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The Effect of Raw and Processed Common Vetch Seed (*Vicia sativa*) Added to Diets of Laying Hens on Performance, Egg Quality, Blood Parameters and Liver Histopathology

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Abbreviation Key: C＝control diet not containing common vetch seed, CV＝common vetch seed.

This study was carried out to determine the effects of raw and physical processed common vetch seed added to diets of laying hens on the laying performance, egg quality traits, blood parameters and liver histopathology. Lohman layers, 46 wk of age as 6 replicate cages each containing 4 hens, were allocated randomly to one of four dietary treatments. Treatment groups were fed diets containing a standard commercial layer diet (Control), basal diet plus 25% raw common vetch seed (CV), 25% autoclaved (at 120℃ for 30 minutes) common vetch seed (ACV) and 25% roasted (at 130℃ for 30 minutes) common vetch seed (RCV) for 12 weeks. Considering the findings of performance traits, differences among the groups were found significant ($P<0,05$); however the effects of treatments were not significant on egg quality and blood parameters. The diets containing raw and different physical processed vetchs reduced feed consumption and egg production.

CV compared to the control caused lipid accumulation in hepatocytes, severe congestion of hepatic blood vessels, inflammation, the increased numbers of Kupffer cells and sinusoidal dilatations. Findings from present study pointed out the risk of increased hepatic damage due to use of raw vetch seed. It is also remarkable that increasing treatments of vetch seed lead to decrease of liver damages.

The results of this experiment indicated that inclusion of CV and ACV in diet affected adversely performance parameters and hepatic damage in laying hens. Alleviation was observed on laying hens fed on diet including 25% RCV.

As a conclusion, it has been determined that 25% RCV is more appropriate to use in diets of laying hens than 25% CV and ACV.

Key words: blood parameters, common vetch, egg quality, performance, laying hens, liver histopathology

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Introduction

Scarcity in animal feed ingredients in some countries has led to investigate the possibility of using locally produced legume seeds in animal diets. One of the most desirable legume species for grain and hay production is common vetch (*Vicia Sativa*) (Sadeghi *et al*., 2004, 2009). Vetch seeds are well adapted to semiarid and arid lands and a cheap source of protein and energy for ruminant and poultry (Lópeze Bellido, 1994). The seeds contain up to 25−30% crude protein, 1−2% ether extract, 5−6% crude fiber and 60% carbohydrate (mainly starch) (Fernandez-Figares *et al*., 1993; Farran *et al*., 1995; Abreu and Bruno-Soares, 1998; Farran *et al*., 2001). However, it contains antinutritional factors (ANF) such as vicine, convicine, β-cyanoalanine and tannin (Cheeke and Shull, 1985; Lalle´s and Jansman, 1998; Wiryaman and Dingle, 1999; Darre *et al.,* 1999). Especially, tannins may result in overestimation of the nutritive value of common vetch seed (*Vicia sativa*) (Gül *et al*., 2005). The presence of 2% tannin in poultry diets was reported to cause reduction in growth and feed consumption of chicks, and thus reducing the feed conversion; however, the presence of low levels of tannin like 0.5% in diets was reported to reduce the growth of hens, and the presence of 5% tannin resulted in 70% of mortality on 7−11th days (Dikicioğlu *et al*., 1996).

Feeding of raw legumes to chickens generally results in lower growth rate and reduced feed efficiency compared with

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feeding processed legumes (Rubio *et al*., 1990). However, each legume produces a different response. Gül *et al*. (2005), for example, reported that inclusion of 22% common into the basal diet of laying hens caused 7.8, 11.6, 18.7 and 4.5% reductions in body weigth (BW), feed intake (FI), egg production (EP) and egg weight (EW) and 16.4% elevation in feed conversion ratio (FCR). Some researchers observed only growth retardation with 34% common vetch in 35 day-old Leghorns (Ocio *et al*., 1980). In addition, detrimental effects on egg produce and feed intake in laying hens were found at levels 15−46% in corn soybean- based diets (Castanon and Perez-Lanzac, 1990). Farran *et al*. (1995) reported that inclusion of CV at more than 25% into the diet of laying hens depressed performance traits such as body weight, feed comsumption, egg production and egg quality traits.

