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Impact of Dietary Corn Germ Meal Inclusion and Bile Acids Supplementation on Growth Performance and Health Status of Japanese Quail

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ABSTRACT:

The shortage and high price of traditional feeds restrict the development of the poultry industry. Therefore, inclusions of nontraditional feedstuffs reduce cost and minimize environmental pollution. The main aim of this study is to determine the impact of corn germ meal inclusion rate without or with dried bile acids supplementation on growth performance, hematology, serum biochemical changes, liver and intestinal histopathology of Japanese quail. A total of 360 - one week old Japanese quail chicks were used in this study and randomly allotted to 6 groups with 3 replicates per group and 20 chicks per replicate based on body weight. Three experimental diets were formulated containing 0.0, 10 and 20% corn germ meal without or with dried bile acids supplementation to produce 6 treatments. Corn germ meal inclusion decreased final body weight, increased feed intake and deteriorated feed efficiency parameters (FCR, PER and EEU) compared to control, while dried bile acids supplementation improved ($P<0.05$) growth and feed efficiency parameters of growing quail chicks compared to groups fed on the same diet without dried bile acids supplementation. Both corn germ and bile acids had no significant effect on quail hematological parameters, while improved phagocytosis. Moreover, 10 or 20% corn germ inclusion decreased serum total protein and globulin concentrations while increasing serum total cholesterol, triglycerides concentration compared to control. Moreover, corn germ addition decreased oxidative capacity while dried bile supplementation improved the activity of these enzymes. Inclusion 10% of corn germ meal in Japanese quail chick's diet with dried bile acids supplementation can be used without negative effects.

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INTRODUCTION:

Generally, corn grains and soybean meal are the main energy and protein sources in poultry diets **(Knudsen, 2014**); however, the shortage and high price of these traditional raw materials restrict the development of the poultry industry. Therefore, this situation increases the pressure on nutritionists to search for and develop nontraditional feedstuffs resources for poultry diets cost reduction and minimize environmental pollution (**Khatun and Khan, 2015).** It is estimated that the production volume of corn in Egypt amounted to 7.44 million metric tons in 2022 (https://www.statista.com/statistics).

Dry grinding and wet milling of corn grains produce many by-products such as distillers' dried grains, corn gluten meal, corn gluten feed and corn germ meal **(Rausch and Belyea, 2006).** Whole corn germ meal (CGM) is a by-product of corn is produced during the extraction of corn oil and considered a good feed ingredient for livestock (**Schilling et al. 2017**). Corn germ meal can be used as an alternative feed instead of corn in animal diets while, its high fiber content limits its inclusion in ruminants' animal ration **(Herold et al. 1998).** Inclusion of CGM in poultry diets previously studied in ducks (**Qi et al. 2022)**, laying hens **(Brito et al. 2009; Brunelli et al. 2010)** and broilers **(Brito et al. 2005; Brunelli et al. 2006 and Lopes et al. 2019).** To our knowledge, very limited information on the inclusion rate of CGM in growing quail diets without negative effects on growth and health.

Local CGM produced in Egypt contains high ether extract (10-15%) we hypothesized that adding bile acid to improve fat digestion in broiler chicken **(Upadhaya et al. 2019)** and improve broiler performance **(Parsaie et al. 2007).** Bile acids composition differs in each species, since the avian bile acids contain mostly cholic acid and chenodeoxycholic acid **(Hofmann and Hagey, 2008).** Bile acids secreted into the duodenum play an essential role in fat digestion and absorption **(Abudabos 2014**). The previous studies using BA in quail diets containing untraditional feeds relatively high in oil content and its effect on quail growth and health is very scarce. Therefore, this study was designed to evaluate the effect of dietary CGM inclusion with BA supplementation on growth performance, serum biochemical changes, antioxidant capacity and intestinal histopathology of Japanese quail.

MATERIALS and METHODS

1. Birds accommodation and management:

Three hundred and sixty-one week Japanese quail chicks were obtained from a private quail farm at Kaferr elsheikh governorate and used in this trial. The chicks were allotted randomly into six equal groups (60 chicks/group and three replicates per group). The chicks were housed in a clean, well-ventilated room previously fumigated with formalin and potassium permanganate. Clean wheat straw was used to form a deep litter of four centimetres' depth on the floor. Suitable feeders and waterers were provided in the house and environmental temperature was adjusted according to the chick's age.

2. Experimental design and feeding program:

Three experimental diets were formulated to produce diets containing 0.0%, 10.0% and 20.0% of corn germ meal (CGM). CGM was included in the diet instead of corn grains and soybean meal to adjust protein% to meet quail chicks' nutrient requirements according to (**NRC**, **1994**) recommendation. The six treatments were arranged as follows; Groups 1-3 were fed the basal diet (BD) containing CGM at 0, 10 and 20 % of the diet, while groups 4-5 had the same levels of CGM as Groups 1-3 respectively, with the addition of 0.5g of dried bile acids (DBA)/kg diet (DBA produced by Shang dong Longchang Animal Health Product Co., Ltd, Jinan, China and mainly contained hyodesoxycholic acid, chenodeoxycholic acid and hyocholic acid at a level of 699.2, 189.2 and 77.5 g/kg BAs, respectively, estimated by HPLC. The ingredient composition and chemical analysis of the experimental basal diets used are presented in Table 1.

Table 1. Ingredient composition and nutrients content of the experimental diets (air dry basis).

Items	Corn germ meal inclusion%		
	0.0	10	20
Ingredients%			
Corn	52.0	44.5	37.0
Soybean meal (44%)	34.5	32.0	29.5
Corn germ meal (CGM)[1]	0.0	10.0	20
Corn gluten meal (60%)	8.0	8.0	8.0
Soybean oil	1.6	1.6	1.6
Dicalcium phosphate (DCP)[2]	1.8	1.8	1.8
Calcium carbonate[3]	1.3	1.3	1.3
Lysine[4]	0.05	0.05	0.05
DL-Methionine[5]	0.1	0.1	0.1
Choline chloride (50%)	0.05	0.05	0.05
Sodium chloride	0.25	0.25	0.25
Mineral & vitamin premix[6]	0.3	0.3	0.3
Mycotoxin binder[7]	0.05	0.05	0.05
Total	100	100	100
Chemical composition:			
Moisture%	11.15	10.98	11.06
Crude protein%	23.89	23.95	23.89
Ether extract%	4.08	4.95	5.85
Crude fiber%	2.56	3.63	4.72
Calcium%[7]	1.06	1.09	1.07
Available phosphorus%[8]	0.39	0.39	0.39
Lysine%[8]	1.16	1.09	1.05
Methionine%[8]	0.53	0.51	0.49
ME (kcal/kg)[9]	2928	2921	2914

