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# Effects of Additives on Laying Performance and Egg Quality of Hens Fed a High Level of Common Vetch Seed (*Vicia sativa*) During the Peak Period

**M. Gül,* M. A. Yörük,* A. Hayirli,*,[1] L. Turgut,† and M. Karaoğlu†**

**Department of Animal Nutrition and Nutritional Disorders, School of Veterinary Medicine, and †Department of Animal Sciences, College of Agriculture, Atatürk University, Erzurum 25700, Turkey*

**Primary Audience:** Poultry Scientists, Veterinarians, Nutritionists

## SUMMARY

**The aim of this study was to determine whether methionine, lysine, choline, or sulfur supplementation ameliorates adverse effects on laying performance and egg quality resulting from inclusion of a high level of common vetch seed (CVS) into diets of hens. Lohman layers, 30 wk of age as 6 replicate cages of 4 hens, were allocated randomly to receive basal diets containing either 12% soybean meal (B) or 22% CVS (BCVS) and diets BCVS plus 0.57% methionine, 0.66% lysine, 0.47% choline, or 0.05% sulfur for 98 d. Feed intake (FI) and egg production (EP) were recorded daily; egg weight (EW) was measured biweekly; and BW was measured monthly. A sample of 12 eggs from each experimental group was collected every month to evaluate egg quality.**

**Inclusion of CVS into the basal diet caused 7.8, 11.6, 18.7, and 4.5% reductions in BW, FI, EP, and EW and 16.4% elevation in feed conversion ratio (FCR). The dietary supplementations did not alleviate depressions in BW, FI, and EW. As compared with hens fed diet BCVS, methionine and choline supplementations partially reduced the adverse effects of CVS on EP (6.2 and 2.9% increases) and FCR (7.4 and 6.6% decreases). The effects of treatments on egg quality were variable. In summary, a high level of CVS adversely affected laying performance and egg quality in peak producing hens. Partial improvements in these parameters by methionine and choline supplementations may suggest that the adverse effects of CVS could be linked to interference with methyl donor metabolism.**

**Key words: laying hen, common vetch seed, methionine, lysine, choline, sulfur**

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## DESCRIPTION OF PROBLEM

Legume seeds are well adapted to semiarid and arid lands and can be important soybean substitutes in poultry diets in these lands [1]. Despite containing relatively high levels of protein (20 to 45%) and carbohydrate (more than 50%) [2], legume seeds are poorly used due to an imbalanced amino acid profile and antinutritional factors [3]. In general, legume seeds are

[1]To whom correspondence should be addressed: ahayirli@atauni.edu.tr or ahayirli_2000@yahoo.com.

deficient in sulfur amino acids and tryptophan and rich in lysine [3] and have a N:S ratio ranging from 1:80 to 1:120 [4]. The adverse effects of legume seeds on growth and laying performance are linked to protein quality and presence of antinutritional factors [5]. Especially, tannins with gallic acid and flavonol structures (about 0.84 g/kg) may result in overestimation of the nutritive value of common vetch seed (CVS) (*Vicia sativa*) [6]. Moreover, CVS contains some allergenic and antigenic substances, such as vicine and convicine [7]. As a result of individual or combined effects of these compounds, hypersensitivity, structural changes in the villus and small intestine, enlargement of the pancreas, and toxicity may occur [8, 9], which may compromise synthesis of digestive enzymes and use of nutrients [10, 11].

Inclusion of CVS into the diets at more than 20% for layers [12] and 15% for broilers [13] depresses performance. Thus, to improve nutritive value and reduce adverse effects of legume seeds, most research has centered on processing and dietary fortification [14, 15]. Extensive reviews on the effects of physical and chemical treatments as well as on nutrient fortification to improve use of cereal [16] and legume [1] grains for poultry are available. Fortification of diets with methionine and choline has been reported to reduce the adverse effects of a high level of sorghum on growth and laying performance [17, 18]. However, data on their additions into a diet containing a high level of legume seeds are limited [19]. It was hypothesized that supplementation of methionine would minimize the adverse effects of CVS on laying performance and egg quality through either being an essential nutrient, playing a role in methyl donor metabolism, as a sulfur source, or all the above. Lysine is also an essential amino acid, and choline contains a methyl group [20]. Therefore, the objective of this experiment was to determine whether supplementation of methionine, lysine, choline, or sulfur ameliorates the adverse effects on laying performance and egg quality, resulting from the inclusion of a high level of CVS into diets of layers during the peak production period.

