



## The Effects of Raw and Physical Processed Common Vetch Seed (*Vicia sativa*) on Laying Performance, Egg Quality, Metabolic Parameters and Liver Histopatology of Laying Hens

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**ABSTRACT :** This experiment was designed to evaluate the effects of the processing method of common vetch seed (CVS) (*Vicia sativa*) on laying performance, egg quality, metabolic parameters and liver histopatology during the peak production period in hens. Lohman layers, 46 wk of age in 6 replicate cages each containing 4 hens, were allocated randomly to one of four dietary treatments. Diets were control (C) diet containing no common vetch and experimental diets containing 25% raw common vetch (RCV), 25% soaked in water for 72 h with exchange of water every 24 h (SCV) and 25% soaked&boiled at 100°C for 30 minute common vetch (SBCV). Inclusion of RCV into the diet deteriorated all laying performance variables. SCV did not alleviate the adverse effect of raw common vetch on feed intake, egg weight, feed conversion, final weight and weight change. SCV partially alleviated egg production ( $p<0.001$ ). SBCV diminished the adverse effect on feed intake, egg weight, feed conversion, final weight and weight change compared to raw *vicia sativa* ( $p<0.001$ ). No significant difference was detected between SBCV and the control group in terms of egg production, feed conversion, final weight and weight change. Regardless of the processing method, all the common vetch groups had lower shell strength compared to the control group. Haugh units did differ between all groups ( $p<0.001$ ). Inclusion of RCV and SCV into the basal diet decreased triglyceride, cholesterol, total protein and serum glucose concentrations ( $p<0.001$ ). However, inclusion of SBCV into the basal diet increased these parameters. Liver samples were stained with Hematoxylin and eosin (HE) and evaluated by light microscopy. A biopsy of native liver tissue was used as a control. No histopathologic finding was present in the control group. Raw *V. sativa* compared with the control caused lipid accumulations in hepatocytes, severe congestion of hepatic blood vessels, inflammation, increased numbers of Kupffer cells and sinusoidal dilatations. Whereas, the livers from groups given treated *V. sativa* showed only different degrees of sinusoidal dilatations. Findings from the present study point out the risk of increased hepatic damage due to use of raw *Vicia sativa*. Increasing treatment of *V. sativa* lead to a decrease of liver damages. Inclusion of raw and soaked vetch seeds in rations affected adversely all parameters examined in laying hens. But alleviation was observed when soaked and boiled vetch seeds (SBCV) were fed. The results of these experiments indicated that soaked&boiled *Vicia sativa* seeds may safely be used at a 25% level in rations of laying hens. (**Key Words :** Common Vetch Seed, Processing Method, Laying Performance, Egg Quality, Metabolic Parameter, Liver Histopatology)

### INTRODUCTION

Common vetch (*Vicia sativa*) seed has been used in animal feeds as an alternative source of protein in poultry diets (Darre et al., 1999). But in the raw state, common

vetch is detrimental for poultry species (Saki et al., 2008) due to presence of certain antinutritional factors (ANF) that interfere with efficient utilization of vetch, such as  $\beta$ -cyanoalanine, vicine, concivine and tannins (Gatel, 1994; Farran et al., 1995; Abdullah et al., 2010). The tannins, especially, may cause a over estimation of the nutritive value of common vetch seed (CVS) (*Vicia sativa*) (Gül et al., 2005).

Tannins are water soluble phenolic metabolites of plant origin that are found in variety of feed ingredients. Tannins are known to inhibit the activity of digestive enzymes and the nutritional effect of tannins is mainly related to their

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interactions with proteins. Ortiz et al. (1993) reported there was a reduction in feed consumption when tannins were fed to chickens. Soaking in water and a cooking process of legume seeds can eliminate phenols and tannins (Onwuka, 2006). Autoclaving and cooking in boiling water reduced the level of tannins in winged bean (Kadam et al., 1987). Ressler et al. (1997) reported that processing common vetch seed by cooking removes toxicity for the young chicks.

The incorporation of untreated common vetch seeds to more than 20% in layer and 15% in broiler diets decreased their performance (Gül et al., 2005). Most research has centered on several detoxification methods including soaking in water at room temperature, acetic acid, sodium bicarbonate and potassium bicarbonate solution and cooking to achieve a high level of common vetch seeds in poultry diets (Farran et al., 1995; Ressler et al., 1997; Darre et al., 1999; Farran et al., 2001a,b; Sadeghi et al., 2004; Saki et al., 2008). However, none of these methods are able to completely remove all the detected ANF that are present in seeds, grains or feed materials. A combination of processing methods is generally more effective than a single method (Sadeghi et al., 2009).

Gül et al. (2005) showed that raw common vetch seeds fed at 22% level are detrimental to laying performance and egg quality. Water and acetic acid soaking or autoclaving processing partially increases utilization of high levels of legume seeds. Farran et al. (2001a) reported that when common vetch was soaked at 1:10 (wt/vol) in water at 40°C for 72 h, with a water change every 12 h (40 WV) or in 1% acetic acid at room temperature (RTAAV), or at 40°C (40AAV) for 24 h, no toxicity symptoms were observed. In addition, the RTAAV treatment resulted in better laying hen performance compared to that of the control.

