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Research Article

Effects of Carbendazim on Plasma Biochemistry, Gross and Microscopic Features of Some Visceral Organs of Japanese Quails (*Coturnix coturnix japonica*) Exposed to Different Protein-Energy Diets

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ABSTRACT

Carbendazim is a fungicidal agent for protecting farm commodities locally but its usage is usually restricted by its associated toxic potential especially when consumed by malnourished wild birds on farm. In this study, we observed the effect of protein-energy malnutrition on the histo-architectural and plasma biochemical changes associated with carbendazim toxicity in adult male Japanese quail. Carbendazim was administered at a single dose of 400 mg/kg by gastric gavage to quails fed two varied diets (Normal Protein-energy and Low Protein-energy diets). The control group received corn oil (vehicle) at 1 ml/200g body weight. The animals were sacrificed on day 8 post administration. Results showed that the relative weight of the gizzard significantly increased ($P<0.01$) in the Low Protein-energy diet-Oil (LPO; Control) treated group compared to the Normal Protein-energy diet-Carbendazim- (NPC) treated groups. Also, the relative weights of the liver and spleen markedly increased ($P<0.01$ and $p<0.05$ respectively) in the Low Protein-energy diet-Carbendazim- (LPC) treated group, compared to the Normal Protein-energy diet-Oil- treated (NPO; Control) group and other groups. The mean triglyceride value of the NPC-treated group was significantly higher ($P<0.05$) relative to the NPO and LPO groups. The liver of Carbendazim-treated groups revealed varying degrees of hepatic (severe diffuse fatty degeneration and necrosis of hepatocytes, as well as a moderate periportal cellular infiltration by mononuclear cells) and renal (severe renal tubular necrosis at the cortical region of the kidneys) histo-architectural disruptions. The severity was relatively higher in the LPC groups. Therefore, malnutrition aggravated the hepatorenal lesions in male quails exposed to Carbendazim.

Key words: Carbendazim, malnutrition, liver, kidney, Japanese quail

INTRODUCTION

Carbendazim is the common name for methyl 2-benzimidazole carbamate, a systemically active benzimidazole fungicide (Nakai *et al*., 1994; Khan *et al*., 2008). It has a broad spectrum systemic fungicidal action and is used for pre- and post- harvest protection of various food crops, fruits and vegetables.

A considerable number of reports of carbendazim on a wide range of laboratory animals. The drug is being reported to show time- and dose-dependent pathological effects, to be gonadotoxic, decreased fertility, affects viability of sperm cells, decreased testes weight and testicular abnormalities in dogs, laboratory mammals and in Japanese quails (Hess and Nakai, 2000; Selmanoglu *et al.,* 2001; Aire, 2005; Rajeswary *et al.,* 2007; Khan *et al.,* 2008).

Increased absolute and or relative liver weights were observed as an effect of short and long term administration of carbendazim (WHO, 1993; Selmangolu *et al*., 2001; Khan *et al*., 2008. Some other studies reported pathological changes featuring central venous and portal congestion, mononuclear cellular infiltration, hydropic degeneration and necrosis of hepatocytes (WHO, 1996; Muthuviveganandavel *et al.,* 2008).

Pathological changes were also observed on chronic administration of carbendazim in the kidney of rats featuring decreased relative weights, congestion, mononuclear cellular infiltration of the interstitium, tubular degeneration and fibrosis (Selmangolu *et al*.,2001); in the thyroid of rats, there was reduction in parafollicular cell population (Barlas *et al*., 2002). In another study of chronic high dose carbendazim administration there was proliferation of the para-

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follicular cells of the thyroid in female Wister rats (WHO, 1996). Nutrition-associated toxicity problems are both of basic and applied interest and a topic of current concern. Malnutrition and diet in general have been understood to play very important roles on bioprocesses, which govern the fate of drug/xenobiotic in the body (Kamala, 1987). It is to be expected that pathologic states induced by protein-energy malnutrition on any organ would alter the intricate process of drug handling (Saroj, 1986). Shankar *et al* (2006) suggested that under nutrition potentiates the foetal toxicity of ethanol in part by disrupting maternal GH-IGF-1, signalling thereby decreasing maternal uterine capacity and placental growth.