## MATERIALS AND METHODS

### *Birds, Diets, and Management*

The Research Animal Ethic Committee of Atatürk University approved this experimental protocol. One hundred and forty-four Lohman layers, 30 wk of age with uniformity of 87% (the number of hens weighing between 0.9 to 1.1% of the mean BW), were blocked according to the location of cages (50 × 46 × 46 cm) and then assigned randomly to receive 1 of 6 isolocaloric and isonitrogenous experimental diets: basal diets containing either 12% soybean meal (B) or 22% CVS (BCVS) and diets BCVS plus 0.57% methionine as DL-methionine, 0.66% lysine as L-lysine monohydrochloride, 0.47% choline as choline chloride, or 0.05% sulfur as inorganic sulfur for 14 wk. Each diet was offered to 6 cages, each containing 4 hens.

The experimental diets (Table 1) were formulated to meet or exceed the NRC recommendations [21]. During the experimental period (98 d), feed and water were provided ad libitum, and eggs were collected once daily at 0830 h. Hens were subjected to a 17L:7D cycle.

### *Sample Collection and Analytical Procedure*

Feed samples were collected monthly and analyzed for DM, CP, crude fiber, and ash [22]. Metabolizable energy, Ca, and P contents and amino acid profile of the experimental diets were calculated from tabular values of feedstuffs for chickens [20]. Feed intake (FI) and egg production (EP) were recorded daily; egg weight (EW) was measured biweekly; and BW was measured monthly. Before determination of EW, a sample of 12 eggs from each experimental group was stored for 24 h at room temperature. Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. Another sample of 12 eggs was randomly collected from each experimental group every month to assess egg quality parameters [23]. Egg quality parameters were shape index, shell strength, shell thickness, albumen index, yolk index, yolk color, and Haugh unit [24]. Data analysis is described elsewhere [25].

## RESULTS

### *Laying Performance*

The effects of the experimental diets on laying hen performance parameters are summarized in Table 2. Partial replacement of soybean meal with CVS in the basal diet caused 7.8% reduction in BW, and none of the supplements altered

TABLE 1. Ingredients and amino acid profiles of the experimental diets

| Ingredient, % | Experimental diets[1] | | | | | |
|---|---|---|---|---|---|---|
| | B | BCVS | BCVSM | BCVSL | BCVSC | BCVSS |
| Corn | 42.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 |
| Soybean meal, 44% CP | 28.00 | 16.00 | 16.00 | 16.00 | 16.00 | 16.00 |
| Common vetch seed | — | 22.00 | 22.00 | 22.00 | 22.00 | 22.00 |
| Wheat | 6.00 | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 |
| Barley | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Wheat bran | 9.30 | 7.00 | 6.43 | 6.34 | 6.53 | 6.95 |
| Molasses | 1.80 | 1.60 | 1.60 | 1.60 | 1.60 | 1.60 |
| Limestone | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Dicalcium phosphate[2] | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Vitamin-mineral premix[3] | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Antioxidant[4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| DL-Methionine | 0.10 | 0.10 | 0.67 | 0.10 | 0.10 | 0.10 |
| L-Lysine·HCl | 0.10 | 0.10 | 0.10 | 0.76 | 0.10 | 0.10 |
| Choline chloride | — | — | — | — | 0.47 | — |
| Sulfur | — | — | — | — | — | 0.05 |
| Amino acid profile[5] | | | | | | |
| Methionine, % | 0.26 | 0.25 | 0.82 | 0.25 | 0.25 | 0.25 |
| Lysine, % | 0.88 | 0.71 | 0.71 | 1.37 | 0.71 | 0.71 |
| Choline, g/kg | 1.24 | 1.07 | 1.06 | 1.06 | 1.53 | 1.07 |
| Sulfur, % | 0.15 | 0.16 | 0.17 | 0.17 | 0.17 | 0.22 |

