TED ANKARA COLLEGE FOUNDATION PRIVATE HIGH SCHOOL

The Effect of Feeding Laying Hens with
0.25%, 0.50% and 1.00% Horse Chestnut Seed Supplement
on the Total Cholesterol Level of Chicken Egg Yolk

BIOLOGY EXTENDED ESSAY

Supervisor: Leyla YAĞUŞ UNGAN

Name of Candidate: Ersan İLKTAN

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ABSTRACT

Egg yolk is considered to be one of the main sources of cholesterol for humans. The purpose of this study was to investigate the effect of feeding laying hens with dried horse chestnut (*Aesculus hippocastanum*) seed grains as 0.25%, 0.50% and 1.00% supplements to their basal diet on egg yolk cholesterol levels.

Although there is a vast amount of researches about the medical and pharmaceutical effects of extracts of horse chestnut bark, leaves and seeds, to my knowledge, there is no academic research on the effect of feeding poultry with horse chestnut seeds without processing them chemically on egg yolk cholesterol level.

My hypothesis predicted that dried horse chestnut seed supplement in non-toxic small amounts of 0.25%, 0.50% and 1.00% in the basal diet of laying hens is going to lower the egg yolk cholesterol level.

Sixty Brown Nick breed laying hens were used in the experiment by dividing in 4 groups of 15 hens and each group in 5 trial groups of 3 hens. The first group was the control group and other three groups were fed with basal diet and water ad libitum for four weeks with dried grains of horse chestnut seed core grains. A total of 160 eggs were collected (10 from each test group at the end of each week) to analyze the egg yolk cholesterol level by using the enzymatic colorimetric test for cholesterol with lipid clearing factor method as described in Boehringer Manheim Gmbh Biochemica, 1989.

At the end of the experiment, no health issues or deaths of hens were observed. The feed efficiency of laying hens were increased by 3.5% when fed with 1.00% dried horse chestnut seed grain supplement. The results showed that 0.25%, 0.50% and 1.00% horse chestnut seed supplement in the basal diet of laying hens does not affect egg yolk cholesterol levels significantly.

Word count: 305

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INTRODUCTION

There is a small city park in front of our house and I really liked it when we first moved to this neighborhood. It was the trees in the park that draw my attention at first. Later, I learned from my parents that they are horse chestnut trees. Because I like to eat grilled chestnuts (Sweet chestnut, *Castenea sativa*), I had also asked why horse chestnut (*Aesculus hippocastanum*) seeds cannot be eaten. The answer I got was that because they taste bitter but it was not very explanatory about the reason. Since then, I have been thinking about a way of using them for a useful purpose instead of letting them wasted on the ground.

When I searched the Internet about the uses of the horse chestnut, thru many public sites and sources, I noticed that there are several uses of mainly its extract from its bark, seeds and leaves in herbal therapy and in traditional medicine as a treatment of several illnesses like phlebitis (swollen veins), diarrhea, and fever as well as some blood circulation problems[1]. I also learned that horse chestnut seeds are poisonous due to their aescin[2] content.

However, because its common name implied, I became curious whether horse chestnut seeds can be used in feeding animals. Thus, I decided to consult to an academic resource and talked to a professor[3] from the Department of Animal Nutrition and Nutritional Diseases of the Faculty of Veterinary Medicine of Ankara University. At the end of our conversation, it was understood that there are only a few academic research on feeding animals with a horse chestnut seed supplement.

In one of the few academic researches (Avcı et. al., 2010)[4], it is mentioned that Swiss albino male mice were fed with horse chestnut seed extract via a stomach tube. The result of this study shows that the aescin from horse chestnut seed extract has a significant effect on obesity by reducing leptin and total cholesterol levels in blood plasma. Also in another study (Williams and Olsen, 1984)[5], the LD50 value (mean lethal dose; the dose that will kill %50 of a test group)[6] of horse chestnut extract for chicks is given as 10.6mg per gram of body weight for one dose and 6.5mg/g for consecutive two-day dose, and for hamsters it is 10.7mg/g for one dose. It was also stated that

[1] WEISS, R.F., Herbal Medicine. (Translated from the 6th. German edition of Lehrbuch der Phytotherapie by A.R.Meuss). The Bath Press. 1986. ISBN 0-906584-19-1
[2] Aescin: main active chemical in horse chestnut. SIRTORI, C. R., 2001. *Aescin: pharmacology, pharmacokinetics and therapeutic profile*. Pharmacological Research. 2001 Sep; 44(3):183-93.
[3] KÜÇÜKERSAN, Seher, PhD. Ankara University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases.
[4] AVCI, G., KÜÇÜKKURT, İ., AKKOL, E, YEŞİLADA, E. 2010. Effects of escin mixture from the seeds of Aesculus hippocastanum on obesity in mice fed a high fat diet. Pharmaceutical Biology, 48(3): 247–252
[5] WILLIAMS, M.C., OLSEN, J.D. 1984. Toxicity of seeds of three Aesculus spp to chicks and hamsters. Am J Vet Res. 45(3):539-542.
[6] LD50. (n.d.) Saunders Comprehensive Veterinary Dictionary, 3rd edition (2007).

feeding with 80mg/g of extract of seeds of American Horse Chestnut (Ohio buckeye) is not toxic to chicks and hamsters.

Two outcomes of those studies sound interesting to me: “reduced total cholesterol level” and, below a certain dose, it will not be toxic to feed animals with horse chestnut seeds.

We all know that high levels of cholesterol in blood may cause various health problems in cardiovascular systems and it is one of the major health issues humans face with in their adulthood. We also know that one of the sources to blame for high level of cholesterol in blood is the chicken egg yolk.

The nutritional values of horse chestnut are given by Majeed et al. (2010)[7], as shown in Table-1 below:

Component	**Level**	**Component**	**Level**	**Component**	**Level**
Crude protein, %	7.21	Potassium, %	0.79	Cupper, ppm	41.2
Oil, %	2.02	Phosphorus, %	0.18	Zinc, ppm	25.6
Sugar, %	9	Sulfur, %	0.07	Manganese, ppm	6.95
Starch, %	30-40	Calsiyum, %	0.08		
Nitrogen, %	1.15	Iron, ppm	159		

Table-1: Nutritional values of horse chestnut seeds by Majeed et al (2010).

Considering that the aescin content of the seeds of various kinds of horse chestnut is between 8-12 % (ANSM, 2015)[8] and it is 9.5% according to Srijayanta et. al. (1999)[9], the horse chestnut seed cores can be mixed into basal feed in certain small amounts to feed laying hens so that the feed will not have any toxic effect on the chicken.

With the above information gathered, I decided to make an experiment with laying hens by using various amounts of dried grains of the raw seeds mixed into the basal diet to feed them and measuring the cholesterol level of the egg yolks obtained from the eggs they lay. I choose to use the dried grains obtained from the raw seeds directly because it does not require any additional chemical process and it will be a simple method to obtain the necessary grains to feed the animals.

[7] MAJEED, M.,. KHAN, M.A.,BASHIR, A., HUSSAIN, A. 2010. Nutritional Value And Oil Content Of Indian Horse-Chestnut Seed. Global Journal of Science Frontier Research, 10(4):17-19

[8] ANSM. (2015). (Agence Nationale de Securite du Medicament et des Produits de Sante). Aesculus hippocastanum, Horse Chestnut.

[9] SRIJAYANTA, S., RAMAN, A., GOODWIN, BL. A Comparative Study of the Constituents of Aesculus hippocastanum and Aesculus indica. J Med Food. 1999;2(2):45-50.

Although there are many researches on medical and pharmaceutical uses of horse chestnut seed extracts, a limited number of them are focused on the effects on animals fed with it; especially, I could not find any on laying hens when I searched via the Ankara University's academic network resources.

According to R. G. Elkin (2007)[10], over the past few decades there were many researches to reduce the egg yolk cholesterol level by changing the diet of laying hens with "various nutrients, natural products, non-nutritive factors, or pharmacological agents". In a review, Aydın et al (2014)[11] states that the content of the diet of laying hens is one of the parameters effecting the egg yolk cholesterol. It was also shown in nutritional trials on mice (Avcı et. al., 2010)[4] that aescin lowers total cholesterol level in blood plasma.

Therefore, I resolved to have the research question for this essay as: **How does feeding laying hens (*Gallus gallus domesticus*[12]*, Nick Brown breed*) with a 0.25%, 0.50% and 1.00% supplement of dried grains of horse chestnut (*Aesculus hippocastanum*) seed core affect the chicken egg yolk cholesterol level measured by the method described in Boehringer Manheim Gmbh Biochemica, 1989[13]?**

Based on the information I learned, my hypothesis is that **supplementing the basal diet feed for laying hens with non-toxic amounts of 0.25%, 0.50% and 1.00% of dried grains of horse chestnut seed core is going to lower the cholesterol level in the chicken egg yolk**. It is predicted that increased non-toxic amount of horse chestnut seeds in the diet will cause lesser chicken egg yolk cholesterol level.

The development of the method, planning of the experiment, the materials and the methods used, how the raw data is processed and the evaluation of the results will be explained in this essay.

[10] ELKIN, R. G. Reducing shell egg cholesterol content. II. Review of approaches utilizing non-nutritive dietary factors or pharmacological agents and an examination of emerging strategies. World's Poultry Science Journal, Vol. 63, March 2007: 5-31

[11] AYDIN, D., RASHID, S. M., AYDIN, R. 2014. Tavuk Yumurtası ve Kolesterol Gerçeği. KSU J. Nat. Sci., 17(3), 2014

[12] AL-NASSER A., Al-KHALAIFA H., AL-SAFFAR A., KHALIL F., ALBAHOUH M., RAGHEB G., AL-HADDAD A. and MASHALY M. Overview of chicken taxonomy and domestication. World's Poultry Science Journal, Volume 63, Issue 02, June 2007, pp 285-300

[13] BOEHRINGER MANHEIM GmbH BIOCHEMICA (1989). Methods of biochemical analysis and food analysis. Manheim, Germany, pp. 26-28.

METHOD DEVELOPMENT AND PLANNING

My aim was to test the effect of horse chestnut feed on chicken egg yolk cholesterol level while keeping the process as simple as possible and easily be applicable later on an industrial scale. For this reason, I have chosen to use raw horse chestnut seed cores to obtain grains which will be used as supplement in the basal diet of laying hens.

To conduct the experiment, a laboratory and a poultry house with enough number of hens were needed. The Department of Animal Nutrition and Nutritional Diseases of the Veterinary Medicine of Ankara University has shown great interest in the experiment and they were kind to support it by providing hens, basal diet and means of using their laboratory and farm environment.

