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Effect of different feed physical forms (pellet, crumble, mash) on the performance and liver health in broiler chicken with and without carbon tetrachloride challenge

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ABSTRACT. A study was carried out to investigate the effects of physical feed form on productive performance and liver function in broiler chickens toxified or not with carbon tetrachloride (CCl_4). The experiment was conducted on 468 ten-day-old broiler chickens fed with mash, crumble and pelleted diets from day 11 to 42 of age. Birds from toxified groups were three times intramuscularly injected with CCl_4 (0.5, 0.5 and 0.75 ml/kg body weight) at day 14, 21 and 28 of age, respectively. The main factors interaction showed that daily weight gain of birds fed with pelleted diet was higher in comparison with those grown on crumble and mash diets but only when birds were challenged with CCl_4. The feed physical form influenced European production efficiency index with higher values observed for birds fed with pelleted diet from day 29 to 42 of age. Liver fat percentage increased by 2.06% in birds fed with pelleted diet compared with those grown on mash one. Broilers offered pelleted diet showed greater serum low-density lipoprotein cholesterol concentration compared with those fed with crumble diet but only when birds were challenged with CCl_4. The frequency of score zero indicating liver with no visible injuries was greater in broilers fed with crumble diet (58.33%) than in those receiving mash and pelleted diets after 31-day feeding period. In summary, the feed physical form can affect broiler growth performance and blood biochemical parameters in broilers subjected to CCl_4 challenge. However, further studies using higher doses of CCl_4 should be conducted to induce more pronounced liver dysfunction in experimental birds.

Introduction

The liver is one of the most important organs that play a critical role in many metabolic functions, including synthesis, degradation and transport of lipids (Li et al., 2014). The liver also performs biotransformation and detoxification of endogenous and exogenous harmful substances (Wang et al., 2013) as well as centralises nutrient metabolism and metabolite excretion (Ozougwu and Eyo, 2014). Therefore, liver injury or dysfunction is defined as a notorious health problem (Shen et al., 2015) resulting in low productivity and economic losses in commercial birds.

In birds, lipogenesis mainly occurs in liver, while adipocytes are the major storage site for triglycerides (TG) (Hünigen et al., 2016). In a healthy bird, the liver rapidly changes the flow of nutrients, but when TG exceed the liver's capacity for excretion, they are accumulated intrahepaticly (Babin and Gibbons, 2009), a phenomenon contributing to the pathology of a precarious metabolic disease known

as fatty liver (Shini, 2014). On the other hand, natural feed contaminants, including toxic substances, may interfere with TG for excretion from the liver and so induce lipid accumulation in the liver (Tedesco, 2001). The role of toxicants in liver function, and in particular with regard to lipid metabolism, must be characterized in detail, as the feedstuffs currently used are constantly considered to be endangered by contamination from environmental or biological sources. Special precautions have to be taken as modern broilers are subject to constant metabolic pressure associated with overfeeding and lack of activity, the two major factors that increase the risk of liver metabolic disorders.

Carbon tetrachloride (CCl_4), as a hepatotoxic proxy, is a useful agent for experimental models to study the effects of natural toxicants on liver function in animals (Wang et al., 2013). Exposure to CCl_4 results in hepatic steatosis, necrosis and ultimately cirrhosis (Bellassoued et al., 2018). In hepatocytes CCl_4 is metabolized by the cytochrome P450, which is accompanied by the production of highly reactive free radicals (Yang et al., 2015). Oxidative stress, defined as a physiological status associated with a destabilized balance between free radicals and antioxidant defence system, plays a key role in the pathogenesis of CCl_4-induced hepatic injury (Shah et al., 2017).

With the rapid development of the poultry industry and due to the physiological sensibility of fast-growing meat-type poultry, it is of urgent necessity to further examine the effects of toxicants on the liver functions. Knowing that CCl_4 may be a useful experimental factor to induce liver damage in experimental animal models (e.g. rodents), it can be suggested that also in birds CCl_4-induced liver injury may be a valuable model to study changes in the liver metabolism, function and structure caused by toxicants. Moreover, a pelleted feed can be a key factor inducing overfeeding leading to fatty liver, in particular, in birds grown on contaminated diets. Therefore, this study aimed to investigate the impact of the physical form of feed on production efficiency and liver function in broiler chickens toxified with CCl_4.