The presence of adverse effects of ingestion of ANF in grain legumes have been investigated by some researchers (Huisman and Tolman, 1992; Gatel, 1994; Wiryaman and Dingle, 1999). It is agreed that most ANF exert their negative effect through interference with normal digestive functions. A number of techniques is available for eliminating ANF, thus improving the nutritional value of grain legumes. The most widely used methods for the reduction of the negative effects of ANF are physical treatments such as roasting, autoclaving, soaking (Brenes *et al*., 1993; Wiryaman and Dingle, 1999; Farran *et al*., 2002). Soaking in water and cooking processes of legume seeds eliminate phenols and tannins (Onwuka, 2006). It was showed that autoclaving and cooking in boiling water reduced the level of tannins in winged bean (Kadam *et al*., 1987).

Evidence is summarized about the fact that the inclusion of vetch seed to 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 occurence of various ANF and to a poor sulphur amino acid content (Martinez *et al*., 1992; Mahmood and Smithard, 1993; Marzo *et al*., 2002). Raw faba bean also produces local reactions in the pancreas and the intestine of chicks (Rubio *et al*., 1989). The possible role of tannins and phytates on some of those alterations is discussed (Longstaff and McNab, 1991). Liver plays a crucial role in metabolism (Yokouchi, 2005). Hepatic metabolizm is, first and foremost, a mechanism that converts campounds into products that are easily excreted (Tolman, 1998). It is recorded that liver is target organ in rats given vetch seed (Santidrian *et al*., 1987; 1994). Therefore, the histologic responses of the liver as relavant with fertilizer treatments of vetch seed seem especially important.

The objective of this study was to investigate the effects of raw or physical treated (autoclaved and roasted) common vetch seeds into diets of hens during the peak production period on performance, egg quality traits, some blood parameters and liver histopathology.

Materials and Methods

Birds, Diets and Management

This study was carried out at the poultry unit of Research and Application Farm, Atatürk University. A total of 96 Lohmann white layers, at age 46 wks, were assigned randomly to four groups, each formed 6 replicate cages as subgroups, comprising of four hens. Experimental groups were fed isocaloric and isonitrogenous standart commercial layer diet (2671 kcal/kg, ME; 17.2% crude protein) (control), basal diet plus 25% raw vetch seed (CV), 25% autoclaved (at 120℃ for 30 minutes) vetch seed (ACV) and 25% roasted (at 130℃ for 30 minutes) vetch seed (RCV) for 12 weeks.

During the experimental period, feed and water were provided *ad libitum*, and eggs were collected once daily at 08:30 h. The diets in Table 1 were formulated to meet nutrient requirements of laying hens according to NRC (1994).

Sample Collection and Analytical Procedure

All vetch seed samples and experimental diets were analyzed for tannin using a spectrophotometric method (UV-visible 2100, Shimadzu, Japan) at 440 nm by Folin Denis reagent according to AOAC (1990) (Table 1).

FI and EP were recorded daily; EW was measured bi-weekly; and BW was measured monthly. Before determination of EW a sample of 10 eggs from each experimental group was stored for 24 h at room temperature. FCR was expressed as kilogram of feed consumed per kilogram of egg produced. Another sample of 10 eggs was randomly collected from each experimental group every month to assess egg quality parameters (Ergün *et al*., 1987). Egg quality parameters were shape index, shell strength, shell thickness, albumen index, yolk index, yolk color and Haugh unit (Gül *et al*., 2005).

Determination of Blood Parameters

About 3 mL of blood samples were drawn from wing vein of two hens from each cage into additive-free vacutainers at the end of the experimental period to determine metabolic profile. Serums were separated by centrifuging blood samples at 3000 g for 15 min at 20℃. Aliquots were kept at −20℃ until laboratory analyses for triglyceride, cholesterol, very low-density lipoprotein, total protein, albumin, globulin creatine, Ca, and P using commercial kits (DDS®, Diasis Diagnostic Systems Co., Istanbul 80270, Turkey) (Beuving and Vonder, 1977).

