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Supplementing growing quail diets with silymarin and curcumin to improve productive performance and antioxidant status and alleviate aflatoxin b_1 adverse effects during the summer season

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ABSTRACT

The current experiment was carried out to test the ability of supplementing growing quail diet with silymarin and curcumin as natural antioxidants to improve antioxidant status of growing Japanese quail and alleviate aflatoxin B_1 adverse effects during the summer season. Three levels each of curcumin and silymarin (zero, 250mg and 500mg/kg diet) were combined to compose 9 experimental diets and the diet that contains zero levels from each natural antioxidant acted as a control basal diet. Three hundred and sixty-one-day-old Japanese quail chicks were distributed randomly into nine experimental groups where each group contained 4 replicates with 10 chicks per each. Each experimental group fed only one diet for five weeks where growth performance parameters were recorded. Carcass characteristics, plasma proteins, liver enzymes and some antioxidant parameters were measured. The following results were obtained. Significant improvement was detected in final body weight and entire body weight gain for quail fed the diet supplemented with 250 mg /kg diet from both curcumin and silymarin compared with quail fed the control diet. Final body weight and entire body weight gain gained more improvement with increasing curcumin and silymarin up to 500mg/kg diet. Curcumin and silymarin supplementation didn't affect significantly the feed intake, feed conversion carcass parameters and plasma proteins fraction. Blood antioxidant parameters were improved by curcumin and silymarin supplementation. The level of aflatoxin B_1 in liver and muscles were significantly decreased by curcumin and silymarin supplementation. From the current experimental results, we can recommend supplementing diets with curcumin and silymarin to improve growth performance and alleviate harms effects of free radicals and aflatoxin B_1.

Keywords: Curcumin, silymarin, aflatoxinB_1, quail performance.

INTRODUCTION

The antioxidant system in poultry involved two main categories exogenous existed in diets such as some vitamins and endogenous such as glutathione enzymes (Surai *et al.,* 2019). Chronic heat stress is an expression referred to stress produced from exposure to partly high temperatures for an extended period like that occurs during the summer season (Abu-Dieyeh, 2006). Lipid peroxidation is created from heat stress exposure (Altan *et al.,* 2000b) and it is initiated when ambient temperature reached 34 °C (Sahin, *et al.,* 2008) and malondialdehyde is a last product for lipid peroxidation (Migliorini *et al.,* 2017).

Aflatoxins are found as natural pollutants in feedstuffs including, corn, Soya beans, barely, sorghum and wheat. Relative humidity around feedstuffs and its moisture content may be the most important factors for producing aflatoxins by *Aspergillus flavus*. When relative humidity is increased than 70% and moisture content of feedstuffs is more than 15% *A. flavus* activate and produces aflatoxins. The best temperature for growing *A. flavus* is ranged from 36 to 38°C, while the greatest temperature for producing toxin is about 25°C. Aflatoxins are the major important mycotoxins formed *by A. parasiticus and A. flavus* strains and bring a threat to animals and humans and animals. Aflatoxins cause four damage effects: acute liver damage and cirrhosis, teratogenic effects and induction of tumors (Pitt and Hocking, 2009; Bhat *et al.,* 2010). Comparative studies in regard sensitivity of poultry species to aflatoxicosis indicated that chickens are the most resistant poultry species to its effects but ducks and turkeys were the greatest sensitive, while quails appear middle sensitivity (Diaz *et al.,* 2008). The term of hormesis used by toxicologists for describing status lay between a low dose and a high toxic dose (Mattson, 2008). Liu, *et al.* 2016 reported that a diet that contains low levels of aflatoxin B_1 (AFB_1) reduced serum content from immunoglobins and decreased feed intake and body gain. They added that low levels of AFB_1 in broiler diets

decreased significantly total antioxidant capacity (TAOC) and glutathione peroxidase (GSH-Px). Moreover, harmful effects of AFB_1 involve lipid peroxidation, producing free radicals and causing cell damage (Surai, 2002).