Material and methods

Animals and diets

All procedures carried out on birds were reviewed and approved by the Animal Care and Use Committee of Lorestan University (Khorramabad, Iran).

A total number of 1000 one-day-old Arbor Acres broiler chicks were provided from a local hatchery (Zarbal, Erak, Iran) and housed in a power ventilated grow out house (Poultry Research Unit, Agriculture Faculty, Khorramabad, Iran). For the first beginning period (days 1–10) chickens were raised in a floor pen and were offered a pelleted starter diet (Table 1) and water *ad libitum*. The chickens were kept under a 23:1 light to darkness lightening regimen. The ambient temperature and relative humidity were 32 ± 1 °C and 60 ± 5% during the raising period, respectively.

Table 1. Ingredients and nutrients composition of the basal diets

Indices	Starter (1–10 day)	Grower (11–42 day)
Ingredients, %		
yellow maize	58.34	63.08
soybean meal	37.70	31.54
soybean oil	1.50	5.10
calcium phosphate	1.24	1.40
$CaCO_3$	1.80	1.34
DL-methionine	0.20	0.28
L-lysine HCl	0.28	0.04
salt	0.14	0.14
mineral premix[1]	0.50	0.25
vitamin premix[2]	0.50	0.25
Nutrient composition		
ME, kcal/kg	3000	3176
crude protein, %	21.50	17.00
lysine, %	1.44	1.00
methionine, %	0.56	0.50
methionine + cystine, %	1.08	0.55
L-threonine, %	0.97	0.72
K, %	0.80	0.76
Ca, %	0.96	0.80
available P, %	0.48	0.41
Na, %	0.20	0.20

[1,2] each kilogram contained: IU: vit. A 12000, vit. D_3 5000, vit. E 80; mg: vit. K_3 3.2, vit. B_1 3.2, vit. B_2 8.6, vit. B_3 20, vit. B_5 65; vit. B_6 4.3, vit. B_9 2.2, vit. B_{12} 0.017, vit. H_2 0.30, choline chloride 1700, antioxidant 1000, manganese 120 000, zinc 110 000, copper 16 000, selenium 300, iodine 1250, iron 20 000

Chickens were sexed at day 11 based on fast/slow feathering plumage pattern and 468 healthy female chickens with the most similar body weight (BW) were chosen and used to examine the effects of six treatments in a 2 × 3 factorial design with a completely randomized block design with 6 replicates per treatment (13 birds per pen). The experimental treatments consisted of a grower diet supplied in three physical forms (mash, crumble and pellet) to birds challenged or not with CCl_4. The CCl_4 challenged chickens were injected three times intramuscularly with CCl_4 (olive oil diluted (1:1, v/v) at a dose of 0.5, 0.5 and 0.75 ml/kg BW at day 14, 21 and 28 of age, respectively.

The primary mash diet was pelleted and then pellets were crumbled in a roller mill, resulting in

a crumbled diet. The pelleting process was performed at a temperature of 90 °C.

A two-phase feeding program was used, with a starter diet from day 0 to 10 of age and a grower diet from day 11 to 42 of age (Table 1). During both phases, the chickens received feed and water *ad libitum*.

