vs. control group in egg production and egg weight during the 1.experiment, but significant decrease of egg productivity and egg weight was found in T group (13.5 % of SB) during the 2.experiment ($P < 0.01$). The color of yolk increased significantly – more orange, in a diet with SB in both experiments ($P < 0.01$). The feed consumption was the highest in the group fed by 13.5 % of SB, but the feed conversion was not better in this group. We can conclude some positive effects of 5 % of SB in a diet for promoting the more orange yolk color, productivity of laying hens, and decreasing number of disorders in eggs. However, 13.5 % of SB in a diet decreased the egg quality and productivity of hens, so we cannot recommend this higher concentration of SB in a diet for laying hens.

Keywords: Seabuckthorn, feed, poultry products, laying hens, quality of eggs

Abstrakt

Rakytník řešetlákový *(Hippophae rhamnoides*) patří do čeledi *Elaeagnaceae* a roste obvykle ve formě keře, používá se v Asii, Evropě a Severní Americe pro lidskou spotřebu, v kosmetickém průmyslu i jako krmení pro zvířata – většinou jako vedlejší produkt po vylisování plodů na džus. Tato studie byla zaměřena na hledání pozitivních vlivů přídavku rakytníku do krmné dávky pro nosnice na jejich produktivitu a kvalitu vajec. Dva pokusy byly realizovány v rámci podniku Mezinárodní testování drůbeže v Ústrašicích. Celkový počet nosnic zařazených do dvou pokusů byl 2160. Do prvního pokusu bylo zařazeno 1440 nosnic, rozdělených do 48 boxů – bylo zkoumáno 7 period snášky. Pokusná skupina nosnic byla krmena krmnou směsí s 5% obsahem rakytníku oproti kontrolní skupině bez rakytníku. Během druhého pokusu bylo testováno 720 nosnic, rozdělených do 12 boxů po dobu dvou snáškových period. Pokusná skupina dostávala krmnou směs s 13,5% obsahem rakytníku. Data byla vyhodnocena pomocí statistického programu SAS System 9.3. Během pokusů byly sledovány parametry jako: produkce vajec, kvalita vajec, živá hmotnost slepic a také spotřeba krmiva. Během prvního pokusu nebyly nalezeny statisticky významné rozdíly v produkci vajec a hmotnosti vajec mezi skupinami, ale při druhém pokusu byl zaznamenán významný pokles ($P < 0.01$) produktivity i hmotnosti vajec v pokusné skupině (13,5 % rakytníku v krmné dávce). Významný rozdíl nastal i v barvě žloutku (sytě oranžový) po podání rakytníku v obou pokusech ($P < 0.01$). Spotřeba krmiva byla nejvyšší ve skupině krmené 13,5% rakytníkem, ale konverze živin u této skupiny nebyla efektivnější. Byly tedy nalezeny pozitivní vlivy přídavku 5 % rakytníku do krmné dávky na sytější (oblíbenější) barvu vaječného žloutku, produktivitu nosnic a také na výskyt abnormálních vajec. Avšak koncentrace 13,5 % rakytníku v krmné dávce způsobila zhoršení kvality vajec i snížení produkce vajec, tudíž tuto koncentraci z hlediska výživy nosnic nemůžeme doporučit.

Klíčová slova: rakytník, krmivo, nosnice, kvalita vajec, drůbeží produkty

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List of acronyms and abbreviations

a.m	ante meridian or before noon
BSc	Bachelor of Sciences
C	control
^{0}C	degree celsius
Ca	Calcium
cm	centimeter
CP	Crude Protein
e.g	*exempli gratia*
g	gram
GRIN	Germplasm Resource Information Network
H	*Hiphopae*
HU	Haugh Unit
Ing	*Inženýr*
I.U	International Unit
ITP	International Testing of Poultry
Kg	kilogram
Lux	Luminous intensity
mg	milligram
MJ	Mega Joule
MSc	Master of Sciences
P	Phosphorous
PhD	Doctor of Philosophy
p.m	post meridian or afternoon
rham	*rhamnoides*
SAS	Statistical Analysis Software
SB	Seabuckthorn
Subsp	subspecies
T	treatment
vs	versus
Wks	Weeks

1. Introduction

There is a gap between demand and availability of feed, due to livestock is mainly fed on by-product which are poor in quality such as energy and essential nutrients. Poultry management is also the key of success in enhancing the quantity and quality of poultry production. In general, worldwide, the poultry industry is one of the fastest growing agricultural sectors and the demand for poultry products is rapidly increasing during the next two decades and this trend is expected to continue. This phenomenon is having an intense on the demand for feed and raw materials. Feed is the most important input for poultry production. However, farmers have used conventional feed sources such as grains, corn, wheat, fats as well as animal by-product which some of those components are consumed for human being. Feeds must be represented 60-70 % of the costs from poultry production, which is a critical point when the production is to remain competitive, in order to meet the demand for animal protein to humans and to evaluate continually the ingredients of feed stuffs.

In addition, feeding program is dependent on balanced nutrition to get energy which is important for the metabolism and animal production as well as the nutrient components that are essential for growth, health and reproduction, such as proteins, amino acids, carbohydrates, fats, water, minerals, and vitamins. Many feed variations were observed for poultry feeding, for example unconventional sources, fruits or vegetable wastes. Even though feed has many characteristic of dietary effects and also some limited factors in the diet. In order to bridge gap between demand and supply Seabuckthorn (*Hippophae rhamnoides*) play an important role to improve efficiency of feed. These plants have been used as an alternative feeding somehow, particularly in poultry to maintain their quantity production, performance and high quality yield.

For decades, the utilization of Seabuckthorn product has been spread widely, particularly in medical and health, they might increase the beneficial and economics (Farias et al., 2009). Lu (1992) said that the Seabuckthorn (SB) is a wonderful plant due to great contents and it has medicinal properties, and these plants have been used for food, and cosmetics (Utioh et al., 2009; Uransanaa et al., 2003). Moreover, studies about the medical effect from the SB has been established that these plants, particularly from the seed has indicated the medicinal uses for human health (Negy et al., 2005). Nowadays, SB is valued much more aspects of human beings and livestock feed, such as soil conservation, reforestation some area, flavonoid contents, and others. However, the most important is it's used as a nutritional and medicinal value (Tang et al., 2002). The objective of this study was to examine the impact of the SB supplement in feed ration on the quality of poultry products, particularly in laying hens.

2. Literature Review

2.1 Characteristics of the Sea Buckthorn

Seabuckthorn (*Hippophae rhamnoides*) is a shrub and berry or small tree 3-4 meter in height, which belong to the family Elaeagnaceae which have been used in Asia and Europe, natural distribution includes such as China (Rongsen, 2009), Rusia (Demidova et al., 2009), Mongolia (Uransanaa et al, 2003), Western Asia, Western Europe, then extends widely in North and South America (Bala L et al., 2011; Zeb et al., 2009; Suryakumar et al. 2011; Shah et al., 2007; Truta et al., 2009). Lu (1992) noticed that Seabuckthorn (SB) is widely grown in the interval of altitude between a few meters to 5200 meters, these plants are tolerant under the temperature up to -40^{0}C (Yadav A et al., 2009) and it also hold the temperatures till 40^{0}C either in under cool or wet climate (McKenzie, 2009). Plants are growing well in pH soil 9.5, even though some species of Seabuckthorn growth so well in 1.1 % of salt due to development in root system even in poor soil (Lu, 1992; Demidova et al., 2009).

Moreover, its roots are able to fix the nitrogen from the air through the nodule, its natural lifespan is about 60 to 70 years, even though the majority of SB tree found in the Yunnan district of China has a lifespan around 300 years of age. SB can divided into male and female. The males are lower growth compared with females and the males are petal and can produce pollen. The atmospheric temperature ranges between 6^{0}C-10^{0}C. Females have the flower without petal and each flower contains one ovary and one ovule, the pollination depends on the wind, the female plants produce ripe Seabuckthorn with yellow or red in color, the range size of this berry is between 3 and 8 mm in diameter (Lu, 1992).

In general, SB is divided into fruits, leaves, roots, seeds and oil. Fruits are used as food additives and cosmetic ingredients or nutraceuticals. Thus, the SB has a unique compound, for example bioactive substances, rich source of valuable compounds, vitamins, carotenoid, flavonoid, organic acids, micro and macro mineral compound. Berries are also rich in fatty acids, for example, saturated 13.7 %, unsaturated 86.3 % and includes palmatic acid, oleic acid, omega 9, omega 7, omega 3, and omega 6. The taste of berries is acidic to eat raw. (Bala L. et al., 2011; Utioh et al., 2009; Wang et al., 2009; Sumita et al., 2009).

Leaves are small, narrow (usually 3-8 cm long and 0.4-1 cm wide), alternate and silver gray in color. Seeds are ovoid to elliptical in shape and 2.8-4.2 mm in size, the color of the seeds is dark and glossy, carbohydrates, proteins and lipids or fats are the major of chemical composition of SB (Suryakumar e al., 2011). The root system of SB has covered with some hairs, branch can grow up. The main function of the root system is to get nutrition in order to growth and adaptation (Lu, 1992).

2.1.1 Taxonomy and distribution of Seabuckthorn

Seabukcthorn belongs to the family Elaeagnaceae, genus is *Hippophae*, class is *Magnoliopsida*. The taxonomy system, the distribution and its utilization of Seabuckthorn are described in table 1 in below and table 2 in the annexes.

Table 1: The taxonomy system of Seabuckthorn (Rajchal, 2008)

Species	Subspecies
Hippophae goniocarpa	-
H. *gyantsensis*	-
H. *litangensis*	-
H. *neurocarpa*	i. Subsp. *neurocarpa* ii. Subsp. *stellatopilosa*
H. *salicifolia*	-
H. *tibetana*	-
H. *rhamnoides*	i. Subsp. *carpatica* ii. Subsp. *caucasica* iii. Subsp. *fluviatilis* iv. Subsp. *mongolica* v. Subsp. *rhamnoides* vi. Subsp. *sinensis* vii. Subsp. *turkestanica* viii. Subsp. *wolongensis* ix. Subsp. *Yunnanensis*

