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The Effects of dietary cysteamine on performance, hatchability, plasma parameters and sex hormone levels in quails (*Coturnix coturnix japonica*)

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ABSTRACT: This study was designed to determine the effects of a single dose of dietary Cysteamine (Cys) on growth performance parameters, meat properties and laying performance, hatchability, plasma glucose, cholesterol, and sex hormone levels of quails. For this purpose, two separated animal experiments have designed as growth period Trial 1 (T1) and laying period Trial 2 (T2) and experiments have conducted one after another.

Trial 1 (T1) : A total of two hundred, 1- day old quail (*Coturnix coturnix japonica*) chicks were randomly allocated to 1 of two treatment groups with 5 replicates, either a control group (basal diet) or a treatment group that contained 90 mg Cys per kg basal diet. During the 5-week experimental period, the performance data regarding the growth period were collected. At the end of T1, a total of 30 quail were slaughtered, and the color values, pH, and meat macronutrients were determined in breast meat. Data from T1 showed that the inclusion of 90 mg Cys in the diet affected neither growth performance nor breast meat parameters of quail.

Trial 2 (T2) : At the end of T1, 40 from each treatment and a total of 80, six-week-old quail were selected randomly to be used in the laying period experiment and allocated to two dietary treatment groups. Forty quails from the control group of T1 were fed with a laying period basal diet. Forty quails from the Cys group of T1 were fed with a laying period diet that contained Cys (90 mg/kg basal diet). During the 10-week laying trial egg production and hatching performance data collected. At the end of the trial, a total of 20 quails were sacrificed and plasma glucose, cholesterol, and sex hormone levels were detected. Results of T2 displayed that 90 mg /kg feed dietary Cys increased egg mass ($P < 0.05$), however, it was ineffective on other laying and hatching performance parameters. Whereas plasma glucose level was not altered, plasma cholesterol level significantly decreased with the presence of Cys in the diet ($P < 0.05$). Moreover, dietary Cys significantly increased plasma sex hormones levels in both sexes. In conclusion, 90 mg dietary Cys did not show any improvement or depressing effect on the growth, laying, or hatching performance of quails however, its cholesterol-lowering impact and boosting effects on plasma sex hormones are noteworthy.

Keywords: meat, egg, cholesterol, estradiol, testosterone

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INTRODUCTION

Cysteamine (Cys) is an amino thiol and has defined as a natural bioactive substance that modulates the endocrine and metabolic status of animals (Rideau et al. 1990b; McLeod et al. 1995; Hu et al. 2008a). Cys occurs by decarboxylation of cysteine amino acid and, entering into the coenzyme-A structure, take the role in energy metabolism (Doğan et al. 2014). With the discovery that Cys is being part of the coenzyme A pathway, different formulas such as cysteamine hydrochloride, phosphocysteamine, and cysteamine bitartrate have used for various therapeutic strategies (Besouw et al. 2013). Furthermore, there are shreds of evidence that Cys rises the growth rate in mammals and poultry by increasing the growth hormone release (Hall et al. 1986; Yang et al. 2006). Recently reported that Cys has been shown to be effective in increasing growth and improving efficiency in a range of livestock species (Sun et al. 2017). On the other hand, Cys was reported to promote the maturation and fertilization of oocytes and to improve embryo developmental rates *in-vitro* (Urdaneta et al. 2004a; Hu et al. 2008a; De Matos and Furnus 2000a). Despite considerable knowledge about the metabolic functions of Cys, its action mechanism still unclear. Cys has rarely investigated as a dietary supplement in domestic animals including poultry. However, reported that a low dosage of Cys (generally ≤ 80 mg) administration may increase the synthesis of glutathione which is a powerful antioxidant. Contrarily, high dosage Cys administration (generally ≥ 100) may cause oxidative stress (Besouw et al. 2013). The quest for effective alternative feed additive to reduce the costs and optimize production in poultry production continues, and examination of bioactive substances such as Cys that may influence performance, and product quality has become more essential. Cys is an example of an additive studied in countries such as China and Thailand with the purpose of establishing beneficial effects on the performance of pigs and broilers (Nunes et al. 2012). The present study conducted in order to provide scientific data regarding usage of Cys as a feed supplement in poultry diets. Herewith, in this study quail was chosen as an experimental animal due to some advantages like early sexual maturity (6-7 weeks of age) and a high reproduction rate (Shim and Vohra 1984) has chosen. Therefore, the effects of dietary Cys (cysteamine hydrochloride) have investigated in quails' growth and laying periods. Thereby, two experiments that followed each other were designed to examine the effects of a single dose, 90 mg/

kg feed dietary Cys on growth performance and meat macronutrients, laying performance, hatching parameters, and plasma cholesterol, glucose, estradiol, testosterone, and Follicle-stimulating hormone (FSH) hormone levels.