Inserting natural antioxidants in diets able to improve antioxidant status of broiler chicks (Selim *et al.,* 2013) and able either to improve antioxidant status of broiler Japanese quail and alleviated adverse effects of impacting aflatoxins (Khaleghipour *et al.,* 2019). Silymarin able to use as natural antioxidant (Abdelazim, 2017).and silymarin is a major pharmacological active substance found in milk thistle herb and able to recover hepatic damage of Japanese quail (Saleemi *et al.,* 2019). Silymarin may be able to inhibit hepatic and cellular damage produced by aflatoxins and prevent lipid peroxidation (Migliorini *et al.,* 2017). Moreover, supplementation of silymarin in quail diets alleviated the adverse effect of aflatoxins on quail performance (Khaleghipour *et al.,* 2019). Silymarin supplementation at a level of 500 mg/kg improved growth performance and hepatic function for quails-fed diet implicated with 2.2 mg aflatoxins (Khaleghipour *et al.,* 2020). In general, silymarin may be a beneficial antioxidant source for eliminating oxidative stressors and harm effects (Behboodi *et al.,* 2017) and existing protection against adverse effects of AFB_1 (Tedesco *et al.,* 2004). Curcumin is natural antioxidant that possesses scavenging activities against free radicals (Zhang *et al.,* 2019) and it able to alleviate oxidative stress that is produced when quail become under heat stress (Sahin *et al.,* 2012). Curcumin may protect liver of Japanese quail from damage (Emadi *et al.,* 2015) and decrease malondialdehyde in the liver, muscle and serum (Sahin *et al.,* 2012). Moreover, curcumin improved liver antioxidant ability and then reduced toxic effect of aflatoxin (Karimi *et al.,* 2020). The experiment aimed to evaluate the ability of silymarin and curcumin as natural antioxidants to improve Japanese quail performance and alleviate harm effects of heat stress during summer season.

MATERIALS AND METHODS

Experimental treatments:

Three levels included 0, 250 and 500 mg/kg diet from each of silymarin (SI) and curcumin (CR) combined with each other to compose 9 mixtures that supplemented Japanese quail basal diets to perform 9 experimental diets. Three hundred and sixty one-day-old Japanese quail chicks were distributed randomly into 9 treatment groups where each group included 4 replicates with 10 chicks per each. The 1st treatment group was fed a control basal diet (CBD) that satisfy Japanese quail requirements according to NRC (1994) **Table (1)** without neither SI nor CR supplementation (zero CR and SI). The 2nd group was fed CBD supplemented with 250mg CR/kg diet and the 3rd group fed CBD supplemented with 500mg CR/kg diet. The 4th, 5th and 6th groups were fed CBD supplemented with 250mg SI/kg combined with zero, 250 or 500mg CR/kg diet respectively. The 7th, 8th and 9th groups were fed CBD supplemented with 500mg SI/kg combined with zero, 250 or 500mg CR/kg respectively.

Table 1. Composition and calculated analysis of control basal diet.

Ingredient	%	Calculated analysis	
Yellow corn	50	CP%	24
Soybean meal (44%)	35.8	ME.KCal/Kg	2900
Corn gluten meal (62%)	5.5	Ca %	0.83
Sunflower oil	0.9	Avail. P%	0.30
Wheat bran	4.5	Meth. %	0.54
Di-Ca-P	1.44	Lysine%	1.36
Limestone	1		
Premix*	0.3		
NaCl (salt)	0.25		
L-lysine-HCL	0.19		
DL-methionine	0.12		
Total	100		

*Each 3 kg contains: 15000.000 IU Vit.A; 4000.000 IU Vit.D3; 50000 mgVit.E; 4000 mg Vit.K3; 3000mg Vit.B1; 8000mg Vit.B2; 5000mg Vit.B6; 16000mg pantothenic acid; 20mg Vit.B12;2000mg folic acid; 4000mg niacin; 150mg cobalt; 1000mg iodine; 150mg selenium; 100000mg manganese and 30000mg iron.

Housing and management:
All chicks were reared in the same house and exposed to the same environmental conditions where ambient and relative humidity was recorded daily and the average ambient temperatures were 37, 38, 36, 37 and 36 for 1st, 2nd, 3rd, 4th, and 5th weeks of age respectively. The average relative humidity was 40%, 45%, 50%, 45% and 50% for 1st, 2nd, 3rd, 4th, and 5th weeks of age respectively (Table, 2). All chicks were exposed to continuous light where light nearly separated equally in the rearing house. All replicates were reared in a wood cage surrounded by wire from two sides with three dimensions of 50 ×50×50cm from 1 day to 35 days of age. Water and feed remained available for all quail chicks during the entire experimental period.

Table 2. Average ambient temperature and relative humidity during experimental periods.

Items	Ambient temperature		Relative humidity	
	Low	High	Low	High
First week	34	36	40	45
Second week	33	35	45	50
Third week	30	37	45	55
Fourth week	28	36	50	55
Fifth week	29	38	50	55

Growth performance parameters:
A sufficient amount of feed was provided daily for all replicates and at the end of each week, the residual was weighed to calculate feed consumption. All quails were weighed individually at 2, 4 and 5 wks of age where body weight (BW) was estimated and body weight gain (BWG) was calculated. For each replicate 10 kgs diet were weighed and put in sacks at the beginning of each week then the remaining diets were weighed at weekend. Feed intake for each week was recorded and the average feed intake for 1st two weeks, 2nd two weeks and last week were calculated. Feed conversion (gram feed/gram gain) for each replicate was calculated by dividing feed intake by (BWG).