Data collection

Body weight (BW) and feed intake (FI) were recorded from day 14 to 42 of age and the obtained data were used to calculate daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR). Mortality was recorded upon occurrence. The European production efficiency index (EPEI) was calculated according to the following equation (Euribrid, 1994):

$$\text{EPEI}(\%) = \frac{\text{body weight (kg)} \times \text{livability (\%)}}{\text{age (days)} \times \text{feed conversion ratio}} \times 100.$$

At the end of the experiment (day 42), two birds from each pen (12 birds per each treatment) with a body weight close to the mean BW of the birds in the same pen were selected and killed by puncturing the jugular veins and carotid arteries. The samples of whole blood were collected and centrifuged at 1800 *g* for 15 min. The collected sera samples were stored at −20 °C pending biochemical assessments. Concentrations of serum glucose (GLU), triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and total protein (TP), and the activity of serum alkaline phosphatase (ALP) were determined using an auto-analyzer (Clima; Ral. Co, Barcelona, Spain). The biochemical analyses were based on enzymatic procedures previously described by Elliott (1984) and conducted using SEPPIM Diagnostic Kits (SEPPIM S.A.S., Sees, France). All analyses were conducted in two replicates at 25 °C.

The livers from all slaughtered birds were weighed and then evaluated macroscopically for colour and apparent health. The liver score was assessed using a 4-point scale in which 'score 3' was assigned to the most severe lesion and colour alteration and 'score 0' to the liver without visible changes as described by Trott et al. (2014) with minor modifications.

After scoring, the liver samples were used for fat extraction using Folch's method (Folch et al., 1957). Briefly, about 1 g of liver tissue was weighed, added to chloroform/methanol (2:1, v/v) mixture in a final volume of 20 times the tissue sample volume, vortexed for a one minute and allowed to stand with agitation for 2 h. The separated liquid was filtered through Whatman No. 1 filter paper into a 100-ml 54 graduated cylinder, and 5 ml of 7.3% potassium chloride solution was added and mixed. After phase separation, the upper layer was completely drained off. Total lipids were measured gravimetrically after solvent evaporation. The samples were then dried and weighed, and the total lipid weight was expressed as a percentage of liver fat relative to the total liver weight.

Statistical analysis

The results of birds performance and blood biochemical analysis were subjected to two-way analysis of variance (ANOVA) using the GLM procedures of SAS ver. 9.1 (SAS Institute, Cary, NC, USA). The statistical model included the main factors effects (feed physical form (mash, crumble and pellet) and CCl_4 injection) and their interaction. The Tukey test was used for multiple treatment comparisons (Kramer, 1956). Liver scores were subjected to the frequency analysis using PROC FERQ with the use of the same statistical analysis software (SAS Institute, Cary, NC, USA). For all tests, the maximum likelihood for type-I error was declared at 5% ($P < 0.05$).

Results

There was no statistical effect of the feed physical form and CCl_4 challenge on DWG, DFI and FCR in chickens (Table 2). The feed physical form exert influence on EPEI ($P < 0.0065$), with higher values observed for birds fed with pelleted diet from day 29 to 42 of age. Also only for the period from day 29 to day 42 of age, the statistically significant feed physical form × CCl_4 interactions for DWG, DFI and FCR were stated (Table 3). Average DWG of birds fed on the pelleted diet was higher compared with those grown on crumble and mash diet but only when birds were challenged with CCl_4 ($P < 0.005$). The CCl_4-injected birds showed greater DFI when were fed with mash diet compared with those fed on crumble diet ($P > 0.005$). Mean FCR was improved in CCl_4-injected birds when they were fed with the pelleted diet compared with those receiving mash diet ($P < 0.005$).

No difference in serum concentration of TG, TC and LDL-C was observed among the birds receiving a grower diet offered in three physical forms from day 14 to 42 day of age or between chickens injected or not with CCl_4 (Table 4).

No effect of feed form and CCl_4 challenge was stated for liver weight express in g and as %

Table 2. Daily weight gain (DWG, g), daily feed intake (DFI, g), feed conversion ratio (FCR, g/g) and European production efficiency index (EPEI) in broiler chickens challenged with CCl_4 and fed with a diet in pelleted, crumble and mash form from day 14 to day 42 of age