It is clear that the inclusion of vetch seed on animal diets brings about a number of undesirable effects such as reduced protein deposition, altered digestibility and absorption of nutrients and impairment of the immune response, which have been attributed to the presence of various antinutritional factors and to a poor sulfur amino acid content (Martinez et al., 1992; Mahmood and Smithard, 1993; Marzo et al., 2002). Raw *Vicia faba* L. minor also produces local reactions in the pancreas and the intestine of chicks (Rubio et al., 1989). The possible role of tannins and phytates in causing these pathologies is discussed by Longstaff and McNab (1991). The liver plays a crucial role in metabolism (Yokouchi, 2005) and hepatic metabolism is, first and foremost, a mechanism that converts compounds into products that are easily excreted (Tolman, 1998). It is recorded that the liver is the target organ in rats given *V. faba* (Santidrian et al., 1981; Santidrian et al., 1987; Santidrian et al., 1994). Therefore, the histologic responses of the liver in relation to fertilizer treatments of *V. faba* is especially important. Thus, in the present study,

histopathological examinations of chicken liver were performed to study the effect of different processing methods of *V. sativa*.

This experiment was designed to evaluate the effects of processing method (soaking and cooking in water) of CVS on laying performance, egg quality, and metabolic parameters and liver histopathology during the peak production period in hens.

## MATERIALS AND METHODS

### Animal, diet, and management

This study was carried out at the poultry unit of the Research and Application Farm of the Agricultural Faculty at Atatürk University. Ninety-six Lohman layers, 46 wks of age, were blocked according to the location of cages (50×46×46 cm) and then assigned randomly to receive one of four dietary treatments for 12 wks. Each dietary treatment was replicated in 6 cages, each containing 4 hens.

Locally produced common vetch seeds were soaked in water at room temperature in the ratio of 1:5 (w/v) for 72 hours with a water change every 24 hours then dried at room temperature and supplemented in diet at 25% level (SCV). One part of SCV was boiled at 100 C for 30 minutes then dried at room temperature and incorporated into the diet at 25% level (SBCV) (Monary, 1989). The experimental diets were formulated to meet the NRC recommendations (NRC, 1994). Treatment groups were fed diets containing a standard commercial layer diet that had no common vetch (C), basal diet plus 25% raw common vetch (RCV), 25% soaked common vetch (SCV) and 25% soaked and boiled common vetch (SBCV) from week 46<sup>th</sup> to 58<sup>th</sup>. Diets were prepared as isonitrogenous (18% CP) and isocaloric (2,850 kcal of ME/kg) based on corn-barley-soybean meal diet (control) (Table 1). Ingredients of the experimental diets are shown in Table 1. During the experimental period, hens were fed *ad libitum* once daily at 08:30 hrs and water was available all the times. Lighting was 17 L:7 D throughout experimental period.

### Sample collection and analytical procedure

All vetch seed samples were analyzed for tannin using a spectrophotometric method (UV-visible 2100, Shimadzu, Japan) at 440 nm by Folin Denis reagent according to AOAC (1990). Methods identified by the AOAC (1990) were used to determine dry matter, crude protein, crude fiber, and ether extract concentrations of feed samples. Feed intake and egg production were recorded daily; egg weight was measured bi-weekly; and body weights were measured at the beginning and the end of the experiment. Feed conversion ratio (FCR) was stated as kilogram of feed consumed per kilogram of eggs produced. Before determination of egg weight, a sample of 12 eggs from each

**Table 1.** Chemical composition of experimental chicken diets and calculated major components

| Ingredient (%)                                | Experimental diets |       |       |       |
|---|--------------------|-------|-------|-------|
|   | 1                  | 2     | 3     | 6     |
| Corn  | 47.50              | 33.75 | 33.75 | 33.75 |
| Soybean meal, 44% CP                          | 14.35              | 7.28  | 7.28  | 7.28  |
| Common vetch seed                             | -                  | 25.00 | 25.00 | 25.00 |
| Wheat   | 3.75               | 9.60  | 9.60  | 9.60  |
| Barley  | 6.80               | -     | -     | -     |
| Soybean oil                                   | 5.00               | 3.75  | 3.75  | 3.75  |
| Vegetable fat                                 | 0.97               | 0.90  | 0.90  | 0.90  |
| Sunflower seed meal                           | 10.00              | 7.50  | 7.50  | 7.50  |
| Fish meal                                     | 0.50               | 1.09  | 1.09  | 1.09  |
| Dicalcium phosphate <sup>1</sup>              | 1.25               | 1.20  | 1.20  | 1.20  |
| Marble  | 9.25               | 9.25  | 9.25  | 9.25  |
| Vitamin-mineral premix <sup>2</sup>           | 0.25               | 0.25  | 0.25  | 0.25  |
| Salt  | 0.30               | 0.19  | 0.19  | 0.19  |
| Methionine <sup>3</sup>                       | 0.05               | 0.12  | 0.12  | 0.12  |
| Lysine <sup>4</sup>                           | 0.03               | 0.12  | 0.12  | 0.12  |
| Chemical composition (analyzed, on DM basis)  |                    |       |       |       |
| Dry matter (%)                                | 90.9               | 89.6  | 89.8  | 90.1  |
| Crude protein (%)                             | 17.2               | 17.4  | 17.4  | 17.6  |
| Crude fibre (%)                               | 6.2                | 6.4   | 6.0   | 6.4   |
| Ether extract (%)                             | 3.8                | 3.3   | 3.3   | 3.4   |
| Crude ash (%)                                 | 9.3                | 9.0   | 9.1   | 9.4   |
| Non-nitrogenous constituents (%) <sup>5</sup> | 54.4               | 53.5  | 54.0  | 53.3  |
| Metabolizable energy (kcal/kg) <sup>5</sup>   | 2,671              | 2,612 | 2,625 | 2,619 |
| Tannin content of the diets (mg/kg)           | 2.74               | 3.29  | 2.91  | 2.42  |
| Tannin content of the vetchs (mg/kg)          | -                  | 4.46  | 3.57  | 1.70  |