Slaughter carcass characteristics and blood samples:
At the end of the 5th week, one chick from each replicate was picked up to determine carcass characteristics and obtain blood samples. During slaughter, two blood samples were collected where one sample was collected in a heparinized tube and another one collected in a non-heparinized tube from each quail chick. With using commercial kits produced by Biodiagnostic diagnostic and research reagents, aspartate transaminase (AST) and alanine transaminase (ALT) were determined. Total protein and albumin were determined and globulin was calculated. Total antioxidant capacity (TAOC) malnodialdhyde (MDA) glutathione peroxidase activity GSH-Px were determined.

Detection of Aflatoxins in basal growing quail diet:
For detecting Aflatoxins in basal quail diet the Thin Layer Chromatography methods reported by Schuller and Egmond (1981) and (AOAC, 2000) were used. From diet sample, 50 grams picked and well ground and mixed in an electric mill. The ground sample transferred to 500 ml Erlynmeyar flasks for dragging and purification of aflatoxins. After purity of aflatoxins, the extract was evaporated pending dryness. The remainder was cooled and stored at zero ºC for chromatography examing on thin layer plates for evaluation of aflatoxin.

Evaluation of AFB1 residue in liver and muscles tissues:
Liver and muscle samples of quails from each group were collected and stored at - 20ºC for further study. The AFB_1 in the tissues was extracted, and purified using an immunoaffinity column and with pre-column derivatization according to Tavčar-Kalcher *et al.* (2007) and Hussain *et al.* (2010). From each sample, 25 g were thawed and ground up in a high speed mixer, followed by the addition of 5 g of NaCl and blending with 100 mL of a mixture (80 methanol:20 water) for 3 min with high speed. After filtration through a paper filter, an aliquot of 10 mL of filtrate (equivalent to 2 g of tissue sample) was diluted with 40 mL of phosphate-buffered saline with 0.1% Tween-20. The procedure was conducted to an immune-affinity column (Vicam, Milford, MA, USA) and approved at a movement rate of 1–2 drops per second at (30 mmHg) pressure on the SPE-10 Manifold apparatus (J.T. Baker, Inc., Phillipsburg, NJ, USA). With 20 mL of distilled water, the immune-affinity columns were washed. Finally, AFB_1 was removed by wishing with 1.0 mL of methanol, at a rate of 1–2 drops per second. The eluted was collected in a glass vial and read in flourometer apparatus (Trucksess *et al.,* 1994).

Statistical analysis:

Statistical Package for the Social Sciences (SPSS, 2007) software program was used to analyse data of the experiment where a general factorial design procedure was used to find the effect of curcumin, silymarin and its interaction. Duncan's multiple ranges were used to compare means (Duncan, 1955).

RESULTS

The aflatoxinB_1 level in the control basal diet was 19ppb for the sample that picked from experimental diet. Half of the experimental sample diet picked at the start of the experiment and another half picked at the end of the experiment. The level of aflatoxinB_1 in experimental diet remained without adding more aflatoxinB_1 in the diet because increasing aflatoxin B_1 level up to a lethal level may be destroyed growth performance. Moreover the level detected in the experimental diet may be the common level in the commercial diets that were used for feeding all flocks in poultry production.

Growth performance:

For the entire experimental period, the results in Table (3) indicated that BW and BWG were influenced significantly by feeding growing quail with diets supplemented with SI, CR and its mixture. Supplementing 250mg SI/kg diets regardless CR to growing Japanese quail diet improved significantly (P≤0.05) BW and BWG for entire growing period. Increasing supplementation levels of SI to 500mg SI/kg diets gained significantly more improvement in BW and BWG. When Japanese quail fed diet contained 500mg SI mixed with 250mg CR/kg diet for entire experimental period BW and BWG recorded significantly (P≤0.05) the highest value.

Table 3. Effect of curcumin, silymarin and interaction (treatments) on body weight and body weight gain