Picture-1: The experimental setup of hen cages in the poultry house for laying hens in the Education and Research Farm of Ankara University

The Ankara University provided sixty, 40-week old, Nick Brown breed laying hens. The setup as shown in Picture-1 above to conduct the experiment was arranged by supplying dividers for the feeders in front of the poultry cages installed in the poultry house so that the feeds mixed with different ratios of additional horse chestnut were separated for different test groups.

The horse chestnut seeds were collected in October 2015 from the botanical gardens of Turkish Grand National Assembly to ensure the species of the horse chestnut trees are indeed *Aesculus hippocastanum*. I had to wait for the autumn season to collect the seeds as they mature during October.

During the design period, the dietary dose of aescin had to be very carefully considered not to exceed toxic level for laying hens. Because of time limitations and not to delay furthermore the start of my experiment, instead of getting from another laboratory a complete and exact chemical content analysis of the seeds I used, I decided to use the values mentioned in scientific literature. By taking into consideration that the average aescin content in the seeds is 10% (ANSM, 2015)[14] as an average, the LD50 value is 10.6mg/g (Williams and Olsen, 1984)[15], the average body weight of a Nick Brown breed laying hen is about 2kg and maximum consumption of basal diet is 130g per hen, I decided to conduct the experiment by mixing an additional 0% (for the control group), 0.25%, 0.50% and 1.00% dried grains of the raw seed cores to the basal diet for laying hens so that the maximal oral intake of aescin will be at most 0,65mg/g per hen per day; which is less than 6.13% of the LD50 value for chicks.

The duration of the experiment should be long enough to see the effects of horse chestnut seed supplement. I decided to feed the hens for four weeks which was a minimal duration as advised by the University.

Because not to decrease the commercial value of hens and eggs, the performance of them was also observed and analyzed and egg quality tests as described in the Appendix-1 were also conducted by the staff of the university.

[14] ANSM. (2015). (Agence Nationale de Securite du Medicament et des Produits de Sante). Aesculus hippocastanum, Horse Chestnut.
[15] WILLIAMS, M.C., OLSEN, J.D. 1984. Toxicity of seeds of three Aesculus spp to chicks and hamsters. Am J Vet Res. 45(3):539-542.

MATERIALS AND PROCEDURE

Variables:
Independent variable: *The amount of horse chestnut seed core mixed as supplement in the basal diet of laying hens.*

In this experiment, to be very cautious about the health of the hens, 0.25%, 0.50% and 1.00% horse chestnut seed mixed in the basal diet are used for three experimental groups respectively; so that the maximum amount of aescin would always be less than 10% of the LD50 dose.

The chemical nutritional composition of the seeds used were analyzed by the University laboratories as shown in the below Table-2:

Components	Dry matter	Ether extract	Crude protein	Crude fibre	Ash	Nitrogen free extract
Horse chestnut	93.00	7.15	8.15	3.00	2.20	72.50

Tabel-2. Chemical nutritional composition of the horse chestnut seeds (%) as analyzed by the Ankara University.

Dependent variable*: The amount of egg yolk cholesterol measured by using the method described in Boehringer Manheim Gmbh Biochemica, 1989*[16]*, which is an enzymatic colorimetric test for cholesterol with lipid clearing factor.*

There are several methods that could be used to measure the egg yolk cholesterol level. One of them is, for example, using the High Performance Liquid Chromatography (HPLC) method[17], which gives highly accurate results but also requires very specialized equipment. HPLC equipment are used by a few laboratories and it would be very expensive for me to use this method. Because of easiness and cost-effectiveness, I chose to use the above method. The HPLC method would also cost about ten times more.

The cholesterol testing kits, HUMAN brand (CHOD-PAP)(4X100 ml)-Biolabs-80106, necessary for cholesterol level tests, were purchased from the dealers locally in Ankara.

Controlled variables: The controlled variables valid for each test groups are explained below:

- Poultry used in the experiment: Laying hens (female chicken)
- The number of laying hens: 60

[16] BOEHRINGER MANHEIM GmbH BIOCHEMICA (1989). Methods of biochemical analysis and food analysis. Manheim, Germany, pp. 26-28.
[17] http://gidaarge.akdeniz.edu.tr/cihazlar.i32.yuksek-performansli-sivi-kromatografisi-hplc-

- The breed of laying hens: All hens were the same breed of Nick Brown laying hens with no known health problems
- The age of laying hens: 40 week-old hens
- The weight of laying hens: average 2kg
- The number of poultry cages: 20; 5 cages for each four of the experimental groups.
- The size of poultry house cages: All the same 50×44×60 cm size cages.
- The same size feeders: Qty.20, 15cm wide feeders along the width (44cm) of the cage
- The same shape feeder dividers: Qty.20, the same shape feeder dividers were used so that the feed did not mix with the other test groups' feed in the next cage.
- The number of hens per cage: Three hens per cage
- The light regimen of the poultry house: 16hr light and 8hr darkness as used in most commercial poultry houses for laying hens.
- The basal diet fed to the laying hens: All hens are fed "ad libitum" (i.e. birds can eat at any time, whenever and as much as they want) with the same composition of basal diet as explained in the below section.
- Feed replacement time: between 10-am and 11am each morning.
- The horse chestnut seeds: seeds of the same species of *Aesculus hippocastanum* trees.
- Egg collection time: between 10-am and 11am each morning.

Horse Chestnut (*Aesculus hippocastanum*) Seeds:

The seeds were collected and cut into a few pieces with a hand shear as shown in Picture-2 below. Their outer brown shell was peeled off. The core part were coarsely grinded with a kitchen rondo. They were layered on a tray and a warm air blower was

Picture-2: Collected horse chestnut seeds and how they were cut into pieces by means of a hand shear.

used to get rid of the excess moisture to prevent rotting and growth of mold. Then they were dried in a drying owen at 60-65°C for 12 hours at the laboratory to obtain dry grains of the seed cores. They were milled at the laboratory to obtain same grain size as the basal diet for the hens because it is also important to add the horse chestnut in the basal chicken feed as the same sized grains so that hens will not be able to select by differentiating according to size which one to eat.

Laying Hens:

The Ankara University provided a total of sixty Nick Brown breed laying hens (*Gallus gallus domesticus*), 40-week old with an average weight of 2kg for the experiment. They were divided into 4 experimental groups of 15 hens. Each group is divided into 5 subgroups of 3 hens so that the experiment would be repeated 5 times with each sub group. Having at least 3 hens for each trial is to minimize error because of individual differences between hens. The structure of the experimental groups is shown in Figure-1 below:

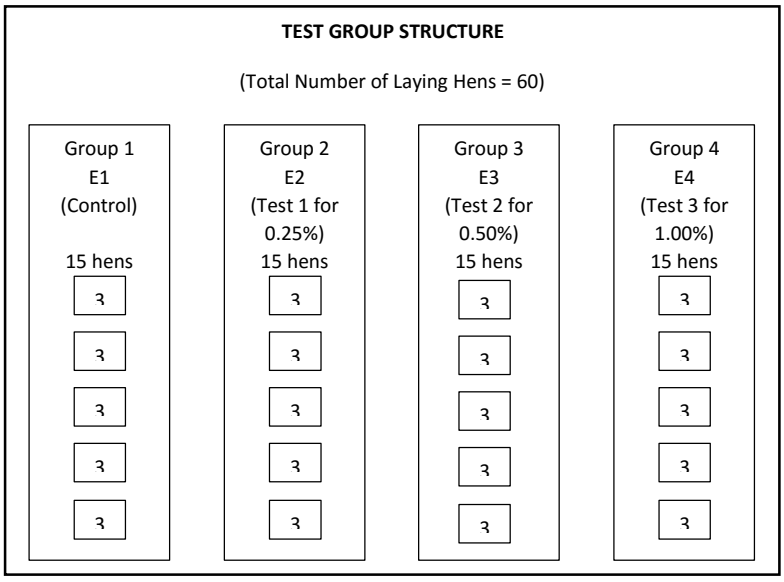


Figure-1: Structure of test groups. Total of 60 hens, 1 control group, 3 test groups, and 5 trials with 3 hens each.

Each trial group of 3 hens is put in cages of same size of 50x44x60cm. The cages were in the windowed poultry house in the animal research farm of the University.

All animal-use protocols in the poultry house and during the experiment were in accordance with the Directive 2010/63/EU of the European Parliament and of the

Council of 22 September 2010 on the protection of animals used for scientific purposes (European Union Directive, 2010)[18].

The first experimental group E1 was the control group and the other three E2, E3 and E4 were the test groups. The first test group E2 was fed with a mixture 0.25% dried grains of horse chestnut seeds to basal diet, the second test group E3 with 0.50% and the third E4 with 1.00% respectively.

Experimental Basal diet feed:

The hens were fed with a basal feed formulated to be isocaloric and isonitrogenic (i.e. with equal calorie and equal nitrogen values) according to the commercial management guide (H & N International, Cuxhaven, Germany)[19] Brown Nick breed laying hen rations.

The nutritional values of the basal diet were determined according to the methods defined in AOAC (2000)[20] .

The ingredients and composition of the experimental basal diet is shown in Table-3 on the right (It is the result of the lab analysis done by the Ankara University labs).

Ingredients, %	**Levels**
Corn	46.20
Barley	10
Soybean Meal	30
Limestone	8.4
DCP	1.5
Vegetable oil	3
Vitamin- premix[1]	0.25
Mineral-premix[2]	0.10
Methionine	0.25
Salt	0.3
Chemical analysis, dry matter (DM) basis	
Dry matter,%	88.80
Crude protein,%	18.20
Ash,%	9.90
Calcium,%	3.65
Phosphorus,%	0.66
Metabolizable energy, kcal/kg*	2744

* : This value was found to with calculation[1]
ME, kcal/kg=53+38 [(crude protein,%)+(2.25×ether extract, %)+(1.1×starch, %)+(sugar, %)]. TSE (1991)
1 Vitamin- premix; each 2.5 kg vitamin premix contained: Vitamin A 12 000 000 IU, Vitamin D3 2 000 000 IU,
Vitamin E 35 000 IU, Vitamin K3 5 000 mg, Vitamin B1 3 000 mg, Vitamin B2 6 000 mg, Vitamin B6 5 000 mg,
Vitamin B12 15 mg, Niacin 20 000 mg, Folic acid 750 mg, D-biotin 45 mg, choline chloride 125 000 mg and
Vit C 50 000 mg, calcium D- pantothenate 6 000 mg
2 Mineral-premix2; each kg mineral premix contained: Mn 80 000 mg, Fe 60 000 mg, Zn 60 000 mg, Cu 5 000 mg,
Co 200 mg, I 1 000 mg, Se 150 mg and Ca 446 925 mg

Table-3. Ingredients and chemical composition of the experimental diets (as-fed basis)

Feed in mash form and water were provided *ad libitum* during the 4 weeks of experimental period. Each morning between 10-11am, the remaining feed in the feeders were weighted and recorded, and then, the feeders loaded with fresh feed in known weights of feed. The feed weight records were used in calculations for feed efficiency.