Diets: C = Control diet; RCV = Basal diet containing common vetch seed (25%), SCV = Soaked in water at room temperature in the ratio of 1:5 (w/v) for 72 hours with water change every 24 hours then dried at room temperature and supplemented in diet at 25% level. SBCV = Soaked in water at room temperature in the ratio of 1:5 (w/v) for 72 hours with water change every 24 hours then dried at room temperature and boiled at 100°C for 30 minute then dried at room temperature and incorporated with diet in 25% level.

<sup>1</sup> Each kilogram contained: Ca, 24% and P, 17.5%.

<sup>2</sup> Each kilogram contained: Vitamin A, 15,000 IU; cholecalciferol, 1,500 ICU; DL- $\alpha$ -tocopheryl acetate, 30 IU; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; pantothenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B<sub>12</sub>, 15  $\mu$ g; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; and Se, 0.15 mg.

<sup>3</sup> DL-methionine. <sup>4</sup> L-lysine hydrochloride. <sup>5</sup> Calculated components.

experimental group were stored for 24 h in room temperature. Twelve egg samples from each experimental group were randomly picked every month to evaluate egg quality parameters. Egg quality parameters were shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Haugh unit. They were assessed according to the method of Hayirli et al. (2005).

At the end of the experimental period, blood samples were collected from the brachial vein of two hens from each cage and put into additive-free vacutainers. Serum was obtained following centrifugation at 3,000 g for 15 min at 20°C and kept at -20°C until laboratory analyses for serum parameters (albumin, triglyceride, cholesterol, very low-

density lipoprotein (VLDL), total protein glucose, creatine, Ca, and P) using commercial kits (DDS<sup>®</sup> Spectrophotometric Kits, Diasis Diagnostic Systems Co., İstanbul 80270, Turkey).

The chicks were killed by ether and immediately dissected at the end of the experiment. Livers were used for slide preparations. The liver samples were fixed in 10% formaldehyde and were embedded in paraffin for microscopic examination and then sections from paraffin blocks (5  $\mu$ m) were stained with HE (Bancrapt and Stevens, 1982).

#### Statistical analysis

Data from the present experiment were statistically analyzed by ANOVA using the GLM procedure of SPSS

software (SPSS 10.01, 1996) and significant differences between means were determined by Duncan's Multiple Range Test. The effects of the dietary treatments on response variables were stated with level of significance at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

According to the results of tannin analysis, raw common vetch contained 4.46 mg/kg DM tannin. All processing methods reduced the tannin content of seeds to a negligible amount (3.57 and 1.70 mg/kg DM tannin SCV and SBCV respectively) (Table 1). Sadeghi et al. (2009) stated that the loss of tannin during processing may be due to either its removal by this treatment or a change in its chemical reactivity.

### Laying performance

Laying performance parameters are summarized in Table 2. Average feed intake of hens fed the SBCV diet was the highest (120.48 g/d) of all treatments. This result may be due to less toxin in soaked and boiled common vetch (Table 1). Layers fed diets containing RCV had lower feed intake than birds fed SCV diets but this difference was not significant (Table 2). The decrease in feed intake of chicks fed with raw common vetch diet could be associated with the presence of high tannin in untreated vetch. This response may be the reaction of bird to this toxic factor in the digestive tract or some unpleasant taste caused by this factor in the diet.

Egg production was the lowest for the RCV group followed by the SCV group ( $p < 0.05$ ). But SBCV group had egg production values that were not different from that of the control group ( $p > 0.05$ ). Cracked egg yield ratio was not affected by any of the dietary treatments ( $p > 0.05$ ). Supplementation by RCV and SCV decreased egg weight.

SBCV partially increased egg weight but did not to that of the control. Inclusion of RCV and SCV into the basal diet decreased the feed conversion ratio. Soaking and boiling of common vetch (SBCV) reduced the adverse effect on FCR. In addition, no significant difference was shown between SBVC and the control group in FCR. Final body weight and body weight change showed a similar trend with the feed conversion ratio. RCV and the addition of SCV to the basal diet reduced final body weight. However, inclusion of soaked and boiled (at 100°C for 30 minute) vetch into the basal diet increased the final body weight. Depressions in BW and laying performance could be related to reduced feed intake or interference of tannins in CVS with protein metabolism or both.

In this study, the results with respect to feed intake, egg production, feed conversion ratio and body weight change were comparable to findings of Farran et al. (1995) in layers fed untreated vetch and soaked in water at a ratio of 1:5 for 24 h. Saki et al. (2008) reported that body weight gains of broilers fed on 20% and 30% vetch seeds either raw or cooked at 100°C for 60 minutes were significantly lower than control but the effects of 10% processed vetch and 20% raw vetch on body weight gain were not statistically different compared with control group.