CGM[1] = corn germ meal chemical composition (DM 93.7%, Moisture 6.3%, CP 16.31%, EE 12.47%, Ash 6.0% and CF 13.31%). Dicalcium phosphate[2]= contain 25% calcium & 16% phosphorus. Calcium carbonate[3]= locally produced lime stone contain 37% calcium. Lysine[4]= lysine hydrochloride (contain 98.5% Lysine). DL-methionine[5]= produced by Evonik Company (99.5% DL- methionine). **Premix**[6]= [3]Vitamin and mineral mix each 3kg contains: Vit A (12000000IU), vit D (2000000IU), vit E(10gr), vit K_3 (2gr), vit B_1 (1gr), vit B_2 (5gr), vitB_6 (1.5gr), vit B_{12} (10gr), nicotinic acid (30gr), pantothenic acid (10gr), folic acid (1gr), biotin (50mg), choline chloride50% (250gr), iron (30gr), copper (10gr), zinc (50gr), manganese (60gr), iodine (1gr), selenium (0.1gr), cobalt (0.1gr) and carrier up to 3kg**. mycotoxin binder**[7]**=** Mycotoxin adsorbent: Beta Mos plus (Zoomaria Sri company). 8= calcium, phosphorus, lysine and methionine content calculated according to NRC, 1994. ME[9] = Metabolizable energy was calculated according to NRC, 1994

3. Growth performance:

Individual bird body weight at the start (one week age) of the trial and at the 2^{nd}, 4^{th} and 6^{th} week of age was recorded. Total weight gain (TWG), total feed intake (TFI), average feed conversion ratio (AFCR), average protein efficiency ratio (APER) and average efficiency of energy utilization (AEEU) throughout the whole experimental period were calculated.

4. Chemical analysis:

Analytical DM contents of CGM and feed samples were estimated by oven-drying at 105°C for 8 h**,** ash contents were determined by ignition at 550°C overnight, crude protein was determined by using Kjeldahl method, and ether extract was obtained by the Soxhlet method **(AOAC, 1995).**

5. Blood picture and immune response measurements:

Blood samples were **collected** with anticoagulant (sodium citrate) from six birds of each group (two of each replicate) at the end of the growth trial and for determination of haematological parameters and differential leucocyte according to **Archer (1965**) and **Gross and Siegel (1983**) respectively, while phagocytosis calculated according to **Kawahara et al. (1991).**

6. Assessment of some blood serum parameters:

At the end of the experiment (42^{th} day, of age) blood samples without anticoagulant were collected from six birds of each group (two from each replicate), and the separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 10 minutes. The clear serum was kept in a freezer (-20^{0}C) until analysis for determination of serum total protein, globulin, albumin, GOT, GPT, serum lipids concentrations (cholesterol, triglyceride, HDL and LDL), antioxidant enzymes (glutothion perioxidase 'GPx', total antioxidant capacity 'TAC' and Malondialdehyde 'MDA') were estimated using specific commercial kits (Roche Diagnostica, Basel, Switzerland).

7. Lymphoid organs weight and some carcass traits:

At the end of the **growing** period (six weeks of bird's age), six birds from each dietary treatment (two from each replicate) were randomly collected, weighed, slaughtered and then weighed after evisceration to determine the relative weight **of** immune organs (spleen, bursa and thymus gland) and some carcass traits (dressing%, liver, gizzard and proventriclus).

8. Intestinal and liver histopathology:

At the end of the experiment, intestinal tissue (ileum) and liver were collected (n=6/ group). Samples were washed with physiological saline and **fixed** in 10% formalin for at least 2 days. Slides were prepared and stained with Hematoxylin and Eosin (H&E) for morphological examination of both tissues **(Bancroft et al. 2013).**

9. Statistical analysis:

The obtained data were statistically analyzed using a two-way analysis of variance (ANOVA) with **Tukey's** multiple comparison test was used. Results obtained were illustrated as mean ± standard error of the mean (SEM) and the differences were considered significant at P< 0.05.

RESULTS

Body weight development and feed efficiency parameters:

Table 2. Showed body weight development of Japanese quail chicks fed on graded levels of CGM without or with DBA supplementation. Statistical analysis of the obtained data revealed that there is no significant (P≥0.05) difference between different groups at the start of the experiment, however, the inclusion of 10% CGM instead of corn and soybean meal in quail chicks' diet was non-significant (P≥0.05) decreased body weight at 2nd, 4th and 6th weeks by about 4.5%, 4.1% and 2.8% respectively while, the inclusion of 20% CGM significantly (P<0.05) decreased body weight at 2nd, 4th and 6th weeks by about 11.7%, 8.6% and 5.9% respectively compared to control. On the other hand, DBA supplementation in 0.0% or

10% CGM-containing diets non-significantly (P≥0.05) improved final body weight by about 3.04% and 3.3% respectively while DBA supplementation with 20% CGM significantly (P<0.05) improved final body weight by about 4.3% compared to quail group fed on the same diet without DBA supplementation

Table 2. Body weight development of Japanese quail chicks (g/chick) fed on graded levels of corn germ meal without or with bile acids supplementation.