Substances		Silymarin (SI)					Curcumin (CR)				
Items	Phase	SI zero (a)	SI 250 (b)	SI 500 (c)	SE±	P. values	CR zero(1)	CR 250 (2)	CR 500 (3)	SE±	P. values
Body weight	2wk	83.26^{b}	85.16^{a}	80.46^{c}	0.92	0.001	79.11^{b}	84.13^{ab}	85.63^{a}	0.93	0.001
	4wk	187.72	189.42	187.53	1.87	N.S.	183.49^{b}	189.25^{ab}	191.93^{a}	1.900	0.006
	5wk	236.4^{c}	240.41^{b}	245.88^{a}	2.95	0.028	237.49^{b}	242.56^{a}	242.64^{a}	3.00	0.039
Body gain	1-2wks	74.26^{b}	76.16^{a}	71.46^{c}	0.89	0.001	70.11^{b}	75.13^{ab}	76.63^{a}	0.94	0.001
	3-4wks	104.46	104.26	107.07	1.46	N.S.	104.38^{b}	105.12^{ab}	106.29^{a}	1.48	0.014
	5wk	48.68^{b}	51.45^{b}	58.35^{a}	2.33	0.023	54	53.31	51.17	2.37	N.S.
	1-5wks	227.4^{c}	231.41^{b}	236.88^{a}	2.95	0.037	228.49^{b}	233.56^{a}	233.64^{a}	3.00	0.046
Treatments (interaction)											
Items	Phase	SI a × CR 1	SI a × CR 2	SI a × CR 3	SI b × CR 1	SI b × CR 2	SI b × CR 3	SI c × CR 1	SI c × CR 2	SI c × CR 3	SE±	P. values
Body weight	2wk	82.74^{c}	83.97^{bc}	83.06^{bc}	83.34^{bc}	83.85	88.28^{a}	71.26^{d}	84.57^{b}	85.56^{b}	1.61	0.001
	4wk	183.18	189.64	190.33	185.74	186.5	196.03	181.55	191.62	189.42	3.28	N.S.
	5wk	232.76^{d}	238.31^{c}	238.12^{c}	235.26^{cd}	240.08^{bc}	245.9^{b}	244.45^{b}	249.3^{a}	243.89^{b}	5.18	0.046
Body gain	1-2wks	73.74^{c}	74.97^{c}	74.06^{c}	74.34^{c}	74.85^{c}	79.28^{a}	62.26^{d}	75.57^{b}	76.56^{b}	1.67	0.001
	3-4wks	100.44	105.67	107.27	102.39	102.65	107.74	110.29	107.05	103.86	2.56	N.S.
	5wk	49.59^{d}	48.67^{d}	47.79^{d}	49.53^{d}	53.58^{c}	51.24^{cd}	62.89^{a}	57.68^{b}	54.47^{c}	4.08	0.039
	1-5wks	223.76^{d}	229.31^{c}	229.12^{c}	226.26^{cd}	231.08^{bc}	236.9^{b}	235.45^{b}	240.3^{a}	234.89^{b}	5.18	0.041

$^{a,b,..}$ Means within the same row with different superscripts are significantly differ (P ≤ 0.05). NS: Not significant. SE=Standard Error of Means

In contrast of BW and BWG, FI and FC didn't influence significantly by nether SI, CR nor its combination (Table, 4). Slight numerical increase in feed intake was observed for growing quail chicks that fed diet supplemented with SI and CR compared with control basal diet.

Table 4. Effect of curcumin (CR), silymarin (SI) and interaction (treatments) on feed intake and feed conversion.

Main effects		Silymarin (SI)					Curcumin (CR)				
Items	Phase	SI zero (a)	SI 250 (b)	SI 500 (c)	SE±	P. values	CR zero (1)	CR 250 (2)	CR 500 (3)	SE±	P. values
Feed intake	1-2wks	167.67	168.90	163.88	3.06	N.S.	163.13	168.62	168.70	3.06	N.S.
	3-4wks	247.73	246.41	246.94	4.97	N.S.	241.48	250.88	248.73	4.97	N.S.
	5wk	138.73	139.00	146.36	3.73	N.S.	145.45	140.97	137.67	3.73	N.S.
	1-5wks	554.12	554.31	557.19	6.26	N.S.	550.05	560.47	555.10	6.26	N.S.
Feed conversion	1-2wks	2.30	2.25	2.20	0.02	N.S.	2.27	2.25	2.23	0.02	N.S.
	3-4wks	2.34	2.38	2.34	0.04	N.S.	2.33	2.38	2.34	0.04	N.S.
	5wk	2.69	2.65	2.71	0.06	N.S.	2.75	2.67	2.63	0.06	N.S.
	1-5wks	2.44	2.43	2.41	0.03	N.S.	2.45	2.43	2.40	0.03	N.S.

Treatments (interaction) effects												
Items	Phase	SI a × CR1	SI a × CR 2	SI a × CR 3	SI b × CR 1	SI b × CR 2	SI b × CR 3	SI c × CR 1	SI c × CR 2	SI c × CR 3	SE±	P. values
Feed intake	1-2wks	169.91	162.90	170.19	160.54	173.73	172.44	158.93	169.24	163.48	5.30	N.S.
	3-4wks	248.26	248.43	246.49	237.52	254.48	247.24	238.65	249.72	252.46	8.61	N.S.
	5wk	143.93	142.21	130.05	145.08	142.20	129.72	147.35	138.50	153.25	6.45	N.S.
	1-5wks	562.10	553.54	546.72	543.14	570.40	549.40	544.92	557.46	569.18	10.84	N.S.
Feed conversion	1-2wks	2.32	2.30	2.29	2.28	2.26	2.21	2.22	2.19	2.18	0.04	N.S.
	3-4wks	2.39	2.32	2.31	2.34	2.44	2.36	2.26	2.39	2.36	0.07	N.S.
	5wk	2.94	2.52	2.61	2.68	2.70	2.57	2.63	2.78	2.72	0.10	N.S.
	1-5wks	2.55	2.38	2.40	2.44	2.47	2.38	2.37	2.45	2.42	0.05	N.S.