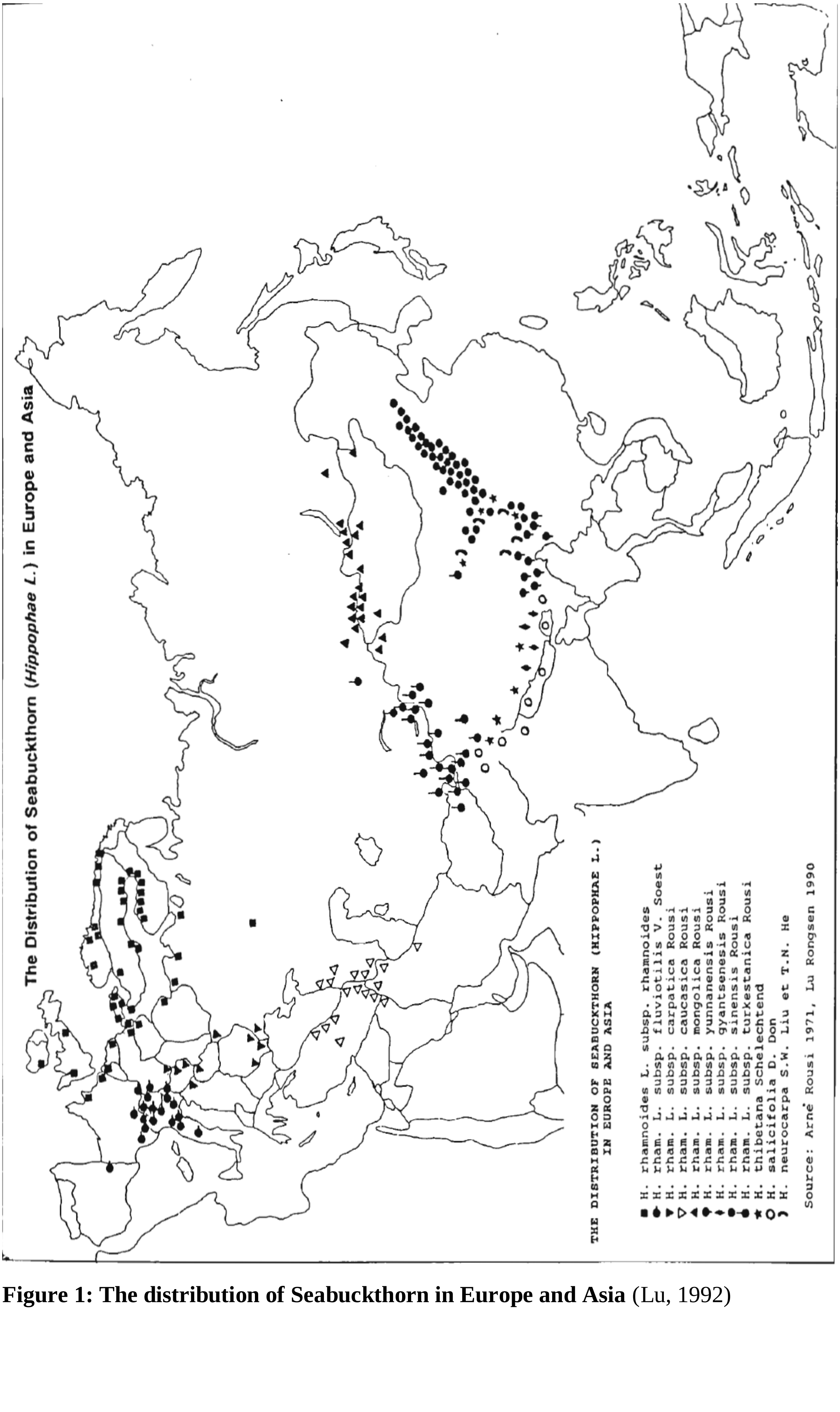

Figure 1: The distribution of Seabuckthorn in Europe and Asia (Lu, 1992)

2. 1. 2 The Utilization of Seabuckthorn (*Hippophae)*

Seabuckthorn (SB) has developed status and it is one of the most hunted plants all over the world due to cosmetics, pharmaceutical industries and having great impact for the health of human beings and these fruits are mainly used in food industry as a nutritional product in the commercial market (Singh et al., 2011; Demidova et al., 2009; Utioh et al., 2009). The fruits of SB (*Hippophae rhamnoides* L.) have been used for human consumption, medical, cosmetic, animals feeding, and fodder for poultry.

SB has two main essential sources from the berries or juices and seed oil (Beveridge et al,. 1999; Oprica et al., 2009). The human is used Seabuckthorn as a food like juice and they more usually mixed together with sweeter substances such as apples or even grapes (Bala L. et al., 2011; Utioh et al., 2009; Wang et al., 2009; Sumita et al., 2009). SB is also used as a source of vitamin C and antioxidant for human health may include tocopherols, tocotrienols, carotenoids, flavoniods, lipids, proteins, minerals and essential fatty acids (Rongsen, 2009; Ecclestona et al., 2002; Shah et al., 2007; Demidova et al., 2009; Oprica et al., 2009).

SB was observed and analyzed for its medicinal, cosmetic and nutritional properties as well as therapeutic potential (Suryakumar et al., 2011; Demidova et al., 2009; Truta et al., 2009; Utioh et al., 2009; Lu, 1992). Seabuckthorn has pharmacognostic and phytochemical profile which is most dependent on environmental and the adaptability of the plants. (Ilango et al., 2013). Owing to Seabuckthorn has a great source of bioactives substances, for example vitamin A, C, E, K, riboflavin, folic acid, carotenoids (α,β,δ-carotene, lycopene), phytosterols (ergosterol, stigmasterol, lansterol, amyrins), organic acids (malic acid, oxalic acid), polyunsaturated fatty acids and some essential amino acids which are used for herbal medical and theurapeutic potential in order to prevent such diseases and are used as anti-stress, anti-microbial, anti-tumor, hepatoprotective, radioprotective, anti-atherogenic, and for tissue generation (Suryakumar et al., 2011).

In animals, the study was observed that the Seabuckthorn were suitable and valuable for healing rat stomach (Xu, 2007). For poultry was reported that SB is used as a fodder with the leaves, seeds, fruit residues of Seabuckthorn (Biswas et al., 2010; Kaushal et al., 2011; Patial et al., 2013) and the results shows that the number of eggs increased 10.3 % and 28.1 % after consumptions with the seeds, leaves and fruit residues of SB

(Biswas et al., 2010; Patial et al., 2013). The leaves were also observed to enhance growth performances and calcium metabolism of broiler.

Seabuckthorn (SB) was evaluated and showed that SB have protective action of poultry, especially, may increase the body weight gain and reduces some poisoned activities during the metabolic process and also Seabuckthorn has natural antioxidant play a major role in detoxification of micotoxin (Solcan et al., 2011). Zhong et al. (2006) reported that the weight of poultry increased after consumption leaves, seeds, and fruit residues of Seabuckthorn. The research showed that the rate of laying hens and the number of eggs increased 10.3 %. The leaves and fruit residues can be treated by such methods, for example silaging or basification in order to decrease the content of crude fiber and increase the digestibility of crude fiber. This experiment also showed that the weight of chicken raising 5.74 % and for hens increase about 7.81 % after 56 days with feed contain of leaves and fruit residues of Seabuckthorn. Moreover, Seabuckthorn could increase meat hybrid chicken or poultry and its action mechanism, and this study also reported that Seabuckthorn may improve feed utilization, reduce feed conversion, promote health and weight gain of broiler (YanMing et al., 2009).

In fact, as we known that the Seabuckthorn is not only acceptable for traditional feeding animals, but those fruits also play a great role in preservatives food, such as used in chicken meat preservation, because it’s antifungal and antibacterial properties. This experiment showed that Seabuckthorn leaves were significantly ($P < 0.05$) decreasing the microbial activity compared with control, prolonging its shelf life and diet in contain of 3 % leaves Seabuckthorn were evaluated may be effected in order to preserve and decontaminate of fresh chicken together with appropriate temperature (Dhanze et al., 2012).

The experiment was established that Seabuckthorn with Glucomannan in combination may also provide some added protection toward toxicity (Ramasamy et al., 2010). Furthermore, the experiment showed that feed with Seabuckthorn leaves supplemented in a diet with 0.25 %, 0.5 %, 1 % respectively were evaluated could be improved the meat flavor and increased the muscle content of poultry under heat stress conditions (Zhao et al., 2012) and extract leaf was examined, it’s utilized antitoxic for small animals (Saggu et al., 2007). Seed is included some good antioxidant and antimicrobial which are used for natural preservation (Chauhan et al., 2007).

The processing of Seabuckthorn as a whole fruit and Seabuckthorn berries are described in figure 2 and figure 3 below:

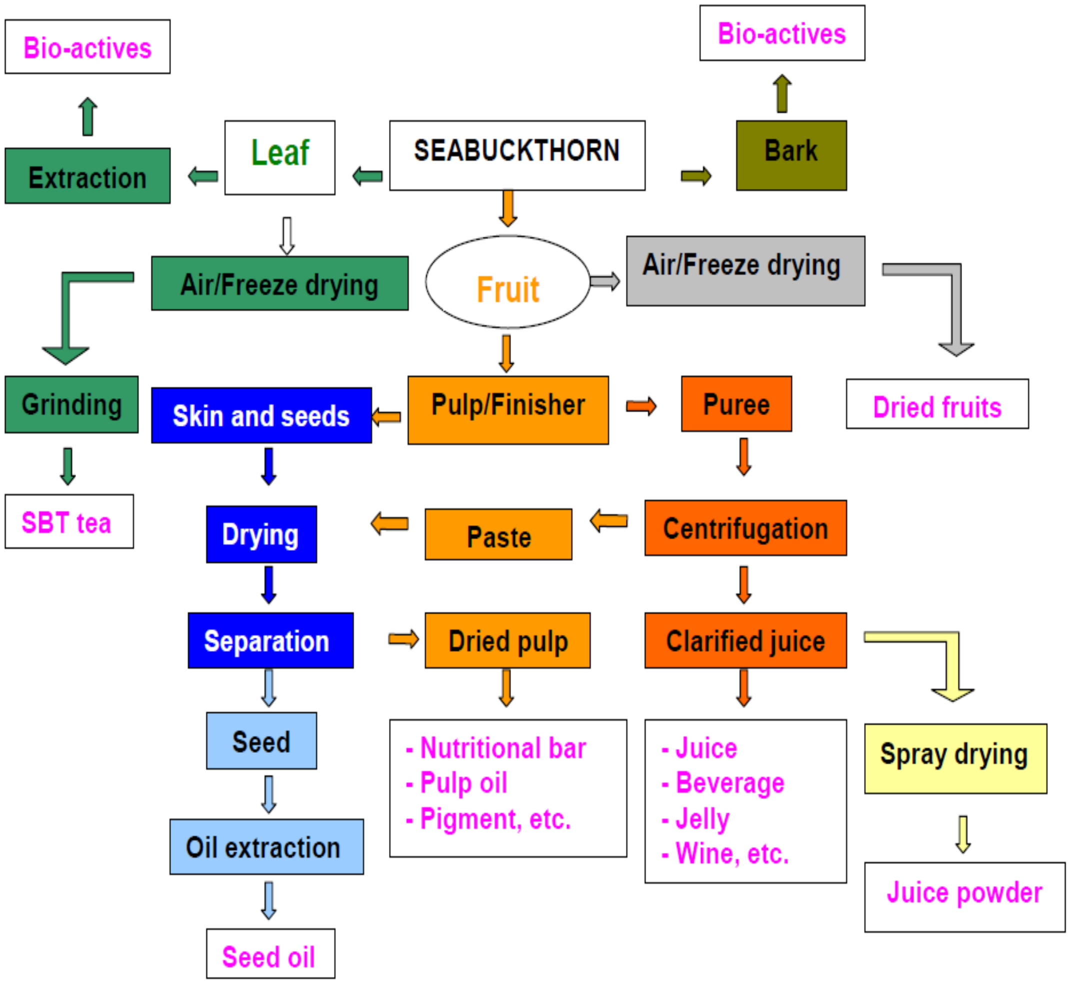

Figure 2: Seabuckthorn processing (Utioh et al, 2009)

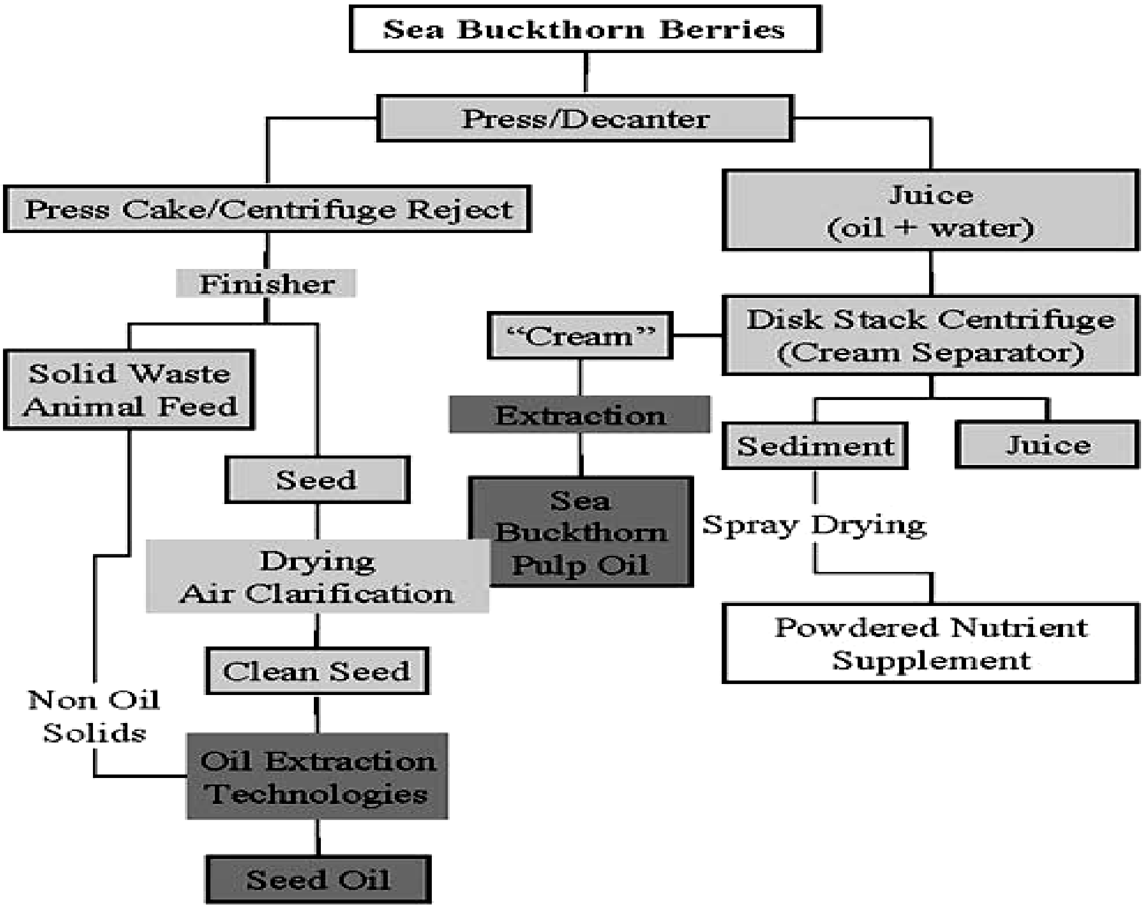

Figure 3: Seabuckthorn berry processing and its products (Utioh et al, 2009)