CGM inclusion rate and DBA supplementation	Age/week Week1	Week2	Week4	Week6
CGM (0.0%) control	25.57±0.55[a]	51.13±0.82[ab]	154.07±3.94[ab]	221.93±4.02[ab]
CGM (10.0%)	25.47±0.62[a]	48.83±0.81[b]	147.70±1.72[bc]	215.67±3.21[bc]
CGM (20.0%)	25.63±1.09[a]	45.17±0.85[c]	140.83±1.30[c]	208.90±1.89[c]
CGM (0.0%) + DBA	25.10±0.69[a]	53.53±1.56[a]	159.10±3.76[a]	228.67±1.05[a]
CGM (10.0%) + DBA	25.53±0.87[a]	51.50±0.70[ab]	152.10±1.35[ab]	222.83±1.80[ab]
CGM (20.0%) + DBA	25.77±1.41[a]	49.30±0.81[b]	149.47±0.81[b]	217.97±1.57[b]
P-values				
CGM[1]	0.925	0.001	0.002	0.002
DBA[2]	0.908	0.002	0.010	0.003
Interaction	0.939	0.639	0.678	0.883

Values means ± SEM. Mean values with different letters at the same column differ significantly at (P < 0.05). [1]CGM =corn germ meal DBA[2]= dried bile acids

Table 3, showed that dietary CGM inclusion at 10 or 20% in the quail diet significantly (P<0.05) decreased total body gain (TBG) throughout the whole experimental period by about 3.1% and 6.7% respectively and increased total feed intake (TFI) by about 3.5% and 8.7% respectively compared to control, while DBA supplementation increased TBG and reduced TFI compared to quail group fed on the same diet without DBA supplementation. Moreover, dietary CGM inclusion at 10 or 20% in quail diet significantly (P<0.05) deteriorated AFCR, APER and AEEU throughout the whole experimental period by about (7.0%, 6.7% and 6.8%) and (16.3%, 14.3% and 16.4%) respectively compared to control while, DBA supplementation significantly (P<0.05) improved AFCR, APER and AEEU compared to quail group fed on the same diet without DBA supplementation.

Table 3. Total weight gain (g/chick) and feed efficiency parameters of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation.

CGM inclusion rate and DBA supplementation	TBG[1]	TFI[2]	Age/week AFCR[3]	APER[4]	AEEU[5]
CGM (0.0%) control	196.37±3.85[ab]	781.0±2.1[cd]	3.98±0.08[c]	1.05±0.02[b]	11.85±0.23[c]
CGM (10.0%)	190.20±3.73[bc]	808.7±2.3[b]	4.26±0.10[b]	0.98±0.02[c]	12.66±0.28[b]
CGM (20.0%)	183.27±0.88[c]	849.0±6.7[a]	4.63±0.03[a]	0.90±0.01[d]	13.79±0.10[a]
CGM (0.0%) + DBA	203.57±0.68[a]	768.0±6.4[d]	3.77±0.03[d]	1.11±0.01[a]	11.23±0.10[d]
CGM (10.0%) + DBA	197.30±2.26[ab]	790.0±2.9[c]	4.00±0.03[c]	1.04±0.01[b]	11.92±0.10[c]
CGM (20.0%) + DBA	192.20±0.55[b]	814.3±4.4[b]	4.24±0.01[b]	0.99±0.01[c]	12.61±0.03[b]
P-values					
CGM[6]	0.001	0.001	0.001	0.001	0.001
DBA[7]	0.002	0.001	0.001	0.001	0.001
Interaction	0.914	0.083	0.247	0.575	0.247

Values means ± SEM. Mean values with different letters at the same column differ significantly at (P < 0.05). TBG[1] = total body gain. TFI[2] = total feed intake. AFCR[3] = average feed conversion ratio. APER[4] = average protein efficiency ratio. AEEU[5] = average efficiency of energy utilization. CGM[6] =corn germ meal DBA[7]= dried bile acids

Blood picture and differential leukocyte count%:

The effect of dietary CGM inclusion and DBA supplementation on some blood pictures and differential leukocyte count of Japanese quail is presented in Tables 4 and 5 respectively. Statistical analysis of the obtained data revealed that CGM inclusion without or with DBA supplementation had no significant ($P \geq 0.05$) effect on RBCs, Hb%, PCV%, WBCs and differential leukocyte count% of growing quail chicks compared to control.

Table 4. Blood picture of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation.

CGM inclusion rate and DBA supplementation	Items			
	RBCs ($x\ 10^6/mm^3$)[1]	Hb%[2]	PCV%[3]	WBCs ($x\ 10^3/mm^3$)[4]
CGM (0.0%) control	2.15±0.20	10.75±1.01	35.48±3.33	30.89±3.02
CGM (10.0%)	2.07±0.32	10.35±1.58	34.15±5.20	29.67±2.44
CGM (20.0%)	1.90±0.09	9.50±0.42	31.34±1.37	31.99±3.11
CGM (0.0%) + DBA	2.01±0.24	10.07±1.18	33.23±3.90	28.79±2.87
CGM (10.0%) + DBA	1.87±0.19	9.37±0.94	30.93±3.09	31.44±2.56
CGM (20.0%) + DBA	1.86±0.17	9.28±0.84	30.61±2.75	32.09±2.95
P-values				
CGM[5]	0.629	0.629	0.622	0.457
DBA[6]	0.456	0.475	0.365	0.456
Interaction	0.937	0.937	0.865	0.875

Values means ± SEM. Mean values with different letters at the same column differ significantly at ($P < 0.05$). RBCs[1]= red blood cells. Hb[2]= hemoglobin. PCV[3]= packed cell volume. WBCs[4]= white blood cell CGM[5] =corn germ meal DBA[6]= dried bile acids

Table 5. Differential leukocyte count% of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation.

CGM inclusion rate and DBA supplementation	Leukocyte type					
	Neutrophil	Basophil	Eosinophil	Monocyte	Lymphocyte	N/L ratio
CGM (0.0%) control	51.85±4.00	4.03±0.32	1.89±0.18	11.30±0.39	30.93±0.72	1.69±0.17
CGM (10.0%)	52.48±2.00	5.00±1.58	1.43±0.20	9.68±1.44	31.43±1.11	1.68±0.09
CGM (20.0%)	52.50±0.42	4.58±1.26	1.63±0.26	8.10±1.42	33.20±1.53	1.59±0.09
CGM (0.0%) + DBA	54.28±0.50	4.93±0.23	1.55±0.22	8.58±0.74	30.68±0.56	1.77±0.05
CGM (10.0%) + DBA	54.30±1.14	5.23±0.22	1.60±0.24	8.20±0.71	30.68±1.01	1.78±0.09
CGM (20.0%) + DBA	54.65±1.07	4.00±0.34	1.85±0.17	7.23±0.45	32.28±0.71	1.70±0.07
P-values						
CGM[1]	0.965	0.696	0.353	0.223	0.940	0.624
DBA[2]	0.196	0.609	0.508	0.102	0.133	0.237
Interaction	0.988	0.799	0.713	0.092	0.438	0.994

Values means ± SEM. Mean values with different letters at the same column differ significantly at ($P < 0.05$). [1]CGM =corn germ meal DBA[2]= dried bile acids

Serum protein profile and phagocytosis:

Table 6, shows that 10% or 20% of CGM inclusion in the Japanese quail diet decreased serum total protein and globulin concentrations by about (3.5% and 23.6%) and (8.5% and 47.3%) respectively compared to control, while CGM inclusion with DBA supplementation increased (P≥0.05) serum total protein and globulin concentrations compared to quail group fed on the same diet without DBA supplementation. Moreover, 10% or 20% of CGM inclusion in the Japanese quail diet improved phagocyte activity by about 8.9% and 12.6% respectively compared to control, while CGM inclusion with DBA supplementation non-significant (P≥0.05) improved phagocyte activity compared to quail group fed on the same diet without DBA supplementation.