NS: Not significant. SE=Standard Error of Means

Carcass characteristics and organs weight percent:

The data in Table (4) indicated that neither, SI, CR nor its interaction affected significantly carcass characteristics. The feather weight% increased numerically when quail feed diet supplemented with each of CR and SI but a numerical decrease was observed in liver weight% when growing quail fed diet supplemented with SI

Table 5. Effect silymarin, of curcumin and interaction (treatments) on carcass characteristics.

Items	Silymarin (SI)					Curcumin (CR)				
	SI zero(a)	SI 250(b)	SI 500(c)	SE±	P. values	CR zero(1)	CR 250(2)	CR 500(3)	SE±	P. values
Blood%	4.41	4.20	4.05	0.18	NS	4.21	4.30	4.14	0.18	NS
Feather%	16.86	17.66	19.60	1.00	NS	17.18	18.44	18.50	1.00	NS
Carcass%	66.82	67.33	64.45	1.14	NS	67.46	64.77	66.37	1.14	NS
Liver%	1.96	1.75	1.58	0.11	NS	1.68	1.74	1.87	0.11	NS
Gizzard%	2.02	1.83	1.98	0.10	NS	1.91	2.03	1.88	0.10	NS
Heart%	0.81	0.82	0.78	0.04	NS	0.78	0.82	0.81	0.04	NS
Tests%	3.53	3.07	3.02	0.23	NS	3.28	3.15	3.19	0.23	NS

Treatments (interaction)										
Items	SI a ×CR 1	SI a ×CR 2	SI a ×CR3	SI b ×CR1	SI b × CR 2	SI b × CR 3	SI c × CR 1	SI c × CR 2	SI c × CR 3	SE±	P. values
Blood%	4.30	4.92	4.01	4.09	4.25	4.26	4.25	3.75	4.16	0.31	NS
Feather%	15.24	17.39	17.96	16.98	18.50	17.49	19.31	19.42	20.06	1.74	NS
Carcass%	66.85	65.59	68.03	69.91	66.72	65.37	65.62	62.01	65.71	1.98	NS
Liver%	1.89	1.72	2.26	1.92	1.58	1.75	1.22	1.93	1.59	0.20	NS
Gizzard %	2.08	1.90	2.07	1.81	1.92	1.76	1.85	2.28	1.81	0.16	NS
Heart%	0.78	0.74	0.93	0.83	0.90	0.74	0.74	0.83	0.77	0.07	NS
Tests %	3.92	3.57	3.11	3.14	2.91	3.15	2.77	2.98	3.32	0.39	NS

NS: Not significant. SE=Standard Error of Means

Plasma proteins profile and Liver enzymes:

Supplementing SI, CR and its mixture to quail diet didn't affect significantly total plasma proteins, albumins and globulins during growing period (Table, 6). The highest numerical total proteins and globulin values recorded when diet supplemented with a mixture of (250mg SI combined with 500mg CR/kg diet)

A significant decrease in AST liver enzyme values was detected when growing quail fed diet supplemented with 500 mg/kg SI compared with quail fed the control diet (Table, 6) nevertheless ALT didn't affect by SI supplementation. On the other hand supplemented diets with different CR levels influenced insignificantly liver enzymes.

Table 6. Effect silymarin (SI), of curcumin (CR) and interaction (treatments) on protein profile and liver enzymes.