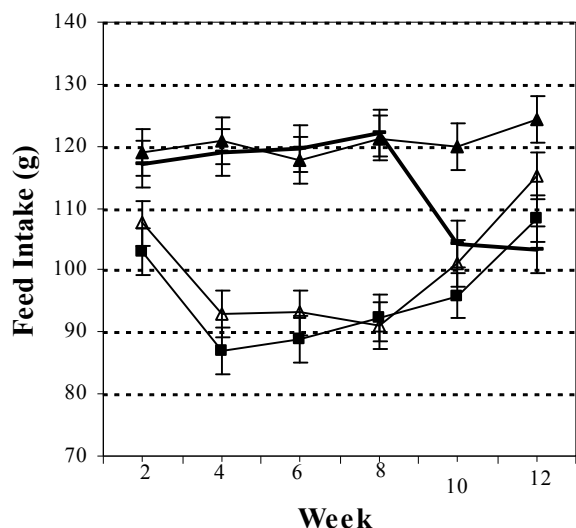
There was a change in feed intake, egg production, egg weight and feed conversion ratio while the present experiment continued. These values were 111.58, 104.89, 104.83, 106.72, 105.32 and 112.76 g/d for feed intake ( $p < 0.004$ ); 77.42, 82.07, 74.85, 74.85, 71.29 and 76.25% for hen-day egg production ( $p < 0.052$ ); 66.44, 65.58, 66.74, 66.30, 64.01 and 64.951 g for egg weight ( $p < 0.049$ ); 2.18, 1.95, 2.15, 2.25, 2.62 and 2.46 kg feed/kg egg for feed conversion ratio on experiment wk 2, 4, 6, 8, 10 and 12 respectively. There was also effect of the experimental diets by time interaction on feed intake ( $p < 0.0001$ ; Figure 1) and

**Table 2.** The effects of processing method of common vetch seed on laying performance parameters

| Treatment | Response variables  |                    |                       |                    |                                   |                    |                       |                      |
|-----------|---------------------|--------------------|-----------------------|--------------------|-----------------------------------|--------------------|-----------------------|----------------------|
|           | Feed intake (g/d)   | Egg production (%) | Cracked egg yield (%) | Egg weight (g)     | FCR <sup>1</sup> (kg feed/kg egg) | Initial weight (g) | Final weight (g)      | Weight change (g)    |
| Control   | 114.23 <sup>b</sup> | 83.52 <sup>a</sup> | 0.75                  | 68.87 <sup>a</sup> | 1.99 <sup>b</sup>                 | 1,658.75           | 1,738.40 <sup>a</sup> | 79.65 <sup>a</sup>   |
| RCV       | 95.88 <sup>c</sup>  | 62.88 <sup>c</sup> | 0.20                  | 63.76 <sup>c</sup> | 2.39 <sup>a</sup>                 | 1,656.88           | 1,517.99 <sup>b</sup> | -138.89 <sup>b</sup> |
| SCV       | 100.15 <sup>c</sup> | 69.34 <sup>b</sup> | 0.25                  | 63.25 <sup>c</sup> | 2.28 <sup>a</sup>                 | 1,690.83           | 1,497.49 <sup>b</sup> | -193.35 <sup>b</sup> |
| SBCV      | 120.48 <sup>a</sup> | 88.20 <sup>a</sup> | 0.25                  | 66.79 <sup>b</sup> | 2.05 <sup>b</sup>                 | 1,658.75           | 1,711.25 <sup>a</sup> | 52.50 <sup>a</sup>   |
| SEM       | 1.53                | 1.94               | 0.16                  | 0.57               | 0.24                              | 25.00              | 40.43                 | 41.49                |
| ANOVA     |                     |                    |                       |                    |                                   |                    |                       |                      |
| Grup (G)  | 0.0001              | 0.0001             | 0.065                 | 0.0001             | 0.0001                            | 0.734              | 0.0001                | 0.0001               |
| Time (T)  | 0.004               | 0.052              | 0.169                 | 0.049              | 0.005                             |                    |                       |                      |
| G×T       | 0.0001              | 0.027              | 0.816                 | 0.251              | 0.111                             |                    |                       |                      |

RCV = Basal diet plus 25% raw common vetch, SCV = 25% soaked common vetch, SBCV = 25% soaked&boiled common vetch.

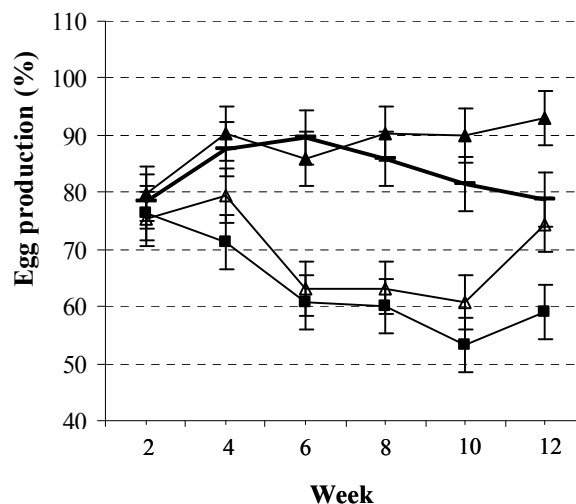
<sup>1</sup> FCR = Feed conversion ratio (kg feed consumed per kg egg produced).