Table 6. Serum protein profile and phagocytosis of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation.

CGM inclusion rate and DBA supplementation	Items				
	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Phagocytic activity%	Phagocytic index
CGM (0.0%) control	6.21±0.05^{a}	4.73±0.09^{a}	1.48±0.13^{ab}	40.82±1.96^{b}	1.17±0.06^{a}
CGM (10.0%)	5.99±0.05^{ab}	4.87±0.23^{a}	1.13±0.18^{abc}	44.46±1.76^{ab}	1.24±0.08^{a}
CGM (20.0%)	5.68±0.18^{b}	4.90±0.05^{a}	0.78±0.23^{c}	45.97±0.43^{a}	1.21±0.06^{a}
CGM (0.0%) + DBA	6.20±0.02^{a}	4.84±0.02^{a}	1.36±0.03^{abc}	43.17±1.17^{ab}	1.19±0.05^{a}
CGM (10.0%) + DBA	6.21±0.01^{a}	4.39±0.21^{a}	1.82±0.21^{a}	47.17±0.93^{a}	1.24±0.05^{a}
CGM (20.0%) + DBA	6.09±0.07^{a}	4.92±0.06^{a}	1.17±0.13^{bc}	46.21±0.82^{a}	1.17±0.03^{a}
P-values					
CGM[1]	0.011	0.125	0.023	0.009	0.468
DBA[2]	0.017	0.281	0.040	0.111	0.884
Interaction	0.100	0.081	0.093	0.595	0.879

Values means ± SEM. Mean values with different letters at the same column differ significantly at (P < 0.05). [1]CGM =corn germ meal DBA[2]= dried bile acids

Serum lipid profile:

Table 7, shows that 10% or 20% of CGM inclusion in the Japanese quail diet increased serum total cholesterol, triglycerides and LDL concentrations by about (1.9%, 2.3% and 3.1%) and (4.9%, 3.4% and 8.9%) respectively and had an effect on HDL concentration and TCHO/HDL ratio compared to control, while CGM inclusion with DBA supplementation increased (P<0.05) serum total cholesterol, triglycerides and LDL concentrations compared to quail group fed on the same diet without DBA supplementation .

Table 7. Serum lipid profile of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation

CGM inclusion rate and DBA supplementation	Items				
	TCHO (mg/dl)[1]	Triglycerides (mg/dl)	HDL (mg/dl)[2]	LDL (mg/dl)[3]	TCHO/HDL ratio[4]
CGM (0.0%) control	190.21±2.34[b]	202.24±3.46[b]	51.55±0.36	98.22±1.36[b]	3.69±0.03
CGM (10.0%)	193.92±5.58[b]	206.84±2.61[ab]	51.25±1.30	101.30±7.41[ab]	3.79±0.21
CGM (20.0%)	199.68±0.50[a]	209.03±1.38[a]	50.85±1.26	107.02±0.61[a]	3.93±0.09
CGM (0.0%) + DBA	200.10±0.23[a]	203.96±0.74[ab]	52.25±0.19	107.06±0.22[a]	3.83±0.01
CGM (10.0%) + DBA	200.32±0.78[a]	207.79±0.95[a]	51.85±0.08	106.91±0.83[a]	3.86±0.02
CGM (20.0%) + DBA	199.84±0.80[a]	207.54±1.72[a]	51.92±0.26	106.41±0.95[a]	3.85±0.02
P-values					
CGM[5]	0.072	0.049	0.736	0.168	0.187
DBA[6]	0.004	0.815	0.184	0.020	0.449
Interaction	0.055	0.697	0.937	0.100	0.267

Values means ± SEM. Mean values with different letters at the same column differ significantly at ($P < 0.05$). TCHO[1]= total cholesterol. HDL[2]= high density lipoprotein. LDL[3]= low density lipoprotein. TCHO/HDL ratio[4]= total cholesterol/high density lipoprotein ratio. CGM[1] =corn germ meal DBA[6]= dried bile acids

Serum oxidative and hepatic enzymes activities:

The effect of dietary CGM inclusion and DBA supplementation on serum oxidative and hepatic enzyme activities of growing quail is presented in Table 8. Statistical analysis of the obtained data revealed that 10% or 20% of CGM inclusion in the Japanese quail diet non-significantly ($P<0.05$) reduced GPx and TAC activities while, increased ($P<0.05$) MDA activity compared to control, while CGM inclusion with DBA supplementation non significantly ($P<0.05$) increased GPx and TAC activities while reduced ($P<0.05$) MDA activity compared to quail group fed on the same diet without DBA supplementation. Moreover, both CGM inclusion and DBA supplementation had no effect on GOT and GPT serum activities.