Substances	**Silymarin (SI)**					**Curcumin (CR)**				
Items	**SI zero (a)**	**SI 250 (b)**	**SI 500 (c)**	**SE±**	**P. values**	**CR zero (1)**	**CR 250 (2)**	**CR 500 (3)**	**SE±**	**P. values**
TP (g/dl)	5.52	5.88	5.67	0.34	NS	5.6	5.61	5.86	0.34	NS
Alb(g/dl)	1.97	1.64	1.46	0.12	NS	1.6	1.9	1.56	0.12	NS
Glb (g/dl)	3.55	4.24	4.21	0.33	NS	4	3.71	4.29	0.33	NS
A/G ratio	0.59	0.4	0.45	0.04	NS	0.42	0.56	0.47	0.04	NS
AST (U/L)	49.72^{a}	38.10^{ab}	32.63^{b}	5.02	0.047	35.54	39.46	45.45	5.02	NS
ALT(U/L)	7.65	7.79	6.91	0.93	NS	6.24	9.46	6.65	0.93	NS
Treatments (interaction)										
Items	**SI a × CR 1**	**SI a × CR 2**	**SI a × CR 3**	**SI b × CR 1**	**SI b × CR 2**	**SI b × CR 3**	**SI c × CR 1**	**SI c × CR 2**	**SI c × CR 3**	**SE±**	**P. values**
TP (g/dl)	5.33	5.3	5.93	5.57	5.7	6.37	5.9	5.83	5.27	0.58	NS
Alb(g/dl)	1.86	2.37	1.68	1.41	1.87	1.63	1.54	1.47	1.38	0.21	NS
Glb (g/dl)	3.47	2.93	4.26	4.16	3.83	4.73	4.36	4.37	3.89	0.5	NS
A/G ratio	0.55	0.81	0.42	0.34	0.5	0.36	0.36	0.35	0.63	0.08	NS
AST (U/L)	52.03^{abc}	55.16^{ab}	41.98^{abc}	11.20^{d}	38.46^{abcd}	64.63^{a}	43.40^{abc}	24.76^{cd}	29.75^{bcd}	8.7	0.006
ALT (U/L)	6.44^{ab}	11.70^{a}	4.82^{b}	4.45^{b}	8.25^{ab}	10.66^{a}	7.83^{ab}	8.44^{ab}	4.47^{b}	1.6	0.028

$^{a,b,..}$ Means within the same row with different superscripts are significantly differ (P ≤ 0.05). NS: Not significant.
SE=Standard Error of Means TP= total proteins Alb= albumin Glb= globulin AST= Aspartate transaminase
ALT= Alanine transaminase A/G ratio = albumin/globulin ratio

Antioxidant status and Aflatoxin in liver and muscle:

Each of TAOC, MDA and GSH-Px was influenced significantly by supplementing quail diet with SI, CR and its mixture (Table, 7). Regarding the effect of SI, supplementing quail diet with 500mg SI/kg diet presented significantly (P≤0.05) the highest TAOC and GSH-Px but recorded significantly (P≤0.05) the lowest MDA values. The effect of CR performed the same manner of SI effects on antioxidant status when it supplemented to growing quail diet.

In the same manner all antioxidant parameters influenced significantly by experimental treatments (interactions) where supplementing a mixture of 500mg/kg diet from each of CR and SI to Japanese quail diet presented significantly (P≤ 0.05) the best antioxidant status. Clearly significant increase in each of TAOC, and GSH-Px was observed in the previous treatment in contrast clearly significant decrease

Aflatoxin residues in the liver and muscle tissues of quail birds for CR, SI and its interaction are presented in (Table, 7). Significant (P≤0.05) decrease in concentration of AFB1 was observed as each of CR, SI levels increased. Regarding effect of treatments on AFB1 concentration in muscle and liver, no AFB1 residues were detected in liver tissues of treatments 8 and 9, and none were detected in muscle tissues of treatments 6, 7, 8 and 9. All residues' values in treated groups were within the acceptable limit of (< 2.0 ng/g) (ppb) in liver and muscles tissues. However, treatment groups have significant efficiency in degradation of AFB1 in the following ascending order frequency as T9, T8, followed by T6, T5, T7, T3 and T2 in comparison with aflatoxicated control group T1. In spite of this, the detected residues in all birds even in aflatoxicated quails are within normal permissible limit of aflatoxins (20 ppb).

Table 7. Effect silymarin (SI), of curcumin (CR) and interaction (treatments) on antioxidant parameters and concentration of aflatoxin B1 in muscles and liver.

Substances		**Silymarin (SI)**					**Curcumin (CR)**				
Items		**SI zero**	**SI 250 (b)**	**SI 500 (c)**	**SE±**	**P. values**	**CR zero**	**CR 250 (2)**	**CR 500 (3)**	**SE±**	**P. values**
TAOC (mmol/l)		0.81^{ab}	0.77^{b}	1.26^{a}	0.16	0.026	0.83^{b}	0.61^{c}	1.41^{a}	0.16	0.005
MDA(nmol/ml		2.86^{a}	1.57^{b}	0.98^{c}	0.31	0.001	2.52^{a}	1.54^{b}	1.35^{b}	0.31	0.030
GSH-		778.12^{b}	935.54^{a}	1142.78	95.23	0.025	797.49^{b}	963.87^{ab}	1095.09^{a}	95.23	0.036
AFB_1	**Liver**	0.372^{a}	0.148^{b}	0.053^{c}	0.038	0.001	0.368^{a}	0.130^{b}	0.076^{c}	0.038	0.001
	Muscl	0.021^{a}	0.011^{b}	0.000^{c}	0.001	0.001	0.018^{a}	0.010^{b}	0.004^{c}	0.001	0.001
Treatments (interaction)											
Items		**SI a × CR 1**	**SI a × CR 2**	**SI a × CR 3**	**SI b × CR 1**	**SI b × CR 2**	**SI b × CR 3**	**SI c × CR 1**	**SI c × CR 2**	**SI c × CR 3**	**SE±**	**P. values**
TAOC(mmol/l)		0.74^{c}	0.58^{d}	1.10^{b}	1.11^{b}	0.64^{cd}	0.55^{d}	0.64^{cd}	0.59^{d}	2.56^{a}	0.27	0.025
MDA(nmol/ml		4.55^{a}	2.27^{b}	1.77^{bc}	2.00^{bc}	1.28^{c}	1.44^{c}	1.03^{d}	1.06^{d}	0.85^{e}	0.55	0.014
GSH-		451.56	898.15	984.64^{c}	960.75	882.51	963.38	980.15	1110.95	1337.25	164.9	0.001
AFB_1	**Liver**	0.700^{a}	0.267^{b}	0.150^{c}	0.243^{b}	0.123^{c}	0.077^{c}	0.160^{c}	0.000^{d}	0.000^{d}	0.065	0.038
	Muscl	0.032^{a}	0.019^{b}	0.013^{c}	0.022^{b}	0.012^{c}	0.000^{d}	0.000^{d}	0.000^{d}	0.000^{d}	0.002	0.001