Utioh et al. (2009) reported that the processing of Seabuckthorn plays a great role to enhance the quality of Seabuckthorn product and determine potential demand and marketing purposes and his studies have been carried out the feeding with supplemented with 1 % of Seabuckthorn leaves in diet was significantly influenced the broiler performances and it can improve deposition of calcium, absorption of calcium and absorption of phosphorous. Moreover, Seabuckthorn leaves has phyto-additive compounds which are used to prevent any diseases and to give the appropriate bacterial environment in digestion functions (Xin et al., 2011). Furthermore, Seabuckthorn contains anti-microbial activity which may improve the immunity response as well as a great beneficial effect of duodenal mucosa of poultry (Ștef et al., 2009). A wide range of research regarding the utilization of Seabuckthorn has been established, Thus, Seabuckthorn is an important plant because of the great compounds as potential for most organisms as well as beneficial functions for therapy and prevention any diseases and other positive effects (Xu et al., 2011).

2. 1.3 Composition of Seabuckthorn

Nutritional and chemical compounds of Seabuckthorn (SB) are more dependent on the environment, regions and its species, especially in medical values. SB is used as potential nutrients for animals, the bioactive subtances of SB fresh berries is presented in table 3, comparison of the vitamin contents of Seabuckthorn compared to other fruits and vegetables is presented in table 4 and comparison of nutritive values of Seabuckthorn and common types of fodder is presented in table 5 as follows:

Table 3: Bioactive Subtances in Seabuckthorn (Hasanuzzaman, 2011)

Bioactive of Seabuckthorn	**Amount (per 100 grams fresh berries)**
Vitamin C	200-1500 mg (typical amount: 600 mg)
Vitamin E (mixed tocopherols)	up to 180 mg (equal to about 270 IU)
Folic acid	up to 80 mg
Carotenoids, including beta carotene, lycopene, zeaxanthine; these contribute the yellow-orange-red color of the fruit	30-40 mg
Fatty acids (oils); the main unsaturated fatty acids; oleic acid (omega-9), palmitoleic acid (omega-7), palmitic acid and linoleic acid (omega-6), and linolenic acid (omega-3); saturated oils and sterols	6-11 % (3-5 % in fruit pulp, 8-18 % in seed); fatty acid composition and total oil content varies with subspecies
Organic acids other than ascorbic (e.g. quinic acid, malic acid)	quantity not determined; the expressed juice has a pH of 2.7-3.3
Flavonoids (e.g. mainly isorhamnetin, quercetin glycosides, and kaempferol)	100-1000 mg (0.1 % to 1.0 %)

Tabel 4: Comparison of the vitamin contents of Seabuckthorn and other fruits and vegetables (mg/100g) (Lu, 1992)

Species	Vitamin A	Vitamin B1	Vitamin B2	Vitamin C	Vitamin K
Seabuckthorn	11.00	0.04	0.56	300-1600	100-200
Cilicrosa roxburghii	4.83	0.05	0.03	1000-3000	-
Kiwi Fruit (*Actinidia sinensis*)	-	-	-	100-470	-
Hawthorn	0.82	0.02	0.05	100-150	-
Orange	0.55	0.08	0.03	50.0	-
Tomato	0.31	0.03	0.02	11.8	-
Carrot	4.00	0.02	0.05	8.0	-

Table 5: Comparison of nutritive values of Seabuckthorn and common types of fodder (Zhong, 2006)

Fodder types	Crude fat	Crude protein	Crude fiber	Lysine	Methionine+cystein	Ca	P
Seabukcthorn leaves	4.1	20.7	15.6	0.73	0.13	1.18	0.18
Seabuckthorn seeds	10.2	26.4	12.3	0.42	0.59	0.31	0.34
Seabuckthorn fruits residues	11.6	18.3	12.7	0.84	0.06	0.19	0.15
Green alfafa (*Medicago sativa*)		5.3	10.7	0.20	0.08	0.49	0.09
Carrot	0	0.9	0.9	0.04	0.06	0.03	0.01
Sorghum seeds	3.3	8.5	1.5	0.24	0.21	0.09	0.36
Maize seeds	3.5	8.5	1.3	0.26	0.48	0.02	0.21
Wheat seeds	1.8	11.1	2.2	0.35	0.56	0.05	0.32
Broad beans	1.4	35.2	5.9	1.82	0.79	0.09	0.38
Soybeans	1.6	37.1	4.9	2.51	0.92	0.25	0.55

Table 5 shows the average nutritive value in leaves, seeds, Seabuckthorn residues, type forage, legumes, carrot, sorghum seeds, maize seeds, wheat seeds and soybeans. Firstly, the content of crude fiber in Seabuckthorn leaves, seeds, and also fruit residues is somewhat higher than most other foods. On the other hand, the crude protein content in the sorghum seeds and maize seeds are lower than soybean and leaves Seabuckthorn are lower in crude protein content compared to beans and peas. Carrots have the lowest crude protein compared with other type fodder. Lysine content in Seabuckthorn fruit residues and seeds are somewhat lower than soybeans, but even so higher than that all the others. The methionine and cysteine in soybeans and also broad beans were very high. Again, carrots have been the lowest nutritive value of them.

2.2 Animals Utilization of nutrients of Seabuckthon

2.2.1 Feeding and management of laying hens

In this research are not discussed all species, but focused on chicken-laying hens in order to meet the egg quality and egg production that is used Seabuckthorn in the diet. Domesticated species, for instances duck, geese, turkey, chicken, game birds (quails and pheasant) and ratites (emus, ostriches) have been used for human utilization. The poultry production plays an important role in human consumption due to a broad spectrum contribution and then very strong demand for human beings, especially in developing countries, this includes feather, skin, manure fertilizer, fuel, meat and egg production (Holik et al., 2009). Worldwide, eggs have high demand and a valuable source of protein as an excellent source of all essential nutrients to maintain our body health.

The nutrition of laying hens requires to improve the nutritive value of eggs. In order to supply good nutrition and health to laying hens, we have to consider according to breed, age, and the purpose of production. The feeding management as a balanced in their feed ration, either energy, proteins, vitamins and minerals should be taken (King'ori AM, 2012).

For instance, crude protein should require 16 grams/day/1 hens, metabolizable energy should be 1.25 MJ. The most important is calcium, it must be provided 3.3-3.8 % of calcium in diet. This nutrient is a basic and critical factor of eggs production and eggshell, if the content of calcium is lower or higher calcium than the requirement, it may result some problem, for example decreasing of egg production, egg size, and mortality. Timing consideration is also important should be fed for laying hens ideally in the afternoon, when the calcium requirement is high (Lesson and Summers, 2008; King'ori AM, 2012). There is feed intake pattern for laying hens based on internal and external factors, internal factors may include genotype, general health, age, production stage and the external factor like rearing programs or housing, nutrition, micro climate and stress levels (Ledvinka et al., 2012).

In order to determine the nutritional requirements to attain egg quality, physical and chemical properties need to be considered such important factors. These are: eggshell quality, nutritional composition, albumen quality, free any defects for instance mottling, blood pot, yolk pigmentation and egg size. Almost these parameters may be influenced by a broad range of dietary feed, poultry management, and by type of housing system (King'ori AM, 2012).

The dietary and nutritional effect of Seabuckthorn compound has been reported that Seabuckthorn compounds can influence the egg quality and poultry products, for example, Rahman et al. (2008) reported that organic acids significantly ($P < 0.05$) enhanced the egg production, eggshell, albumen index and feed conversion. However, organic acids compound in Seabuckthorn did not a significant influence on the egg quality, for example egg mass, egg weight, body composition, laying rate, feed intake as well as feed conversion (Świątkiewicz et al., 2010; Rahman et al., 2008). Vitamin E and vitamin C have a great role to enhance egg production and egg quality. Vitamin C and vitamin E supplemented in a diet were investigated that these vitamins have beneficial effects on egg quality and it can improve body weight gain, egg production and egg weight as well as shell thickness were increased, but mortality significantly decreased (Cifcti et al., 2005; Ajakaiye et al., 2011; Kucuk et al., 2003).

On the other hands the percentage of egg shell, haugh unit, and albumin were not modified somehow. In contrast with Biswas et al. (2010) reported that vitamin E did not affected the egg quality, but this vitamin was beneficial for performance of production and haugh unit score. Moreover the grade of yolk color more orange and carotenoid can increase the weight of yolk or egg mass (Remes et al., 2011; Galea et al., 2011) and omega 3 can be enhance the egg production and positive effect on egg weight, feed intake, and mortality (Yannakopoulus et al., 2005). Seabuckthorn contains great protein and amino acids to promote beneficial value (Uransanaa et al., 2003). Varghese et al (2009) observed that proteins, lipids and essential fatty acids content in diet can increase the number of eggs production and influences egg quality.

Al-Daraji et al. (2011) revealed that omega 3 fatty acids are needed in a diet may influence the feeding consumption and as a result, it also influences the performance, reproduction, and production of poultry. In addition, omega 6 type in poultry diet was observed can influence the efficiency of carcass and promote a good performance.

Seabuckthorn has a great chemical compound due to its bioactive substances including vitamins, particularly vitamin C (Zeb et al., 2009; Shah et al., 2007), vitamin E and carotenoid, sugar, fatty acids, free amino acids, organic compound, volatile compound, and mineral components and oil, these components are used for nutritional and medicinal for human beings and other animals. These studies have shown that Seabuckthorn is kind of amazing plant due to great compounds is used for pharmacological purposes (Zeb et al., 2009).

Seabuckthorn has investigated regarding its industrial application and nutritional effect such as lipid and steroid may be needed during metabolic activity, for example Seabuckthorn is used due to positive effects for health, immune function, safety aspect, antioxidant aspect, may influences skin and mucosa, it can prevent such cardiovascular diseases, improves the immune system especially during chemotherapy. The research has been reported that Seabuckthorn has anti-cancer effect thanks to seed oil of Seabuckthorn (Yang et al., 2002).

According to the table 4, the nutritional of Seabuckthorn has valuable great vitamins A, vitamin C and vitamin K compared to other fruits and vegetables. Vitamin A and vitamin K are linked to blood spots of yolk (Galea et al., 2011). King'ori AM (2012) also reported that blood spots of yolk egg because of lack of vitamin A and vitamin C. Vitamin C was investigated enhanced laying hens performance and reported that this vitamin influenced vitamin E and several parameters of egg quality such as yolk color (Skřivan et al., 2013). Fuhermore, vitamin C and vitamin K play an important role to increase the egg mass and egg production, but unfortunately, haugh unit was not influenced by vitamin C and vitamin K (Park et al., 2005). Fernandes (2009) reported that the vitamin K composition in diet was not significant modified the eggshell quality, egg mass, feed conversion (kg/kg), feed intake, eggshell weight, and percentage of cracked shell. Vitamin K influences the performance as well as bone mineralization.