MATERIALS AND METHODS

The animal experiments were conducted under the Prof. Dr. Orhan Düzgüneş application and research farm facility of Selcuk University Agriculture Faculty. All animal experiments were carried out according to the local ethics committee directives of Selcuk University.

Experimental design

Trial 1 (T1) :A total of 200, one-day-old quail chicks without sex discrimination were used for a 5-week growth period and this experiment named T1. The quail chicks were randomly divided into two treatment groups, each treatment had 100 quails with five replicates; 20 quails each. The chicks were reared in 33 x 40 x 28 cm size cages under the semi-controlled environment terms (ventilation controlling system) as every compartment of cages had a water nipple, manger, and heater. During the trial, 23 - h light - 1 h dark lighting program were applied. Feed and water were provided *ad-libitum*.

Trial 2 (T2) :At the end of the growth period trial (T1), 10 male and 30 female quail chicks from each treatment group, i.e., a total of 80 quails were randomly selected by considering cage dimensions and gender discrimination. Selected birds were allocated in two treatment groups with five replicates for 10 week laying period, and each compartment contained two males and six females (a total of 8 quails). Birds from the T1 control group were placed in the control group of T2 and were fed with the laying period basal diet. Birds from the T1 Cys group were placed in the Cys group of T2 and fed with a laying period basal diet contained 90 mg Cys kg/feed. Feeding of quails with T2 experimental diets was started when quails were 6 week-old age, however, performance data regarding egg production have got to collect after quails have reached 8 week-old age. The performance data and egg samples were collected during the T2. Quails were reared in 28 x 30 x 40 cm size cages, under the semi-controlled periphery terms (ventilation controlling system) as every compartment of cages had a water nipple, manger, and heater. The lighting programme considered as 16 - h light - 8 h darkness. Feed and water provided as *ad-libitum*.

Experimental diets

Feed raw materials was provided by a local feed factory and nutrient contents of experimental diets were formulated as the National Research Council (NRC 1994). Cysteamine (100% purity, crystal form, Cysteaminium chloride) was provided from Merck® (Darmstadt, Germany).

T1: Growth period diets were contained 23.88% crude protein and 2901 kcal metabolizable energy/kg (Table 1). The control group diet of T1 was the basal growth period diet without Cys supplementation. Cys group diet of T1 was a growth period diet with 90 mg cysteamine supplementation per kg basal diet.

T2: Laying period diets were contained 19.16% crude protein and 2900 kcal metabolizable energy/kg (Table 1). The control group diet of T2 was the basal laying period diet without Cys supplementation. Cys group diet of T2 was a laying period diet with 90 mg cysteamine supplementation per kg basal diet.

Table 1. Nutritional compositions of experimental diets used at T1 and T2

Ingredients (g/kg)	Experimental Diets	
	T1	T2
Barley	76.5	77.5
Corn	380	413.4
Soybean meal	409	295
Sunflower meal	50	71
Soybean oil	45	57
Limestone	11	60
DCP	18.5	15.5
Salt	4	4
Vitamin Premix ^A	2.5	2.5
Mineral Premix ^B	1	1
Lysine	1	1.6
Methionine	1.5	1.5
Nutrients (%)		
Crude Protein *	23.88	19.16
Moisture *	7.73	7.81
Ash*	8.04	12.25
Cellulose	7.51	7.48
Metabolizable energy (kcal/kg)	2901	2900
Calcium	0.96	2.85
Available phosphorus	0.47	0.40
DL-Methionine	0.54	0.50
Methionine + Cysteine	0.90	0.82
L-Lysine	1.35	1.14

T1:Trial 1,T2:Trial 2 ,^A: Premix provided the following per kg of diet: Vitamin A, 15,000 IU; Vitamin D3 1500 IU; Vitamin K 5.0 mg; Vitamin B1 3 mg; Vitamin B2 6 mg; Vitamin B6 5 mg; Vitamin B12 0.03 mg; Niacin 30 mg; Biotin 0.1 mg; Calcium D-pantotenat 12.0 mg; Folic acid 1.0 mg; Choline chloride provides 400 mg ^B: Premix provided the following per kg of diet: Manganese 80 mg; Iron 35 mg; Zinc 50 mg; Copper 5.0mg; Iodine 2 mg; Cobalt 0.04mg. *Analyzed value.

