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Effects of selenium and zinc supplementation on cadmium toxicity in broilers

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Abstract: Cadmium (Cd) is a toxic metal regarded as an environmental pollutant. The potential ameliorative effect of simultaneous addition of Se and Zn to broilers against Cd toxicity was examined. A total of 180 as-hatched broilers were used. There were 4 replicate pens of 3 dietary treatments: T1, T2, and T3. In T1, Se, Cd, and Zn were added to a basal diet at 0.3, 0, and 100 ppm, in T2 at 0.3, 50, and 150 ppm, and in T3 at 0.5, 50, and 150 ppm respectively. Selenium, Cd, Ca, Co, Cu, Fe, Li, Mg, Mn, Sb, As, Cr, Pb, Mo, Ni, V, and Zn were determined in breast samples using ICP-MS. Simultaneous addition of Se and Zn to broilers partly ameliorated the negative effects of Cd. The concentrations of Cd, As, and V in breast were significantly affected by the treatments while the concentration of other examined elements remained unaffected.

Key words: Broilers, cadmium, ICP-MS, selenium, toxicity, zinc

1. Introduction

Cadmium (Cd) is a toxic metal and is regarded as an environmental pollutant. It occurs both naturally and from industrial and agricultural sources [1]. Cd often occurs as an inescapable side product of metallurgy of several metals [2] and is considered as a significant public health issue [3]. There is a considerable effort worldwide to reduce Cd discharge and increase Cd-free technology [4]. Animals are exposed to Cd via contaminated water, feed, and air. The presence of Cd in feed-food chain may be a problem for several countries and in some cases exceeds maximum permitted limits [3].

Following absorption from the lung and the gastrointestinal tract, Cd accumulation occurs primarily in liver and kidney where it is bound to metallothionein [5]. Cadmium toxicity is ameliorated by the administration of some trace elements including but not limited to selenium (Se) and zinc (Zn) [6–8]. Counteraction of immunosuppressive effects and oxidative damage of liver and kidney caused by exposure to Cd can be achieved by Se administration [9,10]. Similarly, Zn supplied in conditions of exposure to Cd has been shown to partially protect against Cd induced oxidative stress [11]. Accordingly, when Se and Zn were administered together in rats, Cd toxicity was reduced [12,13]. Information on concomitant administration of Cd, Se, and Zn in poultry is sparse [14,15].

This work was part of a project designed to assess whether Se or Zn can protect against the toxic effects of Cd in broilers. Previously, protection provided by Se against Cd toxicity was investigated [16,17]. The aim of the present study was to investigate the potential ameliorative effect of simultaneous addition of Se and Zn to broilers against Cd toxicity.

2. Materials and methods

2.1. Animals, diets, and experimental design

One hundred eighty, as-hatched, 1-day-old, Cobb 500, chickens (broilers) were used in total. Broiler chickens were purchased from a commercial hatchery. There were 4 replicate pens (2 m length × 1 m width) of 3 dietary treatments namely T1, T2, and T3, randomly allocated in the house. Pen was the experimental unit. There were 15 chickens per pen, 60 per treatment. In T1 treatment, Se, Cd, and Zn were added to a basal broiler diet (only naturally present levels of Se, Cd, and Zn) at a level of

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0.3, 0, and 100 ppm, respectively. In T2 treatment, Se, Cd, and Zn were added to the same basal diet at a level of 0.3, 50, and 150 ppm, respectively, and in T3, at a level of 0.5, 50, and 150 ppm, respectively. Supplemented Se was from a yeast source, Sel-Plex (Alltech Inc., Nicholasville, KY, USA). Cd was added as $CdCl_2$, and Zn as ZnO (Sigma-Aldrich, St Louis, MO, USA).

The experimental duration was 42 days. Care and housing of chickens were performed according to the guidelines of the Ethical Committee of the Faculty of Animal Science of the Agricultural University of Athens. The chickens were raised, according to Cobb's management manual, in a house where light and ventilation were controlled. The chickens were fed a starter diet to the 10th day of their life (21% CP, 12.50 MJ ME/kg), a grower diet to the 22th day (19% CP, 12.90 MJ ME/kg), and a finisher diet to the 42nd day (18% CP, 13.30 MJ ME/kg). Both water and feed were provided ad libitum.