Histopathological Examinations

At the end of the experiment, six animals selected incidentally from each group were killed and immediately dissected. Livers were used for slide preparations. The liver samples were fixed in 10% formaldehyde and embedded in paraffin for microscopic examination, and then, sections obtained from paraffin blocks (5 μm) were stained with Hemotoxylin and eosin (Bancroft and Stevens, 1982).

Statistical Analysis

The results obtained from the experiment were analyzed by an one-way analysis of variance using the general linear model (GLM) procedure of SAS and means were compared

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

Ingredient, (%)	Experimental diets			
	Control	CV	ACV	RCV
Corn	47.5	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	0.5	1.09	1.09	1.09
Dicalcium phosphate[1]	1.25	1.20	1.20	1.20
Marble	9.25	9.25	9.25	9.25
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25
Salt	0.30	0.19	0.19	0.19
Methionine[3]	0.05	0.12	0.12	0.12
Lysine[4]	0.03	0.12	0.12	0.12
Chemical composition (analyzed, DM basis)				
Dry matter, (%)	90.9	89.6	89.9	90.3
Crude protein, (%)	17.2	17.4	17.3	17.4
Crude fibre, (%)	6.2	6.4	6.2	6.0
Ether extract, (%)	3.8	3.3	3.2	3.5
Crude ash, (%)	9.3	9.0	9.2	9.2
Non-Nitrogenous Constituents, (%)	54.4	53.5	54.0	54.2
Metabolizable energy (kcal/kg)	2671	2612	2617	2646
Tannin content of the diets (mg/kg)	2.74	3.29	2.93	2.74
Tannin content of the vetchs (mg/kg)	—	4.46	3.93	1.56

Diets: C=control diet; CV=basal diet containing common vetch seed (25%), ACV=autoclaved (at 120℃ for 30 minutes) vetch seed in diet at 25% level, RCV=roasted (at 130℃ for 30 minutes) vetch seed diet in 25% level.
The common vetch seed contained average of 87.4% DM and 16.7% CP, 3.6% crude fiber, 1.5% ether extract, 7.24% ash, 17.1% ADF, 36.0% NDF.
[1] Each kilogram contained: Ca, 24% and P, 17.5%
[2] The premix provided per 1 kg of diet: Vitamin A, 15.000 IU; cholecalciferol, 1500 ICU; DL-α-tocopheryl acetate, 30 IU; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; panthotenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 μg; 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.
[3] DL-methionine.
[4] L-lysine hydrochloride.

by Tukey's Test (SAS Institute, 1998). Statistical significance was considered at $p<0.05$.

Results

Laying Performance

The effects of the processing method of common vetch seed on laying performance during the peak period are presented in Table 2. In this study, ıt was determined that hens fed on diets including raw, autoclaved and stove-roasted vetch had lower egg production and consumed less feed compared to control group. Hens in the group fed on diets with roasted vetch had higher egg production and consumed more feed compared to other two groups (raw vetch and autoclaved vetch) but these values were found significantly lower compared to control group ($P<0.01$).

No significant differences were determined among the groups considering the rates of damaged egg ($P>0.05$). The mean egg weight of hens fed on diet with raw vetch and physically processed vetch were significantly lower compared to hens in control group ($P<0.01$). Inclusion of CV and ACV into the basal diet adversed the feed conversion ratio. Roasted common vetch (RCV) reduced the adverse effect of CV and ACV on FCR ($P<0.01$). In addition, feed conversion rates of hens in control group and the group RCV fed on diet including vetch roasted at 130℃ found similar to each other. 25% of raw vetch addition to the diets increased feed conversion rate (FCR) by 20.6%.

Addition of raw and processed vetch seed to diets of laying hens brought about significantly reduction in live weight ($P<0.05$) and the changes and mean weights of hens fed on diets including common vetch seed were found as negative. Decrease in live weight was higher in animals fed with

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Table 2. **The effects of diets including raw and processed common vetch seed on laying performance parameters.**