**Figure 1.** The effect of the experimental diets by time interaction on feed intake. (SEM = 3.74 and  $p < 0.0001$ ) (—, hens not fed common vetch seed; ■, hens fed 25% unprocessed common vetch seed; □, hens fed on diet containing 25% of common vetch seed soaked in water at room temperature for 72 hours with water change every 24 hours then dried at room temperature (SCV). ▲, hens fed on diet containing 25% of soaked and boiled (100°C) common vetch (SBCV).

egg production ( $p < 0.027$ ; Figure 2). Feed intake of the control group may have decreased by a change in the basal diet given to hens at 10-12 weeks in the present study.

In this experiment, inclusion of raw and soaked vetch into the basal diet showed similar effect on performance parameters. This may have been due to less diffusion of water-soluble toxins from the hull, which serves as a physical barrier. Soaked and boiled vetch alleviated performance parameters compared to that of soaked vetch. It also may be due to tannin removal by this treatment and/or change in its chemical reactivity.



**Figure 2.** The effect of the experimental diets by time interaction on egg production. (SEM = 4.74 and  $p < 0.027$ ) (—, hens not fed common vetch seed; ■, hens fed 25% unprocessed common vetch seed; □, hens fed on diet containing 25% of common vetch seed soaked in water at room temperature for 72 hours with water change every 24 hours then dried at room temperature (SCV). ▲, hens fed on diet containing 25% of soaked and boiled (100°C) common vetch (SBCV).

### Egg quality

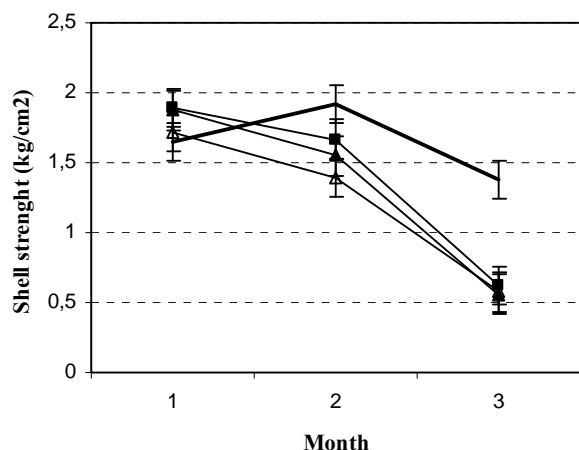
Table 3 summarizes the effects of the experimental diets on egg quality parameters. Except for shell strength and Haugh unit, other egg quality parameters (shape index, shell thickness, shell weight, yolk color, yolk index and albumen index) were not affected by common vetch compared with the control diet ( $p > 0.05$ ). Average shell strength for eggs from hens fed common vetch, whether treated or untreated, was significantly lower than that of the control ( $p < 0.005$ ). The degree of changes was distinct as of the vetch type of supplementation throughout the investigation period ( $p < 0.01$ ; Figure 3).

Haugh unit for hens fed diet SBCV was lower than for

**Table 3.** The effects of processing method of common vetch seed on egg quality parameters

| Treatment | Response variables |                                      |  |                  |            |                |                   |                     |
|-----------|--------------------|--------------------------------------|--|------------------|------------|----------------|-------------------|---------------------|
|           | Shape index (%)    | Shell Strength (kg/cm <sup>2</sup> ) | Shell thickness (mm×10 <sup>-2</sup> ) | Shell weight (g) | Yolk color | Yolk index (%) | Albumen index (%) | Haugh unit          |
| Control   | 73.86              | 1.64 <sup>a</sup>                    | 0.404                                  | 8.32             | 8.28       | 41.85          | 8.20              | 84.85 <sup>a</sup>  |
| RCV       | 73.50              | 1.39 <sup>b</sup>                    | 0.361                                  | 8.04             | 8.06       | 43.56          | 8.91              | 82.36 <sup>ab</sup> |
| SCV       | 74.28              | 1.23 <sup>b</sup>                    | 0.366                                  | 7.76             | 7.72       | 43.36          | 8.31              | 80.42 <sup>bc</sup> |
| SBCV      | 73.11              | 1.33 <sup>b</sup>                    | 0.370                                  | 8.05             | 7.89       | 41.87          | 7.99              | 78.26 <sup>c</sup>  |
| SEM       | 0.64               | 0.08                                 | 0.01                                   | 0.18             | 0.17       | 0.66           | 0.32              | 1.64                |
| ANOVA     |                    |                                      |  |                  |            |                |                   |                     |
| Grup (G)  | 0.618              | 0.005                                | 0.132                                  | 0.192            | 0.164      | 0.116          | 0.233             | 0.001               |
| Time (T)  | 0.924              | 0.0001                               | 0.215                                  | 0.379            | 0.512      | 0.362          | 0.003             | 0.0001              |
| G×T       | 0.466              | 0.010                                | 0.082                                  | 0.075            | 0.031      | 0.048          | 0.365             | 0.302               |

RCV = Basal diet plus 25% raw common vetch, SCV = 25% soaked common vetch, SBCV = 25% soaked&boiled common vetch.