[18] European Union Directive, 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union. 276/33-79, 20.10.2010
[19] H&N International. Management Guide: Brown Nick, Brown Egg Layers. http://www.hn-int.com/eng-wAssets/docs/managementguides/001MG-Brown-Nick_englisch_final.pdf
[20] AOAC (2000). Official Methods of Analysis of AOAC International. 17th Ed., AOAC International, Maryland, USA.

Egg Yolk Cholesterol level measurement

During the experiment, at the end of each week (each 7th day), 10 eggs were randomly selected and collected from each experimental group to determine yolk cholesterol. There were a total of 40 eggs for each week (a total of 160 eggs after 4 weeks) for yolk cholesterol level tests. Egg samples were numbered from 1 to 40 and with their experimental group number E1, E2, E3 and E4 respectively.

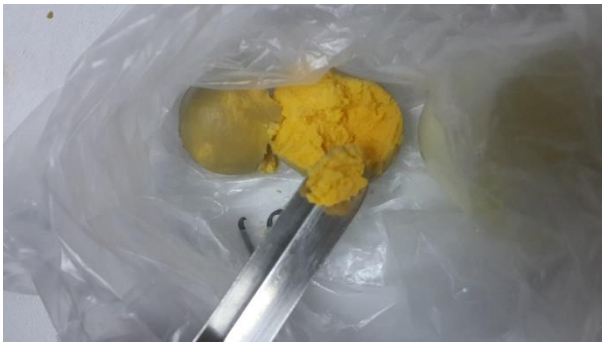
All eggs were boiled for 5 min. within 24 hours they were collected. They were allowed to cool down to room temperature. Then, their shells are peeled off and their albumen and yolk were weighed separately. These samples had to be kept for cholesterol analyses to be carried out at the end of the feeding period.

For this purpose, all egg samples of each week were put in plastic bags separately and kept at in a deep freezer at -18°C until the end of the experiment. A day before conducting cholesterol level tests, all samples were taken out of the deep freezer to allow them to warm up to room temperatures shown in Picture-3.

Picture-3: Boiled egg samples at room temperature in the laboratory.

Then, according to enzymatic colorimetric test for cholesterol with lipid clearing factor by using commercial reagents as described in Boehringer Manheim Gmbh Biochemica, 1989, for each sample of yolk:

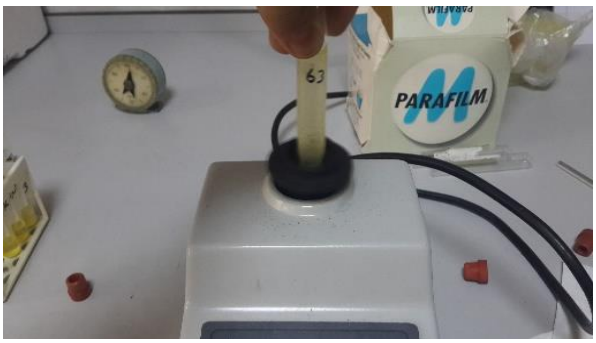
- 0.1g of egg yolk was weighted with a scientific precision scale (±0.1mg) for each sample egg in a test tube suitable to be put in a centrifuge device. (see Picture-4)
- 0.4ml, 99.5% pure isopropyl alcohol added in the tube (Waldroup et al., 1986)[21]



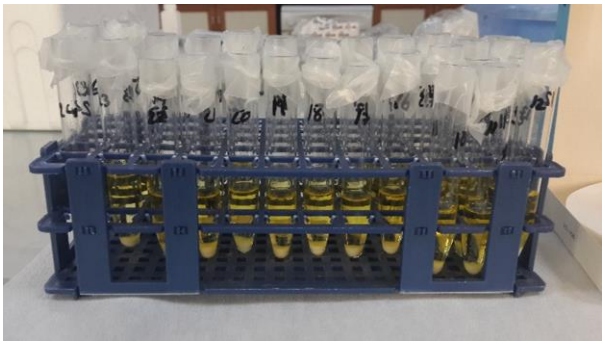
Picture-4: Getting approx.. 0.1g of egg yolk sample to be weighted and put in a test tube.

[21] Waldroup, P.W., Ndide, L.I., Hellwig, H.M., Hebert, J.A., Berrio, L., 1986. Influence of probucol (4,4'-isopropyllidine dithio)-bis(2,6-di-t-butyl-phenol) on egg yolk cholesterol content and performance of laying hens. Poultry Sci. 65, 1949–1954.

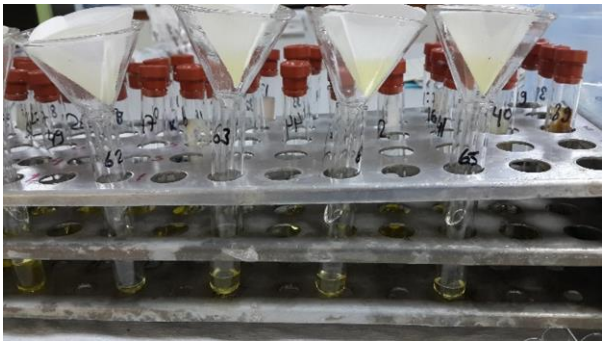
- The contents of the tube were blended with a vortex device at 3000rpm for 10min. to dissolve cholesterol in the alcohol. (see Picture-5)
- They were put in a centrifuge device for 10 minutes to separate solid particles and obtain a clear liquid sample. (see Picture-6)
- Then, the contents were filtered into another test tube thru Whatman 4 filtering paper. (See Picture-7)



Picture-5: Blending the contents of the tubes with a vortex device.



Picture -6: Samples after subjected to centrifuge device for 10min. Solid particles were settled down at the bottom of the test tube.



Picture -7: Filtering samples with Whatman 4 filtering paper to obtain a solution without solid particles.

- 2x1ml of commercial reagent enzyme was put into two separate tubes (1ml for each tube) for two measurement readings for each sample.
- The tubes with reagent were incubated at 37°C for 10 minutes so that the enzyme reacts correctly at correct temperature.
- 10µl of the filtered extract sample was transferred to both test tubes by using a new tip for the pipette each time to prevent incorrect amount transfers.
- The color of the contents of samples changed to very light pink.
- Two tubes are prepared with 10µl of the standard solution of the commercial kit to obtain standard (i.e. known) values.
- Two tubes are prepared with 10µl of distilled water for the “blank test”
- The samples were poured into cuvettes for spectrophotometric measurement.
- There were 320 sample +2 standard +2 blank cuvettes for spectrophotometric reading.
- The spectrophotometer (Shimadzu, UV-VIS UV-1208) was set to 520nm wavelength.

- The blank test was put into the first cell. It was for calibrating the spectrophotometer to zero with the blank test.
- The standard test was put into the second cell.
- The two samples for each yolk were put into the 3rd and 5th cells. (The spectrophotometer was providing erroneous measurements for the 4th and 6th cells, so they were not used for the measurements.)
- The spectrophotometer calibrated to zero with the blank test cuvette.
- The reading for the second cell was for a known level of cholesterol in a sample. ($\Delta A_{standard}$)(It should give a reading of around 0.200 Abs). Average of all readings of standard test were used to lessen measurement errors of the spectrophotometer.
- The readings for the 3rd and 4th cells were the two measurements for the same sample of yolk so that the spectrophotometer were used to measure all 160 samples with two readings to lessen measurement errors of the spectrophotometer. Average of the two readings were used as the measured value of each sample (ΔA_{sample}).

The total levels of egg yolk cholesterol were calculated and expressed as mg per yolk with the below formulae as indicated in the instructions of the cholesterol reagent kit used:

$$Cholesterol\ concentration_{extract} = Standard\ Value\ x\ \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}$$

The standard value with the cholesterol kit I used was 200, so:

$$Cholesterol\ concentration_{extract} = 200\ x\ \frac{\Delta A_{sample}}{\Delta A_{standard} x100}\ (mg/ml)$$

$$Cholesterol_{yolk} = \frac{Cholesterol\ conc._{extract}\ (\frac{mg}{ml})\ x\ 4(ml)}{Weight_{sample}(g)}\ x\ Weight_{yolk}(g) \quad (mg)$$

Method for Statistical analysis

I used the Analysis of Variance (ANOVA) method for the statistical analysis when processing data of the experimental groups and evaluation of the significance of the difference between the mean values. Because there were too much data for me to calculate manually, I have done statistical analyses by using the SPSS program (Version 21.00, SPSS Inc., Chicago, IL, USA). I used “one-way ANOVA” method because there was only one independent variable in the experiment; which was the amount of horse chestnut supplement in the experimental basal diet of the hens.

The results are shown in the following section.

DATA COLLECTION AND PROCESSING

Because hens do not always lay one egg per day at all times, it was not possible to collect 10 eggs for each experimental group for each week.

For each sample of egg yolk, absorbance values were measured by using a spectrophotometer to calculate the total cholesterol level per yolk. Example calculation for the first sample for group E1 is as follows:

Measured Yolk weight = 18,4±0,1g
Measured Yolk weight in the sample: 0,1181±0,0001g
Absorbance value readings at 520nm light:
ΔAstd=0,220 Abs (mean of standard test readings for E1)
Because the standard test was not changed during the measurements, the average of readings for that group was used in the calculations.
ΔA_1=0,044 Abs
ΔA_2=0,047 Abs
Absorbance value of each yolk sample is calculated as the average of two measurements of the same sample: $\Delta A_{sample}= (\Delta A_1+\Delta A_2)/2$=0,0455 Abs

The total cholesterol of egg yolk is calculated by using the above mentioned equations.

By using the data given above, we get:

$$\boldsymbol{Cholesterol\ concentration_{E1/1} = 200\ x\ \frac{0,0455}{0,220x100}\ (mg/ml)}$$

$$\boldsymbol{Cholesterol\ concentration_{E1/1} = 0,41363\ (mg/ml)}$$

$$\boldsymbol{Cholesterol_{yolk} = \frac{0,41363(\frac{mg}{ml})\ x\ 4(ml)}{0,1181(g)}\ x\ 18,4(g) \quad (mg)}$$

$\boldsymbol{Cholesterol_{yolk\ E1/1} = 257,78 \quad (mg)}$ for the first sample of group E1

The calculated values are given in tables in the following pages together with raw data.