Performance parameters

T1: The initial live-weights (LW) of quail were determined at the beginning of the experiment. Thereafter, the average LW was measured weekly throughout 5 weeks. Before every LW measurement, the manger was cleaned and the average feed intake (FI) was calculated as the difference between the given and residual feed. The LW of each subgroup of quail were gauged in plastic containers, and the live-weight gains (WG) of treatment groups were calculated on a weekly basis. At the end of the trial, the LW of quails were recorded as final body weight (FBW). During the experiment, daily mortality was recorded. The feed conversion ratio (FCR) of the groups was calculated using average WG and average FI values. At the end of T1, a total of 30 (15 from each treatment group and 3 from each subgroup) quail were randomly selected and slaughtered. Viscera and feet were removed and carcass weight (CW) and carcass yield (CY) were calculated.

T2: The egg production (EP) was recorded daily while the egg weight (EW) was recorded weekly for 10 weeks period. The egg mass (EM) was calculated as $EM = (EP \times EW) / 100$. The FI was calculated by the difference between the given and residual feed and recorded every week. The FCR was calculated as $FCR = FI / EM$.

Meat macronutrients, color values and pH

Breast muscles were split from carcasses from T1 treatment, and the samples were held until the analyses time (24 hours) in refrigerator +4 °C. The proximate composition of quail meat was determined according to the AOAC method (A.O.A.C. 2000). The moisture value of breast meat was determined after drying in oven at 105 ± 3 °C and the total ash content was analyzed with ash furnace at 550 ± 5 °C. The crude protein content of meat samples was determined with Kjeldahl and fat content was determined with soxhlet device. The pH value of breast meat was determined by portable pH meter (WTW 2A20-1012 Waterproof pH-Meter) (Horwitz and Latimer 2000) and color values (L*, a*, b*) by Minolta Chroma Meter CR 400 (Minolta Co., Osaka, Japan) (Hunt et al. 1991). The L*, a* and b* parameters correspond to the lightness (-100/+100, dark/white), redness (-100/+100, green/red) and yellowness (-100/+100, blue/yellow), respectively.

Hatching parameters

At the last three weeks of T2, 50 eggs from each

group (total of 100 eggs) were collected for determination of hatchability parameters. Eggs were incubated at 37.5°C and 55-60% humidity for 14 days. Then the eggs were transferred to hatching machine- sat 37.2°C and 75% humidity. After the completion of the outputs, embryo deaths and infertility were determined by macroscopic analysis of eggs for calculation of hatchability ratio (HR), fertility ratio (FR), and hatchability of fertile eggs (HOF) (Aygün et al. 2012).

Plasma biochemical analyses

At the end of T2, one male and one female quail from each subgroup; 10 quails from each treatment (total of 20 quails) were slaughtered. Euthanasia of the birds was realized decapitation method. Blood samples were collected from the jugular vein and transferred to heparin-coated glass tubes. The sample tubes were centrifuged at 3000rpm (rpm= round per minute) for 10 minutes and plasma was obtained. Plasma glucose and total cholesterol levels were detected with a Siemens-dimension RLA-MAX (Germany) device using the photometric method. Levels of estradiol, testosterone, and FSH hormones in blood plasma were determined automatically by Siemens-advia centaur XP (Germany) device using the chemiluminescence method (Erdinçler et al. 2004).

Statistical analysis

Statistical analysis was performed using Minitab (Minitab 2000). Growth, laying, and hatching performance and meat properties data differences were determined using by T-test. General linear model, 2x2 factorial design was applied to plasma biochemical and hormone data, and differences were determined by using Tukey test ($P < 0.05$).

RESULTS

Results of T1

In the present study, Cys was used as dietary supplements to investigate its effects on quail's growth performance parameters. Data obtained from T1 are summarized in Table 2. and results showed that 90 mg Cys supplementation in the diet did not affect any parameter on growth period of quails.