$^{a,b,..}$ Means within the same row with different superscripts are significantly differ (P ≤ 0.05).

SE=Standard Error of Means
TAOC= Total antioxidant capacity MDA= malnodialdhyde GSH-Px= glutathione peroxidase activity GSH-Px AFB_1=aflatoxin B_1

DISCUSSION

Growth performance:

The results of SI agreed with Khaleghipour *et al.* (2019) who reported that supplementing SI up to 1000mg SI/kg diets get greater BW and BWG when diet contained zero or 2.2 mg aflatoxin/kg. Improvement of BW by CR supplementation agreed with Karimi *et al.* (2020) who reported that adding CR to quail diets (3gm and 5gm/kg diets) improved BW. Similarly, Kilany and Mahmoud (2014) reported that CR supplementation improved the final body. Improving BW and BWG for the entire experimental period by mixing 500mg SI with 250mg CR/kg diet may be due to combined SI with CR it causes a significant improvement in health (Aboelhadid *et al.,* 2019) they added that relative improvement in rabbit BW was observed by mixing SI with CR. the significant improvement in BW was observed also when SI mixed with CR in broiler (Abu El-Ela *et al.,* 2013). Improving BW and BWG by supplanting SI, CR and its combination to growing Japanese quail diets may be due to the current experiment conducted during summer season. During the experimental period the average ambient temperature (Table, 2 may be unsuitable for growing Japanese quail so improvement of BW and BWG may be due to SI improved growth performance during summer season (Abou-Shehema *et al.*, 2016). Regarding effect of CR on FI and FC, the results agreed with Emadi *et al.,* (2015) who reported that 750 mg CR/kg didn't cause significant differences in feed intake compared diet without CR. Similarly, the insignificant effect of SI in FI and FC were in full agreement with Behboodi *et al.* (2017) who found that SI supplementation didn't influence FI and FC.

Carcass characteristics and organs weight percent:

Regarding slaughter test, the results of SI agreed with Butt *et al.* (2018) who found that SI didn't affect organs weight percent. Moreover Khaleghipour *et al.* (2019) and Behboodi *et al.* (2017) reported that carcass gizzard and liver didn't affect significantly by supplementing 1 g SI/kg diet. The results of CR agree with Basri and Sulastri (2019) who reported that heart, liver and carcass percentage didn't affect by CR supplementation

Plasma proteins profile and liver enzymes:

The result of total protein agreed with Butt *et al.* (2018). Increasing total plasma proteins by increasing supplementation levels of CR in Japanese quail diets was detected by Reda *et al.* (2020). The numerical liner increase of total plasma proteins by increasing CR level in quail diets may be due to CR reduced nitrative alterations of plasma proteins through reducing peroxynitrite formation and reduced oxidation of plasma proteins (Kolodziejczyk, *et al,* 2011). Regarding the results of liver enzymes, the results agreed with (Khaleghipour et al (2020) who reported that supplementing 500 mg/kg SI to quail diets improved hepatic function of quail fed on a diet contaminating 2.2 mg aflatoxins.