The calculated data about egg yolk cholesterol levels for the first week are presented in Table-4 below:

Group No (based on % mass of horse chestnut in feed)	**Egg No**	**Yolk Weight (g)(±0.1g) W_{yolk}**	**Yolk Sample Weighted (g)(±0,0001g) W_{sample}**	**Absorbance (at 520±1nm) (Abs)(±0.001Abs)**			**Absorbance (at 520±1nm)**	**Egg Yolk Cholesterol Level (calculated) (mg)(±0,01mg)**
				of Standard	**of 1st tube**	**of 2nd tube**	**of sample (Abs)(±0.001Abs) $(\Delta A_1+\Delta A_2)/2$**	
				$\Delta A_{standard}$	**ΔA_1**	**ΔA_2**	**ΔA_{sample}**	
E1	1	18,4	0,1181	0,199	0,044	0,047	0,0455	257,78
	2	21,1	0,1217	0,199	0,043	0,043	0,0428	269,52
%0 Horse chestnut	3	16,9	0,1180	0,198	0,041	0,041	0,0410	213,53
	4	17,8	0,1235	0,198	0,048	0,040	0,0438	229,30
	5	15,8	0,1251	0,197	0,038	0,053	0,0455	208,97
	6	18,8	0,1231	0,198	0,042	0,033	0,0370	205,48
	7	17,5	0,1155	0,198	0,035	0,042	0,0385	212,12
	8	19,2	0,1239	0,197	0,038	0,031	0,0343	193,00
E2	1	15,6	0,1135	0,197	0,045	0,045	0,0450	224,91
	2	17,7	0,1280	0,198	0,043	0,044	0,0433	217,48
%0.25 Horse chestnut	3	17,0	0,1193	0,197	0,054	0,042	0,0478	247,43
	4	17,0	0,1179	0,196	0,031	0,062	0,0463	242,50
	5	17,7	0,1250	0,228	0,042	0,043	0,0420	216,26
	6	16,8	0,1255	0,229	0,038	0,041	0,0393	191,06
	7	17,3	0,1263	0,228	0,034	0,048	0,0405	201,73
	8	16,0	0,1214	0,228	0,035	0,049	0,0420	201,29
E3	1	15,1	0,1231	0,228	0,047	0,063	0,0550	245,33
	2	16,8	0,1128	0,228	0,043	0,035	0,0390	211,22
%0.50 Horse chestnut	3	16,0	0,1263	0,220	0,064	0,064	0,0635	292,52
	4	16,7	0,1263	0,219	0,048	0,031	0,0395	189,92
	5	18,2	0,1157	0,214	0,025	0,036	0,0305	174,47
	6	15,4	0,1280	0,197	0,030	0,037	0,0335	146,56
	7	15,3	0,1224	0,213	0,040	0,035	0,0373	169,32
	8	16,1	0,1185	0,216	0,040	0,038	0,0388	191,45
E4	1	16,3	0,1130	0,214	0,034	0,055	0,0443	232,11
	2	16,7	0,1190	0,216	0,040	0,064	0,0520	265,36
%1.0 Horse chestnut	3	14,3	0,1282	0,214	0,046	0,035	0,0400	162,25
	4	16,6	0,1227	0,212	0,043	0,053	0,0475	233,68
	5	19,1	0,1217	0,211	0,041	0,052	0,0463	263,95
	6	10,9	0,1180	0,197	0,040	0,038	0,0385	129,32
	7	17,3	0,1165	0,210	0,039	0,041	0,0398	214,65
	8	15,3	0,1268	0,209	0,040	0,042	0,0408	178,80

Table-4: The calculated data about egg yolk cholesterol levels for Week 1. Uncertainties in yolk weight and the weighted yolk samples are ±0,1g and ±0,0001g respectively because different scales used the weigh them. A high precision scale is needed to weigh the yolk samples.

The calculated data about egg yolk cholesterol levels for the second week are presented in Table-5 below:

Group No (based on % mass of horse chestnut in feed)	Egg No	Yolk Weight (g)(±0.1g) W_{yolk}	Yolk Sample Weighted (g)(±0,0001g) W_{sample}	Absorbance (at 520±1nm) (Abs)(±0.001Abs) of Standard $\Delta A_{standard}$	of 1st tube ΔA_1	of 2nd tube ΔA_2	Absorbance (at 520±1nm) of sample (Abs)(±0.001Abs) $(\Delta A_1+\Delta A_2)/2$ ΔA_{sample}	Egg Yolk Cholesterol Level (calculated) (mg)(±0,01mg)
E1 %0 Horse chestnut	1	18,0	0,1119	0,2380	0,031	0,031	0,0310	199,47
	2	21,9	0,1259	0,1880	0,026	0,042	0,0338	234,83
	3	18,2	0,1282	0,2000	0,040	0,038	0,0388	220,05
	4	14,3	0,1185	0,1900	0,058	0,041	0,0493	237,73
	5	14,3	0,1232	0,2000	0,030	0,039	0,0345	160,18
	6	17,3	0,1226	0,2000	0,035	0,037	0,0358	201,79
	7	19,8	0,1253	0,2260	0,046	0,049	0,0473	298,66
E2 %0.25 Horse chestnut	1	15,2	0,1208	0,2000	0,040	0,039	0,0393	197,55
	2	15,0	0,1195	0,1960	0,051	0,036	0,0433	217,16
	3	18,8	0,1226	0,1890	0,045	0,035	0,0400	245,35
	4	15,8	0,1238	0,2380	0,040	0,047	0,0433	220,79
	5	18,1	0,1277	0,2380	0,037	0,042	0,0395	223,95
	6	18,6	0,1208	0,2380	0,039	0,040	0,0393	241,74
	7	16,5	0,1155	0,2380	0,032	0,048	0,0398	227,14
	8	18,9	0,1175	0,1970	0,032	0,053	0,0423	271,84
	9	15,8	0,1255	0,2380	0,036	0,051	0,0433	217,80
E3 %0.50 Horse chestnut	1	15,6	0,1254	0,1960	0,033	0,038	0,0353	175,41
	2	17,9	0,1187	0,1960	0,037	0,037	0,0365	220,17
	3	17,4	0,122	0,1950	0,037	0,057	0,0470	268,13
	4	17,8	0,1222	0,1950	0,040	0,046	0,0428	249,08
	5	18,5	0,1284	0,1970	0,029	0,041	0,0348	200,27
	6	15,1	0,1276	0,1950	0,029	0,040	0,0340	160,94
	7	19,6	0,1236	0,1960	0,034	0,049	0,0413	261,65
	8	17,6	0,1297	0,1970	0,037	0,036	0,0365	198,12
	9	17,6	0,1268	0,1980	0,035	0,065	0,0498	276,22
E4 %1.0 Horse chestnut	1	17,9	0,1161	0,1970	0,029	0,058	0,0433	266,73
	2	14,5	0,1163	0,1950	0,035	0,049	0,0418	208,21
	3	19,2	0,1206	0,1950	0,039	0,045	0,0420	267,46
	4	16,1	0,1154	0,1970	0,042	0,058	0,0500	279,03
	5	18,6	0,1189	0,1990	0,045	0,043	0,0438	273,76
	6	17,1	0,1185	0,2000	0,042	0,046	0,0438	252,53
	7	15,8	0,1137	0,2000	0,040	0,032	0,0358	198,72

Table-5: The calculated data about egg yolk cholesterol levels for Week 2. Uncertainties in yolk weight and the weighted yolk samples are ±0,1g and ±0,0001g respectively because different scales used the weigh them. A high precision scale is needed to weigh the yolk samples.

The calculated data about egg yolk cholesterol levels for the third week are presented in Table-6 below:

Group No (based on % mass of horse chestnut in feed)	**Egg No**	**Yolk Weight (g)(±0.1g)** W_{yolk}	**Yolk Sample Weighted (g)(±0,0001g)** W_{sample}	**Absorbance (at 520±1nm) (Abs)(±0.001Abs)**			**Absorbance (at 520±1nm)**	**Egg Yolk Cholesterol Level (calculated) (mg)(±0,01mg)**
				of Standard	**of 1st tube**	**of 2nd tube**	**of sample (Abs)(±0.001Abs)** $(\Delta A_1+\Delta A_2)/2$	
				$\Delta A_{standard}$	ΔA_1	ΔA_2	ΔA_{sample}	
E1 %0 Horse chestnut	1	16,6	0,1172	0,224	0,033	0,037	0,035	196,88
	2	16,0	0,1157	0,223	0,036	0,037	0,037	201,90
	3	15,7	0,1135	0,216	0,027	0,036	0,031	171,53
	4	20,3	0,1107	0,205	0,038	0,039	0,039	282,40
	5	14,5	0,1169	0,200	0,030	0,040	0,035	173,65
	6	15,1	0,1172	0,201	0,043	0,036	0,039	202,28
	7	17,8	0,1134	0,210	0,046	0,052	0,049	304,52
E2 %0.25 Horse chestnut	1	14,9	0,1116	0,210	0,035	0,038	0,036	193,59
	2	18,2	0,1195	0,203	0,037	0,032	0,034	207,13
	3	17,4	0,1164	0,206	0,039	0,045	0,042	251,13
	4	17,4	0,1251	0,206	0,051	0,051	0,051	282,35
	5	18,3	0,1253	0,207	0,049	0,050	0,049	287,72
	6	17,7	0,1147	0,203	0,038	0,041	0,039	242,28
	7	16,2	0,1155	0,198	0,030	0,047	0,038	214,60
E3 %0.50 Horse chestnut	1	17,7	0,1234	0,198	0,045	0,035	0,040	228,06
	2	16,4	0,1107	0,195	0,038	0,039	0,038	226,67
	3	17,0	0,1273	0,197	0,038	0,035	0,036	193,64
	4	17,7	0,1109	0,198	0,031	0,037	0,034	213,87
	5	16,1	0,1201	0,198	0,061	0,043	0,052	277,49
	6	18,0	0,1234	0,197	0,038	0,035	0,036	211,51
	7	18,5	0,1173	0,198	0,028	0,038	0,033	205,03
	8	15,1	0,1104	0,195	0,034	0,030	0,032	173,71
	9	19,0	0,1128	0,194	0,040	0,022	0,031	207,18
	10	16,6	0,1199	0,198	0,052	0,049	0,050	278,28
E4 %1.0 Horse chestnut	1	18,3	0,1195	0,195	0,041	0,036	0,038	232,77
	2	17,0	0,1273	0,216	0,039	0,036	0,038	200,31
	3	16,3	0,1151	0,223	0,043	0,035	0,039	218,09
	4	17,2	0,1147	0,223	0,034	0,034	0,034	200,94
	5	12,3	0,1142	0,221	0,036	0,039	0,037	160,48
	6	18,9	0,117	0,223	0,031	0,028	0,029	189,00
	7	18,2	0,1217	0,222	0,037	0,037	0,037	221,33
	8	15,2	0,1221	0,220	0,058	0,036	0,047	234,04

Table-6: The calculated data about egg yolk cholesterol levels for Week 3. Uncertainties in yolk weight and the weighted yolk samples are ±0,1g and ±0,0001g respectively because different scales used the weigh them. A high precision scale is needed to weigh the yolk samples.