Treatment	Response variables							
	Feed consumption	Egg production	Cracked egg yield	Egg weight	FCR[1]	Body weigth		
						Initial	Final	Change
	(g/d)	(%)	(%)	(g)	(kg feed/ kg egg)		(g)	
Control	112.11a	84.68a	0.76	66.64a	1.99b	1657.29	1706.85a	49.56a
CV	95.88b	62.88c	0.20	63.76b	2.39a	1656.88	1517.99b	−138.89b
ACV	93.68b	61.70c	0.52	63.26b	2.40a	1673.33	1387.11b	−286.22b
RCV	99.13b	69.41b	0.24	63.63b	2.25ab	1695.00	1486.21b	−208.79b
SEM	1.59	2.06	0.33	0.57	0.24	22.51	46.43	54.49
P value	0.0001	0.0001	0.185	0.0001	0.008	0.600	0.001	0.002

[1] FCR=feed conversion ratio (kg feed consumed per kg egg produced).
[a–c] Means within columns with different superscripts differ significantly at $p<0.05$.

Table 3. **The effects of diets including raw and processed common vetch seed on egg quality parameters**

Treatment	Response variables							
	Shape index (%)	Shell strenght (kg/cm^2)	Shell thickness ($mmx10^{-2}$)	Shell weight (g)	Yolk color	Yolk index (%)	Albumen index (%)	Haught unit
Control	72.21ab	1.28	0.36	8.01	8.31	42.65	9.39a	84.76
CV	73.50ab	1.39	0.36	8.04	8.06	43.56	8.91ab	82.36
ACV	72.14b	1.54	0.36	8.07	8.06	42.37	8.05ab	79.50
RCV	74.61a	1.36	0.35	8.02	7.66	41.42	7.48b	79.87
SEM	0.57	0.10	0.01	0.20	0.19	0.69	0.43	1.61
P value	0.012	0.324	0.960	0.967	0.130	0.197	0.013	0.098

[a–c] Means within columns with different superscripts differ significantly at $p<0.05$.

autoclaved vetch than other groups.

Egg Quality Parameters

Egg quality parameters are summarized in Table 3. No significant difference was detected between the groups considering the egg quality traits except for shape index and albumen index. Egg shape index is an important quality feature, for eggs having normal index is highly important for marketing. Normally, egg shape index is expected to change between 72−78%. In this study, there were significant differences amoung the groups considering the egg shape index values ($P<0.01$); however, they were within the normal limits.

No significant differences were established considering the following egg quality parameters; shell strenght, shell thickness, shell weight, yolk color, yolk index and haugh unit ($P>0.05$). The lowest egg albumen index value was determined in roasted vetch group, while the highest value was obtained from control group.

Blood Parameters

Table 4 summarizes the effects of the experimental diets on blood parameters. Albumen (Alb), Triglyceride (TG), Cholesterol (CHOL), Very low-density lipoprotein (VLDL), Total protein (TP), Alkaline phosphatase (ALP), Globulin (Glo), Creatine (Cre), Calcium (Ca) and Phosphorus (P) concentrations of blood samples taken from laying hens, and the effects of diets including raw and processed vetch seeds on these parameters were investigated in the present study.

There were numerical differences among the groups in triglyceride, VLDL and cholesterol values; however, the differences were not statistically significant.

Liver Histopatalogy

The effects of raw and processed common vetch seed on liver histopatology are presented in Fig. 2, 3 and 4. The livers of hens in the control group showed a normal lobuler architecture with central veins and radiating hepatic cords (Fig. 1). Severe liver damages in CV group were established such as: clear sinusoidal dilatations, congestion of central vein, increased numbers of Kupffer cells, lipid accumulation and lymphocyte infilitration (Figs. 2a and b). Whereas, diet inclusion at 120℃ 30 minute autoclaved vetch caused slight sinusoidal dilatations (Fig. 3). Moreover, hens fed at treatment with at 130℃ for 30 minute roasted vetch seed was observed liver sections similar to control (Fig. 4).