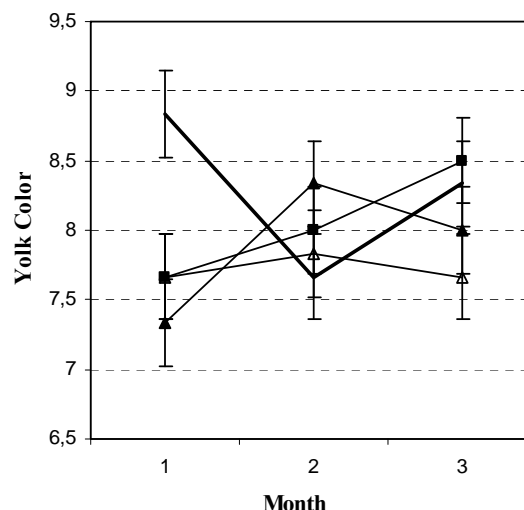
**Figure 3.** The effect of the experimental diets by time interaction on shell strenght. (SEM = 0.14 and  $p < 0.01$ ) (—, hens not fed common vetch seed; ■, hens fed 25% unprocessed common vetch seed; △, hens fed on diet containing 25% of common vetch seed soaked in water at room tempareture for 72 hours with water change every 24 hours then dried at room temperature (SCV). ▲, hens fed on diet containing 25% of soaked and boiled (100°C) common vetch (SBCV).

hens fed the control diet. Inclusion of RCV and SCV into basal diet partially increased the Haugh unit but did not bring it up to the level of the control group. There were variations in shell strenght, albumen index and Haugh unit as the experiment continued ( $p < 0.0001$ ) and average shell strenght was 1.78, 1.63 and 0.784 kg/cm<sup>2</sup>, albumen index 8.87, 8.67 and 7.53% and Haugh unit 82.97, 82.35 and 75.34 on experiment month 1, 2, and 3, respectively. Moreover, the experimental diets affected yolk color and yolk index in different ways during the experimental period ( $p < 0.031$ , Figure 4;  $p < 0.048$ , Figure 5 respectively).

In contrast with the current findings Gül et al. (2005) showed that shell strength and Haugh unit obtained by use of the high levels of vetch diet increased. Similarly, Farran et al. (1995, 2001a) reported a positive increase in Haugh unit in a group fed on a diet containing soaked vetch (25%, 60%). Ergün et al. (1987) reported that laying hens at peak production fed diets containing an increasing level (0, 5 and 10%) of common vetch seed for a period of 4 mounths had linear decreases in yolk index but no changes in other egg quality parameters (egg weight, shape index, shell strength, albumen index, yolk color, Haugh unit, shell thickness, and shell weight).

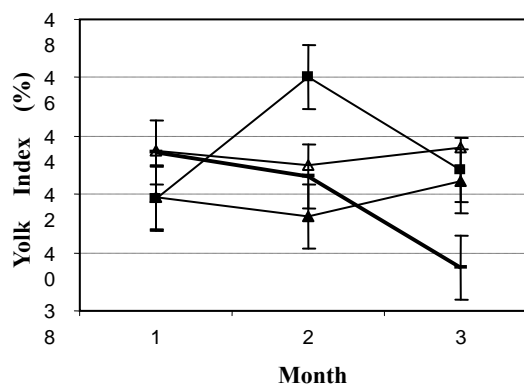
#### Metabolic parameters

The effects of the processing method of common vetch seed on metabolic parameters during the peak period are presented in Table 4. Inclusion of RCV and SCV into the basal diet decreased triglyceride ( $p < 0.012$ ), cholesterol ( $p < 0.028$ ), total protein ( $p < 0.040$ ) and serum glucose ( $p < 0.03$ ) concentrations. However, inclusion of SBCV into



**Figure 4.** The effect of the experimental diets by time interaction on yolk color. (SEM = 0.31 and  $p < 0.03$ ) (—, hens not fed common vetch seed; ■, hens fed 25% unprocessed common vetch seed; △, hens fed on diet containing 25% of common vetch seed soaked in water at room tempareture for 72 hours with water change every 24 hours then dried at room temperature (SCV). ▲, hens fed on diet containing 25% of soaked and boiled (100°C) common vetch (SBCV).

the basal diet did not decrease these parameters. Alkaline phosphatase (Alp) concentrations of blood samples taken from hens fed RCV ( $p < 0.007$ ) at the end of the experimental period decreased whereas SCV and SBCV groups were not different from the control group.



**Figure 5.** The effect of the experimental diets by time interaction on yolk index. (SEM = 1.10 and  $p < 0.048$ ) (—, hens not fed common vetch seed; ■, hens fed 25% unprocessed common vetch seed; △, hens fed on diet containing 25% of common vetch seed soaked in water at room tempareture for 72 hours with water change every 24 hours then dried at room temperature (SCV). ▲, hens fed on diet containing 25% of soaked and boiled (100°C) common vetch (SBCV).