At weekly intervals, chickens were weighed, their body weight was recorded and the mean body weight gain was calculated. Furthermore, feed intake was measured, mean feed intake and feed to gain ratio were calculated. Broilers were inspected daily and mortality was recorded on the appropriate data capture form. Total mortality was calculated as the number of broilers that died throughout the study compared to the initial number of broilers placed. At the end of the trial, 3 chickens per pen (12 per treatment) were individually weighed and were slaughtered. Breast muscle samples were collected for trace element analysis and blood for determination of selected haematological parameters, and total protein concentration.

2.2. Trace element determination

The determined elements by inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer, Elan 9000, Waltham, MA, USA) were As, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, V, and Zn. ICP-MS provides high-throughput, ultra-trace level analysis [18].

For the total digestion of the samples, a microwave digestion system (CEM, Mars X-Press, Matthens, NC, USA) was used. Breast muscle samples (1 g wet weight) were soaked in 10 mL HNO_3 (65% w/v, Suprapur, Merck, Darmstadt, Germany). The program of microwave digestion system was: the power was ramped during 20 min from 100 W to 1200 W and then was held for 15 min. A maximum of 200 °C was reached following by a 15 min for cool down. Afterwards, a filtration step was followed through disposable syringe filters (Chromafil, Macherey-Nagel, Germany) and appropriate dilution with Milli-Q Water. For the preparation of calibration curves, high purity standards (Inorganic Ventures, VA, USA) were used.

2.3. Haematological analyses

Standard haematological analyses included determination of haematocrit, blood protein concentration, and the leukocyte type (% of lymphocytes, heterophiles, monocytes, eosinophils and basophiles). An ABX Pentra 400 bench top analyser (Horiba-ABX, Montpellier, France) was used for the determination of blood protein concentration and haematocrit. Leukocyte type (% of different white blood cells) was determined manually by light microscopy using a Neubauer chamber following a 1:20 dilution with the diluting solution (Turk's solution; 2% acetic acid v/v with a few drops of gentian violet) [19]. The counting was performed by 1 haematologist who was blinded to the blood probes examined. Lymphocytes, heterophiles, monocytes, eosinophils, and basophiles were counted and expressed as a percentage of total white blood cells.

2.4. Statistical analysis

The SAS software (SAS Institute Inc., Cary NC, USA) was used for statistical analysis. All variates were analysed by ANOVA. Descriptive statistics including mean and standard error of the mean (SEM) are presented. The statements of significance presented in this study were based on $P \leq 0.05$, unless otherwise stated.

3. Results

3.1. Performance of broilers

Body weight was significantly reduced ($P<0.001$) in broilers fed diets with 50 ppm of Cd added (T2 and T3) compared to broilers fed the control diet (T1) (Table 1). Mortality of broilers did not differ ($P > 0.05$) between the 3 dietary treatments at any interval point (not shown) or the whole period (Table 1). For the whole experimental period, the mean feed intake of broilers fed diets with Cd was significantly lower ($P < 0.001$) compared to that of broilers fed no added Cd (T1). Similarly, the mean body weight gain of broilers fed diets with added Cd (T2 and T3) was significantly lower ($P < 0.001$) compared to that of broilers fed no added Cd. Overall, broilers fed no added Cd levels showed a better feed conversion ratio ($P < 0.001$) compared to that of broilers fed diets with 50 ppm of Cd added (Table 1).

3.2. Haematological parameters

Haematocrit and total blood protein of broilers did not differ between the 3 dietary treatments (Table 2). No significant differences were noted in leukocyte type indicating no treatment effect except for eosinophils values. In detail, eosinophils values of broilers of the T2 treatment were lower ($P = 0.018$) compared to those of broilers of the control treatment. Similarly, lower eosinophils percentage ($P < 0.05$) was noted in broilers fed the T3 diet compared to that of broilers fed the control diet. The overall percentage representation of each white cell type was in the following order: lymphocytes > heterophiles > monocytes > eosinophils > basophiles.

Table 1. Performance of broilers during the total experimental period (0–42 days).

Parameter	Treatments[1]			SEM	P-value
	T1	T2	T3		
BW[2] (g)	2425.26[a]	1853.46[b]	1590.00[c]	41.23	<0.001
Mortality (%)	3.33	5.00	3.33	2.89	NS[3]
MFI[4] (g)	3950.68[a]	3402.49[b]	2839.39[c]	50.24	<0.001
MBWG[5] (g)	2384.74[a]	1813.81[b]	1549.67[c]	41.32	<0.001
FCR[6]	1.657[a]	1.880[b]	1.833[b]	0.036	0.004

Values are the means of 4 replicate pens (n = 4). Means with different superscripts (a, b, c) in each row indicate significant differences between treatments.
[1]In T1 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.3, 0, and 100 ppm, respectively. In T2 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.3, 50, and 150 ppm, respectively. In T3 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.5, 50, and 150 ppm, respectively.
[2]BW: Body weight of 42 days old broilers.
[3]NS: Statistically not significant (P > 0.05).
[4]MFI: Mean feed intake of the total experimental period (0–42 days).
[5]MBWG: Mean body weight gain of the total experimental period (0–42 days).
[6]FCR: Feed conversion ratio of the total experimental period (0–42 days).