The extent of protein binding to plasma and tissue proteins also influences the distribution characteristics of drugs (Saroj, 1986). Hence it is necessary to consider the role of nutrients in xenobiotic / drug metabolism, disposition and its effects on nutrient absorption, metabolism and excretion (Kamala, 1987).

There is a need to study the effects of carbendazim acting in concert with protein- energy malnutrition; because the seasons of protein-energy deprivation, especially in developing countries, coincides with periods pesticide-treated grains are prematurely released into the market. Carbendazim toxicity in wild birds is attributed to pesticide-dressed crop fields.

The Japanese quail, with a relatively high crude protein requirement of 23.08% (Soares *et al*., 2003) is a good candidate for this study which investigates the interaction between ecotoxicity and protein-energy malnutrition.

MATERIALS AND METHODS

Experimental animals and their feeding

Forty adult, seven week old, male Japanese quails (*Cortunix cortunix japonica*), free from any observable ailment, were obtained from the Quail Breeding Unit of the Nigeria Veterinary Research Institute, Ikire Substation. The birds weighed between 120-151g. They were kept in galvanized wire mesh cages, under hygienic conditions, in the same environment in four groups of ten animals each. Two poultry feed formulations; normal diet (24.6% total protein, 3151.60 MCal. metabolisable energy) and Low protein-energy diet (4% total protein, 1026 MCal. metabolisable energy) were used throughout the experiment. The feed rations and drinking water were supplied *ad libitum.* The birds were stabilized on their assigned diet for seven days before the commencement of drug dosage.

Experimental Design

The two treatment groups (NPC and LPC) were administered by oral gavage with 400 mg/kg body weight of Carbendazim (Aldrich Chemical Company Inc., Milwaukee, USA) suspended in corn oil. The control groups (NPO and LPO) received the vehicle, corn oil, only at 1ml/200g body weight.

Blood collection, sacrifice and harvest of organs

On day 8 post Carbendazim administration, the gross weights of the birds were taken using a digital balance (Scout Pro. SPU 402, OHAUS Corporation, Pine Brook, New Jersey, USA) and then deeply anaesthetized with Ketamine and Diazepam. Blood samples were collected by cardiac puncture into Lithium heparinised tubes, centrifuged at 3000rpm for 15 minutes using a centrifuge (CF-405 Gallenhamp, England). The supernatant was collected for biochemical assay for hepatic (aspartate amino transferase, alanine aminotransferase cholesterol and triglycerides) and renal (blood urea nitrogen and creatinine) injury markers using commercially available kits.

The visceral organs viz., liver, kidney, heart, lungs were removed from each bird. The organs were wiped dry of fluid, using a filter paper and weighed on the digital balance. The relative weight of each organ was calculated as a percentage of the body weight. Each organ was also examined for the presence of gross lesions.

Tissues obtained from birds in each group were fixed in 10% neutral buffered formalin and processed for histopathological examination using the routine paraffin-wax embedding method. 5 µm thick sections were made and stained with Haematoxylin and Eosin according to standard procedures described by Bancroft and Layton (2013), and observed under the light microscope for histopathological changes.

Statistical analysis

Comparisons between groups were achieved by subjecting the data obtained to 2 by 2 random block design and analysis of variance (ANOVA) followed by Duncan's Multiple Range Test. The level of significance was set at $P \leq 0.05$. Results are presented as mean ± standard error of the mean (SEM).

RESULTS

Behavioural alterations

Within few minutes of Carbendazim administration, quails of the Low Protein-energy-diet- Carbendazim-treated and Normal Protein-energy diet-Carbendazim-treated groups showed depression which lasted for about two to three hours. Other observable alterations included a reduced attraction towards feed and water as well as decrease in the frequency of crowing. These signs were also seen in quails given corn oil only but they were very mild. In both cases the signs returned to normal within a day after administration.