The calculated data about egg yolk cholesterol levels for the fourth week are presented in Table-7 below:

Group No (based on % mass of horse chestnut in feed)	**Egg No**	**Yolk Weight (g)(±0.1g) W_{yolk}**	**Yolk Sample Weighted (g)(±0,0001g) W_{sample}**	**Absorbance (at 520±1nm) (Abs)(±0.001Abs)**			**Absorbance (at 520±1nm)**	**Egg Yolk Cholesterol Level (calculated) (mg)(±0,01mg)**
				of Standard	**of 1st tube**	**of 2nd tube**	**of sample (Abs)(±0.001Abs) $(\Delta A_1+\Delta A_2)/2$**	
				$\Delta A_{standard}$	**ΔA_1**	**ΔA_2**	**ΔA_{sample}**	
E1	1	20,9	0,1113	0,250	0,038	0,039	0,039	254,79
	2	17,8	0,1243	0,224	0,025	0,041	0,033	166,54
%0 Horse chestnut	3	16,5	0,1225	0,225	0,037	0,040	0,039	182,76
	4	17,3	0,1151	0,222	0,033	0,024	0,029	150,97
	5	20,5	0,1152	0,210	0,050	0,026	0,038	238,31
	6	19,4	0,1463	0,210	0,055	0,039	0,047	219,64
	7	18,2	0,1203	0,211	0,055	0,038	0,047	247,93
	8	15,7	0,1155	0,213	0,043	0,048	0,046	217,97
E2	1	16,8	0,1226	0,215	0,046	0,040	0,043	207,66
	2	18,5	0,1130	0,215	0,028	0,041	0,035	199,06
%0.25 Horse chestnut	3	17,7	0,1211	0,215	0,031	0,036	0,034	172,56
	4	19,7	0,1372	0,232	0,045	0,039	0,042	212,53
	5	18,7	0,1196	0,233	0,042	0,041	0,042	228,68
	6	17,8	0,1298	0,232	0,038	0,035	0,037	176,40
	7	18,0	0,1195	0,237	0,028	0,035	0,032	167,22
E3	1	15,4	0,1244	0,233	0,059	0,042	0,051	220,32
	2	17,8	0,1294	0,224	0,049	0,046	0,048	230,27
%0.50 Horse chestnut	3	15,9	0,1124	0,224	0,029	0,021	0,025	124,63
	4	15,3	0,1205	0,206	0,045	0,041	0,043	192,41
	5	19,1	0,1132	0,207	0,042	0,042	0,042	249,75
	6	18,6	0,1228	0,210	0,064	0,034	0,049	261,56
	7	19,7	0,1151	0,216	0,048	0,033	0,041	244,29
	8	14,3	0,1131	0,221	0,048	0,048	0,048	213,89
E4	1	15,6	0,1112	0,225	0,046	0,046	0,046	227,43
	2	17,2	0,1122	0,261	0,053	0,014	0,034	180,99
%1.0 Horse chestnut	3	17,7	0,1230	0,262	0,047	0,008	0,028	139,47
	4	19,3	0,1275	0,238	0,092	0,023	0,058	306,75
	5	16,2	0,1138	0,237	0,040	0,028	0,034	170,58
	6	15,8	0,1286	0,244	0,050	0,016	0,033	142,89
	7	17,6	0,1178	0,246	0,052	0,023	0,038	197,45
	8	15,6	0,1154	0,249	0,068	0,040	0,054	257,26

Table-7: The calculated data about egg yolk cholesterol levels for Week 4. Uncertainties in yolk weight and the weighted yolk samples are ±0,1g and ±0,0001g respectively because different scales used the weigh them. A high precision scale is needed to weigh the yolk samples.

Statistical analysis resulting tables of SPSS about calculated egg yolk cholesterol values:

Descriptive statistical calculations and one-way Analysis of Variance of calculated data for each week were carried out by using the SPSS software. The results are presented in the following tables.

Descriptive statistics of calculated egg yolk cholesterol level values of week 1

Group Name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
E1	8	223,7125	26,78315	9,46927	201,3212	246,1038	193,00	269,52
E2	8	217,8328	19,95307	7,05448	201,1516	234,5140	191,06	247,43
E3	8	212,3736	41,22636	14,57572	177,9075	246,8397	169,32	292,52
E4	8	210,0156	49,00360	17,32539	169,0476	250,9836	129,32	265,36
Total	**32**	**215,9836**	**34,73839**	**6,14094**	**203,4591**	**228,5081**	**129,32**	**292,52**

Table-8: The descriptive statistical values of the calculated values (in mg) of total egg yolk cholesterol levels from Table-4 representing Week 1.

One-way ANOVA analysis of calculated egg yolk cholesterol level values of week 1

			Sum of Squares	df	Mean Square	F	Sig.(P)
Between Groups	(Combined)		894,441	3	298,147	0,229	0,876
	Linear Term	Contrast	866,761	1	866,761	0,665	0,422
		Deviation	27,680	2	13,840	0,011	0,989
Within Groups			36514,994	28	1304,107		
Total			37409,434	31			

Table-9: One-way ANOVA results of the data of total egg yolk cholesterol levels from Table-4 representing Week 1 and descriptive statistics of Table-8.

Descriptive statistics of calculated egg yolk cholesterol level values of week 2

Group Name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
E1	7	221,8139	42,87677	16,20590	182,1595	261,4683	160,18	298,66
E2	9	229,2576	21,25839	7,08613	212,9170	245,5983	197,55	271,84
E3	9	223,3331	42,28180	14,09393	190,8324	255,8338	160,94	276,22
E4	7	249,4921	32,59067	12,31812	219,3508	279,6335	198,72	279,03
Total	32	230,3893	35,40075	6,25803	217,6260	243,1527	160,18	298,66

Table-9: The descriptive statistical values of the calculated values (in mg) of total egg yolk cholesterol levels from Table-5 representing Week 2.

One-way ANOVA analysis of calculated egg yolk cholesterol level values of week 2

		Sum of Squares	df	Mean Square	F	Sig.(P)
Between Groups (Combined)		3528,825	3	1176,275	0,932	0,438
Linear Term	Unweighted	2128,394	1	2128,394	1,687	0,205
	Weighted	1935,432	1	1935,432	1,534	0,226
	Deviation	1593,393	2	796,697	0,632	0,539
Within Groups		35320,781	28	1261,456		
Total		38849,606	31			

Table-10: One-way ANOVA results of the data of total egg yolk cholesterol levels from Table-5 representing Week 2 and descriptive statistics of Table-9.

Descriptive statistics of calculated egg yolk cholesterol level values of week 3

Group Name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
E1	7	219,0225	52,76406	19,94294	170,2239	267,8211	171,53	304,52
E2	7	239,8289	36,69415	13,86909	205,8925	273,7654	193,59	287,72
E3	10	221,5443	33,53986	10,60624	197,5514	245,5373	173,71	278,28
E4	8	207,1212	24,76408	8,75542	186,4179	227,8244	160,48	234,04
Total	32	221,3866	37,35117	6,60282	207,9201	234,8532	160,48	304,52

Table-11: The descriptive statistical values of the calculated values (in mg) of total egg yolk cholesterol levels from Table-6 representing Week 3.

One-way ANOVA analysis of calculated egg yolk cholesterol level values of week 3

		Sum of Squares	df	Mean Square	F	Sig.
Between Groups (Combined)		4048,235	3	1349,412	0,964	0,424
Linear Term	Unweighted	1098,433	1	1098,433	0,785	0,383
	Weighted	1170,537	1	1170,537	0,836	0,368
	Deviation	2877,698	2	1438,849	1,028	0,371
Within Groups		39200,164	28	1400,006		
Total		43248,399	31			

Table-12: One-way ANOVA results of the data of total egg yolk cholesterol levels from Table-6 representing Week 3 and descriptive statistics of Table-11.

Descriptive statistics of calculated egg yolk cholesterol level values of week 4

Group Name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
E1	8	209,8640	38,76316	13,70485	177,4572	242,2708	150,97	254,79
E2	7	194,8730	23,23627	8,78248	173,3830	216,3629	167,22	228,68
E3	8	217,1415	43,31930	15,31569	180,9257	253,3574	124,63	261,56
E4	8	202,8509	57,96667	20,49431	154,3895	251,3122	139,47	306,75
Total	31	206,5472	41,81855	7,51083	191,2080	221,8863	124,63	306,75

Table-13: The descriptive statistical values of the calculated values (in mg) of total egg yolk cholesterol levels from Table-7 representing Week.

One-way ANOVA analysis of calculated egg yolk cholesterol level values of week 4

		Sum of Squares	df	Mean Square	F	Sig.
Between Groups (Combined)		2049,244	3	683,081	0,366	0,778
Linear Term	Unweighted	,600	1	,600	0,000	0,986
	Weighted	,021	1	,021	0,000	0,997
	Deviation	2049,222	2	1024,611	0,549	0,584
Within Groups		50414,501	27	1867,204		
Total		52463,745	30			

Table-14: One-way ANOVA results of the data of total egg yolk cholesterol levels from Table-7 representing Week 4 and descriptive statistics of Table-13.