Table 8. Serum oxidative and hepatic enzymes activities of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation

CGM inclusion rate and DBA supplementation	Items				
	GPx (u/ml)[1]	TAC (mM/L)[2]	MDA (mmol/ml)[3]	GOT (μ/L)[4]	GPT (μ/L)[5]
CGM (0.0%) control	333.90±97.41	0.99±0.07[ab]	9.57±0.44[bc]	40.33±3.71	8.27±0.96
CGM (10.0%)	293.15±63.56	0.92 ±0.10[ab]	11.10±0.30[a]	52.50±10.5	9.70±0.10
CGM (20.0%)	252.90±107.15	0.77±0.11[b]	11.40±0.59[a]	48.33±5.36	8.50±0.32
CGM (0.0%) + DBA	282.47±73.57	1.25±0.11[a]	8.70±0.42[c]	47.67±5.17	8.67±0.33
CGM (10.0%) + DBA	304.07±71.80	0.98±0.07[ab]	10.13±0.34[ab]	60.23±0.88	8.83±0.12
CGM (20.0%) + DBA	299.57±74.01	0.86±0.12[b]	10.47±0.30[ab]	55.67±1.20	7.93±0.64
P-values					
CGM[6]	0.806	0.026	0.003	0.060	0.205
DBA[7]	0.683	0.284	0.024	0.072	0.462
Interaction	0.478	0.309	0.993	0.998	0.507

Table 9. Dressing and internal organs % of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation

CGM inclusion rate and DBA supplementation	Items						
	Dressing%	Liver%	Spleen%	Bursa%	Thymus%	Gizzard%	Provent.%
CGM (0.0%) control	65.34±0.19	2.13±0.23	0.08±0.02	0.06±0.02[b]	0.05±0.01	1.59±0.12[c]	0.37±0.11
CGM (10.0%)	67.81±0.34	2.62±0.30	0.09±0.04	0.05±0.02[b]	0.11±0.01	1.83±0.08[bc]	0.42±0.07
CGM (20.0%)	66.09±0.18	2.95±0.27	0.08±0.02	0.06±0.01[b]	0.07±0.02	2.45±0.10[a]	0.41±0.12
CGM (0.0%) + DBA	69.07±0.21	1.97±0.11	0.10±0.02	0.16±0.03[a]	0.06±0.02	1.87±0.06[bc]	0.29±0.10
CGM (10.0%) + DBA	68.76±0.09	2.78±0.06	0.12±0.03	0.11±0.01[ab]	0.12±0.02	2.12±0.11[ab]	0.36±0.11
CGM (20.0%) + DBA	67.15±0.06	2.46±0.11	0.09±0.02	0.11±0.02[ab]	0.09±0.02	2.44±0.07[a]	0.38±0.06
P-values							
CGM[1]	0.354	0.661	0.117	0.296	0.056	0.001	0.116
DBA[2]	0.110	0.057	0.097	0.002	0.127	0.130	0.112
Interaction	0.402	0.052	0.116	0.503	0.060	0.531	0.965

Values means ± SEM. Mean values with different letters at the same column differ significantly at (P < 0.05).
[1]CGM =corn germ meal DBA[2]= dried bile acids

Liver fat content and histopathology:

Liver moisture and ether extract (EE) content and hepatic tissue histopathologies of Japanese quail chicks fed on graded levels of CGM without or with DBA supplementation are presented in Table 10 and Figures 1-2 respectively. It was found that CGM inclusion instead of corn and soybean meal had no significant (P<0.05) effect on hepatic tissue moisture content while DBA supplementation did not significantly (P<0.05) increase liver moisture% compared to the quail group fed on the same diet without DBA supplementation. Moreover, dietary CGM inclusion in a growing quail diet led to hepatic steatosis and fat vacuoles compared to the control while DBA supplementation showed a marked decrease in hepatic steatosis and fat vacuoles compared to quail group fed on the same diet without DBA supplementation.

Table 10. Liver moisture and ether extract (EE) content of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation

GM inclusion rate and DBA supplementation	Items			
	Dry matter%	Moisture%	EE% (On DM Basis)	EE% (On fresh Basis)
CGM (0.0%) control	40.26 ± 0.01^{a}	59.74 ± 0.21^{b}	19.81 ± 0.78^{a}	7.94 ± 0.95^{a}
CGM (10.0%)	38.49 ± 0.02^{ab}	61.51 ± 0.03^{ab}	22.71 ± 0.80^{a}	8.74 ± 1.72^{a}
CGM (20.0%)	40.53 ± 0.06^{a}	59.47 ± 0.10^{b}	22.29 ± 1.16^{a}	9.04 ± 0.91^{a}
CGM (0.0%) + DBA	31.77 ± 0.18^{b}	68.23 ± 5.58^{a}	17.48 ± 2.61^{a}	5.55 ± 1.30^{a}
CGM (10.0%) + DBA	37.95 ± 0.13^{ab}	62.05 ± 0.50^{ab}	22.70 ± 1.38^{a}	8.82 ± 1.26^{a}
CGM (20.0%) + DBA	35.37 ± 0.15^{ab}	64.63 ± 2.04^{ab}	20.70 ± 1.73^{a}	7.34 ± 0.51^{a}
P-values				
CGM[1]	0.607	0.607	0.276	0.375
DBA[2]	0.035	0.035	0.525	0.289
Interaction	0.040	0.040	0.891	0.516

Values means ± SEM. Mean values with different letters at the same column differ significantly at P < 0.05).
[1]CGM =corn germ meal DBA[2]= dried bile acids