Vitamin B and vitamin D are linked to calcium as well as phosphorus lead to egg shell quality and shell thickness (Galea et al., 2011; King'ori AM 2012). Vitamin B, particularly vitamin B6 was observed on the effect of egg quality, the results show that vitamin B6 significantly increases the egg weight and yolk weight, but not significantly influence the albumen thickness (Horrocks, et al., 2011).

Seabuckthorn contains high level of vitamin C, approximately in berries contains 75 % of vitamin C or it ranges about (191-295.6 mg/100 g). High amount of antioxidants including phenolic compounds, such as flavonoids, ascorbic acids, tocopherols and some health fatty acids. For this reason the Seabuckhorn gains popularity around the world (Bala et al., 2011; Shah et al, 2007). The main phenolic compound is also important for example, antioxidant and health beneficial effect. This content may influence the color even though, depends on processing and storage (Sa´nchez et al., 2008). Phenolic compound can influence and improve the food quality of egg protein properties (Hassan et al., 2012). Main compounds of Seabuckthorn described into structural biochemistry such as phenolic, vitamin C, vitamin K, and vitamin E are presented in the figure 4 in the annexes.

In addition, Tarasewicz (2006) reported that crude protein (CP) level in a diet did not influence the final body weight and the mortality and Kwari (2011) also observed that there was not any effect of CP in diet to feed utilization, egg production, egg weight and shell quality. In contrast with Uddin et al. (1991) reported that the diet with high level of CP enhances egg weight, however egg quality parameters like albumen index, yolk index, shell thickness, albumin protein, and others were not significant. Adeyemo et al. (2012) also investigated 17 % of CP in diet have positive impact on egg quality compared to 14 % CP, 15 % CP, 16 % CP, these contents were able to promote the poultry performance and increase the egg production, but did not significantly influence egg quality.

The experiment investigated by Imik (2006) reported that methionine can influence the egg quality parameters, for example, increases the number of egg production and shell thickness. The lysine content can influence the albumin index and the number of eggs was higher. The high calcium content found in powdered leaves of *Sophora*, dried alfalfa, and Seabuckthorn leaves. Thus, the phosphorous content shows Seabuckthorn seed and peas are good sources of calcium, but even soybeans were the highest phosphor compared to other fodder.

King'ori AM (2012) reported that calcium together with phosphorous (P) are important factors in order to get good egg shell quality and egg production. Skřivan (2010) observed that phosphor can influence the feed intake and performance of poultry, however, excess of P can reduce the egg production, eggshell and fed intake, moreover, those influences depend on the concentration of phosphor in diet. For instance, diet containing 0.3 % of P did not influence the number of egg production and shell strength, even though haugh unit of the egg was highest. The eggshell structure can influence the embryo mortality, moreover the main influence of embryo mortality due to improper incubation (Mróz et al., 2007). Egg quality and performance of laying hens also is influence by the type of housing system. The effect of housing system and egg quality are described in the table 6 below

Table 6: The relation between egg quality and housing system (Ledvinka et al., 2012)

Egg quality	Conventional cage	**Enriched cage**	Aviar	Litter
Albumen share (%)	60.9	62.0	62.3	60.9
Yolk share (%)	26.8	25.4	25.3	26.8
Shell share (%)	12.3	12.6	12.6	12.3
HU	90.3	81.3	78.2	85.4
Shell thickness (mm)	0.355	0.380	0.387	0.358

$P \leq 0.05$

From Table 6 shows that enriched cage system was significantly influenced by egg quality, the albumen content was higher about 62 %, the shell share is about 12.6 % and yolk share is about 25.4 %. The environment and the cage system can cause the egg thickness. The cage system, environmental and welfare must be considered in order to get good quality of egg (Barbosa et al., 2006). However, each housing system has a diverse influence on egg quality, and it depends on the purposes of production. Table 7 shows the effect housing on laying hens performance.

Table 7: The effect housing on laying hens performance (Ledvinka et al., 2012)

Parameters	Conventional cage	**Enriched cage**	Aviar	Litter
Egg production (monthly/hen)	272	**287**	268	198
Feed intake (g)	143	140	172	195
Egg weight (g)	60.1	**63.3**	62.2	59.5

$P \leq 0.05$

From Table 7 shows that the enriched cage has a higher number of eggs than other housing system, and there were also heavier eggs. Egg productions is influenced by species or breed of laying hens, the breed of egg type can produce approximately 260-300 eggs and the meat type breeds produce approximately 150-170 eggs. The production is much more depending on the organic nutrients content, such as crude protein and lysine and it also can be declined with age. The egg weight is influenced by methionine and linoleic acid content and egg weight is also influenced by age, it means that during the onset of laying period the hens have low weight about 40-43 g and at the end of laying period the hens have higher weight about 70-75 g. In the second cycle period the egg weight will increase about 10 % (Lesson and Summers, 2008).

Moreover, the eggs laid in the morning are heavier than eggs laid later, for examples at 6 a.m. in the morning the egg weight is 63 g, at 10 am the egg weight is 61.6 g, at 2 p.m. the egg weight is 61.2 g. The egg shape affects the hatchability, and it is influenced by age of laying hens. Shape may be circular if eggs laid at the onset of laying and eggs can be longer if laid at the end of laying (Tumova et al., 2005). Egg quality and yolk color also depend on the oxycarotenoids content in diet.

Dumbrava et al. (2006) reported that carotenoids found in the yolk due to feed was added by Seabuckthorn is about 4 % in a diet and in the same period was observed an increased number of eggs as well. Ben-Mahmoud (2013) was observed 2 experiments with different broiler slow growing genotype NL-JA757 and broiler chicks ROSS 308, these results shown that experiment 1 with 5 % in a diet did not significant influence on mortality and health, however it can promote the color of broiler skin. Experiment 2 with diet contains 15 % of Seabuckthorn promoted mortality and alter the color to a more yellowish the skin pigmentation and decrease a feed conversion. The composition of Seabuckthorn is actually different depends on the origin, altitude, species, climate, time of harvesting, type of soil, the method of processing and variable locations (Shah et al., 2007; Oprica et al., 2009).

Clearly, poultry need well-balanced diets and the important thing is to know the daily nutrient requirement, adequate and precise nutrition and proper management. Many nutrients may be determined by direct way like chemical analysis for example fats, calcium, sodium, and crude protein also metabolizable energy and amino acid should be established (Lesson and Summers, 2008). This is a critical factor for the laying hens management, for instance considering the adequate diet or feeding of birds based on the nutritional requirements aspects is sometimes more costly, especially during the rearing program and even though we know that it has influence on egg quality as well. Several alternative feeding in small holder such as by product of agriculture is used in the tropics and subtropics. Some alternative components can be used in feed mixture, for example plant of medicinal herbs or Seabuckthorn in poultry nutrition is one of the great solutions to solve those problems. Table 8 provides examples of such specifications for the layers.

Table 8: Diet specification for layers (Lesson and summer, 2008)

Approximate age	18-32 wks		32-45 wks		45-60 wks		60-70 wks	
Feed intake (g/bird/day)	90	95	95	100	100	105	100	110
Crude Protein (%)	20	19	19	18	17.5	16.5	16	15
Metabolize Energy (kcal/kg)	2900	2900	2875	2875	2850	2850	2800	2800
Calcium (%)	4.2	4	4.4	4.2	4.5	4.3	4.6	4.4
Available Phosphorus (%)	0.5	0.48	0.43	0.4	0.38	0.36	0.33	0.31
Sodium (%)	0.18	0.17	0.17	0.16	0.16	0.15	0.16	0.15
Linoleic acid (%)	1.8	1.7	1.5	1.4	1.3	1.2	1.2	1.1
Methionine (%)	0.45	0.43	0.41	0.39	0.39	0.37	0.34	0.32
Methione+Cystine (%)	0.75	0.71	0.70	0.67	0.67	0.64	0.6	0.57
Lysine (%)	0.86	0.82	0.80	0.76	0.78	0.74	0.73	0.69
Threonine (%)	0.69	0.66	0.64	0.61	0.60	0.57	0.55	0.52
Arginine (%)	0.88	0.84	0.82	0.78	0.77	0.73	0.74	0.70
Valine (%)	0.77	0.73	0.72	0.68	0.67	0.64	0.63	0.60
Leucine (%)	0.53	0.50	0.48	0.46	0.43	0.41	0.40	0.38
Isoleucine (%)	0.68	0.65	0.63	0.60	0.58	0.55	0.53	0.50
Histidine (%)	0.17	0.16	0.15	0.14	0.13	0.12	0.12	0.11
Phenylalanine	0.52	0.49	0.46	0.44	0.42	0.41	0.39	
Vitamins (per kg of diet):								
Vitamin A (I.U)				8000				
Vitamin D3 (I.U)				3500				
Vitamin E (I.U)				50				
Vitamin K (I.U)				3				
Thiamin (mg)				2				
Riboflavin (mg)				5				
Pyridoxin (mg)				3				
Pantothenic acid (mg)				10				
Folic acid (mg)				1				
Biotin (µg)				100				
Niacin (mg)				40				
Choline (mg)				400				
Vitamin B12 (µg)				10				
Trace minerals (per kg of diet)								
Manganase (mg)				60				
Iron (mg)				30				
Copper (mg)				5				
Zinc (mg)				50				
Iodine (mg)				1				
Selenium (mg)				0.3				

3. Aims of the thesis

To find the impact of the Seabuckthorn (*Hippophae rhamnoides*) supplement in feed ration on the productivity of laying hens and egg quality.

Hypothesis

1. Supplemented Seabuckthorn will increase the productivity of laying hens products.
2. Seabuckthorn in feeding supplementation will influence the yolk color of the egg.

4. Material and Methods

4.1 Methodology

a) Writing of literature review from scientific sources found in scientific databases based on the keywords; keywords: Seabuckthorn, feed, poultry products, laying hens, quality of eggs.

b) Two experiments were conducted at the ITP (International Testing of Poultry) on winter 2012 to autumn 2013 in Ústrašice, Czech Republic. The total number of laying hens used in the experiments was 2160 animals.

First experiment, there were 1440 hens divided into 48 pens within 7 periods- (1 period = 4 weeks). A diet containing 5 % of Seabuckthorn (*Hippophae rhamnoides*) (Treatment). Diet without Seabuckthorn (*Hippophae rhamnoides*) (Control).

Second Experiment, there were 720 hens used- divided into 12 pens within 2 period (period 6 and period 7). A diet containing 13.5 % of Seabuckthorn (*Hippophae rhamnoides*) (Treatment). Diet without Seabuckthorn (*Hippophae rhamnoides*) (Control).

The external and internal quality of eggs was determined at 28th week and 32nd week of age of the poultry layer during 3 periods (period 3, 5, and 7). The parameters observed during this period were as in table 11 the parameters observed (see table 12)

Table 9: Parameter observed in eggs of layers

Traits	Units
Egg production	%
Egg weight	g
Egg shape index	
Haugh unit	
Yolk weight	g
Yolk color	grade from 1-15
Shell thickness	mm
Disorder eggs	
Blood spot	
Live weight	g
Feed consumption	g

During both experiments were measured these parameters:

- Egg production was collected daily at the same time, every day by hand, individually. Laying was divided into 2 periods: first experiment 6 periods and second experiments 2 periods (1 period= 4weeks) (production per 1 hen per each period).
- Quality of eggs such as egg weight, yolk weight, yolk color, shell color and eggs with a blood spot, strength of shell as well as live weight of animals, and mortality.
- Feed consumption (per hen and per feed day), Feed efficiency = Feed consumed in g / Egg mass production in g.
- Live weight was measured in age 840 days or 28 weeks, individually.
- Egg weight (the average weight for each period, the average weight for the whole testing and measured classification of eggs).
- Percentage of abnormal eggs (grading of eggs to exclude non-standard eggs, cracked eggs, broken eggs, eggs with double yolk or membranes).
- The individual Haugh unit score was calculated with the egg weight and albumen height by using the following equation:
  HU = 100 log (H – 1.7W0.37 + 7.6), Where, HU = Haugh unit; H = Observed height of the albumen in mm; W = Weight of egg (g).