Descriptive statistics of calculated egg yolk cholesterol level values representing whole duration of the experiment

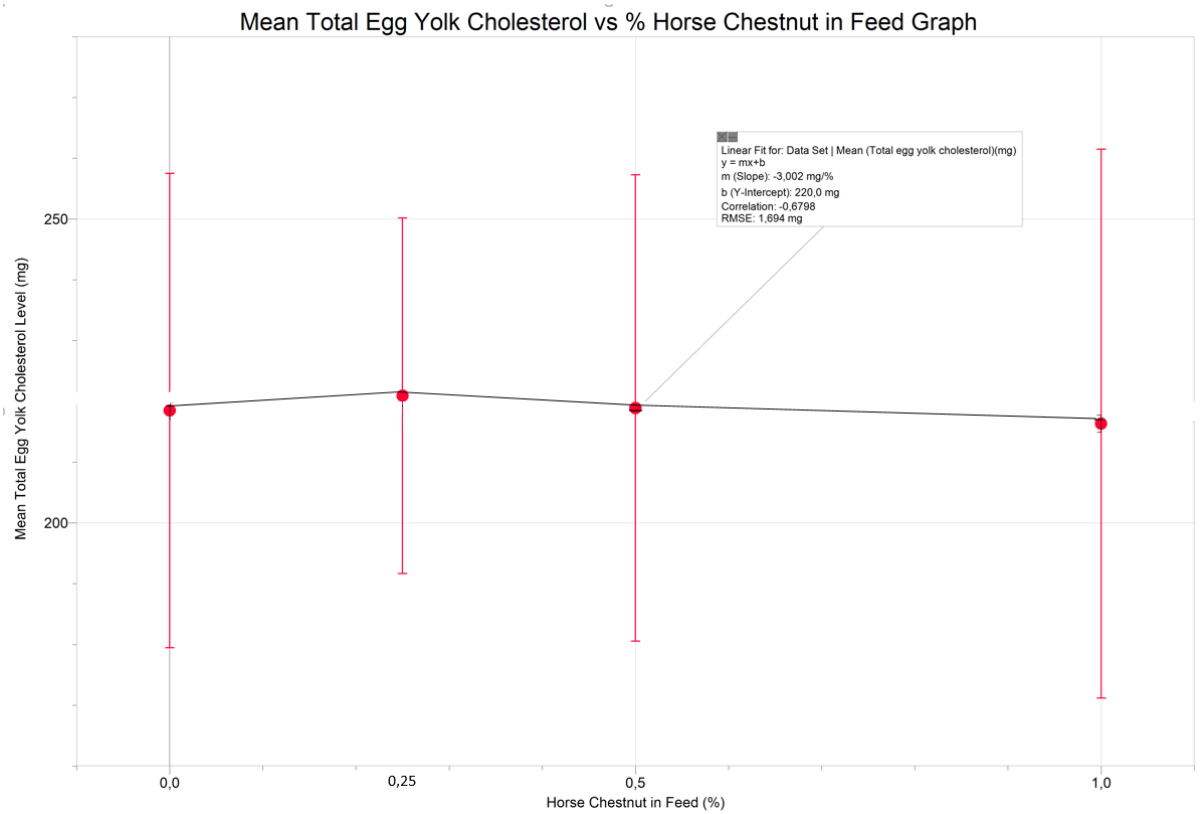
Group Name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
E1	30	218,4822	39,02563	7,12507	203,9098	233,0546	150,97	304,52
E2	31	220,9321	29,25429	5,25423	210,2015	231,6626	167,22	287,72
E3	35	218,9018	38,37578	6,48669	205,7192	232,0843	124,63	292,52
E4	31	216,3337	45,14610	8,10848	199,7740	232,8935	129,32	306,75
Total	127	218,6714	37,94201	3,36681	212,0086	225,3342	124,63	306,75

Table-15: The descriptive statistical values of the calculated values (in mg) of total egg yolk cholesterol levels from Table-4, Table-5, Table-6 and Table-7 representing the whole duration of the experiment.

One-way ANOVA analysis of calculated egg yolk cholesterol level values of values representing whole duration of the experiment

			Sum of Squares	df	Mean Square	F	Sig. (P-value)
Between Groups	(Combined)		330,768	3	110,256	0,075	0,973
	Linear Term	Unweighted	110,326	1	110,326	0,075	0,785
		Weighted	112,023	1	112,023	0,076	0,783
		Deviation	218,744	2	109,372	0,074	0,928
Within Groups			181058,339	123	1472,019		
Total			181389,107	126			

Table-16: One-way ANOVA results of the data of total egg yolk cholesterol levels from Table-4, Table-5, Table-6 and Table-7 representing whole duration of the experiment and descriptive statistics of Table-15. Note that the combined P-value between groups is 0,973 which is >0.05.



Graph-1: Graphical representation of mean values of calculated total egg yolk cholesterol levels for all experimental groups for the whole duration of the experiment (data from Table-15). Calculated by using Logger Pro Program.

CONCLUSION

The aim of this experiment was to explore the effect of horse chestnut seed supplement on egg yolk cholesterol levels of laying hens. I had decided to find an answer to the question “how feeding laying hens with a 0.25%, 0.50% and 1.00% supplement of dried grains of horse chestnut seed core affects the chicken egg yolk cholesterol level measured by the enzymatic colorimetric test for cholesterol with lipid clearing factor described in Boehringer Manheim Gmbh Biochemica, 1989”. My hypothesis was that increased amount of horse chestnut supplement would cause much lowered egg yolk cholesterol levels.

I used “Analysis Of Variance, ANOVA” method because there were more than two experimental groups in the experiment. ANOVA tests whether the mean values obtained from 3 or more groups are equal or not. It also decreases the probability of rejecting the null-hypothesis that “feeding laying hens with dried horse chestnut grains does not affect the egg yolk cholesterol level” even if it is true. This is indicated with the P-values: if the P-values are greater than 0,05, it means the results of the experiment are within normal values. And, because there was only one independent variable, I used one-way ANOVA.

The egg yolk cholesterol level results of this experiment does not support my hypothesis. The slight change shown in Graph-1 above in the egg yolk cholesterol levels are statistically insignificant. The one-way ANOVA analysis (see Table-16) shows that the combined P-value between groups is 0,973 which is a value very close to 1 indicating that there was almost no change of the mean values of the egg yolk cholesterol levels between groups.

Also, because the standard deviations of the results are too high as shown in Table-23, the experiment should be repeated by applying more robust methods to reduce measurement errors for each step of the enzymatic colorimetric test for cholesterol with lipid clearing factor.

Within my knowledge, this experiment was the first time trial on the effect of dried horse chestnut seed grain supplement on the egg yolk cholesterol levels of laying hens. The results shows that 0,25%, 0,50% and 1,00% horse chestnut seed supplement are not harmful to laying hens. This information can be used as a starting point for new experiments on the subject.

Because of lack of scientific evidence on long term health and performance effects of feeding laying hens with dried horse chestnut seed grains, further research is necessary on the subject. It should be conducted with a team of researchers, with more number of laying hens, for a longer period of time of at least 3 months, and by supplementing the basal feed with carefully increased amount of horse chestnut seed supplementation.

EVALUATION

When I searched for a similar experiment with horse chestnut seed supplement on laying hens, I could not find any academic paper in scientific databases that can be accessible via the academic network of the Ankara University. For this reason, I could only evaluate the results of this experiment by comparing the values for the control group with the ones for the three experimental groups.

First of all, there were no health issues or deaths of the hens of all experimental groups during the experiment.

Because there were no health issues or deaths observed on the hens during the experiment, it can be said that the doses of 0.25%, 0.50% and 1.00% of horse chestnut seed supplement were not toxic to laying hens when they are fed for a duration of one month.

However, the experiment was conducted for a relatively short duration of one month only. We cannot be sure about the long term health effects of feeding laying hens with small amounts of horse chestnut supplement. Extensive research should be conducted on the long term effects of horse chestnut supplement to laying hens.

Because the focus was on the egg yolk cholesterol level for this experiment, the detailed data on performance and egg quality tables can be seen in Appendix-2 and Appendix-3.

The summary table of the data on the effect of horse chestnut supplement on the egg yolk cholesterol level (mg of total cholesterol per yolk) of laying hens is shown in Table-15 in the previous section of data collection and processing. The maximum mean value of ~220,9mg is with the group fed with 0.25% horse chestnut and the minimum value of ~216,3mg is with 1.0% group. The means are almost the same for groups 0.0% and 0.5% at ~218.4mg and ~218.9mg respectively.

Graph-1 is the graphical representation of values from Table-15. It is observed that standard deviation bars are too large when compared with the very small negative slope of the graph.

Table-15 and Graph-1 indicates that there is no significant change in the egg yolk cholesterol levels of the groups E2, E3 and E4 when they are compared with the group E1. The decrease in the mean value of the total egg yolk cholesterol of 2,15mg for E4 is just about 1%. Table-15 also shows that the standard deviations of the results are too high for all groups; they are between about 13-20%.

This can be due to some limitations I faced during the measurements. The main one was with time that I had to conduct the cholesterol level measurement tests during a weekend when no one was using the laboratory. To prepare 320 samples for

colorimetric analysis and make a measurement with the spectrophotometer for each took more than 20 hours distributed to two days for me. Although I was very careful during each step of the cholesterol level tests described in the materials and methods section, because the number of steps were too much, errors should have accumulated to the final resulting values. These tests should be conducted with a team of people to prevent errors that might have occur due to time pressure.

The second major limitation was the number of hens used in the experiment. To minimize differences originating from individual hens, the experiment should be conducted with more laying hens.

A reason of the “no significant effect of horse chestnut supplement” can be short the duration of the experiment: One month of feeding might not have shown its effect on the metabolism of the laying hens. To see a significant effect the experiment may be conducted for a 3-month or more duration as suggested by the faculty of veterinary medicine. Other two major reasons maybe human errors during measurements and the number of calculations.

APPENDICES

Appendix 1

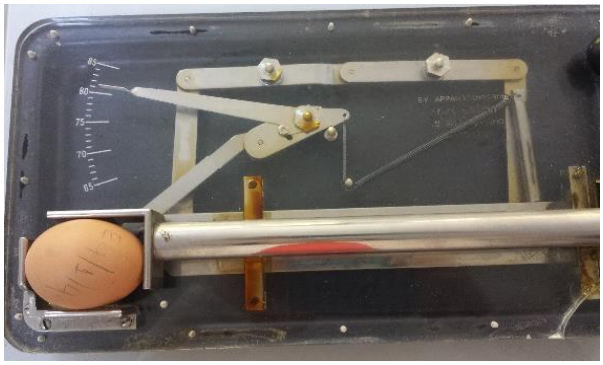
Egg Quality Measurements

Although the focus of this experiment was on the egg yolk cholesterol levels, conducting egg quality measurements were necessary as an indicator of the effect of horse chestnut seed supplement on the health of the hens. The details of egg quality measurement are provided in the Appendix.

Picture 3: Weighing the eggs.

To determine the egg internal and shell quality characteristics, eggs laid at 0900 to 1200 h were collected randomly from each group once a week.

Each egg was weighed in grams (see Picture 3) and their shape index was measured with a special instrument (manufactured by B.V. Apparatenfabreik Van Doorn, No: 75 135/2, De Bilt, The Netherlands) (see Picture 4). The egg shape index is the percent ratio of its width to its length. The special instrument is designed to give a reading of the index without making calculations.



Picture 4: Measuring the shape index of the eggs.

Egg shell breaking strength was measured in kg/cm^2 by using an egg breaking tester (static compression device, Dr,-Ing. Georg Wazau Mess-+Pruftechnick, Berlin, Germany). (Raunch 1965)[22].

Picture 5: Measuring in kg/cm^2 the shell breaking strength of the eggs with a static compression device specially designed by Dr,-Ing. Georg Wazau Mess-+Pruftechnick, Berlin, Germany .

Picture 6: Visual inspection of albumen and egg yolk on a glass topped table.

The egg content was broken onto a glass-topped table (see Picture 6) so that it will be easier to inspect the yolk and albumen of the eggs visually for any visible defects also from underneath the table.