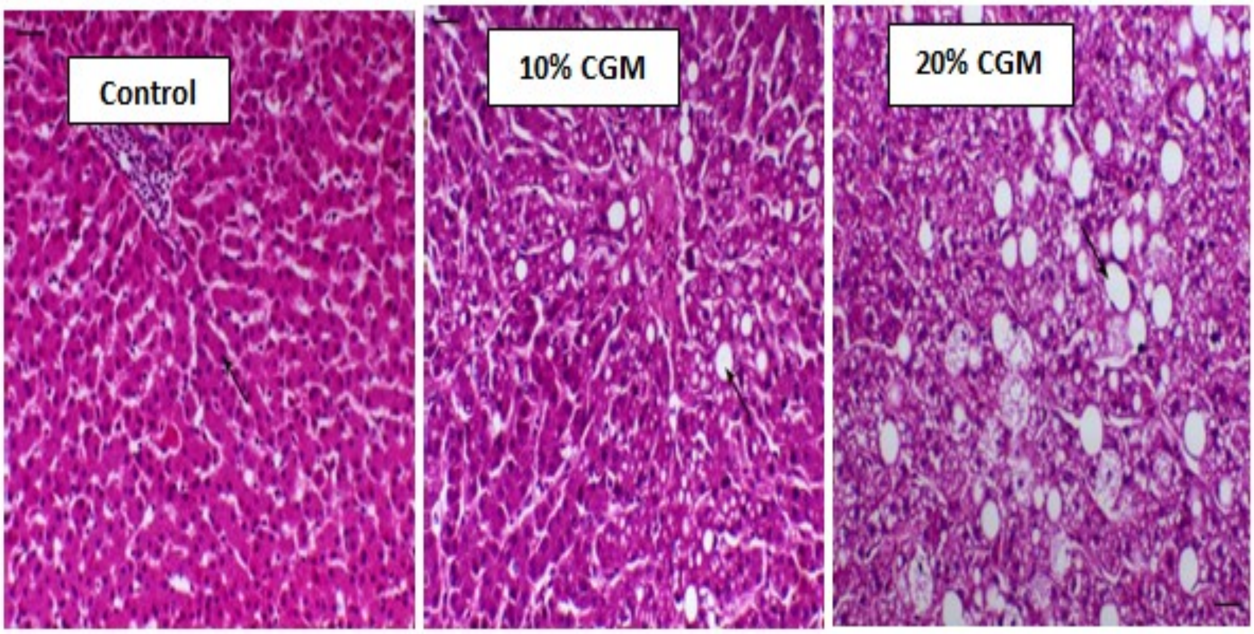

Figure (1): Hepatic histopathology of Japanese quail chicks showing normal hepatocytes around the portal area (control at left side), or showing hepatic steatosis (arrow indicates different fat vacuoles mostly centrolobular) of 10% CGM fed group (middle) and showing marked hepatic steatosis (arrow indicates large fat vacuoles) of 20% CGM fed group (right side).

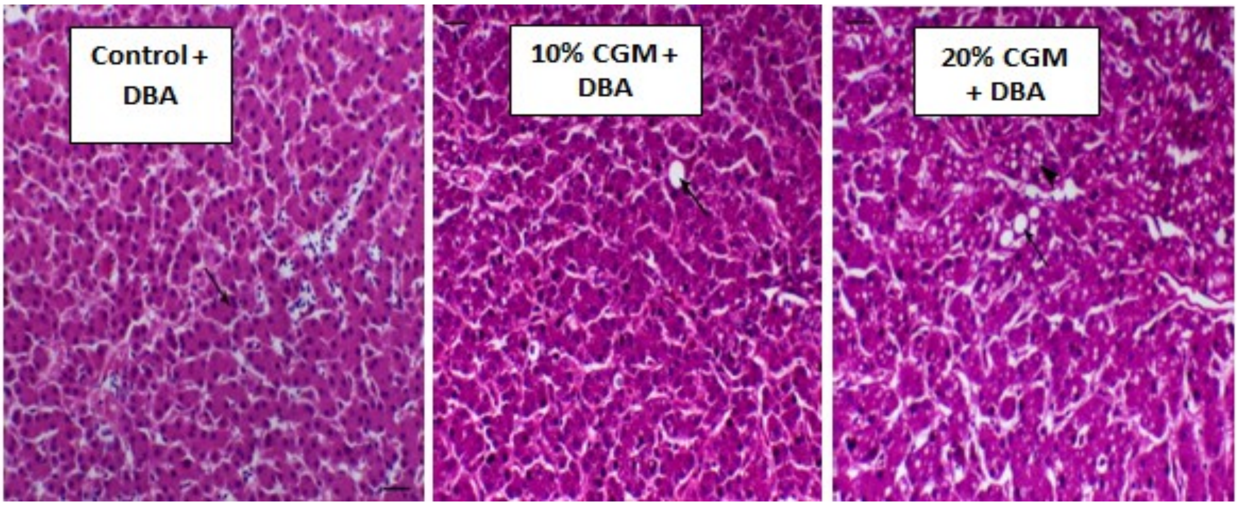

Figure (2): Hepatic histopathology of Japanese quail chicks showing normal hepatocytes around the portal area (control + DBA at left side), or showing marked decrease of hepatic steatosis (arrow indicates one fat vacuoles within normal parenchyma), of 10%CG M with DBA fed group (middle) and showing marked decrease of hepatic steatosis (arrow and arrowhead indicate macrovesicular and microvesciular fat vacuoles respectively), of 20% CGM with DBA fed group (right side).

Intestinal histopathology:

The effect of dietary CGM inclusion without or with DBA supplementation on ileum histopathology of Japanese quail is presented in Table 11 and Figures 3-4. Statistical analysis revealed that 10 or 20% of CGM inclusion decreased villi length by about 9.7% and 31.5% respectively and non-significantly (P<0.05) increased villi width and crypt depth compared to control, while DBA supplementation non-significantly (P<0.05) increased villi length, width, crypt depth and V/C ratio compared to quail group fed on the same diet without DBA supplementation. On the other hand, 10 or 20% of CGM inclusion increased inter villi space by about 32.9% and 37.1% respectively while, DBA supplementation non-significantly (P<0.05) reduced inter villi space compared to the quail group fed on the same diet without DBA supplementation.

Table 11. Ileum morphometric measurement of Japanese quail chicks fed on graded levels of corn germ meal without or with bile acids supplementation

CGM inclusion rate and DBA supplementation	Items				
	villi length (µm/mm)	Villi width (µm/mm)	Crypt depth (µm/mm)	Inter villi space	V/C ratio[1]
CGM (0.0%) control	730.00±32.15^{bc}	78.33±6.12^{a}	86.67±6.22^{a}	56.67±4.63^{d}	8.54±0.86^{ab}
CGM (10.0%)	659.33±40.01^{c}	81.00±4.16^{a}	92.67±8.57^{a}	75.33±4.81^{ab}	7.21±0.71^{b}
CGM (20.0%)	500.00±32.15^{d}	94.33±4.18^{a}	95.67±4.33^{a}	77.67±5.24^{a}	5.23±0.32^{c}
CGM (0.0%) + DBA	854.00±19.08^{a}	81.67±3.53^{a}	94.33±3.18^{a}	45.00±3.79^{cd}	9.06±0.14^{a}
CGM (10.0%) + DBA	761.33±25.05^{bc}	85.00±4.36^{a}	97.00±5.29^{a}	62.33±2.91^{bc}	7.90±0.56^{ab}
CGM (20.0%) + DBA	683.00±11.53^{b}	80.67±9.91^{a}	100.33±4.91^{a}	65.33±5.46^{abc}	6.85±0.42^{bc}
P-values					
CGM[2]	0.356	0.437	0.139	0.006	0.001
DBA[3]	0.001	0.252	0.298	0.001	0.06
Interaction	0.134	0.585	0.675	0.989	0.456