Indices	14–28 day			29–42 day			
	DW	DFI	FCR	DWG	DFI	FCR	EPEI
Feed physical form							
pellet	63.7	75.8	1.28	108.3	161	1.49	288[a]
crumble	59.2	80.3	1.21	87.4	144	1.64	221[b]
mash	62.7	80.3	1.28	99.1	157	1.58	257[ab]
SEM	1.982	1.645	0.132	3.823	5.930	0.061	17.6
CCl_4[1]							
+	62.9	77.4	1.24	98.9	151	1.54	251
−	60.9	83.2	1.37	97.7	165	1.67	244
SEM	1.618	2.813	0.172	2.495	3.986	0.132	14.3
P-value							
feed	0.2518	0.8431	0.4542	0.2518	0.8431	0.4542	0.0065
CCl_4	0.3902	0.1019	0.5508	0.3902	0.1019	0.5508	0.8568
feed × CCl_4	0.8663	0.4285	0.8810	0.0063	0.0285	0.0333	0.0563

[1] CCl_4 was intramuscularly injected at a dose of 0.5, 0.5 and 0.75 ml/kg body weight diluted in olive oil at a ratio of 1:1 (v/v) at day 14, 21 and 28 of age, respectively; [a-b] – means with different superscript within the same column (for each main effect separately) differ significantly ($P < 0.05$); SEM – standard error of the mean

Table 3. Feed physical form × CCl_4 interaction for daily weight gain (DWG, g), feed intake (DFI, g), and feed conversion ratio (FCR, g/g) in broiler chicken challenged with CCl_4 and fed with a diet in pelleted, crumble and mash form from day 29 to day 42 of age

Indices		DWG	DFI	FCR
Feed physical form	CCl_4[1]			
pellet	−	111.0[ab]	160[ab]	1.448[bc]
pellet	+	121.0[a]	197[ab]	1.401[c]
crumble	−	96.3[b]	149[ab]	1.546[ab]
crumble	+	96.3[b]	152[b]	1.512[ab]
mash	−	103.3[ab]	161[ab]	1.557[bc]
mash	+	102.7[b]	189[a]	1.852[a]
SEM		5.362	6.152	0.085
P-value		0.006	0.029	0.033

[1] CCl_4 was intramuscularly injected at a dose of 0.5, 0.5 and 0.75 ml/kg body weight diluted in olive oil at a ratio of 1:1 (v/v) at day 14, 21 and 28 of age, respectively; [a-b] – means with different superscript within the same column differ significantly ($P < 0.05$); SEM – standard error of the mean

Table 4. Mean serum concentration of triglyceride (TG), cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in broiler chickens challenged with CCl_4 and fed with a diet provided in three physical forms (pelleted, crumble or mash) from day 14 to day 42 of age

Indices	TG, mg/dl	TC, mg/dl	LDL-C, mg/dl
Feed physical form			
pellet	128	177	48.2
crumble	145	170	44.5
mash	156	168	47.7
SEM	12.52	8.93	2.95
CCl_4[1]			
+	137	163.	62.1
−	149	181	74.9
SEM	10.09	7.20	2.38
P-value			
feed	0.2957	0.6752	0.8156
CCl_4	0.3947	0.1128	0.1590
feed × CCl_4	0.0326	0.0207	0.0044

[1] CCl_4 was intramuscularly injected at a dose of 0.5, 0.5 and 0.75 ml/kg body weight diluted in olive oil at a ratio of 1:1 (v/v) at day 14, 21 and 28 of age, respectively; SEM – standard error of the mean

of body weight (Table 5). Liver fat percentage increased by 2.06% in the birds fed with the pelleted diet compared with those grown on mash diet ($P < 0.004$) at day 42 of age; however no effect of CCl_4 challenge was stated. There was also a statistically significant feed physical form × CCl_4 interaction for liver fat percentage (Table 6), showing that broilers fed with the pelleted diet without CCl_4 injection had greater liver fat content compared with birds receiving mash or crumble diet without CCl_4 injection ($P < 0.038$). Moreover the birds fed mash diet with simultaneous CCl_4 challenge had higher liver fat parentage compared to not challenged one.