4.2 Management of laying hens

4.2.1. Breed, age, and their placement

Type of hens is ISA Brown from 267 to 322 of age. All hens were kept in coincident environment conditions.

4.2.2 Housing system

Hens were kept in a windowless house with full control of the environment. They were kept in the enriched cage batteries. Enriched cages provided 756 cm^2 of floor space per hen. Enriched cages were equipped with a perch, a nest, a roosting ash place and claw shortening devices in addition to feeders and drinkers. The feed was manually filled in the feeders, water was supplied by automatic nipple drinkers. Droppings were removed from the conveyor belt. Eggs were collected by hands.

4.2.3. Living conditions

The temperature was kept between 18 – 20 °C. Relative humidity was 60 – 70 %. The temperature was regulated by transversal automatically controlled ventilation (fans and air inlets on the opposite side of the house). In cold weather a gas heater was used. Ventilation provided minimum ventilation rate of 3 m^3/hour/kg live weight in winter and 5 m^3/hour/kg live weight in the summer.

4.2.4. Lighting regime

Hens were kept in windowless house. All the birds were submitted to the following lighting program. The lighting regime was 16 hours from 05.00 a.m till 21.00 p.m and the Luminous intensity is 15 – 20 lux.

4.3 Feeding

4.3.1 Chemical analysis of Seabuckthorn

Seabuckthorn (*Hippophae rhammonides*) is used as a dried by-product after fruit processing for juices. The sample was analyzed by local company SEVAC Star, s.r.o, Bohladov-Czech Republic and for chemical composition was analyzed in the department of Chemistry / State Veterinary Institute in Prague as described in table 10

Table 10: The chemical composition the sample of Seabuckthorn

Ingredients	Percentage	Unit	Analysis/Methodology
Fat	17.14	g/100g	SOP NO: 21 (Gravimeter)
N-substances	20.87	g/100g	SOP NO: 23 (Kjeldahl)
Calcium	400.0	mg/kg	SOP NO: 02 (AAS- flame)
Phosphate	3208	mg/kg	Photometry
Starch	1.79	g/100g	SOP NO: 36 (Polarimetry)
Sugar	3.58	g/100g	SOP NO: 35
Fiber	18.13	g/100g	CSN EN ISO 6865
Lysine	7.85	g/kg	GC-FID
Methionine.	2.82	g/kg	GC-FID
Ash	2.02	g/100g	SOP NO: 26 (Gravimeter)
Dry weight	93.43	g/100g	SOP NO: 25 (Gravimeter)

Hens were fed with two types of feed: diet with Seabuckthorn and a diet without SB as a control. Both complete feeds were in mash form and fed *ad libitum*. The feed was supplied by local company SEVAC Star, s.r.o, Bohladov, Czech Republic. Diet was ISO caloric and ISO nitrogenous and methionine requirement of (NRC, 1994). Feeding mixture (rations) for laying hens (see in table 10 and table 11).

4.3.2 Feeding mixture (rations) for laying hens

Feeding mixture (rations) for laying hens in the experiment 1 and experiment 2 (see in table 11 and table 12**)**

Table 11: Feeding mixture for laying hens in the experiment 1

Ingredients	**Seabuckthorn (%)**		**Dynin-control (%)**	
Wheat	23.25		28.25	
Fish meal	2.00		2.00	
Corn	39.68		39.68	
Soybean extracts meal	18.80		18.8	
Vegetable oil	1.00		1.00	
DL-methionine	0.13		0.13	
Salt	0.25		0.25	
Limestone	8.00		8.00	
MCP – monocalciumphosphate	1.30		1.30	
Seabuckthorn dried	5.00		-	
Supplement of biofactor	0.50		0.50	
Nutrients	**Amount**		**Amount**	
Crude protein	172.10	g/kg	172.10	g/kg
ME	11.35	MJ/kg	11.35	MJ/kg
Lysine	9.19	g/kg	9.19	g/kg
Methionine	4.79	g/kg	4.79	g/kg
Calcium	37.10	g/kg	37.10	g/kg
Phosphorus	6.70	g/kg	6.70	g/kg
Sodium	1.50	g/kg	1.50	g/kg

Table 12: Feeding mixture for laying hens in the experiment 2

Ingredients	Seabuckthorn (%)		Lysa-control (%)	
Wheat	35.00		40.00	
Barley	9.200		18.500	
Corn	13.50		10.00	
Soybean extracts meal	16.80		20.00	
Vegetable oil	1.50		1.00	
DL-methionine	0.05		0.05	
Salt	0.35		0.35	
Limestone	8.40		8.40	
MCP – monocalciumphosphate	1.20		1.20	
Seabuckthorn dried	13.50		-	
Supplement of biofactors	0.50		0.50	
Nutrients	**Amount**		**Amount**	
Crude protein	172.83	g/kg	173.10	g/kg
ME	10.86	MJ/kg	10.91	MJ/kg
Lysine	7.98	g/kg	8.32	g/kg
Methionine	3.74	g/kg	3.76	g/kg
Calcium	37.69	g/kg	37.80	g/kg
Phosphorus	6.09	g/kg	6.20	g/kg
Sodium	1.65	g/kg	1.70	g/kg

4.4 Statistical Analysis

Data collected was processed and analyzed by statistical software SAS Version 9.3. The data was subjected to the ANOVA procedure Duncan's multiple range tests.

5. Results

Two experiments were processed during our study on the total number of 2160 laying hens. No statistical significant differences between groups of hens fed by 5 % of Seabuckthorn in a diet versus control group without SB were found in egg production and egg weight during the 1.experiment (7 periods of laying). But significant differences were found in the 2.experiment (2 periods) with higher content of SB (13.5 %) in treatment group (P < 0.01). The results showed that the production of eggs and egg weight were higher – better, in the control group, so our first hypothesis was not confirmed.

According to our results in the table 13 and 14 the yolk weight was also significantly higher in laying hens fed by control feed mixture during breeding periods in both experiments. However, interestingly compared with control, the color of yolk increased significantly – more orange, in a diet with Seabuckthorn also in both experiments (P < 0.01), respectively both concentrations of SB (5 % and 13.5 %) in a diet (shown in Figure 6 and 8). This was probably due to the carotenoid compounds in Seabucthorn. The carotenoids in Seabuckthorn contains all pigments such as beta carotene, lycopene, and zeaxanthine (Table 3). So the second hypothesis was confirmed by both experiments.

Experiment 1.

Table 13: Statistical significant differences between Seabuckthorn (5 % in diet) against control (C)

Parameters	Seabucktorn		Control	Probability
Hens live weight	1951.47 ± 233.80	<	2007.47 ± 232.21	P < 0.01
Egg weight	61.96 ± 4.72	<	62.31 ± 4.68	P < 0.05
Egg length	56.09 ± 2.08	<	57.33 ± 1.93	P < 0.01
Yolk weight	16.49 ± 1.55	<	17.34 ± 1.22	P < 0.01
Yolk color	9.76 ± 1.63	>	8.27 ± 0.83	P < 0.01

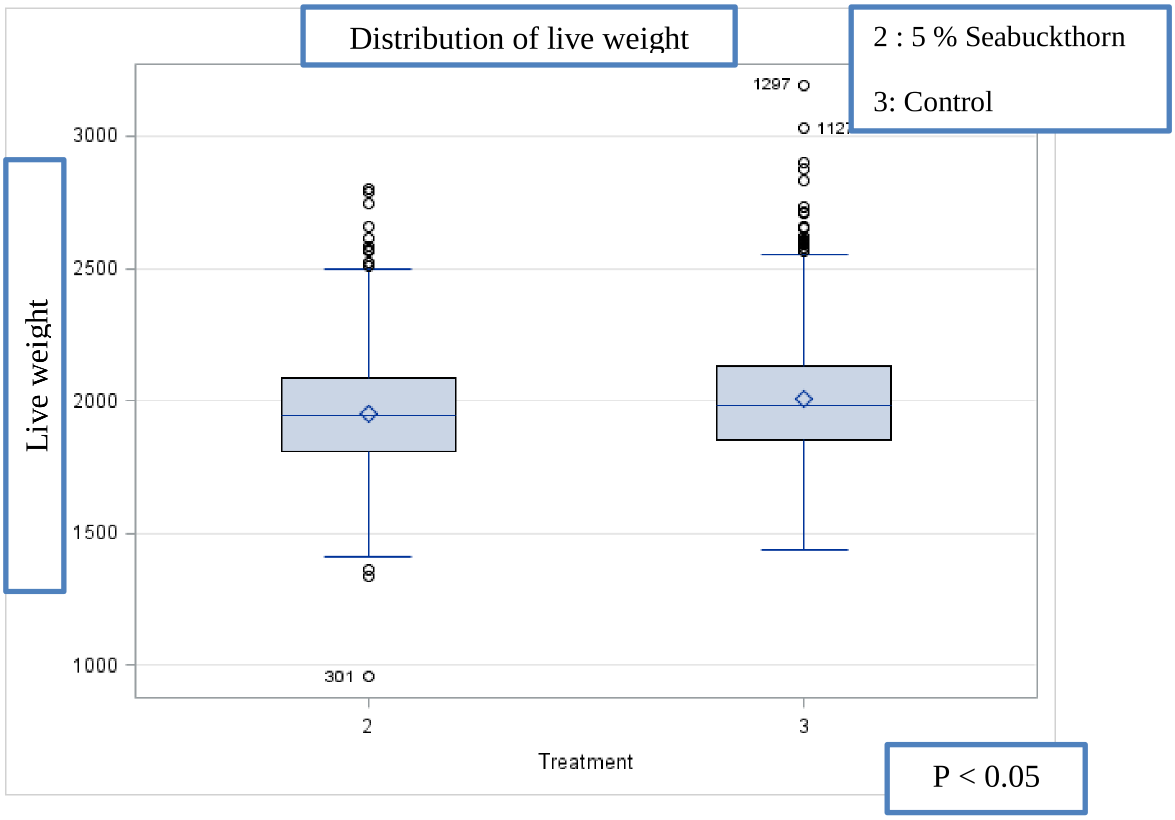

Figure 5: The distribution of live weight under 5 % of Seabuckthorn

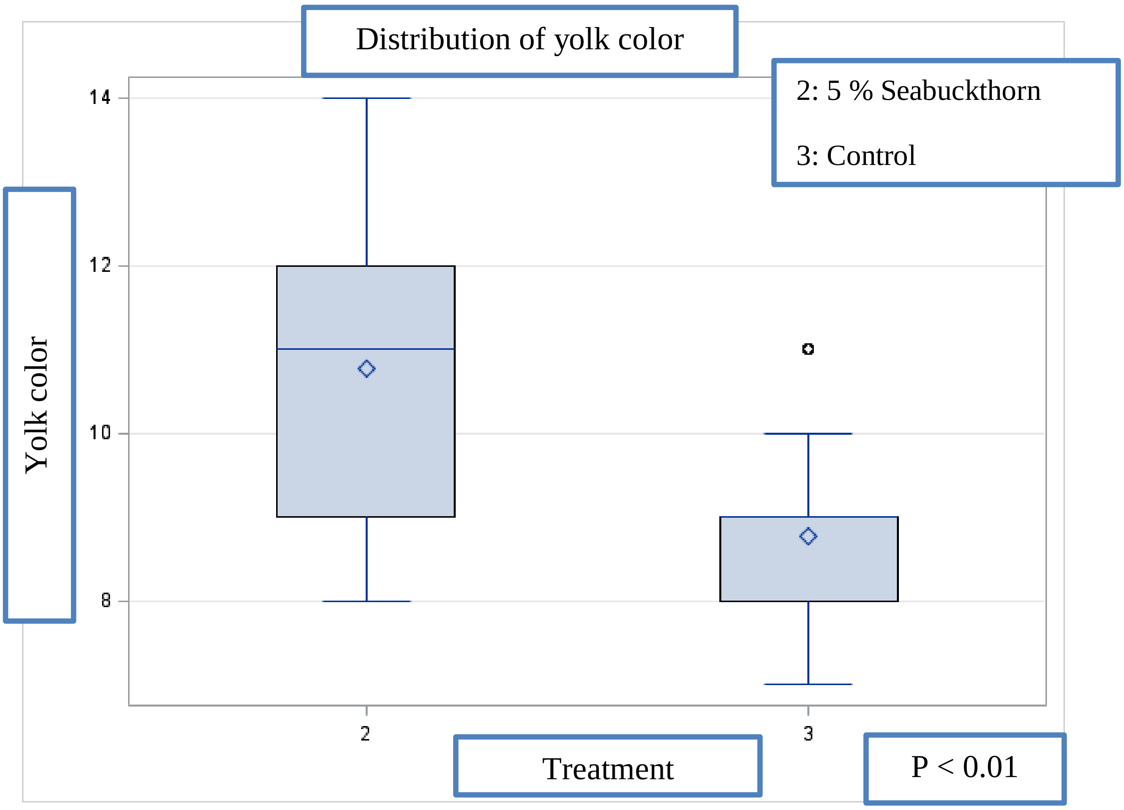

Figure 6: The distribution of yolk color under 5 % of Seabuckthorn

There were found significant differences between supplemented SB and control group in the live weight of hens, which was significantly lower in treatment SB compared with control ($P < 0.01$) in both experiments, see the Figure 5 and 7.