Values means ± SEM. Mean values with different letters at the same column differ significantly at (P < 0.05). V/C ratio[1]= villi length/crypt depth ratio. CGM[2] =corn germ meal DBA[3]= dried bile acids

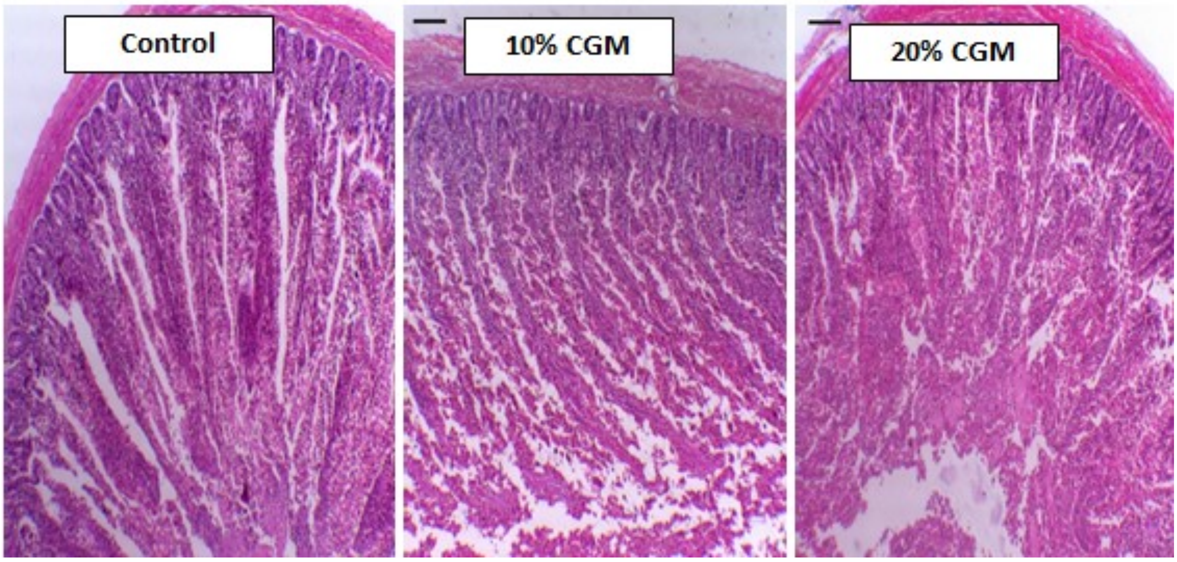

Figure 3. Quail chicks fed on 0.0% CGM diet (at left side) showing normal villi, Quail chicks fed on 10.0% CGM diet (middle) showing decrease of villi length, and Quail chicks fed on 20.0% CGM diet (right side) showing marked decrease of villi length associated with sloughing of their mucosa

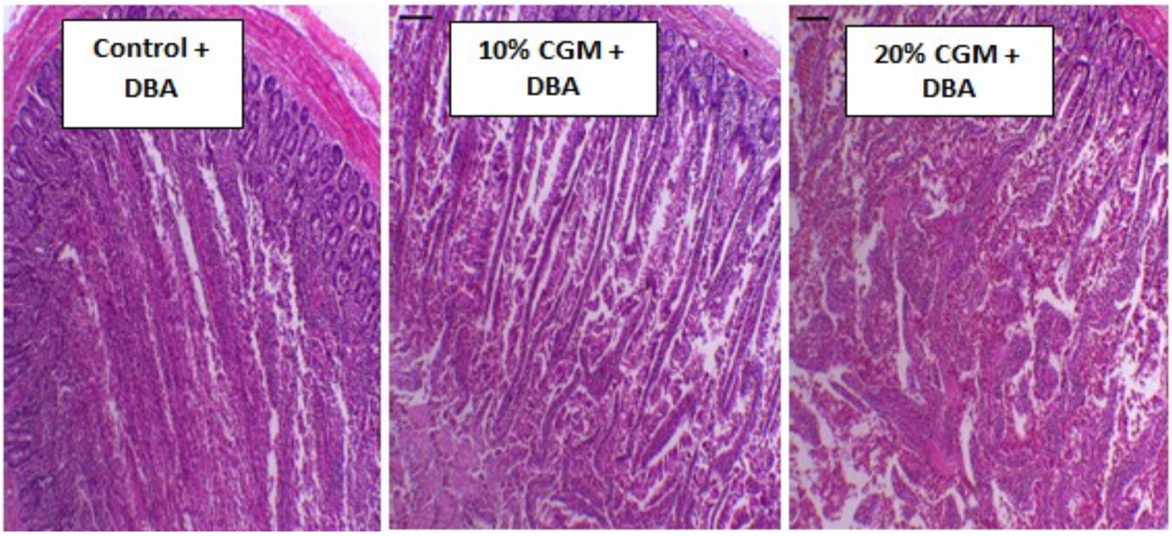

Figure 4. Quail chicks fed on 0.0% CGM diet (left side) with bile acids showing increase of villi length, Quail chicks fed on 10.0% CGM diet (middle) with bile acid showing increase of villi length to the normal limits, and Quail chicks fed on 20.0% CGM diet (right side) with bile showing increase villi length

DISCUSSION:

The main aim of this study was to determine the maximum inclusion level of CGM in growing Japanese quail chicks diets based on growth performance, some serum biochemical changes and carcass traits as well as liver and intestinal health status. Reduction of final weight and TBG with increasing feed intake and deterioration of feed efficiency parameters (AFCR, APER and AEEU) in a level-dependent manner was observed in growing Japanese quail chicks with dietary inclusion of 10 or 20% CGM may be related to the high fiber content of CGM. Our results are in contrast with those obtained by **Qi et al. (2022)** stated that the inclusion of CGM at 3, 6, 9 or 12% in growing duck diet had no effect on growth performance but deteriorated FCR. Also, **Lopes et al. (2019**) observed a non-significant ($P<0.05$) reduction of broiler weight fed on whole corn germ (up to 20%) containing diets compared to control. The difference may be related to species used in each trial, CGM inclusion rate and chemical composition. **Jaworski et al. (2015)** indicated that non -starch polysaccharides components of CGM limit its inclusion in poultry diets. However, **Tejeda et al. (2021)** stated that inclusion 4% CF in broiler ration had no adverse effect on growth compared to control. Growth reduction with CGM inclusion in quail diet may be related to lower nutrient utilization compared to nutrient utilization of corn or soybean meal. On the other hand, improvement of quail growth performance and feed efficiency parameters with DBA supplementation may be related to improvement of nutrients digestibility and utilization **(Mohamed et al. 2020b).**