Discussion

Vetch groups consumed less feed compared to control group ($P<0.01$). The values obtained for feed consumption were lower than the results of Farran *et al*. (1995). The mean values determined by Fernandez *et al*. (1972) on raw and autoclaved beans were higher than the results of present

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Table 4. **The effects of diets including raw and processed common vetch seed on blood parameters of hens (mg/dL)**

Treatment	Response variables[1]									
	Alb	TG	Chol	VLDL	TP	Alp	Glo	Cre	Ca	P
Control	1.87	1118.58	236.0	153.63	5.95	962.8	341.3	1.72	14.42	4.63
CV	1.65	890.67	221.8	169.17	5.53	652.8	314.8	1.30	14.37	5.17
ACV	1.73	1240.0	259.7	172.00	5.67	1092.5	379.2	1.72	14.67	5.23
RCV	1.68	832.67	225.8	143.00	5.03	1135.8	287.7	1.12	14.35	4.40
SEM	0.09	189.38	27.34	21.67	0.26	141.6	30.86	0.25	0.30	0.55
P value	0.389	0.404	0.765	0.758	0.062	0.100	0.221	0.25	0.620	0.512

[1] Alb=Albumin; TG=Triglyceride; Chol=Cholesterol; VLDL=Very low-density lipoprotein. TP=Total protein; Glo=Globulin; Cre=Creatine; C=Calcium; P=Phosphor. All are in mg/dL

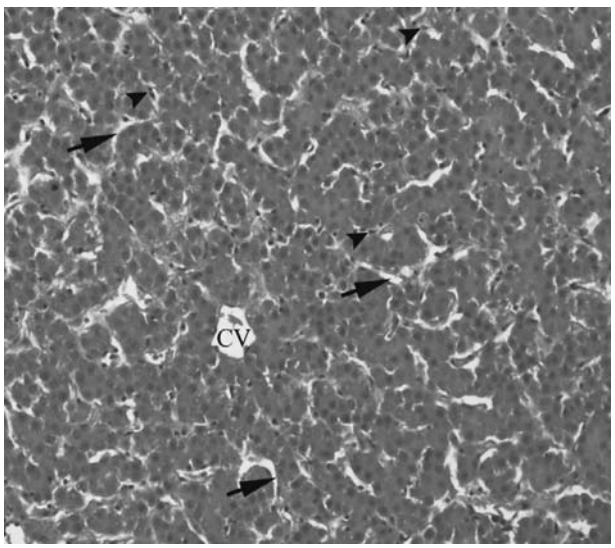


Fig. 1. **The liver of control hens, arrow, Sinusoid (black arrows), Kupffer cell (arrow heads), Central vein (CV), (HE, x68).**

studies, but lower than the results determined by Iyayi and Taiwo (2003). The differences among the studies related to results of feed consumption could be caused by certain factors such as animal race, age, live weight, egg laying period, energy level of diet, environment temperature and health sitatus of birds. Fernandez *et al*. (1972) reported that egg production was lower in group fed on diets including high levels of bean compared to control group and the group fed on diets with autoclaved bean diets but hens in group fed on diets with autoclaved bean had similar egg production to hen fed on control diet. Similarly, Gül *et al*. (2005) reported that egg production was reduced in laying hens fed on diets with high levels of vetch.

Hens fed on diets including vetch seed had significantly lower egg weight compared to control group. It may be hypothesized that vicine causes the production of small yolk which results in the production of small eggs. Vicine may act in the following ways: by reducing the amount of precursor material available to the granulosa cells; by damaging the granulosa cells, and hence their activity; or by destroying the ovum (Muduuli *et al*., 1981). Wyburn *et al*. (1965) suggested that the granulosa cells of the yolk may be involved in the transport of the large quantities of precursor material required to form the yolk and that it may be actively involved in yolk synthesis. Carmen *et al*. (1999) reported that chicken fed on 20 and 30% of roasted Velvet Bean (VB) demonstrate better growth performance and feed conversion as compared to chickens fed on diets with the same levels of raw VB; however, these values were significantly lower compared to control group.

In this study, inclusion of vetch seed significantly reduced live weight. Özcan and Demir (2000) determined that the effects of processed vetch and raw vetch on live weight gain of broilers were insignificant. In another study, Ergün *et al*. (1987) reported that the highest live weight was obtained in the group fed on diet with 5% of not autoclaved common vetch seed. In the present study, autoclaving of vetch caused reduction in live weight (−286.22 g), while in another study, Farran *et al*. (1995) reported increase in live weight gain (59 g) in group fed on diet with autoclaved vetch. The findings of the present study were compatible with the results of Farran *et al*. (2001).