Table 2. Blood total protein and selected haematological parameters of broilers.

Parameter	Treatments[1]			SEM	P-value
	T1	T2	T3		
Total Protein (g/dL)	3.68	3.68	3.75	0.198	NS
Haematocrit (%)	34.00	35.75	32.00	1.16	NS[2]
Heterophiles (% total white blood cells)	36.75	46.50	48.00	4.26	NS
Lymphocytes (% total white blood cells)	40.00	39.00	39.50	4.61	NS
Monocytes (% total white blood cells)	14.00	8.25	9.75	2.11	NS
Eosinophils (% total white blood cells)	7.75[a]	5.25[b]	2.50[b]	1.04	0.018
Basophiles (% total white blood cells)	1.50	1.00	0.25	0.4	NS

Values are the means of 4 replicate pens (n = 4). Means with different superscripts (a, b) in each row indicate significant differences between treatments.
[1]In T1 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.3, 0, and 100 ppm, respectively. In T2 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.3, 50, and 150 ppm, respectively. In T3 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.5, 50, and 150 ppm, respectively.
[2]NS: Statistically not significant (P > 0.05).

3.3. Treatment effects on the concentration of 17 elements in breast muscle

Comparison of the elements' concentration in breast muscle between T1 (no Cd added) and T2, and T3 treatments (50 ppm of added Cd) revealed that the concentration of Cd, As, and V were significantly affected by the dietary treatments while the concentration of all other examined elements did not alter. In detail, broilers fed the diets with added Cd had significantly increased (P = 0.017) tissue Cd concentration compared to that of broilers fed diet with no added Cd (Table 3). The concentration of As and V were significantly lower in T2 and T3 treatments compared to the T1. Specifically, As concentrations in breast tissue of broilers of the T2 and T3 treatments were

Table 3. Determined concentration (ppb) of 17 elements in broilers breast muscle.

Element	Treatments[1]			SEM	P-value
	T1	T2	T3		
Se	217.1	207.4	239.6	10.21	NS[2]
Cd	21.6[a]	331.8[b]	240.0[b]	62.10	0.017
Ca	25534	21233	22924	4280	NS
Co	3.4	4.2	6.7	1.363	NS
Cu	335.7	317.1	384.7	50.32	NS
Fe	12731	11081	35277	13837	NS
Li	3.2	2.7	2.8	0.16	NS
Mg	282702	282746	296240	10749	NS
Mn	174.4	151.5	238.1	34.4	NS
Sb	1.004	0.899	0.978	0.051	NS
As	7.8[a]	6.6[b]	6.7[b]	0.295	0.032
Cr	30.7	30.9	33.9	1.996	NS
Pb	17.7	15.9	16.1	0.496	NS
Mo	63.5	60.3	56.0	13.05	NS
Ni	17.2	6.1	12.2	3.15	NS
V	2.7[a]	2.4[b]	2.4[b]	0.079	0.034
Zn	4519	4403	4744	509	NS

Values are the means of 4 replicate pens (n = 4). Means with different superscripts (a, b) in each row indicate significant differences between treatments.

[1]In T1 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.3, 0, and 100 ppm, respectively. In T2 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.3, 50, and 150 ppm, respectively. In T3 treatment, Se, Cd, and Zn were added to a commercial broiler diet at a level of 0.5, 50, and 150 ppm, respectively.

[2]NS: Statistically not significant (P > 0.05).

approximately 17% reduced compared to that of broilers of the T1 treatment (P = 0.032). Similarly, V was significantly reduced by 12.5% in T2 and T3 treatments compared to the T1 (P = 0.034). Se and Zn supplementation in T2 and T3 treatments resulted in numerically higher values of concentration in breast muscle although statistically not significant (P > 0.05).