The obtained data indicated that neither CGM nor DBA addition in growing quail rations had no significant effect on hematology and all values within normal range. Moreover, numerical increase of neutrophil%, basophil% and phagocytosis with CGM inclusion and DBA supplementation indicated that there was improving of cellular immunity. Total proteins in the blood stream are a currently used parameter to estimate body condition in birds, and when taken together, total protein, albumin and globulin are indicative of the protein synthesis **(Piotrowska et al. 2011).** In the present study quail groups fed on CGM containing diets showed lower serum total protein and globulin levels compared to control which indicating lower protein availability in CGM containing diets. Moreover, DBA addition improve nutrients digestion and absorption and this improvement reflected on higher serum total protein and globulin levels compared to group fed on the same diet without DBA supplementation. In support **Mohamed et al. (2020a)** reported that bile salts addition in broiler diets increased ($P<0.05$) serum protein concentration compared to control.

Lipid metabolites in the blood are related to energy metabolism **(Piotrowska et al. 2011).** Generally, increasing circulating lipids levels indicates an improvement of de novo lipolysis, while the reduction in serum lipid profile reflects an improvement of amino acid transportation and increased lipid metabolism with fat deposition reduction **(Zhao et al. 2009).** The serum lipid profile of the current study revealed that reduction of lipid availability in quail chick's blood fed diets without DBA supplementation. **Alzawqari et al. (2010)** stated that dietary DBA supplementation significantly increased serum triglycerides, cholesterol, LDL and HDL concentrations of broiler chickens compared to control.

Increasing the intake of diets rich in antioxidants may maintain high levels of antioxidant enzymes in the cell **(Saleh et al. 2018).** In our study quail feeding on CGM that contains high levels of corn oil may increase the number of free radicals in the animal biological system after consumption consequently lowering serum TAC and GPx and increasing MDA activities compared to the control. The obtained data agree with **Tari et al. (2021)** reported that chickens fed diet containing corn oil had lower SOD and GPx activities compared to those fed diets containing other oils (palm, flaxseed). Moreover, antioxidant capacity improvement with DBA supplementation supported those obtained by **Mohamed et al. (2020a)** stated that improvement in SOD and CAT activities of chicken fed on bile salt supplemented diets.

Improvement of some immune organs relative to weight (mainly thymus gland) of growing quail fed on diets containing different levels of CGM confirmed the immune stimulation effect of a high fibre diet. Our data come in consistence with **Sabour et al. (2019)** who documented that dietary inclusion of insouluble fiber in broilers diet not influenced the spleen and bursa of Fabricius relative weights. Moreover, DBA supplementation improved ($P\geq0.05$) immune organs relative weights compared with the group fed the same diet without DBA supplementation. Our data agreed with those obtained by **Alzawqari et al. (2016)** indicating that bursa and spleen relative weight did not affect broiler by bile acid addition in broiler ration, the stimulatory effect of the bile acids on evolution and growth of the lymphoid organs may be perfect of improving immunity. The higher relative weight of gizzard and proventriculus of growing quail fed on diets containing different levels of CGM instead of corn and soybean meal can be explained by the high concentrations of crude fibre in CGM. This explanation supported by **Hetland et al. (2003)** reported that high-fiber diets lead to an increase in the gizzard's relative weight due to fibre requires more ground than other nutrients. Also, **Lopes et al. (2019)** reported that the dietary inclusion of whole corn germ in broiler chicken diet increased gizzard and proventriculus weights compared to control.

Both liver chemical and histopathological examination of the current study indicated that liver fat increased with CGM inclusion in quail chick's diet, while DBA supplementation ameliorated this effect and improved hepatic health. This effect may be related to DBA stimulating fat catabolism and reduced fat synthesis by regulating lipid-metabolism-related gene expression **(Ge et al. 2019 Yin et al. 2021).** The obtained data supported by **Lai et al. (2018**) found that DBA addition to the broiler chicken diet improved liver health with no histological changes. Also, **El-Katcha et al. (2019**) observed mild vacuolation of hepatocytes or even normal hepatocytes with DBA supplementation compared to control. This lipid-lowering effect of DBA supplementation is especially important in the high inclusion level of CGM accumulating fat in the liver (Figures 1 and 2).

Dietary inclusion of the CGM instead of corn grains and soybean meal negatively affected the intestinal morphology, especially with the highest level used. Feeding of growing quail chicks on a 20 % CGM-containing diet significantly ($P<0.05$) reduced villi length and villi length/crypt depth ratio, while increased ($P<0.05$) inter-villi space which correlated with poor nutrient absorption which could explain the lower growth performance and poor feed utilization obtained at this level. The obtained result could be attributed to the high oil and fiber content of the CGM-containing diets, which negatively influenced intestinal morphology. The present data disagree with those obtained by **Tavakkoli et al. (2019)** indicated that CGM inclusion at 5, 10, 15 or 20% in broiler chicken diets had no significant effect on intestinal histology compared with control and the difference may be related to breed and age difference. Improvement of ileum morphometric measurement was observed with DBA supplementation in the growing quail diet, and confirmed by growth rate improvement compared to the quail group fed on the same diet without DBA supplementation. The current results indicated that DBA had an intestinal protective effect against injury caused by CGM. The obtained data supported by **Li et al. (2023)** revealed that dietary DBA addition increased intestinal villi length, decreased inter villi space and improved beneficial microflora of broiler chicken. Also, **Mohamed et al. (2020b)** stated that bile salt supplementation to the broiler diet had a significant ($P<0.01$) effect on the histological structure of broiler chicken small intestine through increasing villi length compared to control.

CONCLUSION

In conclusion, dietary inclusion of 10% or 20% CGM reduced growth rate, deteriorated feed efficiency parameters and reduced both liver and intestinal health of growing Japanese quail while, DBA supplementation improved the negative effect produced by CGM inclusion. Therefore, the obtained data suggested that 10% of CGM can be included in Japanese quail chick's diet with DBA supplementation without negative effects.

Competing interests: The authors have no conflict of interest to declare.

Ethical approval: The present study was affirmed by the Ethics of Animal Experiments Committee, Agricultural Research Center, Egypt.

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