Effects of the feeding of quails with 90 mg additional dietary Cys during T1 on meat color, pH, and meat nutrients content are presented in Table 3. Data showed that at the end of 5 weeks T1, Cys supplementation did not change meat color and pH values and not affect the macronutrients composition of meat compared with the control group.

Table 2. Effects of dietary cysteamine on growth performance parameters in T1

	Control group	Cysteamine group	P value
FCR	3.06±0.051	2.95±0.028	0.103
FI (0-5 Week) (g)	472.30±13.0	459.87±1.80	0.410
AWG (0-5 Week) (g)	154.29±2.50	156.01±1.40	0.569
FBW (g)	173.8±4.50	181.72±2.90	0.189
CW (g)	123.47±2.4	130.43±2.2	0.070
CY (%)	71.1±0.58	71.89±2.00	0.727

T1: Trial 1, FCR: Feed conversion ratio, FC: Feed intake, AWG: Average weight gain, FBW: Final body weight, CW: Carcass weight, CY: Carcass yield

Table 3. Effects of dietary cysteamine on meat color, pH value and nutrient composition at the end of T1

	Control group	Cysteamine group	P value
Meat color values			
L*	42.37±0.58	41.77±1.1	0.634
a*	6.21±0.32	6.81±0.16	0.156
b*	3.59±0.39	3.09±0.31	0.364
Meat pH	5.68±0.01	5.64±0.02	0.205
Meat macronutrients (%)			
Dry matter	29.31±0.46	30.47±0.27	0.070
Ash	1.35±0.05	1.36±0.03	0.829
Fat	2.69±0.33	3.12±0.21	0.311
Protein	23.78±0.39	24.67±0.28	0.105

T1: Trial 1

Results of T2

Data regards on impact of diet Cys on laying performance parameters of quails are demonstrated in Table 4. Outcomes of T2 displayed that long term Cys feeding of quails was not effective on FI, FCR, EW and EP in laying period however, with the inclusion of Cys in diet, EM increased significantly ($P<0.05$).

Determined hatching parameters during T2 are summarized in Table 5. None of the hatching parameters did not affected by 90 mg dietary Cys significantly.

Alterations of plasma glucose, cholesterol, estradiol, FSH, and testosterone levels detected at the end of T2 are shown in Table 6. Plasma glucose levels of quails were similar between sexes and, Cys in diet did not affect plasma glucose levels of animals. On the other hand, both, sex, and treatment were effective on plasma cholesterol levels of quails and, it was higher in male quails than females and Cys inclusion in diet significantly decreased blood cholesterol in both sex-

es ($P<0.05$). Plasma estradiol levels as expected was significantly higher in the female quails than male and Cys in diet increased plasma estradiol level ($P<0.05$). Additionally, Cys caused higher plasma estradiol level in male whereas decreased in female quails ($P<0.05$). Difference of plasma FSH levels between two sexes was insignificant but, dietary Cys raised up plasma FSH level according to control group and, the highest plasma FSH level detected in Male \times Cys group ($P<0.05$). Plasma testosterone levels of quails were dramatically different between two sexes and, testosterone levels of males quite higher than females ($P<0.05$). Dietary Cys caused significantly high plasma testosterone level than control group, and with the inclusion of 90 mg in diet the plasma testosterone was not change in female whereas significantly boost in male quails ($P<0.05$). Results demonstrated that 90 mg dietary Cys per kg basal diet independently gender displayed cholesterol- lowering and boosting-sex hormones effects in quail as a bioactive component.

Table 4. Effects of dietary cysteamine on laying performance parameters in T2

	Control group	Cysteamine group	P value
FI (Weekly) (g)	238.4 \pm 14.0	231.50 \pm 8.80	0.698
FCR	3.03 \pm 0.28	2.70 \pm 0.24	0.401
EM (g)	10.26 \pm 0.15 ^B	11.35 \pm 0.38 ^A	0.045
EW (g)	12.44 \pm 0.25	12.71 \pm 0.18	0.417
EP (%)	82.67 \pm 2.80	89.15 \pm 2.00	0.102

^{A, B, C} Means in the same row with a different superscript differ significantly ($P<0.05$). T2: Trial 2, FCR: Feed conversion ratio, FC: Feed intake, EM: Egg mass, EW: Egg weight, EP: Egg production