Egg shell thickness was measured in three different parts (upper and lower ends and middle of shells) by using a micrometer in mm (see Pi,cture 7) (Mitutoyo, IP65, Coolant Proof Micrometer, No:293-230, 0–25 mm, 0.001mm, Japan). (Card and Nesheim 1972)[23]

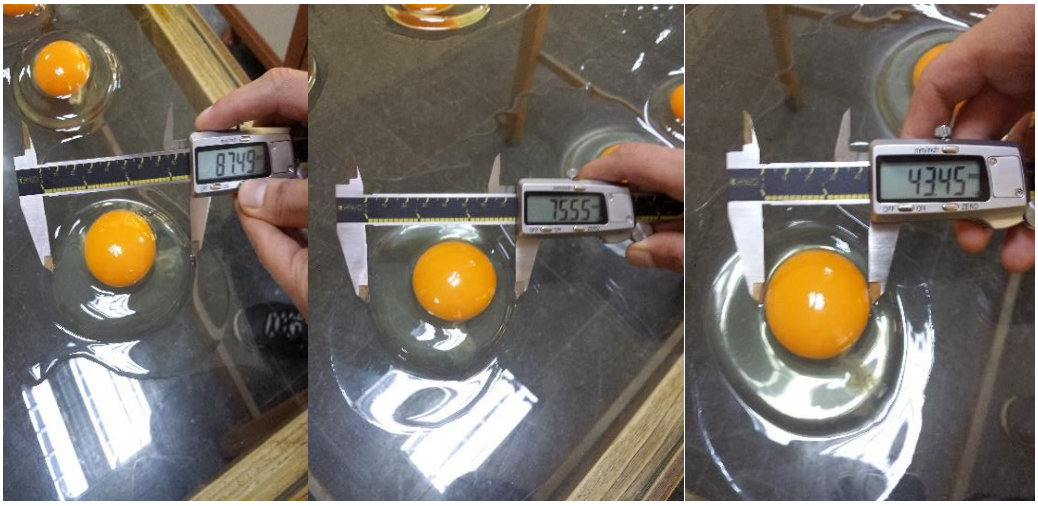
Picture 7: Measuring egg shell thickness with a micrometer.

Then, the height of the yolk and the albumen was measured with a tripod micrometer in mm (see Picture 8)(Mitutoya, No. 2050–08, 0.01–20 mm; Kawasaki, Japan). (Wells, 1968)[24]

Picture 8: Measuring egg yolk (left) and albumen (right) heights in mm with a tripod micrometer.

[23] Card, L.E., Nesheim, M.C., 1972. Poultry Production, 11th ed. Lea and Febiger, Philadelphia.
[24] Wells R.G. : A study of the hen's egg. In: Carter, T.C. British Egg Marketing Board Symposium, Edinburgh, pp. 207-249. 1968.

The length and width of the albumen and the diameter of the yolk were measured using a digital caliper. (See Picture 9) (Card and Nesheim 1972)[21]



Picture 9: Measuring egg albumen width (left), albumen length (middle) and yolk radius (right) in mm with a caliber.

By using the above mentioned measurements (Card and Nesheim, 1972)[22]:

- The albumen index is calculated as follows (higher values indicates better quality eggs because of albumen consistency):

Albumen index (%) = [Albumen height (mm)/(albumen length + albumen width) (mm)] x100

- The Haugh unit is calculated as follows (>80 values means better quality eggs):

Haugh Unit = 100 log (H+7.57-1.7 G 0.37)

Where; H= Albumen height (mm) G= egg weight (g)

- The yolk index is calculated as follows (higher values indicates better quality eggs):

Yolk index (%)= [Yolk height (mm)/Yolk radius (mm)] x100

The egg yolk color was evaluated visually by means of generally used La Roche scale (see Picture 10) (today also named as DSM Yolk Color Fan) (RYCF; DSM Nutritional Products, Kaiseraugst, Switzerland)[25]

Picture 10: Visual determination of egg yolk color by using DSM Yolk Color Fan.

[25] DSM Egg Yolk Color Fan. http://www.dsm.com/markets/anh/en_US/products/products-solutions/products_solutions_tools/Products_solutions_tools_EggYolk.html

Appendix 2

Hen performance indicators

As an indicator of healthy hens, feed consumption and egg production records were also kept for each day per test group.

Detailed tables of processed data on hen performance tests:

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	126.76	3.51	127.56	2.56	125.09	1.22	124.27	2.70	0.804^{-}
2.	123.27	2.35	123.13	1.80	122.68	1.90	120.48	2.68	0.794^{-}
3.	126.36	2.85	127.30	2.23	125.74	2.00	124.72	2.93	0.906^{-}
4.	123.56	4.05	127.85	1.97	125.37	2.12	120.50	2.66	0.341^{-}
Mean	**124.98**	**1.54**	**126.46**	**1.09**	**124.72**	**0.89**	**122.49**	**1.34**	**0.153^{-}**

-: not significant; p>0.05
n: 5

Table-A2.1. Feed consumption values of experimental groups (g/day/hen) (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	99.05	0.95	98.10	1.90	98.10	1.17	98.09	1.90	0.962^{-}
2.	98.89	1.11	96.67	2.22	97.78	1.36	96.67	2.22	0.790^{-}
3.	92.38	2.43	96.19	0.95	91.43	2.78	97.14	2.86	0.283^{-}
4.	96.19	3.81	97.14	1.17	97.14	1.90	96.19	1.78	0.983^{-}
Mean	**96.63**	**1.25**	**97.02**	**0.77**	**96.11**	**1.08**	**97.02**	**0.52**	**0.916^{-}**

-: not significant p>0.05
n: 5

Table-A2.2. Egg production values of experimental groups (%) (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	65.45	1.43	63.59	1.43	64.98	1.20	64.28	1.52	0.767^{-}
2.	63.48	1.08	63.95	0.86	66.51	1.43	65.76	1.14	0.233^{-}
3.	64.70	1.58	66.21	1.10	68.40	1.02	67.40	1.28	0.231^{-}
4.	67.53	2.31	64.69	1.78	66.39	1.20	65.73	0.74	0.662^{-}
Mean	**65.29**	**0.80**	**64.61**	**0.66**	**66.57**	**0.63**	**65.79**	**0.61**	**0.916^{-}**

-: not significant; p>0.05
n: 5
Table-A2.3. Egg weight values of experimental groups (g) (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	1.96	0.05	2.05	0.06	1.97	0.05	1.98	0.08	0.681^{-}
2.	1.97	0.07	2.00	0.05	1.89	0.05	1.90	0.05	0.431^{-}
3.	2.12	0.07	2.00	0.04	2.02	0.07	1.91	0.04	0.110^{-}
4.	1.91	0.05	2.04	0.03	1.95	0.07	1.91	0.04	0.243^{-}
Mean	**1.99**	**0.03**	**2.02**	**0.02**	**1.96**	**0.03**	**1.92**	**0.02**	**0.077^{-}**

-: not significant; p>0.05
n: 5
Table-A2.4. Feed efficiency values of experimental groups (kg feed/ kg egg) (mean±standard error)

	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
Feed consumption (g/day/hen)	124.98	1.54	126.46	1.09	124.72	0.89	122.49	1.34	0.153^{-}
Egg production (%)	96.63	1.25	97.02	0.77	96.11	1.08	97.02	0.52	0.916^{-}
Egg weight (g)	65.29	0.80	64.61	0.66	66.57	0.63	65.79	0.61	0.916^{-}
Feed efficiency (kg feed/ kg egg)	1.99	0.03	2.02	0.02	1.96	0.03	1.92	0.02	0.077^{-}

Table-A2.5: Effect of horse chestnut supplement on performance characteristics in laying hens ($\bar{x} \pm S\bar{x}$ = mean±standard error)

The Table-A2.5 indicates that there is no decrease in the performance of the hens in all experimental groups. Since all P values are greater than 0.05, there is no statistically significant change in the performances of the hens.

The Table-A2.5 also shows us that there is about 2% decrease in feed consumption but about 3.5% increase in feed efficiency for the experimental group fed with 1.00% horse chestnut. It means that the hens fed with this amount of horse chestnut will be producing 3.5% more eggs but consuming 2% less feed. This corresponds to more than 5% cost saving commercially.

Appendix 3

Detailed tables of processed data on egg quality tests:

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	80,38	0.59	79.34	1.24	78.10	1.29	80.00	0.53	0.403-
2.	79.06	0.59	80.40	0.96	81.14	1.08	80.02	0.98	0.472-
3.	80.38	0.81	79.34	0.90	78.10	0.23	80.00	0.70	0.446-
4.	80.16	0.67	79.37	0.99	79.34	0.49	79.45	0.34	0.798-
Mean	**79.65**	**0.34**	**79.51**	**0.49**	**79.42**	**0.48**	**79.97**	**0.32**	**0.815-**

-: not significant p>0.05, n:10

Table-A3.1. Egg shape index of experimental groups (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	5.44	0.23	5.70	0.29	5.38	0.52	5.69	0.49	0.911-
2.	4.91	0.41	5.09	0.51	5.21	0.59	5.28	0.27	0.943-
3.	5.16	0.16	5.23	0.26	4.79	0.34	4.50	0.29	0.234-
4.	4.34	0.29	4.43	0.26	3.54	0.24	3.88	0.11	0.054-
Mean	**4.96**	**0.72**	**5.11**	**0.19**	**4.73**	**0.27**	**4.83**	**0.22**	**0.356 -**

-: not significant p>0.05, n: 10

Table-A3.2. Egg breaking strength of experimental groups (kg/cm2) (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	363	1.12	368	1.53	353	1.06	388	0.65	0.231-
2.	350	1.86	346	0.71	346	1.88	359	0.05	0.267-
3.	383	0.83	379	0.54	383	0.78	373	1.42	0.891-
4.	380	0.83	369	0.54	373	0.46	390	0.65	0.791-
Mean	**369**	**6.64**	**366**	**5.03**	**364**	**6.38**	**377**	**4.58**	**0.222-**

-: not significant p>0.05, n: 10

Table-A3.3. Egg shell thickness of experimental groups (µm) (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	12.60	0.24	12.60	0.24	12.20	0.37	12.00	0.00	0.284-
2.	12.80	0.37	12.80	0.37	13.00	0.00	12.00	0.32	0.145-
3.	12.00	0.00	12.10	0.19	12.10	0.10	12.00	0.00	0.828-
4.	12.60	0.37	12.90	019	12.80	0.12	12.70	0.20	0.828-
Mean	**12.50**	**0.15**	**12.60**	**0.14**	**12.53**	**0.13**	**12.18**	**0.11**	**0.118-**