The broilers grown on the mash diet and challenged with CCl_4 and those fed with the crumble diet without CCl_4 injection showed higher serum concentration of TG, compared with birds receiving the pelleted diet without simultaneous CCl_4 challenge ($P < 0.05$; Table 6) after 31-day period of feeding. Mean serum TC concentration was altered by feed physical form × CCl_4 interaction, with TC concentration lowered in the broilers given crumble diet with CCl_4 injection and mash diet without CCl_4 injection compared with those fed on the pelleted diet and injected simultaneouly with CCl_4 ($P < 0.05$; Table 6). When birds were challenged with CCl_4 serum LDL-C concentration was increased in

Table 5. Liver weight in g and as a % of body weight, and liver fat content (%) in broiler chickens challenged with CCl_4 and fed with a diet provided in three physical forms (pelleted, crumble or mash) from day 14 to day 42 of age

Indices	Liver weight, % of body weight	Liver weight, g	Liver fat content, %
Feed physical form			
pellet	2.06	24.0	7.15[a]
crumble	2.56	21.4	5.63[ab]
mash	2.30	22.9	5.09[b]
SEM	0.18	1.04	0.68
CCl_4[1]			
–	2.30	22.2	6.02
+	2.31	23.4	5.90
SEM	0.26	0.85	0.55
P-value			
feed	0.1468	0.2267	0.0044
CCl_4	0.9349	0.3056	0.1230
feed × CCl_4	0.3858	0.8672	0.8739

[1] CCl_4 was intramuscularly injected at a dose of 0.5, 0.5 and 0.75 ml/kg body weight diluted in olive oil at a ratio of 1:1 (v/v) at day 14, 21 and 28 of age, respectively; [a-b] – means with different superscript within the same column differ significantly ($P < 0.05$); SEM – standard error of the mean

Table 6. Feed physical form × CCl_4 interaction for serum concentration of triglyceride (TG), cholesterol (TC) and low-density lipoprotein (LDL-C) and liver fat parentage in broiler chickens challenged with CCl_4 and fed with a diet provided in three physical forms (pelleted, crumble or mash) from day 14 to day 42 of age

Indices		TG, mg/dl	TC, mg/dl	LDL-C, mg/dl	Liver fat content, %
Feed physical form	CCl_4[1]				
pellet	–	104[b]	154[b]	39.3[c]	8.44[a]
pellet	+	153[ab]	201[a]	57.2[a]	5.86[abc]
crumble	–	163[a]	182[ab]	49.7[abc]	5.19[b]
crumble	+	128[ab]	159[b]	39.2[c]	6.07[ab]
mash	–	145[ab]	154[b]	44.1[b]	4.42[c]
mash	+	167[a]	182[ab]	51.3[abc]	5.75[ab]
SEM		18.28	13.03	4.31	0.952
P-value		0.032	0.021	0.004	0.038

[1] CCl_4 was intramuscularly injected at a dose of 0.5, 0.5 and 0.75 ml/kg body weight diluted in olive oil at a ratio of 1:1 (v/v) at day 14, 21 and 28 of age, respectively; [a-c] – means with different superscript within the same column differ significantly ($P < 0.05$); SEM – standard error of the mean

the broilers offered pelleted diet compared with the birds fed with the crumble diet. Serum concentration of LDL was also declined in the birds receiving the pelleted and mash diets without injection of CCl_4 ($P < 0.05$; Table 6).

No effects of feed physical form and CCl_4 administration were found on serum activity of ALP and glucose concentration ($P > 0.05$; Table 7) after 31-day period of feeding.