Antioxidant status and Aflatoxin in liver and muscle:

Regarding the results of antioxidant parameters, it agreed with Çeribaşı *et al.* (2020) who reported that disturbance in antioxidant balance was significantly prevented by supplementation of 10 g milk thistle/kg diet that contains 500mg SI. On the contrast, the insignificant difference between zero and 250mg SI/kg diet levels agreed with Baradaran *et al.* (2019) who reported that supplemented broiler diet with 100 mg SI /kg diet not affect significantly TAOC. Karimi *et al.* (2020) reported that the addition of CR in quail diets improved antioxidant status. Kilany and Mahmoud (2014) reported that CR supplementation caused a significant decrease in MDA, on the other hand, it cause significant increase in GSH-Px. Feeding quail diet contains different levels of CR in a nano form bringing beneficial impacts on antioxidant indices (Reda *et al.*, 2020). Aflatoxin B1 was classified as a carcinogen group1 by the International Agency for Research on Cancer (IARC, 1993). Many countries have established tolerance AFB1 levels in food in order to reduce toxin exposure. For animal feed, the European Union established maximum limits of AFB1 ranging from 5 to 20 µg/kg (ppb) depending not only on the type of product but also of the animal fed (Commission EC, (2006). The obtained present residues of AFB1 in liver and muscle tissues were significantly lower than the permissible limits of 15 ppb (FDA and WHO) and 20 ppb (FAO, 2011) and are safe for human consumption. Previous studies revealed that, as the toxin level in diet increased, its residual level in the liver increased (Oliveira et al., 2000, Rizzi et al., 2003; Bintvihok & Kositcharoenkul, 2006). This could be attributed to the difference in metabolism of each used dose of AFB1 by different birds. Hence, the lower level of toxin in liver might be accompanied by a greater level of metabolic derivatives in the liver, tissues and/or eggs. AFB1 derivatives as 8,9-epoxides are responsible of the toxin carcinogenic effects (CAST 2003).In general, stress-induced changes in the liver detoxification mechanism could be responsible for a reduction in AFB1 carryover in liver during aflatoxicosis. Further studies should perform in order to clearly elucidate the underlying physiological mechanism.

CONCLUSION

An improvement in growth performance, antioxidant system and a decrease in muscles and liver aflatoxin B_1 were detected when growing Japanese quail was fed diet supplemented with silymarin and curcumin at a level up to 500mg/kg diet. So we can recommend that silymarin and curcumin can supplement to growing quail diet during summer season.

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اضافة السيليمارين و الكركمين لعلائق السمان النامي لتحسين الاداء الإنتاجي ، حالة مضادات الأكسدة وتقليل الاثار الضارة للافلاتوكسين ب$_1$ خلال فصل الصيف

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أجريت هذه التجربة لتحديد مقدرة اضافة الكركمين والسيليمارين كمضادات أكسدة طبيعية على تحسين حالة مضادة الأكسدة السمان الياباني النامي. تم الخلط بين ثلاثة مستويات من كل من الكركمين والسيليمارين (صفر ، 250 مجم و 500 مجم / كجم علف) لتكوين 9 علائق تجريبية ، حيث كونت عليقة المقارنة القاعدية بدون اي اضافات من مضادات الاكسدة وهو المستوى صفر من كل من مضادتي الأكسدة الطبيعية (الكركمين والسيليمارين). وزعت عشوائيًا ثلاثمائة وستون كتكوت سمان ياباني عمر يوم إلى تسع مجموعات تجريبية وتكونت كل مجموعة من 4 مكررات يحتوي كل مكرر على 10 كتاكيت. غذيت كل مجموعة تجريبية على عليقة واحد فقط لمدة خمسة أسابيع. تم تقدير الأداء الإنتاجي خلال فترة النمو و صفات الذبيحة و بروتينات البلازما وأنزيمات الكبد وبعض مقاييس مضادات الأكسدة. وقد تم الحصول على النتائج التالية:

لوحظ تحسن معنوي في الوزن النهائي للجسم وكذلك زيادة وزن الجسم المكتسبة خلال المدة الاجمالية للتجربة للسمان النامي المغذي على عليقة مضاف لها 250مجم لكل من الكركمين و السيليمارين/ كجم عليقة مقارنة بالسمان المغذى على عليقة المقارنة القاعدية. زاد التحسن في الوزن النهائي للجسم وكذلك زيادة وزن الجسم المكتسبة خلال المدة الاجمالية للتجربة بزيادة مستوى الكركمين و السيليمارين 500 مجم / كجم عليقة. لم تؤثر اضافة الكركمين و السيليمارين معنوياً على الغذاء المستهلك ولا معامل التحويل الغذائي ولا صفات الذبيحة ولا بروتينات بلازما الدم . لوحظ تحسن في مقاييس مضاد الاكسدة المقدرة في الدم بإضافة الكركمين و السيليمارين. أدى اضافت الكركمين و السيليمارين لنقص معنوياً في تركيز للافلاتوكسين ب$_1$ في الكبد والعضلات. ومن التجربة الحالية يمكن التوصية بإضافة الكركمين و السيليمارين لعلائق السمان النامي لتحسين الأداء الانتاجي للسمان النامي وتقليل الاثار الضارة للجزور الحرة و للافلاتوكسين ب$_1$ خلال فصل الصيف.

الكلمات المفتاحية: كركمين، سيليمارين، السموم الفطرية ب$_1$ ، اداء السمان.