**Table 4.** The effects of processing method of common vetch seed on metabolic profile

| Treatment | Response variables <sup>1</sup> |                       |                    |        |                    |                      |                     |                    |       |       |
|-----------|---------------------------------|-----------------------|--------------------|--------|--------------------|----------------------|---------------------|--------------------|-------|-------|
|           | Alb                             | TG                    | Chol               | VLDL   | TP                 | Alp                  | Glo                 | Cre                | Ca    | P     |
| Control   | 1.82                            | 1,429.50 <sup>a</sup> | 282.5 <sup>a</sup> | 195.83 | 6.33 <sup>a</sup>  | 966.7 <sup>a</sup>   | 374.2 <sup>a</sup>  | 2.48 <sup>a</sup>  | 14.78 | 5.15  |
| RCV       | 1.65                            | 890.67 <sup>b</sup>   | 221.8 <sup>b</sup> | 169.17 | 5.53 <sup>ab</sup> | 652.8 <sup>b</sup>   | 314.8 <sup>ab</sup> | 1.30 <sup>b</sup>  | 14.37 | 5.17  |
| SCV       | 1.87                            | 751.33 <sup>b</sup>   | 214.8 <sup>b</sup> | 133.63 | 5.33 <sup>b</sup>  | 1,224.0 <sup>a</sup> | 276.2 <sup>b</sup>  | 0.90 <sup>b</sup>  | 14.43 | 4.73  |
| SBCV      | 1.83                            | 1,374.17 <sup>a</sup> | 281.5 <sup>a</sup> | 184.50 | 6.32 <sup>a</sup>  | 950.2 <sup>ab</sup>  | 386.2 <sup>a</sup>  | 1.72 <sup>ab</sup> | 14.65 | 5.47  |
| SEM       | 0.08                            | 172.89                | 22.98              | 19.77  | 0.26               | 217.89               | 27.04               | 0.30               | 0.12  | 0.36  |
| p         | 0.276                           | 0.012                 | 0.028              | 0.099  | 0.040              | 0.007                | 0.03                | 0.015              | 0.097 | 0.570 |

RCV = Basal diet plus 25% raw common vetch, SCV = 25% soaked common vetch, SBCV = 25% soaked and boiled common vetch.

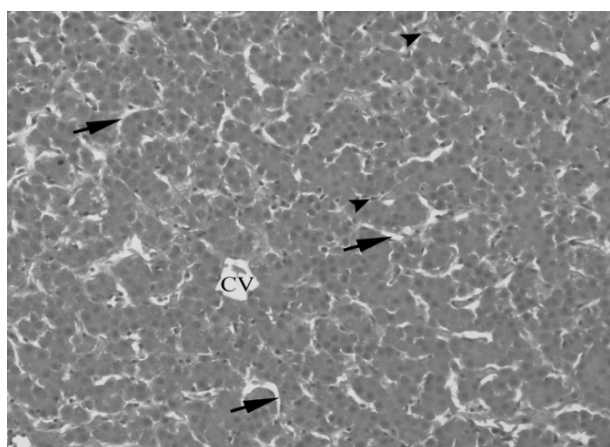
<sup>1</sup> Alb = Albumin; TG = Triglyceride; Chol = Cholesterol; VLDL = Very low-density lipoprotein. TP = Total protein; Alp = Alkaline phosphatase; Glo = Globulin; Cre = Creatine. All are in mg/dl.

Regardless of the processing method, supplementation of common vetch reduced the level of creatine ( $p < 0.015$ ).

The negative effects of legume seeds on growth and laying performance are accompanied by a decrease in serum protein concentration (Wyckoff et al., 1983). In the present study, decreasing body weight and laying performance of hens fed common vetch could be related to reduced serum protein concentration. Decreasing serum protein concentration may be due to an interference of tannins in CVS with protein metabolism. Yalçın et al. (1998) reported that serum total protein and lipid concentration decreased in Japanese quails (*Coturnix coturnix japonica*) fed diets containing 0, 5, and 10% common vetch seed. Vicin at 1% level fed to layers was reported not to affect total protein concentration and increased plasma lipid and lipid peroxide levels (Muduuli et al., 1981). Avcı et al. (2003) stated that adding 4% and 8% vetch seed (*V. sativa L.*) to turkey rations did not have a significant effect on the values of serum total cholesterol and protein.