Table 5. Effects of dietary cysteamine on hatching performance parameters in T2

(%)	Control group	Cysteamine group	P value
HR	89,10 \pm 4,5	92,28 \pm 1,3	0,541
HOF	94,52 \pm 2,8	94,10 \pm 1,4	0,897
FR	94,48 \pm 2,2	98,11 \pm 0,78	0,200

HR: Hatchability ratio, HOF: hatchability of fertile eggs, FR: fertility ratio

Table 6. Effects of dietary cysteamine on plasma biochemical and sex hormone levels at the end of T2

	Glucose (mg/dL)	Cholesterol (mg/dL)	FSH (mIU/mL)	Estradiol (ng/dL)	Testosterone (ng/dL)
Sex	<i>Male</i>	271.4	276.5 ^A	0.129 ^A	2.925 ^B
	<i>Female</i>	268.9	163.6 ^B	0.111 ^A	7.690 ^A
Treatments	<i>Control</i>	271.4	226.5 ^A	0.069 ^B	5.221 ^B
	<i>Cysteamine</i>	268.9	213.6 ^B	0.171 ^A	5.393 ^A
Sex \times Treatment	<i>Male \times control</i>	271.8	284.0 ^A	0.044 ^C	2.393 ^D
	<i>Female \times control</i>	271.0	169.0 ^B	0.094 ^B	8.049 ^A
	<i>Male \times Cysteamine</i>	271.0	269.0 ^A	0.214 ^A	3.456 ^C
	<i>Female \times Cysteamine</i>	266.8	158.2 ^B	0.128 ^B	7.330 ^B
<i>SEM</i>		1.631	13.24	0.013	52.572

^{A, B, C} Means in the same column with a different superscript differ significantly ($P<0.05$). SEM: Standard error meaning, T2: Trial 2

DISCUSSION

Growth performance and meat properties

Studies regarding the effects of Cys on growth have reported that the positive effects of Cys supplementation for broiler chickens (Yang et al. 2006; Hu et al. 2008b). Moreover, some studies declared that improving effects of dietary Cys depends on its dosage (Jie et al. 2006; Ma et al. 2009). Many studies reported similar positive effects of dietary Cys on growth (Zhengkang 1993; Nunes et al. 2012). On the other hand, despite evidence Cys presence induces better growth, our results revealed that 90 mg/kg dietary Cys was ineffective on the growth performance of quails. These results may be due to that Cys can decrease the activity of digestive enzymes and can negatively affect the growth (Yang et al. 2005; Yang et al. 2006). Additionally, Jeitner and Lawrence (2001) reported that Cys may cause formation of hydrogen peroxide molecules and oxidative stress, and also decreases the activity of glutathione peroxidase. Clearly, in the present study, the dosage of dietary Cys for growing quails was not at the toxic levels that may negatively affect the growth performance. Nevertheless, 90 mg/kg diet Cys did not display the expected improving impact on the growth of quails.

Meat color is being the main factor that governs consumers' prefers decisions (Bai et al. 2018) and meat pH value is an indicator for the shelf life as it may influence the water holding capacity (Allen et al. 1998). It has been reported that dietary Cys supplementation could improve antioxidant status and delay meat discoloration by improving glutathione levels and antioxidant activity (Bai et al. 2018). Researches related to dietary Cys and its' effect on pH value and meat color have conducted in pigs and Zhu et al. (2018) reported that dietary Cys was ineffective on meat pH. Another study stated that Cys presence in piglet diets was not changed color values of meat (Tao et al. 2020). However, Bai et al. (2018) noticed that whereas dietary Cys reduces color discoloration depend on the administered dose, ineffective on meat pH value. To our knowledge, there are no studies related to the effects of dietary Cys on meat macronutrients that may contribute to this section. However, some researchers reported that Cys could increase the carcass lean percentage (Tao et al. 2020). Although little is known about the role of Cys on protein and lipid metabolism. In this study, 90 mg Cys inclusion to diet did not change dry matter, protein, fat, or ash contents of quail meat.