Relative organ weights

Gizzard: The mean relative weight of the gizzard in the LPO group was significantly increased ($P<0.01$), compared to the mean relative weights of the NPO and NPC treated groups (Figure 1).

Liver: The relative weights of the liver of LPC-treated group significantly increased ($P<0.01$) compared to the untreated groups (NPO and LPO) (Figure 1).

Others: The relative organ weights of the kidney, proventriculus, heart and lungs in all the groups were not significantly different from one another (Figure 1).

Plasma biochemistry

The plasma concentrations of cholesterol (CHOL), triglycerides (TG), aspartate amino transferase (AST), alkaline phosphatase (ALP), creatinine (CK) and blood

urea nitrogen (BUN) of Quails that received either Low Protein-energy or Normal Protein-energy diets and administered either Oil or Carbendazim were as shown in Figures 2-7. The mean triglyceride and AST values of the Normal Protein-energy diet-Oil-treated and Normal Protein-energy diet-Carbendazim-treated groups (NPO and NPC) were higher than that of the low protein groups. Only the triglyceride (TG) values of the Normal Protein-energy diet-Carbendazim-treated group (NPC) were significantly higher than that of the Low Protein-energy diet groups (LPO and LPC).

Histopathology

Liver: The liver of the Low Protein-energy diet-Carbendazim-treated group (LPC) showed severe diffuse, fatty degeneration and necrosis of hepatocytes. There was also moderate portal congestion with periportal infiltration by mononuclear cells. (Figure 8). In the Normal Protein-energy diet-Carbendazim-treated group (NPC), there was also necrosis of hepatocytes, but the lesions were multifocal and appears limited to the periportal area with mild cellular infiltration. In the Low Protein Oil-treated group (LPO), the liver presented mild fatty degeneration of hepatocytes. No visible lesions were observed in the Normal Protein-energy diet-Oil-treated group (NPO) (Figure 8).

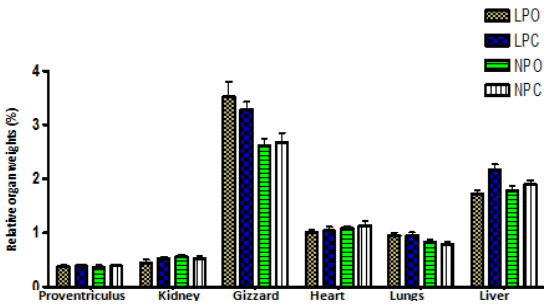
Kidney: There was severe renal tubular necrosis at the cortical region in the kidney of LPC group (Figure 9). There were few protein casts in the tubular lumen and mild interstitial mononuclear cellular infiltration. The tubular epithelial cells of NPC appear swollen. There were no visible lesions observed in LPO and NPO (Figure 9).

DISCUSSION

Quails and other food birds get exposed to carbendazim through consumption of post harvest dressed seeds. Apart from deleterious effect on the general well being of the birds, this could also be another route of carbendazim into the food chain.

The behavioural alterations characterized by depression, reduced crowing, feeding and drinking which occurred after a few minutes of carbendazim administration by the treated groups has been reported previously (Aire, 2005; Khan *et al.,* 2008). However, some of these could be attributed to the stress of handling and the Oil-based oral infusion, as the untreated control groups given Corn oil also manifested some slight alterations after dosing. There were no diet-related differences in these behavioural alterations

The observed significant increase in relative weights of the gizzard of the low protein groups (LPO, LPC) compared to their normal protein counterparts could be suggested not to be related to the treatment with carbendazim but rather an adaptive mechanism to the low protein energy diets, this adaptation appears to be adversely affected by the drug. However, the effects on the relative weights of the liver appear to be elicited by the treatment and aggravated by protein-energy deficiency. Further studies using longer duration of protein-energy malnutrition and multiple doses of carbendazim may support this.