[1]Diets: B = basal diet containing additional soybean meal (12%); BCVS = basal diet containing common vetch seed (22%); BCVSM = diet BCVS plus methionine (0.57%); BCVSL = diet BCVS plus lysine (0.66%); BCVSC = diet BCVS plus choline (0.47%); BCVSS = diet BCVS plus sulfur (0.05%). The experimental diets contained average of 89.9% DM and 2,901 ± 13 kcal/kg ME, 19.1 ± 0.1% CP, 7.4 ± 0.8% crude fiber, 3.18 ± 0.07% ether extract, 10.3 ± 1.1% ash, 2.15 ± 0.01% Ca, and 0.65 ± 0.04% P (mean ± SD).
[2]Per kilogram contains Ca, 24% and P, 17.5%.
[3]Per kilogram contains vitamin A, 15,000 IU; cholecalciferol, 1,500 ICU, vitamin E (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 $B_{12}$, 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.
[4]Ethoxyquin.
[5]Calculated values [20].

BW depression. Feed intake for hens fed diet BCVS was 11.6% lower than for hens fed diet B. Lysine and sulfur supplementations did not alter FI depression, whereas methionine and choline supplementations further depressed FI. Due to inclusion of CVS into the basal diet, FI remarkably decreased during the first 4 wk and then increased at different magnitudes, depending upon the type of supplementation toward the end of the experiment ($P < 0.05$; Figure 1A).

Inclusion of 22% CVS into the basal diet caused 18.7% reduction in EP. Only methionine supplementation significantly alleviated the depression in EP. As the experiment continued, magnitude of changes in EP was affected by the experimental diets ($P < 0.001$; Figure 1B). Except for hens fed diet B, EP for other groups sharply decreased during the first 4 wk and then gradually increased at different magnitudes, depending upon the type of supplementation toward the end of the experiment but did not reach the EP of hens fed diet B. Egg weight also decreased by 4.5% due to inclusion of 22% CVS into the basal diet, and none of the supplements alleviated reduction in EW.

Inclusion of CVS into the basal diet caused 16.4% increase in FCR. Supplementation of methionine and choline, but not of lysine and sulfur, decreased elevation in FCR. Moreover, as a result of this improvement, there were no differences in FCR for hens fed diets B, diets BCVS plus 0.57% methionine as DL-methionine, and diets BCVS plus 0.47% choline as choline chloride. Due to inclusion of CVS into the basal diet, FCR varied as the experiment continued ($P < 0.004$; Figure 1C). Except for hens fed diet B, FCR for other groups increased dramatically

TABLE 2. The effects of supplemental methionine, lysine, choline, and sulfur on laying performance and egg quality parameters of hens fed diets containing a high level of common vetch seed during the peak production period (30 to 44 wk of age)

| | Experimental diets[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameter | B | BVS | BVSM | BVSL | BVSC | BVSS | SEM |
| Laying performance | | | | | | | |
| BW, kg | 1.67$^{a}$ | 1.54$^{bc}$ | 1.54$^{bc}$ | 1.53$^{bc}$ | 1.50$^{c}$ | 1.56$^{b}$ | 0.01 |
| Feed intake, g | 129.9$^{a}$ | 114.8$^{b}$ | 110.5$^{c}$ | 111.2$^{bc}$ | 110.1$^{c}$ | 112.7$^{bc}$ | 2.0 |
| Egg production, % | 93.4$^{a}$ | 75.9$^{c}$ | 80.6$^{b}$ | 75.1$^{c}$ | 78.1$^{bc}$ | 76.1$^{c}$ | 1.4 |
| Egg weight, g | 63.9$^{a}$ | 61.0$^{b}$ | 60.4$^{b}$ | 61.2$^{b}$ | 60.5$^{b}$ | 60.7$^{b}$ | 0.5 |
| Feed:egg, kg:kg | 2.20$^{a}$ | 2.56$^{b}$ | 2.37$^{a}$ | 2.53$^{b}$ | 2.39$^{ab}$ | 2.50$^{b}$ | 0.06 |
| Egg quality | | | | | | | |
| Shape index, %[9] | 77.5$^{a}$ | 74.5$^{c}$ | 75.8$^{bc}$ | 76.1$^{abc}$ | 76.2$^{ab}$ | 76.3$^{ab}$ | 0.6 |
| Shell strength, kg/cm$^{2}$ | 2.12$^{b}$ | 2.65$^{a}$ | 2.39$^{ab}$ | 2.49$^{ab}$ | 2.66$^{a}$ | 2.60$^{ab}$ | 0.15 |
| Shell thickness, mm*10$^{-2}$ | 1.214 | 1.197 | 1.172 | 1.114 | 1.191 | 1.174 | 0.032 |
| Albumen index, % | 9.4$^{b}$ | 10.9$^{a}$ | 11.0$^{a}$ | 10.5$^{a}$ | 10.6$^{a}$ | 11.1$^{a}$ | 0.4 |
| Yolk index, % | 42.3$^{c}$ | 43.8$^{b}$ | 45.3$^{a}$ | 43.8$^{b}$ | 43.4$^{bc}$ | 44.4$^{ab}$ | 0.7 |
| Yolk color | 11.2$^{a}$ | 10.4$^{b}$ | 10.4$^{b}$ | 10.5$^{b}$ | 10.1$^{b}$ | 10.6$^{b}$ | 0.2 |
| Haugh unit | 84.7$^{b}$ | 90.2$^{a}$ | 90.0$^{a}$ | 88.0$^{a}$ | 88.2$^{a}$ | 89.2$^{a}$ | 1.3 |