Farran *et al*. (1995) reported that autoclaved vetch in the proportion of 25% could be used safely in diets. Ergun *et al*. (1987) stated that vetch addition in diets had no harmful effect on the health of laying hens. Gül *et al*. (2005) reported that inclusion of 22% CV (Common Vetch Seed) adversely affected laying performance, but no death related to the experimental diets.

Egg quality parameters except for albumen index were not affected by experimental diets. The study findings were compatible with the results of Farran *et al*. (1995) and İyayi and Taiwo (2003). Gül *et al*. (2005) stated that vetch addition to the diets increased egg albumen index compared to control group. Albumen quality is dependent on the quantity of β-ovomucin secreted by magnum, and β-ovomucin is essentially responsible for the gelatinous height of egg white (Anonymus, 2000). Low egg albumen of vetch groups could be caused by the oppressive effect on β-ovomucin quantity secreted by magnum.

Raw and processed vetch seeds did not have significant effect on blood parameters. Yalçın *et al*. (1998) reported

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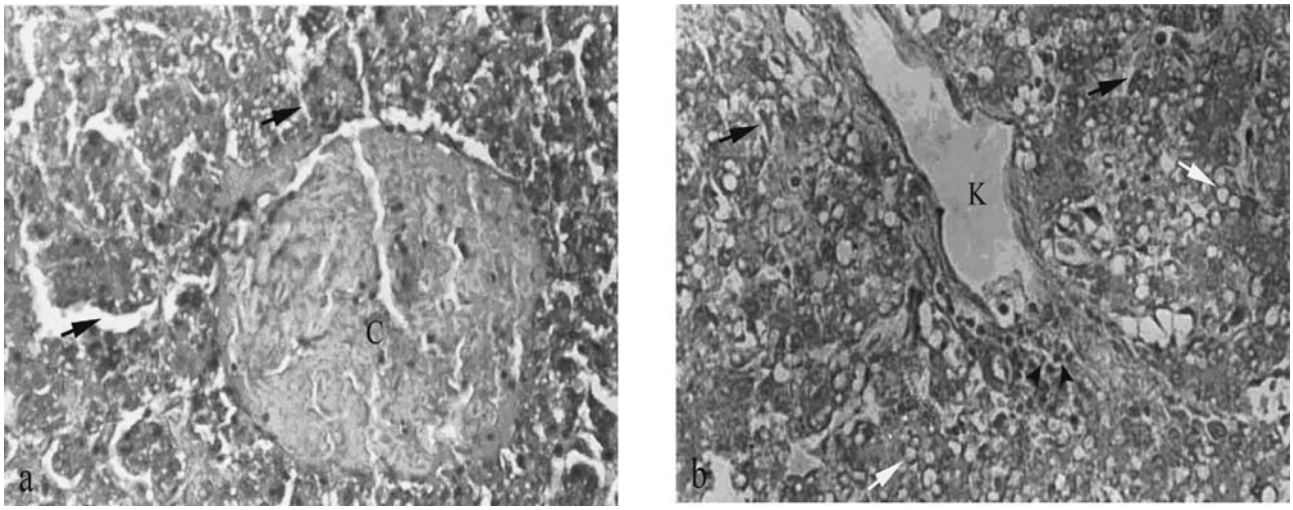


Fig. 2. **The liver of hens fed diet including raw common vetch a) Sinusoidal dilatation (black arrows), and congestion (C), b) Lipid accumulation (white arrows), Capillary (K) Lymphocyte infiltration (arrowheads), (HE, x68).**

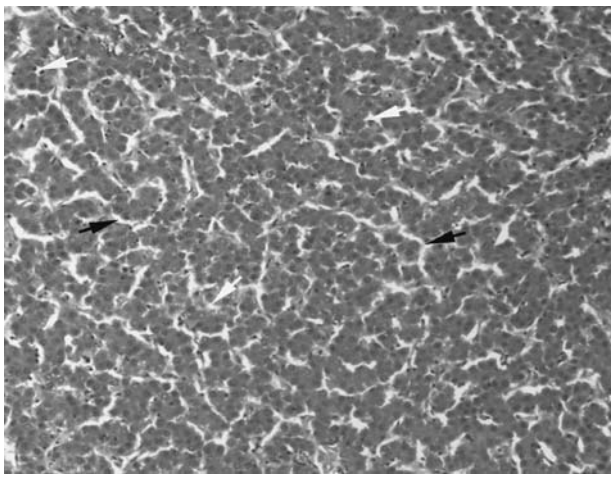


Fig. 3. **The liver of hens fed diet including autoclaved common vetch seed.** Sinusoidal dilatations (arrows), Central vein (white arrows) (HE; x 68).