-: not significant p>0.05, n: 10

Table-A3.4. Egg yolk colour of experimental groups (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	8.73	0.14	9.68	0.41	8.98	0.45	9.18	0.30	0.295-
2.	8.54	0.20	8.08	0.39	9.31	0.71	8.99	0.51	0.684-
3.	8.05	0.29	8.51	0.52	8.95	0.76	8.84	0.48	0.337-
4.	7.10	0.30	7.92	0.41	8.39	0.39	7.88	0.33	0.646-
Mean	**8.10**	**0.18**	**8.55**	**0.26**	**8.91**	**0.28**	**8.72**	**0.22**	**0.111-**

-: not significant p>0.05, n: 10

Table-A3.5. Egg albumen index of experimental groups (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	45.18	0.54	46.64	0.51	42.37	0.50	44.39	1.00	0.055-
2.	43.84	0.66	42.32	0.73	44.04	1.13	41.71	0.77	0.179-
3.	44.30	0.68	44.05	0.79	44.42	0.64	44.10	0.49	0.975-
4.	44.06	0.44	44.35	0.45	44.36	0.48	43.15	0.28	0.183-
Mean	**44.34**	**0.29**	**43.59**	**0.34**	**43.80**	**0.39**	**43.34**	**0.40**	**0.239-**

-: not significant p>0.05, n: 10

Table-A3.6. Egg yolk index of experimental groups (mean±standard error)

Periods (weeks)	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
1.	82.64	0.67	87.55	1.60	85.45	1.88	84.74	1.22	0.149-
2.	82.54	0.73	82.04	0.74	84.79	2.09	84.41	1.72	0.474-
3.	80.94	0.32	82.09	2.17	83.85	2.66	82.73	2.20	0.787-
4.	76.04	0.85	79.05	1.52	81.62	1.47	79.31	1.18	0.053-
Mean	**80.54**	**0.69**	**82.69**	**1.01**	**83.93**	**1.00**	**82.80**	**0.89**	**0.074-**

-: not significant p>0.05, n: 10

Table-A3.7. Egg Haugh unit of experimental groups (mean±standard error)

	Horse chestnut supplementation (%)								P
	0		0.25		0.5		1.0		
	$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		$\bar{x} \pm S\bar{x}$		
Egg shape index	79.65	034	79.51	0.49	79.42	0.48	79.97	0.32	0.815-
Egg breaking strength (kg/cm^2)	4.96	0.72	5.11	0.19	4.73	0.27	4.83	0.22	0.356-
Egg shell thickness (µm)	369	6.64	366	5.03	364	6.38	377	4.58	0.222-
Egg yolk color	12.50	0.15	12.60	0.14	12.53	0.13	12.18	0.11	0.118-
Egg albumen index	8.10	0.18	8.55	0.26	8.91	0.28	8.72	0.22	0.111-
Egg yolk index	44.34	0.29	43.59	0.34	43.80	0.39	43.34	0.40	0.239-
Egg Haugh unit	80.54	0.69	82.69	1.01	83.93	1.00	82.80	0.89	0.074-

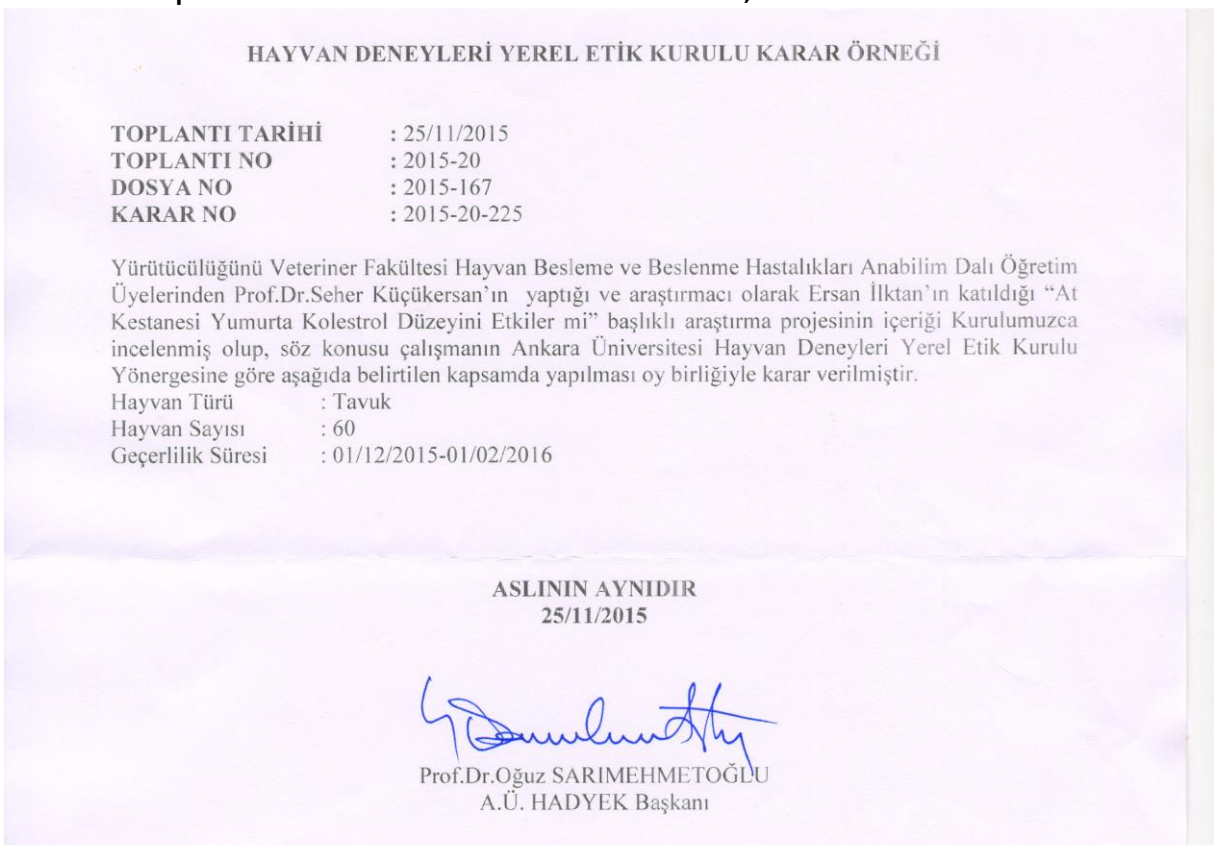
Table-A3.8: Effect of horse chestnut supplement on egg quality characteristics in laying hens ($\bar{x} \pm S\bar{x}$ = mean±standard error)

The summary Table-A3.8 indicates that there is no statistically significant change in the egg quality of the hens, since all P values are greater than 0.05. It shows us that in this experiment, horse chestnut supplement did not have toxic effect because the egg quality data do not differ significantly compared with the control group.

Table-A3.8 also shows that the experimental group E4, fed with 1.00% horse chestnut supplement, produced eggs with slightly more consistent albumen; which is a desirable effect as it is perceived by people as more fresh egg.

Appendix 4

The Decision of the Ankara University Local Ethical Committee on Animal Experiments dated November 25th, 2015.



HAYVAN DENEYLERİ YEREL ETİK KURULU KARAR ÖRNEĞİ

TOPLANTI TARİHİ : 25/11/2015
TOPLANTI NO : 2015-20
DOSYA NO : 2015-167
KARAR NO : 2015-20-225

Yürütücülüğünü Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı Öğretim Üyelerinden Prof.Dr.Seher Küçükersan'ın yaptığı ve araştırmacı olarak Ersan İlktan'ın katıldığı "At Kestanesi Yumurta Kolestrol Düzeyini Etkiler mi" başlıklı araştırma projesinin içeriği Kurulumuzca incelenmiş olup, söz konusu çalışmanın Ankara Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu Yönergesine göre aşağıda belirtilen kapsamda yapılması oy birliğiyle karar verilmiştir.

Hayvan Türü : Tavuk
Hayvan Sayısı : 60
Geçerlilik Süresi : 01/12/2015-01/02/2016

ASLININ AYNIDIR
25/11/2015

Prof.Dr.Oğuz SARIMEHMETOĞLU
A.Ü. HADYEK Başkanı

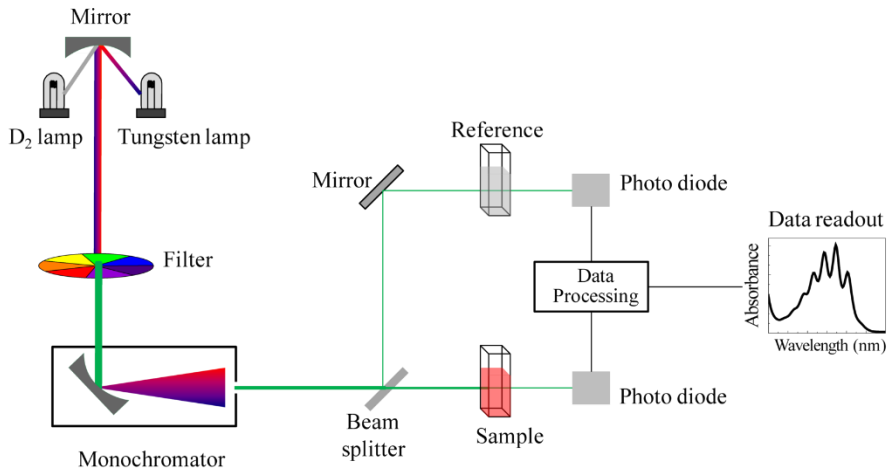
Picture-A4.1: Scanned image of the decision of the Ankara University Ethical Comittee about Experiences on Animals, dated November 25th, 2015, indicating the acceptance of the experiment to be conducted.

Appendix 5

Enzymatic colorimetric test for cholesterol with lipid clearing factor:

It is a method which is based on forming a dye from cholesterol after some chemical reactions and then measuring the concentration of the dye by using a spectrophotometer as it is directly proportional to the concentration of cholesterol. Using enzymes is to quicken the chemical reactions to form the dye. In my experiment HUMAN brand reagents for cholesterol were used. They produce a pinkish color which is then used to measure the absorbance of the sample to determine the amount of cholesterol in the sample.

Spectrophotometer uses ultraviolet light in the visible range to measure transmittance of materials at certain wavelengths. Its working principle is show in the below Picture-A5.1.



Picture-A5.1. Schematic of UV-Visible Spectrophotometer. Public domain: https://commons.wikimedia.org/wiki/File:Spetrophotometer-en.svg#/media/File:Spetrophotometer-en.svg

Data readout of the spectrophotometer is in Absorbance Units calculated according to the following formula:

$$A = -Log(\%T/100\%)$$

Where T, Transmittance is the ratio of the intensity of light after it passed thru the sample to the intensity of the light before.

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