Values with different superscripts are significantly different. *P<0.05; ** P<0.01

Fig. 1: Relative organ/body weight (%) of Carbendazim-treated Quails on normal and low protein-energy diet.

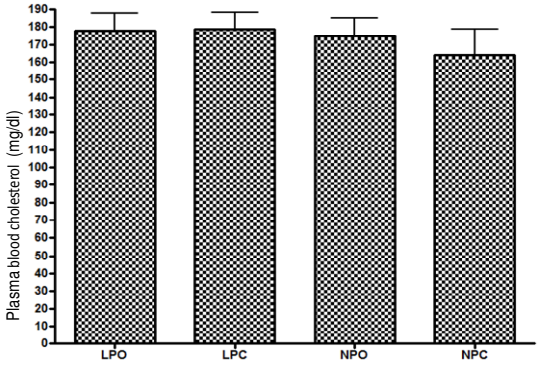


Fig. 2: Plasma cholesterol concentrations of adult male quails that received either Carbendazim or Corn Oil and fed either Normal protein-energy or Low protein-energy diets

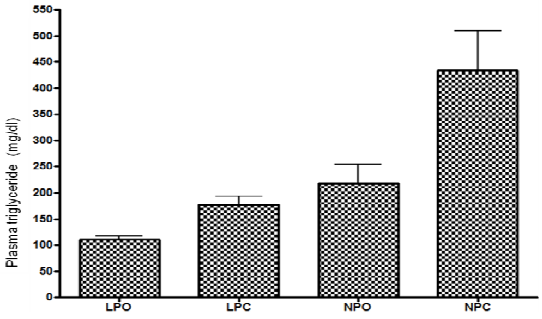


Fig. 3: Mean ± SEM plasma triglyceride concentrations of adult male quails that received either Carbendazim or Corn Oil and fed either Normal protein-energy or Low protein-energy diets. P<0.05.

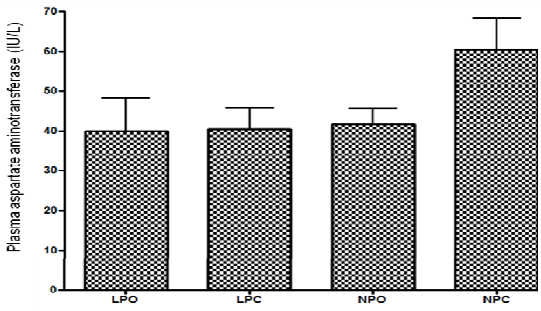


Fig. 4: Mean±SEM plasma aspartate amino transferase concentrations of adult male quails that received either Carbendazim or Corn Oil and fed either Normal protein-energy or Low protein-energy diets.

Table 1: Effects of diets and or Carbendazim on the plasma biochemistry and relative weight of some visceral organs of Japanese quail

Variables	Diet effect				Drug effect		Diet-drug inter-action
	Np		Lp				
	Oil	Carb	Oil	Carb	Oil	Carb	
				Relative weights (%)			
Proventriculus	0.36	0.39	0.37	0.39	0.37	0.39	Ns
Kidney	0.55	0.53	0.45	0.53	0.5	0.53	Ns
Gizzard	2.61	2.67	3.52	3.29	3.07	3.1	Ns
Heart	1.08	1.14	1.01	1.04	0.54	1.09	Ns
Lungs	0.83	0.79	0.95	0.95	0.89	0.87	Ns
Liver	1.78	1.9	1.72	2.17	1.75	2.04*	Ns
Plasma concentrations							
Chol (mg/dl)	178	179	175	164	175	172	Ns
Tg (mg/dl)	111	177	218	434	208	306*	Ns
Ast (iu/l)	40	40.5	41.7	60.4	45.2	50.5	Ns
Alt (iu/l)	14.8	15.8	17.8	18.4	16	17.1	Ns
Urea (mg/dl)	47.4	50.7	78.2	56.4	50.5	53.6	Ns
Bun (mg/dl)	21.6	23.2	36	25.8	23.1	24.5	Ns

Keys: ns-(not significantly different), * ($p<0.05$), NP (Normal protein), LP (Low protein).