There were no differences between groups in egg width, shell strength, albumen height, shell color, and shell thickness, as well as egg production in the first experiment, but the egg length was significantly lower in treatment 5% SB compared with control ($P < 0.01$).

The table 14 demonstrates important results from the second experiment; live weight of hens, egg weight, also yolk weight, and egg length were significantly lower in SB treatment compared with control ($P < 0.05$).

Experiment 2

Table 14: Statistical significant differences between Seabuckthorn (13.5 % in diet) against control (C)

Parameters	Seabuckthorn		Control	Probability
Hens live weight	1848.98 ± 185.73	$<$	1897.02 ± 173.76	$P < 0.05$
Egg weight	59.96 ± 4.55	$<$	63.14 ± 4.23	$P < 0.01$
Egg length	56.09 ± 2.08	$<$	57.33 ± 1.93	$P < 0.01$
Yolk weight	16.00 ± 1.35	$<$	17.34 ± 1.36	$P < 0.05$
Yolk color	8.58 ± 1.41	$>$	6.28 ± 0.94	$P < 0.01$

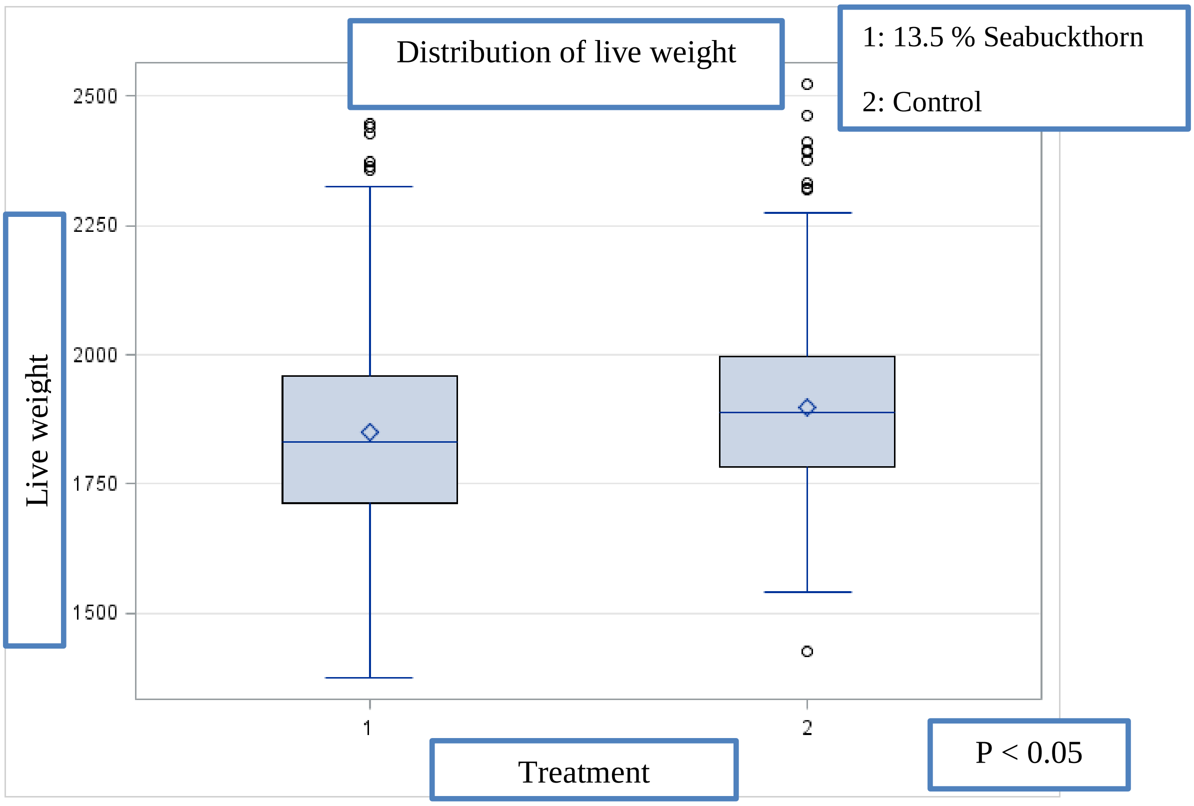

Figure 7: The distribution of live weight under 13.5 % of Seabuckthorn

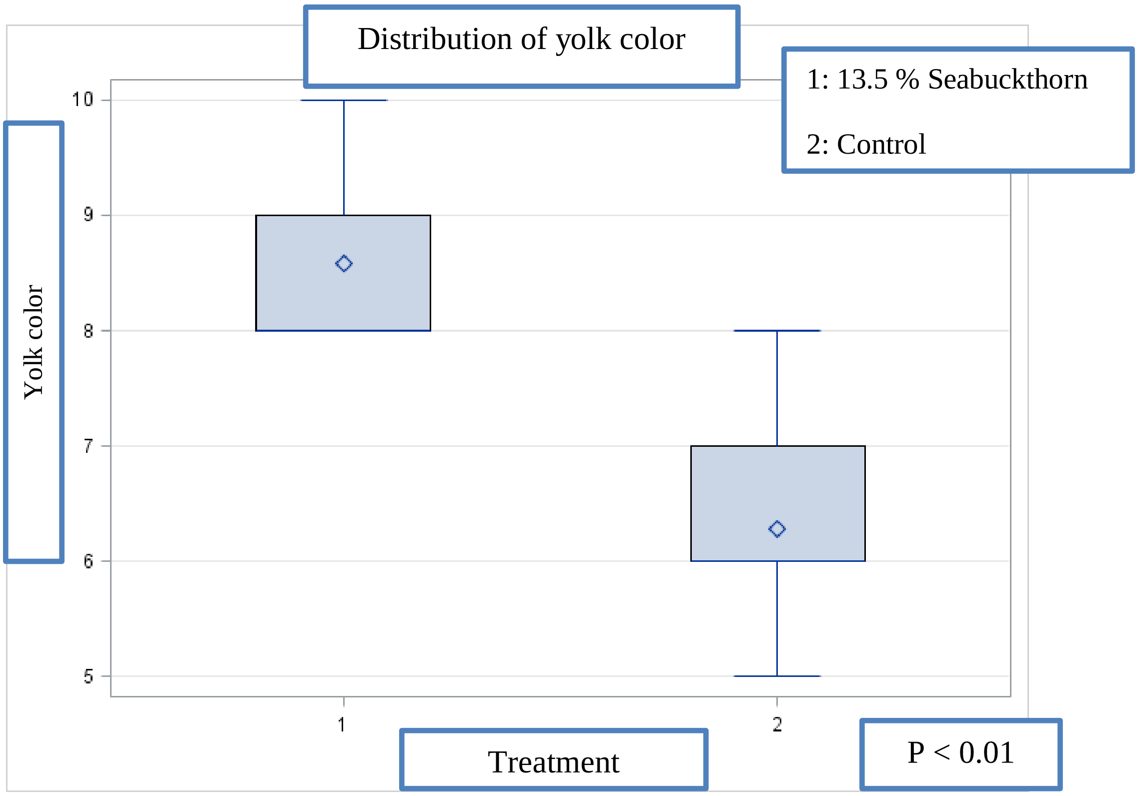

Figure 8: The distribution of yolk color under 13.5 % of Seabuckthorn

There were no differences in egg width, shell strength, albumen height, shell color, and shell thickness in the second experiment.

The feed consumption was highest in group fed by 13.5 % of SB, but the feed conversion was not better in this group, probably due to the higher content of crude fiber in SB. However, we found higher number of some egg disorders in the control group (P < 0.01), as shows the Figure 9

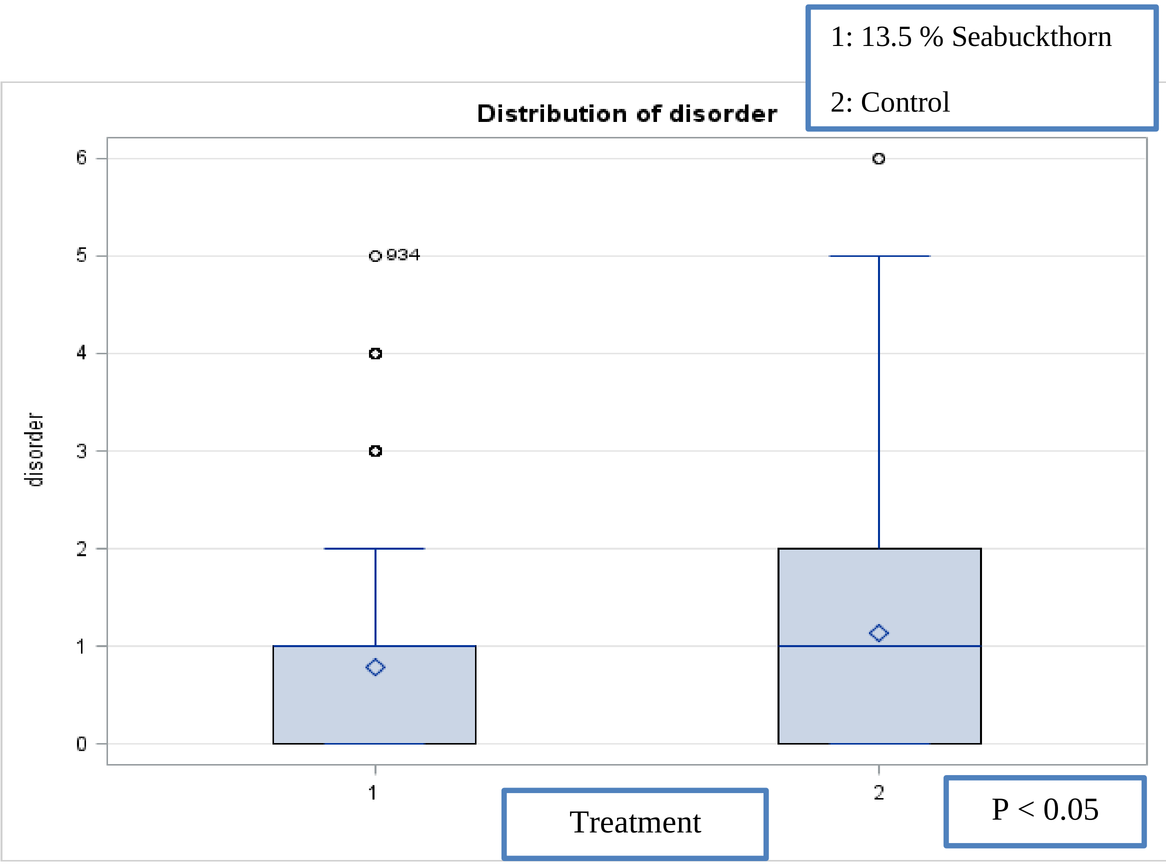

Figure 9: Distribution of abnormal egg under 13.5 % Seabuckthorn

6. Discussion

The fruits of Seabuckthorn (*Hippophae rhamnoides*) have been used for human consumption, medical, cosmetic, animals feeding, and fodder for poultry (Singh et al., 2011; Demidova et al., 2009; Utioh et al., 2009). However, the rest of Seabuckthorn or by-product from Seabuckthorn has variable contents of nutritive value, influence some parameters of egg quality and performance of laying hens. Even though, genotype, general health, age, production stage and the external factor like rearing programs or housing, and nutrition, micro climate and stress levels are also influences of performance and egg quality of laying hens (Ledvinka et al., 2012).