Laying and hatching performance

Few researches were conducted regarding the effects of dietary Cys on the laying poultry performance. However, some studies showed that Cys in the diet may improve the laying performance. For example, Hu et al. (2008a) reported that Cys at level of 400 mg / kg feed of breeder broiler ration significantly increased the average laying rate by 2.24% but egg weight was not affected. Li and Liu (2005) reported that Cys inclusion to the diet significantly increased the egg production and egg weight were in laying hens in the late production period. The results of the current study showed that 90 mg Cys per kg basal diet increased the in egg production per bird and this parameter was found significantly higher than the control group ($P < 0.05$). However, unfortunately, there is no scientific clarification or sufficient confirming data to explain the relationship between the increasing in the EM and dietary Cys. *In-vitro* studies reported that Cys has regulatory effects on oocyte maturation and fertilization and also increased embryo growth rates (Urdaneta et al. 2004b; De Matos and Furnus 2000b). It has been thought that dietary Cys may improve the incubation parameters of poultry. Similar to our study, Hu et al. (2008b) reported that addition of Cys to diet has not affected on fertility and hatchability in breeder broiler. Clearly, administration methods (*in-ovo* or oral) and dosage may effective factors or the effect of Cys on hatchability parameters. This study proved that 90 mg Cys in the diet was not effective on hatching performance of quails.

Plasma metabolites and sex hormones

Birds maintain higher plasma glucose concentrations than other vertebrates and smaller bird species have higher blood glucose level (Braun and Sweazea 2008). In this study, both sexes of laying quails had similar plasma glucose levels, and among the plasma parameters, only glucose level not changed by the addition of Cys in the diet. Rideau et al. (1990a) demonstrated that inoculation of 300 mg Cys per kg body weight to day-old male chicks increased plasma glucose level. Apparently, the dosage and administration method may modulate the effects of Cys on the plasma glucose level of poultry. On the other hand, in this study, the changes in plasma cholesterol levels of laying quails were dramatic and the differences were significant in both sexes and treatments ($P < 0.05$). Our results revealed that the plasma cholesterol level of quails are dissimilar between sexes and coherently with the current study Marks and Washburn (1991)

reported that cholesterol levels in quail may show differences between sexes or lines. Compatible with our results, there is evidence that the addition of Cys in the diet decrease the blood cholesterol in poultry and Xiao-jie et al. (2004) reported that 100 mg Cys per kg body weight in the geese diet decreased the cholesterol level in plasma and influence the lipid metabolism. Wittwer et al. (1987) confirmed a significant reduction of plasma cholesterol after supplementing Cys in the diet of cholesterol-fed rabbits, while and Zhu et al. (2007) reported that Cys lowered plasma low-density lipoprotein (LDL) levels in rats. Moreover, a study suggested that oral supplementation of Cys at level of 340 mg per kg body weight inhibited cholesterol secretion into bile (Salam et al. 2005). Data of the present study supported the cholesterol-lowering effects of dietary Cys.

Former studies, regard on Cys administration in avian species generally have focused on blood growth hormone alterations. In this study growth hormone level of birds, could not be detected. But sex hormone levels did alter significantly ($P < 0.05$) (Table 6). Data of this study displayed that the effects of dietary Cys on the plasma FSH levels depended on the sex. Consequently, the alterations of FSH level affected testosterone and estradiol levels differently in the two sexes. The effect of FSH on male and female reproductive hormones in poultry can be explained as follows: Vanmontfort et al. (1995) reported that there is a positive correlation between FSH and testosterone hormones in male animals and Baird et al. (1981)

stated that estradiol secretion is controlled by LH (Luteinizing hormone). It is not found any experimental or *in-vivo* study show the impact of dietary Cys on plasma sex hormone levels, however, obviously, this study proved 90 mg in the diet showed boosting effect on sex hormones levels of quails.

CONCLUSIONS

In a conclusion, according to data from the two experiments (T1 and T2), 90 mg Cys in quails' diet was ineffective on growth, laying, and hatching performance parameters as well as meat properties. However, the cholesterol-lowering effect and sex hormone-raising effects of dietary Cys were remarkable. In a nutshell, it looks that using Cys at the level of 90 mg per kg feed has not a growth promoter effect on the growth or laying performance of quails. Studies regarding cysteamine and its effects on poultry performance and metabolism are inadequate. The results of the current study may contribute to further research on this topic and clearly is needed more research on cysteamine usage in poultry diets.

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CONFLICT OF INTEREST

None declared by the authors.

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