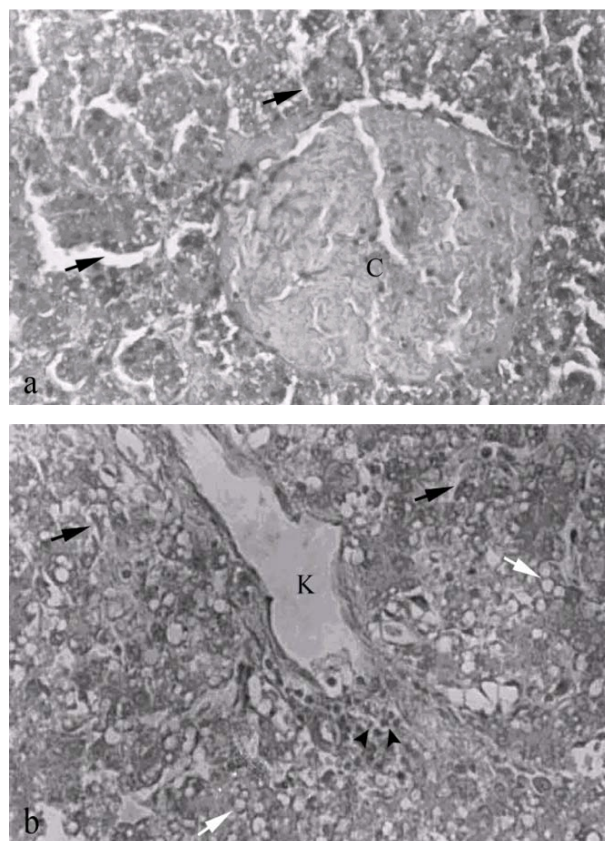
### Histopatology

In the controls, microscopic examination showed liver

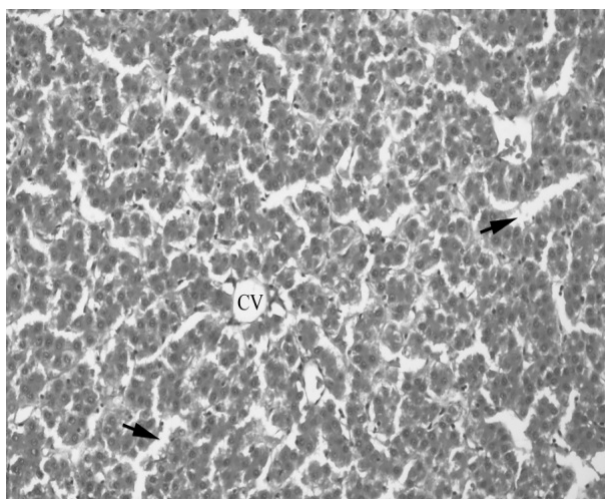


**Figure 6.** The liver of control hens fed on basal diets, arrow, Sinusoid (black arrows), Kupffer cell (arrow heads), Central vein (CV), (HE,  $\times 68$ ).

paranchyma consisting of hepatocytes. Sinusoids, Kupffer cells, and the central vein were also clearly visible (Figure 6). The livers in the raw common vetch seed group revealed severe pathological damages such as: sinusoidal dilatation, congestion of the central vein, increased numbers of Kupffer cells, lipid accumulation and lymphocyte infiltration (Figure 7a, and b). Sinusoidal dilatations were also prominent in liver samples of group fed SCV (Figure 8). Whereas, SBCV caused less sinusoidal dilatation (Figure 9).



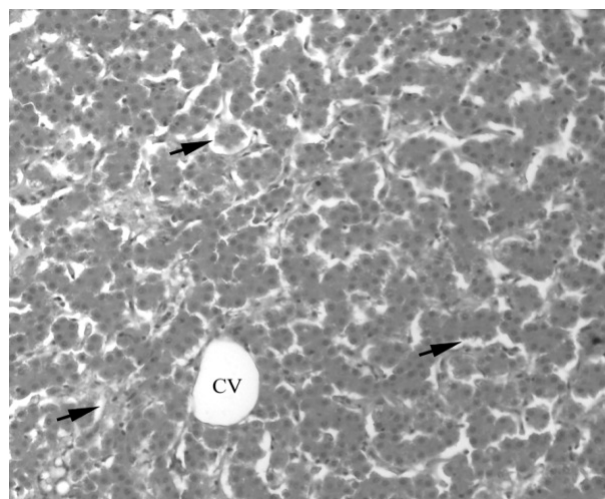
**Figure 7.** The liver of hens fed on 25% of raw common vetch (RCV) a) Sinusoidal dilatation (black arrows), and congestion (C), b) Lipid accumulation (white arrows), Capillary (K) Lymphocyte infiltration (arrowheads), (HE,  $\times 68$ ).



**Figure 8.** The liver of hens fed on diet containing 25% of common vetch seed soaked in water at room temperature for 72 hours with water change every 24 hours then dried at room temperature (SCV). Sinusoidal dilatation (arrow), Central vein (CV), (HE,  $\times 68$ ).

Our observations on control livers were in agreement with previous reports (Wight and Siller, 1975; Whitehead, 1979). The livers of chicks fed common vetch grown under different fertilizer treatments showed only sinusoidal dilatations. Whereas, vascular disorders and other hepatic lesions were seen as the effect of raw common vetch. It is reported that increased arterial flow leads to sinusoidal dilatations (Nobuyoshi et al., 2005). Irregular sinusoidal structures also give signs of congestions (Ozturk et al., 2005). The obstruction of hepatic venous outflow causes intrahepatic venous congestion and portal hypertension (Masaaki et al., 2004). Kupffer cells, macrophages of the liver, play an important role in liver damage and regeneration. It is proposed that Kupffer cells are stationary and regenerate after liver trauma by local proliferation (Bair et al., 2005). A study describes, apparently for the first time in mice, the involvement of raw *V. faba* intake in some immunological disturbances affecting both humoral and cell-mediated aspects of the immune response (Martinez, 1992). In conclusion, intrahepatic lymphocytes are believed to be directly involved in the immunopathogenesis of chronic liver diseases (Wang et al., 2004). On the other hand, the excessive accumulation of fat in the cytoplasm is associated with the inhibition of protein synthesis (Hinton and Lauren, 1990). A significant reduction of liver protein was found in growing male rats with the anti-nutritive effect caused by the raw field bean (*V. Faba*) (Santidrian et al., 1981). In the present study, lipid accumulation in chick liver seems important. Thus, it is suggested that raw common vetch may also lead to a metabolic dysfunction in liver.

Our results show that the increasing treatments of *V. sativa* lead to a decrease in liver damage. Whereas, raw *V.*



**Figure 9.** The liver of hens fed on diet containing 25% of soaked and boiled common vetch (SBCV). Sinusoidal dilatation (black arrows), Central vein (CV), (HE,  $\times 68$ ).

*sativa* is capable of inducing histopathological alterations in chicken liver.

In conclusion, the present study investigating the performance, egg quality, blood parameters and histopathology showed that feeding raw and soaked common vetch seeds at 25% level is detrimental to laying hens. However, the effects could be alleviated when soaked and boiled common vetch seeds were fed. Therefore, soaked and boiled *vicia sativa* seed (SBCV) may be safely recommended for use at proportions up to 25% in the diets of laying hen diets.

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