6.1 The first experiment: Productivity of laying hens and egg quality under supplemented 5 % of Seabuckthorn in the diet

The experiment 1 was conducted during 7 periods of laying with 1440 ISA Brown hens, 1 period is equal 4 weeks. There was measured the effect of 5 % Seabuckthorn supplemented in a diet, on the number of eggs and egg quality such as egg weight, egg length, egg width, shell strength, albumen height, yolk weight, shell color, and shell thickness; also the live weight of hens, disorders of eggs, feed consumption, and mortality. As shown in table 13, parameters like live weight of hens, also egg weight, egg length, and yolk weight were significantly lower in treatment SB compared with control ($P < 0.01$). These results were closely in coincident with (Hazzanuzaman, 2013). Interestingly, the parameters of shell color were not significantly influenced after consumption the diet containing Seabuckthorn. Because of the eggshell color needs three main pigments - zinc chelate, biliverdin, and protoporphyrin in order to put in place the color pigment onto the egg shell (Butcher G, 2011) whereas those components does not appear in Seabuckthorn.

However, yolk color was efficient ($P < 0.01$) and significantly higher with 5 % of SB meal compared to control, the color of the yolk was more orange (see figure 6). This result is connected to those of (Dumbrava et al., 2006) who reported a yellow-orange color of yolk due to the carotenoids added by Seabuckthorn. The effect of 5 % of Seabuckthorn on the live weight of hens was significantly lower in SB treatment compared with control ($P < 0.05$). These results are connected to those of (Zhong et al., 2006; Solcan, 2011) who reported a weight of chicken was higher with supplemented Seabucthorn from 7 % to 10 %, so our 5 % of Seabuckthorn in a diet is not good enough to increase the weight of hens.

Lower effect after consumption of Seabukcthorn due to these experiments used by-products of Seabuckthorn after utilization for human consumption it has obviously lower nutritive value.

There were not significant differences in egg length, egg width, shell strength, albumen height, shell thickness and blood spots after supplemented with Seabuckthorn compared with control. As shown in figure 9, the number of abnormal eggs, as cracked egg, double yolk egg, and broken eggs were higher in control compared with treatment ($P < 0.05$). These results were closely in coincident with Zhong (2006), because of crude protein plays an important role in order to maintain the quality of the egg and avoid disorders of egg. These results are advantages in order to increase the benefit of SB. Fernandes (2012) is also reported that vitamin K which is contained in Seabuckthorn was able to maintain disorders of egg.

The results did not show any significant influences in feed consumption in the diet with Seabuckthorn, this is probably due to palatability and characteristics of feed. This is clear that the first hypothesis was not confirmed for laying hens productivity.

6.2 The second experiment: Productivity of laying hens and egg quality under supplemented 13.5 % of Seabuckthorn in the diet

In the experiment 2, the productivity of laying hens and egg quality under supplemented 13.5 % Seabuckthorn were significantly influenced in egg length ($P < 0.01$) and yolk weight ($P < 0.05$) compared with control (see table 14). However, there were not significantly influenced other egg quality parameters like albumen height, shell color, Haugh unit and blood spots. The yolk color was also significantly higher in treatment SB compared with control ($P < 0.01$), see figure 8. This result was the same like experiment 1 (figure 6) and both experiments were closely in coincident with experiment Dumbrava et al. (2006) and Biswas et al. (2010) who reported that vitamin E contained in Seabuckthorn did not influence the egg quality. However, supplemented Seabuckthorn in a diet significantly influenced the color of egg yolk, hens weight and was able to decrease the number of egg disorders; also promoted hens performance - the number of mortality was higher in control compared with treatment ($P < 0.05$).

Appearance of yolk color or yolk pigmentation plays a great role for consumer purposes. Mostly, the consumers prefer to choose strong and fresh color of the yolk. They believe that more orange and fresh in colorant is much more benefit because of it may contain antioxidants for human health and it also prevent some diseases (Baker et al., 2004). In general, the people prefer more orange due to their assumption that more orange yolk is similar to egg from organic farming and hens were kept independently or in free range system.

Addition of Seabuckthorn for laying hens in this experiment had a negative influence on yolk weight and hens weight compared with control ($P < 0.05$), see table 14. Regarding to this result, there were found low values during treatment compared with control. These phenomena was associate with Uddin, et al. (1991) who reported that content of crude protein in a poultry diet did not influence parameters of egg quality. The weight of hens was lower after consumption of Seabuckthorn, probably it may be working very slowly during digestion in the gastrointestinal tract, because of high content of fiber or cellulose in Seabuckthorn (Kotrbacek et al., 2013). Ben-Mahmoud (2013) reported that lowering of hens weight compared with control is also likely due to the higher content of crude fiber (CF), about 13.5 % in a diet ($P < 0.05$). Feed conversion in this experiment was low compared with control ($P < 0.05$). As we have already known that hens are monogastric animals which have limited digestion for fiber content and it may have a negative effect on the parameters of feed conversion.

As shown in figure 9, number of abnormal eggs in this experiment was significantly decreased in treatment compared with control ($P < 0.05$), these results have positive effect on laying hens performance, because the lower number of disorders is correlation the more advantages that we would get it. Clearly, there are several positive effects of Seabuckthorn in diet - high in vitamins, particularly vitamin C and A which was increasing the egg performances (Cifcti et al., 2005; Ajakaiye et al., 2011; Kucuk et al., 2003).

Feed consumption of laying hens in this experiment was significantly higher compared to control, it could be due to omega 3 and omega 6 fatty acids content in Seabuckthorn. This content is likely influences the palatability and feed consumption. Al-Daraji et al. (2011) revealed that omega 3 and omega 6 fatty acids are needed in a diet and they might influence the feeding consumption and as a result, it also influences the performance, reproduction, and production of poultry. But it could be also due to the higher content of crude fiber and less concentrated nutrients in SB.

The performance of hens, as mortality after consumption 13.5 % Seabuckthorn was slightly higher compared to control, maybe due to lack of some nutrients or some human factor in management during the rearing period. The type of housing system can influence the egg productivity as well, the enriched cages are used in this experiment, which were the same correspondence with Ledvinka et al. (2012) who reported higher egg production in the enriched housing system.

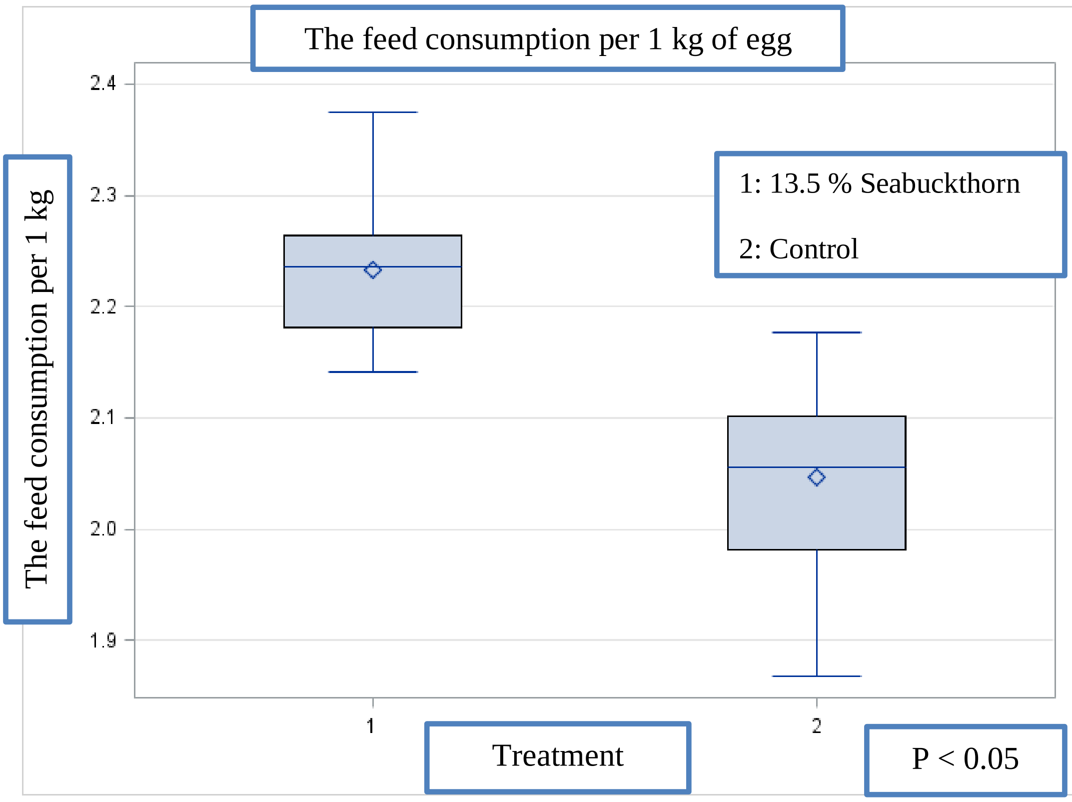

Figure 10: The feed consumption per 1 kg of egg mass (13.5 % of SB)

At the same time, the feed consumption per 1 kg of egg mass was higher ($P < 0.05$) in treatment compared with control (see figure 10). It could be influenced by higher content crude fiber in SB by-product, so maybe lower digestibility and not so concentrated nutrients in by-product used; or on the other hand, because of vitamin C and vitamin K in a diet with SB, because vitamin C and vitamin K plays an important role to increase the egg mass and egg production (Park et al., 2005).

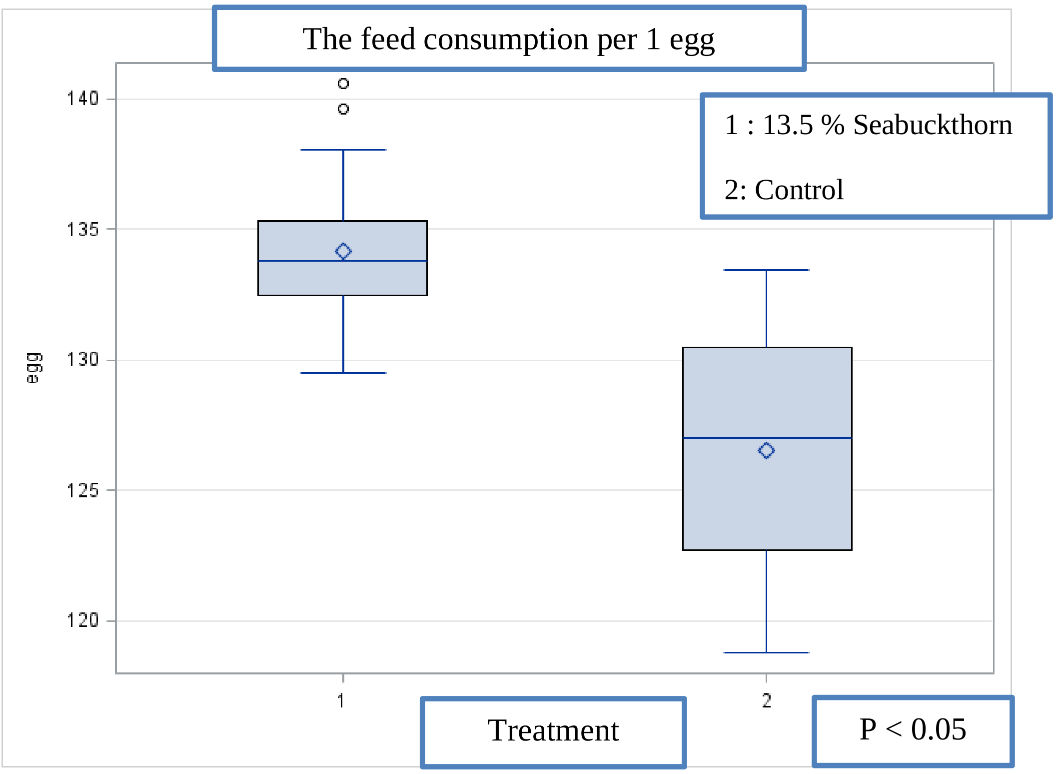

Figure 11: The feed consumption per 1 egg (13.5 % of SB)

As shown in Figure 11, the feed consumption per production of egg supplemented with 13.5 % of SB was significantly higher ($P < 0.05$) than control in correspondence with (Biswas et al., 2010; Zhong et al., 2006) who reported that the number of eggs increased after consumptions with the seeds, leaves and fruit residues of Seabuckthorn under 10% of Seabuckthorn and these results are in coincidence with Zhong et al. (2006) who reported that the rate of laying hens and the number of eggs increased 10.3% and decreasing if the portion of Seabuckthorn up to 10 %. This is clear that the first and second hypothesis were confirmed for laying hens productivity and increases the color of yolk egg.