The liver score results showed that the frequency of score zero indicating a healthy liver was

Table 7. Serum activity of alkaline phosphatase (ALP), and serum concentration of glucose (GLU) and protein (TP) in broiler chickens challenged with CCl_4 and fed with a diet provided in three physical forms (pelleted, crumble or mash) from day 14 to day 42 of age

Indices	ALP, U/L	GLU, mg/dl	TP, g/dl
Feed physical form			
pellet	2071	233	5.54
crumble	2001	234	5.20
mash	1988	264	5.26
SEM	42.06	17.49	0.19
CCl_4[1]			
–	1997	233	5.24
+	2043	255	5.42
SEM	33.91	14.10	0.15
P-value			
feed	0.4187	0.2614	0.7610
CCl_4	0.3040	0.3411	0.8338
feed × CCl_4	0.3686	0.3412	0.6723

[1] CCl_4 was intramuscularly injected at a dose of 0.5, 0.5 and 0.75 ml/kg body weight diluted in olive oil at a ratio of 1:1 (v/v) at day 14, 21 and 28 of age, respectively; SEM – standard error of the mean

Table 8. Frequency of a 4-point liver scores in broiler chickens challenged with CCl_4 and fed with a diet provided in three physical forms (pellet, crumble or mash) at day 42 of age

Indices	Liver score, %[1]			
	score 0	score 1	score 2	score 3
Feed physical form				
pellet	8.33	46.7	29.4	39.3
crumble	58.3	33.3	11.8	35.7
mash	33.3	20.2	58.8	25.0
CCl_4[1]				
–	66.7	66.7	29.4	46.4
+	33.3	33.3	70.6	53.6
P-value				
feed	<.0001	<.000	<.0001	<.0001
χ^2	24.00	30.00	34.00	56.00
CCl_4	<.0001	<.000	<.0001	<.0001
χ^2	12.00	15.00	17.00	28.00
feed × CCl_4	0.6342	0.917	0.0838	0.5379
χ^2	0.91	0.17	4.95	1.24

[1] the frequency of occurrence of each liver score, where 'score 3' was assigned to the most severe lesion and colour alteration and 'score 0' to the liver without visible changes; [2] CCl_4 was intramuscularly injected at a dose of 0.5, 0.5 and 0.75 ml/kg body weight diluted in olive oil at a ratio of 1:1 (v/v) at day 14, 21 and 28 of age, respectively

higher in broiler fed with crumble diet (58.33%) than in the broiler receiving mash and pelleted diet after 31-day feeding period ($P < 0.05$; Table 8). Higher frequency for score 1 (46.67%) and score 3 (39.29%) was observed in the birds receiving the pelleted diet than those fed with crumble and mash diets. Birds grown on crumble diet had the

highest frequency of score 2 (58.82%) compared with those grown on the mash or pelleted diets ($P < 0.05$).

Discussion

The effects of feed presentation form and ingredients particle size on broiler performance are still of major interest in the poultry industry (Ebrahimi et al., 2010). Svihus et al. (2004) reported that feeding broilers with a pelleted diet increased weight gain and feed intake, and improved FCR compared with those maintained on a mash diet, such results are in line with the findings of the present study. In almost all reports, performance improvement with pelleted diets are alike attributed to increased nutritional density, improved starch digestibility due to chemical changes during pelleting, better nutrient intake, changes in physical feed form, reduced feed waste, and decreased energy expenditure in eating (Amerah et al., 2008; Jafarnejad et al., 2010). It is well known that the use of carbohydrate enriched easily digestible diets in the commercial feeding of broilers with minimal mobility in the densely crowded houses immensely enhances the risk of liver disorders. Under the same circumstances, liver injury, even indistinctly, can lead to low birds performance and great economic losses (Wang et al., 2013). We have hypothesized that this condition will worsen and will be more detrimental when birds receive a diet contaminated with harmful toxicants such as mycotoxins or environmental chemicals, in particular when diet is presented in pelleted form, since a pelleted diet may promote FI. Indeed, we stimulated the challenging situation that commercial birds' breeding around the world is facing.