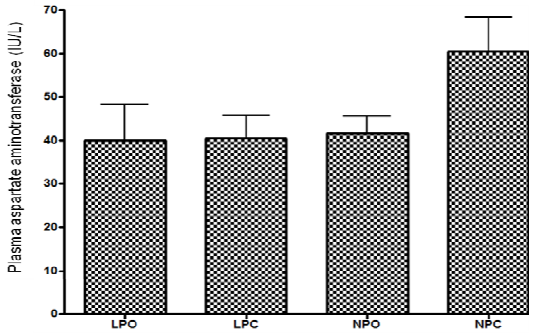


Fig. 5: Mean ± SEM plasma alkaline phosphatase concentrations of adult male quails that received either Carbendazim or Corn Oil and fed either Normal protein-energy or Low protein-energy diets.

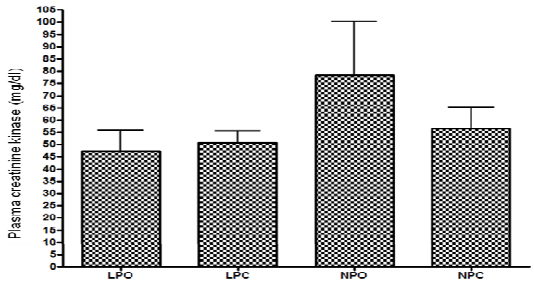


Fig. 6: Mean ± SEM plasma creatinine kinase concentrations of adult male quails that received either Carbendazim or Corn Oil and fed either Normal protein-energy or Low protein-energy diets.

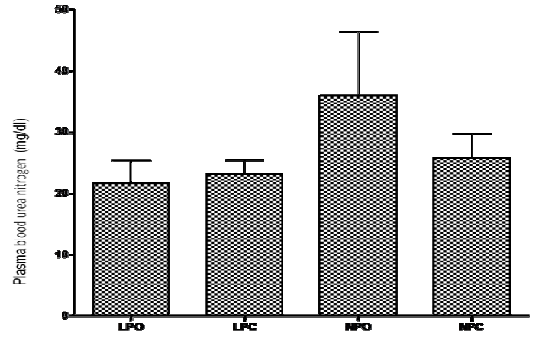


Fig. 7: Mean ± SEM plasma blood urea nitrogen concentrations of adult male quails that received either Carbendazim or Corn Oil and fed either Normal protein-energy or Low protein-energy diets.

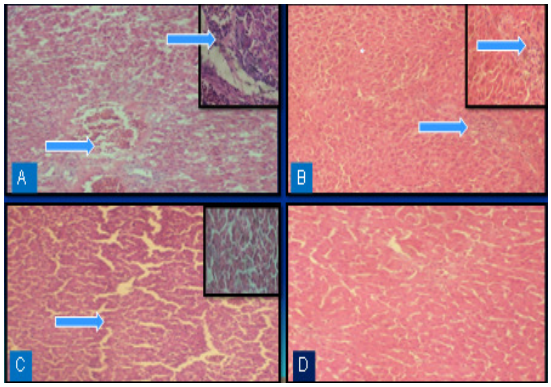


Fig. 8: Photomicrographs of the liver of quails that received either low protein-energy diet or normal protein-energy diet A (LPC): Diffuse hepatic vacuolar degeneration and necrosis, severe portal congestion with cellular infiltration of the portal area by mononuclear cells (arrowed) B (NPC): Periportal hepatic necrosis and cellular infiltration (arrowed). C (LPO): Mild diffuse vacuolar degeneration (arrowed). D (NPO): Normal hepatic parenchyma (Haematoxylin & Eosin; Magnification: x 100; Insets show the pictures at magnification x400).