$^{a–c}$Means with different superscripts within the same row differ ($P < 0.05$).
[1]The least squares means. B = basal diet containing additional soybean meal (12%); BCVS = basal diet containing common vetch seed (22%); BCVSM = diet BCVS plus methionine as DL-methionine (0.57%); BCVSL = diet BCVS plus lysine as L-lysine monohydrochloride (0.66%); BCVSC = diet BCVS plus choline as choline chloride (0.47%); BCVSS = diet BCVS plus sulfur as inorganic sulfur (0.05%).

during the first 4 wk, then remained constantly low and unchanged for the second 4 wk, and then increased at greater magnitudes toward the end of the experiment.

### *Egg Quality*

The effects of experimental diets on egg quality parameters are summarized in Table 2. Shape index for hens fed diet BCVS was lower than for hens fed diet B. Supplementation of choline and sulfur, but not of methionine and lysine, improved shape index. Inclusion of CVS into the basal diet increased shell strength by 25%. Except for choline, other supplements tended to decrease shell strength. The magnitude of changes was different depending upon the type of supplementation during the experimental period ($P < 0.01$; Figure 2A).

There was a 16% increase in albumen index due to inclusion of 22% CVS, and all of the supplements maintained this increase. The experimental diets affected albumen index in different ways as the experiment continued ($P < 0.02$; Figure 2B). Yolk index also increased about 3.6% due to inclusion of 22% CVS into the basal diets. All of the supplements maintained this increase and, in particular, methionine supplementation increased yolk index further compared with diet BVCS. In response to the experimental diets, changes in yolk color were opposite of those in albumen index. The yolk color for hens fed diet BCVS was 7.1% lower than for hens fed diet B, and none of the supplements ameliorated the adverse effect of CVS on yolk color. Moreover, the type of supplementation affected yolk color in different ways during the experimental period ($P < 0.05$; Figure 2C). Changes in Haugh unit in response to the experimental diets were similar to those in albumen index. Haugh unit increased about 6.5% due to partial replacement of soybean meal with CVS in the basal diet, and all of the supplements maintained this increase. Moreover, the experimental diets affected Haugh unit in different ways during the experimental period ($P < 0.03$; Figure 2D).

## DISCUSSION

Antinutritional factors and an amino acid imbalance in legume seeds interfere with protein metabolism [1] and consequently adversely affect growth and laying performance [27, 28]. The minimum level of antinutritional factors that does not lower performance is un-