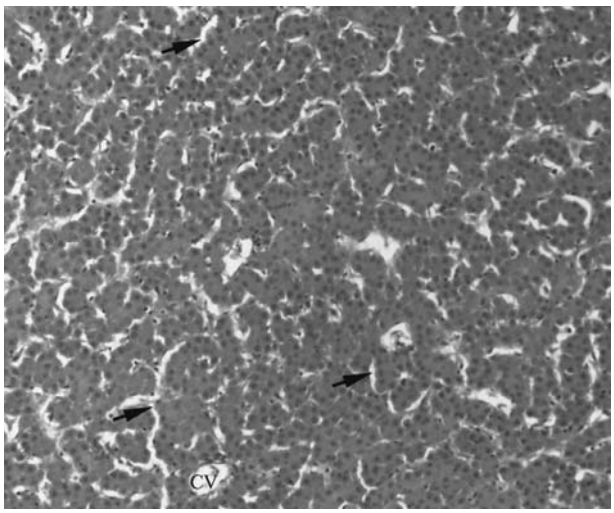


Fig. 4. **The liver of hens fed diet including roasted common vetch seed.** Sinusoidal dilatation (arrows), Central vein (CV), (HE; x 68).

that the values of serum total protein and total lipid decreased in quails when the amount of vetch in diets increased. Similarly, Ologhobo *et al*. (1993) found that serum total protein level was reduced by 20% of raw bean was added to broiler chicken diets. Serum protein concentration was proposed to be an indicator for reflecting the adverse effect of legume seeds on growth and laying performance (Wyckoff *et al*., 1983). In another study, Muduuli *et al*. (1981) stated that 1% of vicin in the diet for laying hens didn't change blood plasma protein and increased plasma lipid level ($P<$ 0.05).

The differences among the results from present study and findings of other researchers related to blood parameters could be caused by certain factors such as animal spice, treatment applied to vetch seed and the vetch seed level in the diets.

In the present study, lipid accumulation in chick liver seems important. Thus, it is suggested that raw vetch seed may also lead to metabolic dysfunction and capable of inducing histopathological alterations in hen livers. Our observations on livers from control group were in agreement

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with the reports of some researchers (Wight and Siller, 1975; Whitehead, 1979). The livers of hens as relevant with fertilizer treatments of vetch seed showed only sinusoidial dilatations. Whereas, vascular disorders and another hepatic lesions revealed by effect of raw vetch seed. 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). As regards, the obstruction of hepatic venous outflow causes intrahepatic venous congestion and portal hypertension (Masaaki *et al*., 2004). Again, 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 proliferations (Bair *et al*., 2005). A study describes, apperantly for the first time in mice, the involvement of raw vetch seed 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 (Hinton and Lauren, 1990). A significiant reduction of liver protein was found in growing male rats with the antinutrive effect caused by the raw field bean (Santidrian *et al*., 1982).

Conclusions

In generally, common vetch seed is well adapted to semi arid or arid regions, cheaper than other protein sources and it can be partially used as raw material instead of soybean meal or other protein sources being more expensive than common vetch seed in diets of laying hens. As a result of these phisical treatments there were not significant differences in FCR being the most important factor of performance for hens fed diets including 25% CV and ACV, and FCR and egg production in control group were fed basal diet better than other treatment groups. But FCR and egg production values of RCV group were closer to control group than those of CV and ACV groups. In addition, RCV did not have adverse effect on performance, egg quality, blood parameters and liver histopathology. So it has been determined that 25% RCV is more appropriate to use in diets of laying hens than 25% CV and ACV. But, future studies should focus on physical treatment of CV and these treatments that do not change performance and egg quality traits.

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