7. Conclusion

This study was aimed to find any positive effect of the Seabuckthorn (*Hippophae rhamnoides*) supplement in feed ration on the poultry productivity and quality of eggs.

Two experiments were conducted at the ITP (International Testing of Poultry) in Ústrašice in the Czech Republic, with a total number of laying hens 2160 animals. In the first experiment the effect of the diet containing 5 % of Seabuckthorn (SB treatment) was compared with the diet without SB (Control). The laying hens were kept under the same conditions during the second experiment, only the concentration of Seabuckthorn was higher in the diet for hens (13.5 %). Parameters of the production of eggs, quality of eggs, live weight of hens and also feed consumption were measured.

On the basis of our results, there were not found any statistical significant differences between groups of hens fed by 5 % of Seabuckthorn in a diet versus control group without SB in egg production and egg weight during the 1.experiment, but significant differences were found in the 2.experiment (13.5 % of SB) in treatment group ($P < 0.01$). The results showed that the production of eggs and egg weight were higher – better, in the control group, so our first hypothesis was not confirmed.

The yolk weight was also significantly higher in laying hens fed by control feed mixture during breeding periods in both experiments. However, interestingly compared with control, the color of yolk increased significantly – more orange, in a diet with Seabuckthorn also in both experiments ($P < 0.01$), respectively both concentrations of SB (5 % and 13.5 %) in a diet. This qualitative parameter of egg was probably influenced due to the carotenoid compounds in Seabuckthorn. So the second hypothesis was confirmed by both experiments. It is the advantage of SB in preferences of consumers, because they like more orange egg yolks, they look like they come from organic farming.

There were also found significant differences between supplemented SB and control group in the live weight of hens, which was significantly lower in treatment SB compared with control ($P < 0.01$) in both experiments. However the results did not show any differences between groups with both concentrations of SB in egg width, shell strength, albumen height, shell color, and shell thickness.

The feed consumption was the highest in the group fed by 13.5 % of SB, but the feed conversion was not better in this group, probably due to higher content of crude fiber in SB. However, we found a higher number of some egg disorders in the control group (P < 0.01), this result could be quite positive information for farmers.

In general, the Seabuckthorn has a great status due to the wide range of positive effects for human consumption as well as animal feeding. Indeed, supplemented Seabuckthorn 5 % and 13.5 % in a laying hens diet significantly influenced the egg quality and performance of hens. It seems to be a positive significant effect of 5 % of Seabuckthorn in a diet for promoting the productivity of laying hens, and decreasing the number of disorders in eggs, as well as more orange color of yolk owing to carotenoid compounds in Seabuckthorn. However, 13.5 % of Seabukcthorn in a diet were able to decrease the egg quality and productivity of hens, so we cannot recommend this higher concentration of SB in a diet for laying hens as suitable feedstuff.

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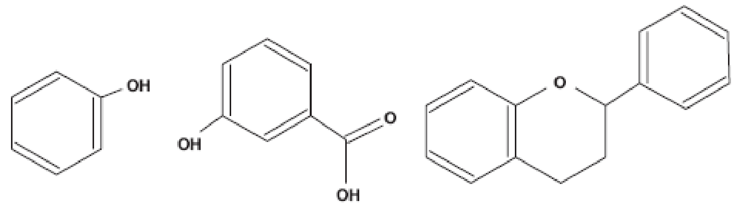
Annexes

Table 2: The distribution and utilization of *Hippophae* (Rajchal, 2008)

Taxons	The Areas of Distribution	Utilization
1. H. *rhamnoides.* Subsp. *rhamnoides*	Scandinavian countries, Baltic Sea Countries, Germany, Belgium, Netherlands, Ireland, Poland, U.K., France, Russia	Many varieties are cultivated in some European countries and Canada
2. H. *rham.* Subsp. *sinensis*	The North, Northwest, Southwest of China	Wild resources are used for ecological restoration and berries are processed for products. Some new varieties are in tests.
3. H. *rham*. Subsp. *Yunnanensis*	Sichuan, Yunnan, Tibet of China	Wild resources are used for.ecological restoration only.
4. H. *rham*. Subsp. *Mongolica*	Siberia of Russia, Mongolia, Xinjiang of China	More than 60 varieties are cultivated in Russia, Mongolia, many East European countries. Many West European countries, Canada and China introduced the Varieties for test.
5. H. *rham*. Subsp. *turkestanica*	India, Pakistan, Afghanistan, Turkmenistan, Kyrgyzstan, Uzbekistan, Kazakhstan, Iran, Turkey, Xinjiang , Tibet of China	Wild resources are used for ecological restoration and berries are processed for various products on a commercial level in India for the production of food, medicine and cosmetics.
6. H. *rham*. Subsp. *fluviatilis*	Around Alps Mountains: Germany, France, Switzerland, Austria, Czech, Slovakia, Italy,	Most of wild resources are protected as forest species. Some berries are collected for processing products
7. H. *rham*. Subsp. *carpatica*	The Capathinan Mountains, TranssylvanianAlps, the valley and the mouths of the Danube and its tributary	Most of wild resources are protected as forest species. Some varieties are cultivated for processing products
8. H. *rham*. Subsp. *caucasica*	TheCaucasusMountains, Georgia, Azerbaijan, Armenia, Ukraine, Romania, Turkey, Bulgaria, Iran, Russia.	Most of wild resources are protected as forest species. Some selected varieties are cultivated for test.
9. H. *goniocarpa*	Sichuan, Qinghai of China	Most of wild resources are protected as forest species. Very few studies have been done.
10. H. *goniocarpa*	Sichuan, Qinghai of China	Most of wild resources are

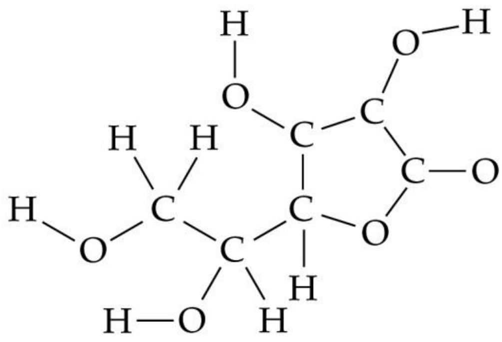
Continue from table 2

Subsp. *litangensis*		protected as forest species. Very few studies have been done.
11. H. *Neurocarpa*	Sichuan, Qinghai, Gansu of China	Most of wild resources are protected as forest species. Very few studies have been done.
12. H. *neurocarpa* Subsp. *Stellatopilosa*	Sichuan, Qinghai, Tibet of China	Most of wild resources are protected as forest species. Very few studies have been done.
13. H. *Tibetana*	Sichuan, Qinghai, Gansu, Tibet of China, Nepal, India	Most of wild resources are protected as grassland species. Very few studies have been done.
14. H. *Gyantsensis*	Tibet of China	Most of wild resources are protected as forest species. Some berries are collected for producing Tibetan medicine.
15. H. *Salicifolia*	The southern slope of Himalayan Mt. Tibet of China, Bhutan, Nepal, India	Most of wild resources are protected as forest species. Some the berries are collected for producing products.

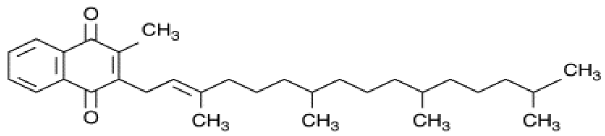


Phenols Phenolic acids Flavonoids

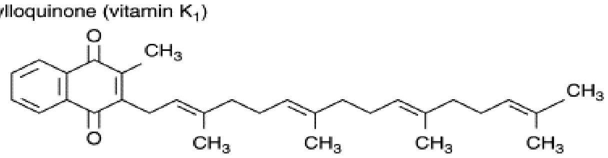
Structures of common phenolic compounds.



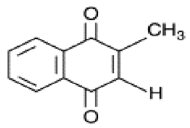
Vitamin C



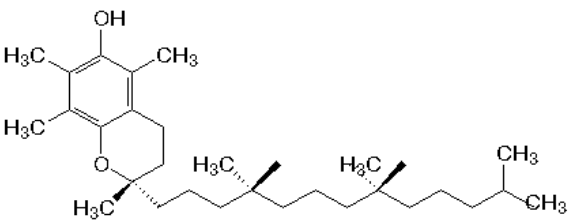
Phylloquinone (vitamin K_1)



Menaquinone-4 (vitamin K_2)



Menadione (vitamin K_3)

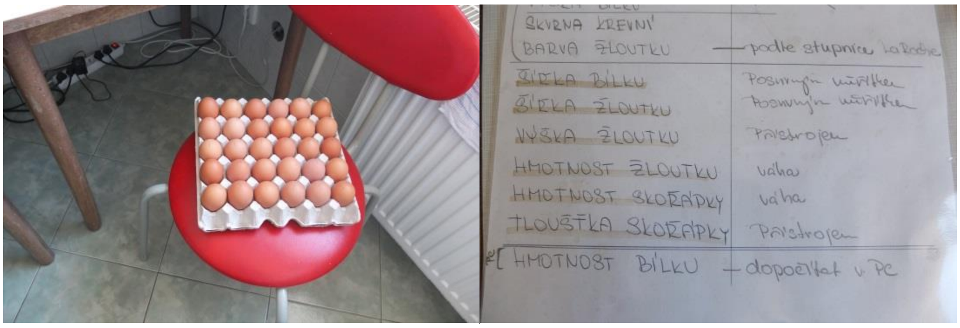


Vitamin E (α-tocopherol)

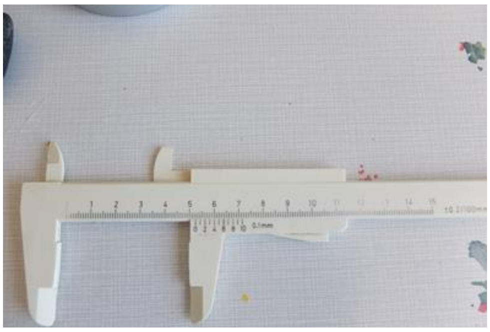
Figure 4: Main compounds of Seabuckthorn (Source: http://chemistry.tutorvista.com/ Accessed 2014-03-26)

Figure 12: Research activities (author: Pebriansyah, 2013)

a) The Poultry/laying hens

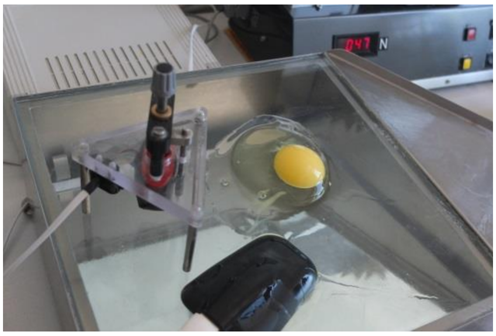


b) Sample of eggs and egg shell color



c) Eggs are shaped

d) Egg weight



e) Blood spot

f) Yolk weight

g) Yolk color

h) Shell thickness