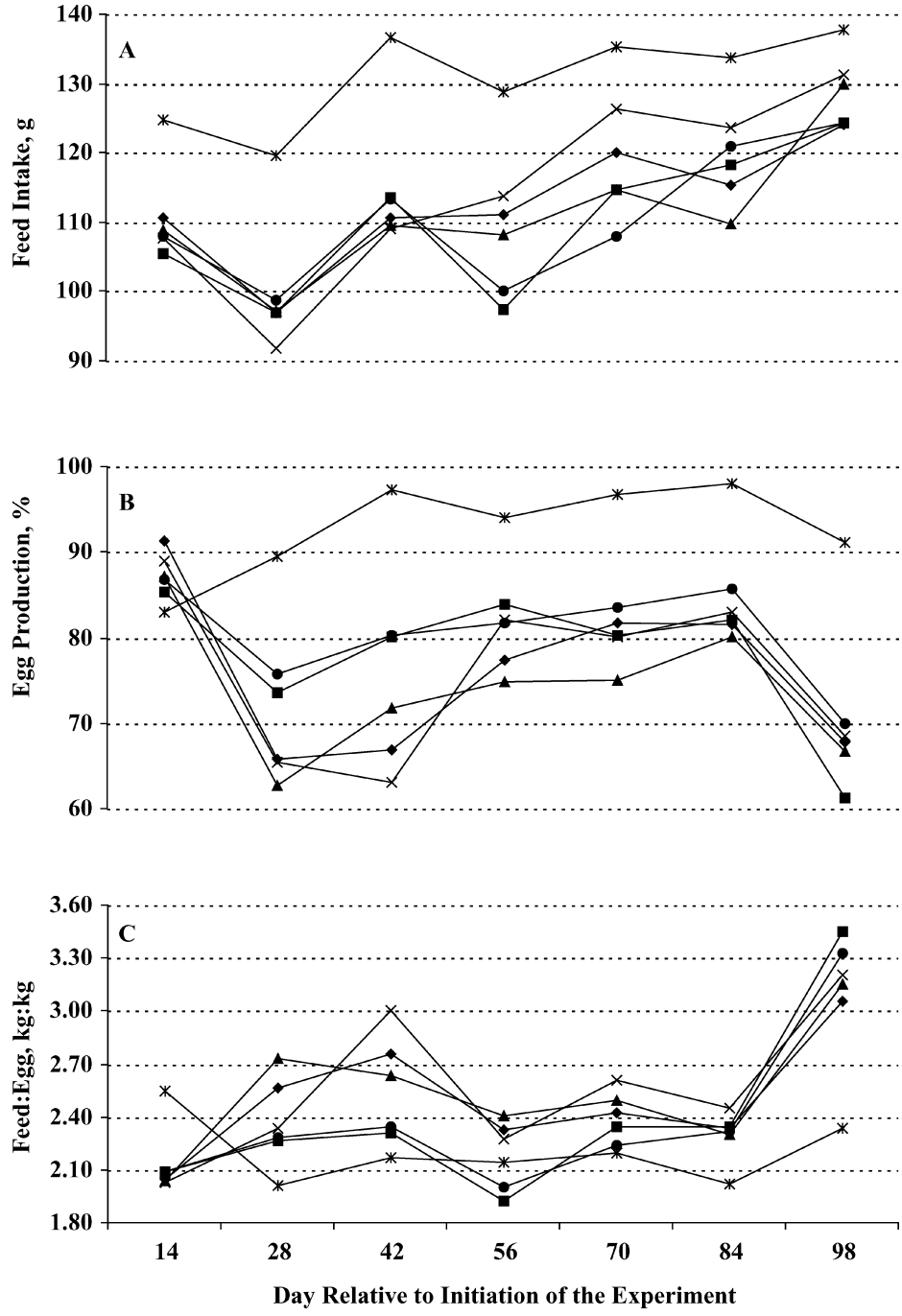



**FIGURE 1.** The effects of supplemental methionine, lysine, choline, and sulfur on laying performance parameters of hens fed diets containing a high level of common vetch seed during the peak production period (30 to 44 wk of age). Hens fed basal diets containing additional 12% soybean meal (—*—); 22% common vetch seed (—x—); and diets containing common vetch seed plus supplemental methionine (0.57%, —●—), lysine (0.66%, —▲—), choline (0.47%, —■—), and sulfur (0.05%, —◆—). Panel A: Probabilities for the effects of time and experimental diet by time interaction on feed intake (g) were less than 0.0001 and 0.05, respectively, with SEM of 3.7. Panel B: Probabilities for the effects of time and experimental diet by time interaction on egg production (%) were less than 0.0001 and 0.004, respectively, with SEM of 0.16. Panel C: Probabilities for the effects of time and experimental diet by time interaction on feed conversion ratio (feed consumption:egg production, kg:kg) were less than 0.0001 and 0.001, respectively, with SEM of 0.38.