Reports suggested that serum TC, LDL-C and TG and low HDL-C levels were enhanced by CCl_4 detoxification in animal models (Lin et al., 2008; Yang et al., 2008). Carbon tetrachloride enhances the synthesis of fatty acids and TG by the use of acetate as a substrate. This process results from an increased transfer of acetate into liver cells, followed by an increase in blood lipids (Boll et al., 2001). Earlier studies showed that an increase in serum cholesterol level in a liver-injured bird is associated with a weakening of the liver's role in removing circulating cholesterol (Owen, 1990). Sonkusale et al. (2011) and Khodadust et al. (2015) revealed that CCl_4 injection improved serum TG, TC and LDL-C levels in animal models. Liu et al. (2019) recently reported that administration of CCl_4 in Japanese quail and mice, respectively, resulted in lower concentrations of calcium and TG and higher concentration of glucose. However, no effect on total protein and TC was observed in the former report. These reports, like many others (Sobrane Filho et al., 2016), confirm evidently inconsistent modifications in certain blood biochemical constituents as well as liver function in animals toxified with CCl_4.

In spite of many reports confirming the deleterious effects of CCl_4, particularly in birds overfed with pelleted diets, the results obtained in the present study partly did not confirm the above-mentioned effects. In this study administration of CCl_4 at the relevant doses exerted no effects on productive performance of the birds. Previous studies have shown that poultry, unlike other laboratory animals such as mice, is resistant to the necrogenic effects of CCl_4-induction. This lack of sensitivity in poultry is discussed by the fact that their liver does not modify CCl_4 to active metabolites, including free radicals such as CCl_3^-. This low capacity for CCl_4 activation might be associated with a lower content of cytochrome P-450 in the liver of poultry compared with more susceptible species such as mice. Cytochrome P-450 plays a key role in the transformation of CCl_4 and other toxins to more active metabolites in hepatocytes. On the other hand, the dosage of CCl_4 used in the present study might have been too low and it was only adequate to induce a generalized immune response in birds, an event which may eventually lead to beneficial metabolic alterations in favour of improved performance.

Some enzymes are usually located in the cytoplasm of hepatocytes; therefore, a decrease in the structural integrity of these cells is reflected by a surge in the serum levels of these enzymes (Li et al., 2014). Radicals originated from CCl_4 catabolism induce lipid peroxidation, damage the liver cells membrane and organelles, lead to edema and necrosis of hepatocytes and cause the release of cytosolic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) into the blood circulation (Parmar et al., 2012). However, no increase in ALP levels as an indicator of hepatic injury was observed in the present study.

Samudram et al. (2008) in studies on the harmful effects of CCl_4 on rat liver function reported a lower serum protein level, which is partly confirmed by our results, since interaction of the pelleted diet and CCl_4 decreased serum protein level numerically (but not statistically). It was shown that decreased serum levels of total protein and albumin in CCl_4 injected birds could be due to impaired liver function toward reduced protein biosynthesis occurring through impairment of ribosomal function in endoplasmic reticulum (Clawson, 1989).

It is generally accepted that CCl_4-induced liver cell damage is initiated by the conversion of CCl_4 to a reactive free radical (trichloromethyl free radical, CCl_3^-) by the cytochromes P-450. Then CCl_3^- reacts with oxygen and transforms into proxy trichloromethyl (Khoramshahi and Samadi, 2010). The proxy trichloromethyl attacks the endoplasmic reticulum membrane and causes lipid peroxidation, loss of cellular calcium, reduced protein synthesis and increased activity of liver enzymes as well as eventual destruction of liver cells (Panovska et al., 2007). However, the results obtained in the present study did not show all the adverse effects of CCl_4 administration on broiler chickens fed with a pelleted diet, while much more deleterious effects were expected.

Conclusions

Based on the findings of the present study, it can be concluded that the physical form of feed can be considered as a factor of a significant impact on broiler growth performance and biochemical blood parameters. Presentation of a pelleted diet to birds challenged with CCl_4 did not, however, exert significant harmful effects on the liver in a way that would inhibit the metabolism of the whole organism. Such results may be due to the natural resistance of birds to necrogenic effects of CCl_4, resulting from the fact that the liver of birds to a much lesser extent metabolizes CCl_4 to its active metabolites, including CCl_3^- free radial. Therefore, further studies using higher doses and a different CCl_4 injection schedule should be conducted to induce more pronounced liver dysfunction in experimental birds.

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