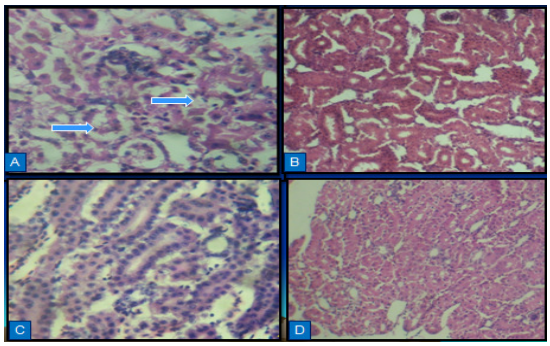


Fig. 9: Photomicrographs of the kidney of quails that received either low protein-energy diet or normal protein-energy diet. A (LPC): Severe renal tubular necrosis, cellular debris in tubular lumen (arrowed). B (NPC): Swollen tubular epithelium. C (LPO) and D (NPO): Normal renal tubules. (Haematoxylin & Eosin; Magnification: x 100).

The significantly elevated mean alanine amino-transferase (AST) value of the normal protein- treated group (NPC) relative to its normal protein counterparts

and most especially the low protein groups (LPO and LPC) suggests that there is a treatment effect which potentiated the differences in the plasma AST values caused by the diet.

The marked increase in triglyceride (TG) value in Carbendazim treated birds, especially of the normal protein-energy diets (NPC) could be attributed to several factors. Micronutrients and nutritional factors present in the balanced diet could be in vital interactions with Carbendazim. This synergism then would likely derange other factors responsible for metabolism of triglycerides in the animal body.

Carbendazim is an established endocrine disruptor (Barlas *et al.*, 2002; Shui-Yuan Lu *et al.*, 2004). Hormones like insulin, ACTH, TSH, glucagon and thyroid hormone are known to regulate the release of triglycerides from fat tissue so that they meet the body's needs for energy between meals. Carbendazim may in different degrees affect the regulation of these hormones and indirectly impact on the triglyceride levels, though further studies are needed to corroborate this fact.

The observed insignificant difference in renal (BUN and creatinine) and hepatic (ALT) injury markers levels could suggest that carbendazim concentrations may impact blood plasma biochemistry in a way that indicates effects on metabolism more particularly in the normal protein groups that had higher mean BUN, Creatinine and ALT values while there may not be direct impact on the visceral organs at this dose. In order to elaborate further on these relationships and mechanisms, a larger study in which levels of certain micronutrients are altered alongside carbendazim treatment is necessary.

The varying degree of hepatic lesions (diffuse fatty degeneration, necrosis of hepatocytes with moderate portal congestion and periportal infiltration by mononuclear cells) observed in this study is consistent with documented features of liver pathology seen on carbendazim administration, especially in the low protein-energy diet group (Selmagnolu *et al*, 2001; Muthuviveganandavel *et al.*, 2008). The lesions seen in this study appear to be accentuated by the protein-energy malnutrition. Also, the renal lesions (moderate cortical renal tubular necrosis, few tubular luminal protein casts and mild interstitial mononuclear cellular infiltration) observed in the low protein-energy diet group in this work is incongruent with the stable data on the plasma BUN and creatinine values. Although there have been reports in mammals (WHO, 1996), renal lesions have not been previously reported in Japanese quails treated with carbendazim. Further studies on this would be necessary.

In summaryof this study, while one can infer the significantly deliterious effect of treatment on relative liver weight and plasma triglyceride values it could also be histopathologically shown that inadequate protein-energy diet potentiates the deleterious effects of carbendazim at 400mg/kg body weight in Japanese quails. In conclusion, the present study confirms that protein-energy malnutrition is capable of aggravating the pathology of Japanese quails exposed to carbendazim.

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