4. Discussion

The aim of the present study was to investigate whether concomitant administration of Se together with Zn, elements shown to protect against Cd toxicity [11,15], could ameliorate the negative effects of 50 ppm of added Cd to the diet. However, solely by examining performance parameters, simultaneous administration of Se and Zn could not protect against toxicity caused by Cd. It was hypothesised that the high availability of organic Se together with Zn may protect the body during stress conditions induced by Cd. In a previous work [17], it was shown that broilers supplemented with organic Se added at concentrations ranging from 0.3 ppm up to 3 ppm, could tolerate 10 ppm of Cd but addition of 100 ppm Cd led to an impairment of broilers' performance. In the present study, broilers fed 50 ppm of Cd had reduced body weight compared to those fed the control diet with no Cd present despite the presence of additional levels of Zn and Se in the diet. Similarly, in a study by Li et al. [20], cocks fed a diet with 10 ppm of inorganic Se or 150 ppm of $CdCl_2$, or their combination, had reduced final body weight in the Cd group compared to the other groups. In contrast, in rodents, no significant differences have been observed for the body weight gain among 5 groups (Cd, Cd + Zn, Cd + Se, or Cd + Zn + Se) following 35 days of exposure to 200 ppm Cd in the drinking water [21]. In laying hens, egg production was reduced relative to control when birds were exposed to 153 ppm of dietary Cd [14]. The same authors reported that dietary Zn at 803 ppm was shown to protect against the negative effects of 153 ppm of dietary Cd regardless of the level of supplemental Se. The exposure to Cd induces oxidative stress which can partially be ameliorated by Zn by restoration of the CuZn-SOD activity in rats [11], while Se and Zn can have a cooperative effect in the protection against Cd-induced rat liver damage [21]. The contradictory results between studies may be related to the ratio of the added elements, namely Se, Cd, and Zn. Furthermore, it is not clear whether these differences could also be attributed to the formation of complexes or other indirect interrelations that need to be elucidated.

Despite the lack of protective effects of Se and Zn on body weight reduction due to Cd addition, addition of both of these elements to broilers' feed maintained mortality and haematological parameters to levels similar to those of broilers fed no added Cd. Avian haematology possesses several distinctive characteristics such as short life span of red blood cells that have a nucleus and a heterophil function similar to that of mammalian neutrophil function. In the present study, haematocrit (PCV) ranged between 32% and 35.75%, values that are within the physiological values (23%–55%) [22]. The same applied for the examined blood total protein level (3.5–5.5 g/dL). Regarding the white blood cells, they ranged within the physiological values. Furthermore, the treatment effects observed for eosinophils cannot be regarded as eosinopenia (low eosinophils counts) since they ranged within the normal values (0%–16% total white blood cells). The overall percentage representation of each white cell type was in the following order: lymphocytes ≥ heterophiles > monocytes > eosinophils > basophiles.

Although the intention of the study was to focus on accumulation on edible muscle tissue and not to examine potential interactions between elements, the results from the 17 analysed elements indicate that bioaccumulation and potential toxicity of a metal can be modulated by the interaction with other toxic or essential metals. The present study revealed that the concentrations of Cd, As, and V in breast muscle were significantly affected by the dietary treatments while the concentration of all other examined elements remained unaffected. The extend of increase of Cd level in breast muscle is in line with previous studies [16,17] indicating that breast muscle is a tissue that accumulates relative lower amounts of Cd compared to other nonedible tissues. Regarding the Cd level in breast muscle between the 2 treatments that had 50 ppm of added Cd in the diet, no statistically significant differences were noted. Given that the 2 treatments had similar Zn and Cd added levels, the numerically lower accumulated Cd may be attributed to the higher supplemental Se present. Similarly, Lazarus et al. [23] reported that Se supplementation reduced Cd concentrations in the liver and kidney of rat pups. Similarly, Pappas et al. [24] showed that Se added in broilers could reduce the tissue deposition of the low naturally occurring Cd levels. Formation of insoluble compounds that are excreted in the faeces may be a mode of action when Se is administered as a remedy for heavy metal toxicity [25]. Previous studies have found significant associations between As, Cd, Se, and Zn [26–28]. Under this context, the reduction of V concentration in treatments with 50 ppm of added Cd, in the presence of Zn and Se, compared to control may indicate an association between V and Cd. In mice, it has been shown that a pentavalent V compound can induce metallothionein synthesis thus, it seems possible that Cd and V are linked via the action of metallothionein [29].

In conclusion, simultaneous addition of Se and Zn to broilers, partly ameliorated the negative effects of added Cd. The level of supplemental elements, their chemical form, the duration of exposure, and their antagonistic or synergistic effects are important factors determining the detected concentration in the edible tissues. Future studies with focus on elements' absorption, distribution and retention may further elucidate heavy metal detoxification.

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Conflict of Interest
The authors declare no conflict of interest.

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