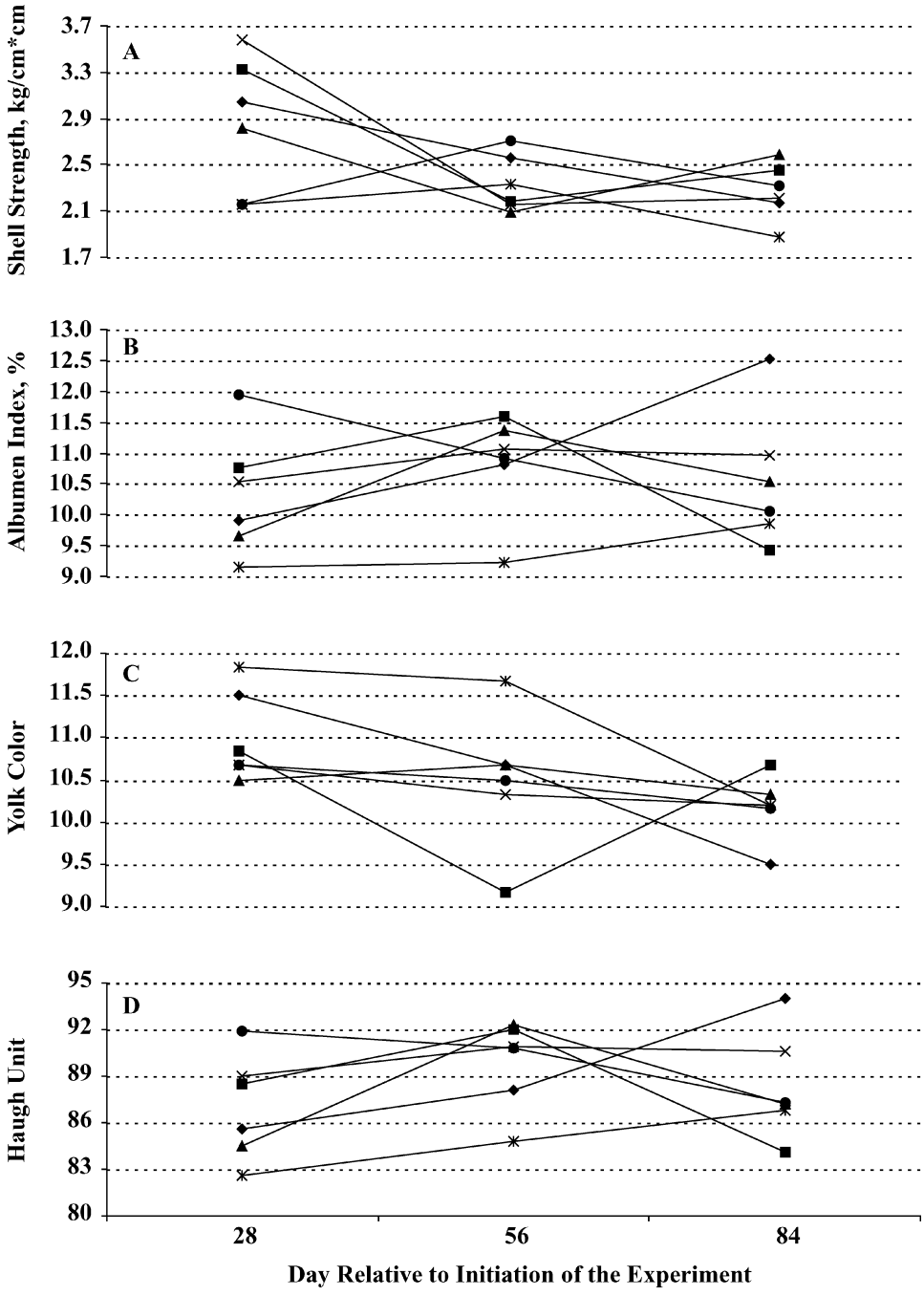



**FIGURE 2.** The effects of supplemental methionine, lysine, choline, and sulfur on egg quality parameters of hens fed diets containing a high level of common vetch seed during the peak production period (30 to 44 wk of age). Hens fed basal diets containing additional 12% soybean meal (—*—); 22% common vetch seed (—x—); and diets containing common vetch seed plus supplemental methionine (0.57%, —●—), lysine (0.66%, —▲—), choline (0.47%, —■—), and sulfur (0.05%, —◆—). Panel A: Probabilities for the effects of time and experimental diet by time interaction on shell strength (kg/cm$^2$) were less than 0.001 and 0.01, respectively, with SEM of 0.27. Panel B: Probability for the effect of experimental diet by time interaction on albumen index (%) was less than 0.02 with SEM of 0.64. Panel C: Probabilities for the effects of time and experimental diet by time interaction on yolk color were less than 0.007 and 0.05, respectively, with SEM of 0.43. Panel D: Probability for the effect of experimental diet by time interaction on Haugh unit was less than 0.03 with SEM of 2.16.

known. A 1% tannin level in the diet was shown not to adversely affect laying performance in hens [17]. However, feeding broilers more than 20% vetch containing 0.53% $\beta$-cyanoalanine, 0.73% vicine, and 0.08% convicine was shown to cause neurotoxicty and to increase FCR [7]. In general, feeding hens at more than 20% legume seeds adversely affects laying performance [29, 30]. Although inclusion of 22% CVS adversely affected laying performance (Table 2), no death related to the experimental diets was noted in the present study. Serum protein concentration has been proposed to be an indicator for reflecting the adverse effect of legume seeds on growth and laying performance [31].

In the present study, depressions in BW and laying performance could be related to reduced FI or interference of tannins in CVS with protein metabolism or both. Quadratic decreases in BW and serum total protein concentration and quadratic increase in FCR were reported in Japanese quails (*Coturnix coturnix japonica*) fed diets containing 0, 5, and 10% CVS [32]. Inclusion of vicin, a hemolytic agent present in legume seeds, however, was reported to not affect total protein concentration in laying hens [33]. Moreover, significant experimental diet by time interaction effects on FI (Figure 1A), EP (Figure 1B), and FCR (Figure 1C) in this experiment may indicate an adaptation to antinutritional factors consumption when CVS was included in the diet at 22%. However, in an earlier study [13], linear decreases in growth and mortality rates were reported in broilers fed diets containing 0, 20, 30, or 40% CVS (*Vicia sativa* L. var. *Wilamette*) for 4 wk. In peak-producing hens, increasing the level of CVS from 0 to 10% for 4 mo was shown to decrease FI, EP, specific gravity, and yolk index linearly but not affect FCR and other egg quality parameters [23]. Moreover, in agreement with the present study, the lack of change in FCR was related to greater reduction in feed consumption than in EP and EW.

Dietary fortification with amino acids may eliminate the adverse effects of legume seeds. However, the dietary fortification has been used when the diet contained high-cereal grains but not legumes in most research. A lack of sulfur amino acids in the diet was shown to depress laying performance; inclusion of sulfur amino acids alleviated depression within 1 wk [34]. This may suggest that the adverse effects of legume seeds could be linked to low methionine and cysteine levels [35]. Feeding legume seeds also may interfere with sulfur containing amino acid metabolism and reduce laying performance and consequently may increase demand for choline [36]. Although 0.1% choline supplementation did not affect growth performance during the growing period, continuing supplementation during the laying period increased EP and egg size in chickens, suggesting that choline requirement may increase when diet is methionine deficient [36].

Methionine, via S-adenosylmethionine, alleviates the adverse effects of tannins by acting as a methyl donor for hydrolysis of tannins into gallic acid, which is excreted via urine as 4-*O*-methyl gallic acid [37]. To elucidate the role of methionine on alleviation of the adverse effect of tannins, we focused on its essentiality and having the sulfur and methyl group. Levels of the supplements were arranged to be higher in this study compared with studies involving high-sorghum diet [18, 38, 39], because the tannin level is greater in CVS (about 0.85 g/kg) [1, 6] than in sorghum (about 0.33 g/kg) [16, 18]. None of the supplements alleviated BW, FI, and EW depressions in the present study (Table 2). However, supplementation of methionine and choline, but not of lysine and sulfur, partially reduced depression in EP and elevation in FCR (Table 2), suggesting that methionine may play a role as methyl donor in alleviating the adverse effect of tannins. In chicks fed a diet containing 70% sorghum, supplementation of methionine (0.31%) alone or choline (0.20%) and lysine (0.20%) in combination with methionine (0.15%), but not alone, were shown to alleviate the adverse effects of tannins on FI and daily gain [18, 38, 39]. No differences in growth parameters of broilers fed diets containing 57% corn and 57% Serena sorghum containing 1.21% tannin plus 0.51% methionine [40]. In another study, after addition of 0.47% methionine into diets of hens, it was shown that increasing level of pea (*Pisum sativum*) from 0 to 30% using 10% increments did not affect BW, mortality rate, FI, EP, EW, and shell thickness [41]. However, there were

increases in expenditure of energy and ratio of methionine to cysteine per 100 g of egg and decrease ratio of methionine to lysine as pea consumption per animal and day increased. This result may suggest that pea could be fed up to 30% when the diet was fortified with methionine without decreasing performance. However, this was not ascertained for CVS in the present study.

## CONCLUSIONS AND APPLICATIONS

1. A partial replacement of soybean meal with CVS (22%) in the basal diet caused 7.8, 11.6, and 4.5% reductions in BW, FI, and EW, respectively; and the supplements (0.57% methionine, 0.47% choline, 0.66% lysine, or 0.05% sulfur) did not significantly reduce the depression in these parameters.
2. The inclusion of 22% CVS into the basal diet caused 18.7% reduction in EP and 16.4% increase in FCR.
3. Only methionine supplementation significantly lowered the depression in EP (6.2% increase) and alleviated elevation in FCR (7.4% decrease). Choline supplementation tended to ameliorate the adverse effect of CVS on EP (2.9% increase) and FCR (6.6% decrease).
4. The effects of CVS and supplements on egg quality parameters were variable.
5. Partial improvements in EP and FCR by supplementation of methionine and choline may suggest that the adverse effects of CVS could be related to interference with methyl donor metabolism. This, however, has to be supported by biochemical parameters.
6. Future studies should focus on optimizing levels of CVS and these supplements that do not alter laying performance and egg quality.

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24. Egg quality parameters were assessed using the following formulas [23]: Shape index (%) = [(egg width (cm)/egg length (cm)] × 100; shell strength (kg/cm × cm) was determined by using a machine with spiral pressure system; shell thickness (mm) was determined in 3 different parts (upper and lower ends and middle) by using a micrometer; albumen index (%) = [(albumen height (mm)/average of albumen length (mm) and albumen width (mm)] × 100; yolk index (%) = [(yolk height (mm)/yolk diameter (mm)] × 100; yolk color was determined by using commercially available yolk color fan according to the CIE standard colorimetric system; Haugh unit = $100 \times \log(H + 7.57 - 1.7 \times W^{0.37})$, where H = albumen height (mm) and W = egg weight (g).

25. In a complete randomized block design experiment, the location of cages was considered as a blocking factor. Feed intake and EP data were reduced to weekly means before statistical analyses. Data were subjected to 1-way ANOVA using the mixed procedure as repeated measures with time being a subplot [26]. The linear model to test the effects of the experimental diets on laying performance and egg quality parameters was as follows: $Y_{ijk} = \mu + B_i + TRT_j + T_k +$ error A + $(TRT \times t)_{jk}$ + error B, where $Y_{ijk}$ = response variable, $\mu$ = population mean, $B_i$ = block (i = 1 cage at lower level by corridor side to 6 cage at upper level by window side), $TRT_j$ = experimental diet (j = diet B to diet BVCS plus 0.05% sulfur as inorganic sulfur), $T_k$ = time (k = wk or mo relative to initiation of the experiment), error A = whole plot error, $(TRT \times t)_{jk}$ = experimental diet j and time k interaction, and error B = subplot error. For determining the effects of additives on response variables, diet B served as positive control, whereas diet BCVS served as negative control. Significant differences ($P < 0.05$) among the experimental groups were attained using Duncan's multiple range test. The experimental diet by time interaction term was dropped from the linear model when $P > 0.25$.

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