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Afr. J. Biomed. Res.Vol.15 (May 2012); 117 - 121

Research Article

Effects of Dietary Protein-Energy Malnutrition on the Testes of Japanese Quails (*Coturnix coturnix japonica*) Exposed To Carbendazim

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ABSTRACT: This study was aimed at observing the effects of protein-energy malnutrition on the morphometrical, histological and hormonal changes associated with the testicular toxicity of Carbendazim (methyl 2-benzimidazole carbamate) in the adult male Japanese quail. Carbendazim was administered at a single dose of 400 mg/kg by gastric lavage to quails fed either normal protein-energy or low protein-energy diets. The birds were monitored for eight days post-administration. Significant decreases in the relative weight of the testis ($p<0.05$) were observed in the Carbendazim-treated groups, irrespective of their dietary protein-energy status, compared to the Normal Protein-energy diet-Oil-treated (control) group. There were similarly significant decreases in the plasma testosterone levels ($p<0.05$) of Normal Protein-energy diet-Carbendazim- and Low Protein-energy diet-Carbendazim- treated groups compared to the Normal Protein-energy diet-Oil-treated (control) group. Histopathology of the testes of the Carbendazim-treated groups revealed germinal epithelial sloughing and occlusion of tubular lumen by immature germinal cells, the severity of the lesions were relatively higher in the Low Protein-energy diet-Carbendazim-treated group. Protein-energy malnutrition aggravated the reproductive toxicity of the male Japanese quail exposed to Carbendazim.

Key words: Carbendazim Effects, Protein-energy Malnutrition, Testis Structure, Testosterone, Quails

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.

Carbendazim has been shown to be gonadotoxic and to induce a decrease in fertility, viability of sperm cells, decreased testes weight and testicular abnormalities in dogs, laboratory mammals and Japanese quails (Hess and Nakai, 2000; Selmanoglu *et al*.,2001; Aire, 2005; Rajeswary *et al*., 2007; Khan *et al*., 2008).

Primarily the gonadotoxic effects of carbendazim is through disruption of microtubule and intermediate filament integrity leading to stage-dependent sloughing of germ cells and abnormal development of the head of elongating spermatids (Hess and Nakai, 2000). Impaired Sertoli cell function and diminished Leydig cellular activities have also been reported through the same mechanism of action and through increased oxidative stress (Markelewicz *et al*., 2004; Moffit *et al*., 2007; Rajeswary *et al*., 2007).

Carbendazim is an endocrine disruptor. There is evidence that reproductive toxicity induced by carbendazim is carried out by androgen and androgen receptor-receptor mechanisms (Lu *et al*., 2004).

Nutrition-associated toxicity problems are both of basic and applied interest and a topic of current concern. The role of malnutrition and diet in general on bioprocesses, which govern the fate of drug/xenobiotic in the body, is beginning to be understood (Kamala, 1987). It is to be expected that pathologic states induced by protein-energy malnutrition on any organ

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Received: November 2011; Accepted (Revised): April, 2012

would alter the intricate process of drug handling (Saroj, 1986).

Studies of the effects of carbendazim acting in concert with protein-energy malnutrition have not been reported previously. There is a need to contribute to filling this gap in the study of carbendazim toxicity, especially for birds, which have the greatest probability of access to pesticide-treated products by virtue of their position in the food chain. Also, the seasons of protein-energy deprivation, especially in developing countries, coincides with periods during which this pesticide-treated feedstuff are prematurely released into the market The Japanese quail, whose crude protein requirement of 23.08% is relatively high (Soares *et al*., 2003), is a good candidate for this study which investigates the interaction between ecotoxicity and protein malnutrition.

MATERIALS AND METHODS

Experimental animals

Forty male Japanese quails (*Cortunix cortunix japonica*), weighing between 120-151g, aged seven weeks and free from any observable ailment, were purchased from the Quail Breeding Unit of the Nigeria Veterinary Research Institute, Ikire Sub-Station and used for this study.

Feeding and housing

The basic diet consisted of a mixture of maize, bone meal, salt and a mineral/vitamin/trace element broiler premix. Soya, fish meal, limestone and methionine were added to the normal protein-energy diet (24.6% total protein and 3151.60 MCal. metabolisable energy) only. Wheat bran, wheat offal and maize offal were added to the low protein-energy diet (4% total protein and 1026 MCal. metabolisable energy) only. The proportions of the components of both diets are presented in Table 1 and the mean proximate analyses of the two diets are shown in Table 2. The feed rations and drinking water were supplied *ad libitum* to the birds.

The birds were kept in galvanized wire mesh cages, under hygienic conditions, in four groups of ten animals each.

Experimental design

The 40 male Japanese quails were randomly assigned to two dietary protein-energy groups; Normal Protein-energy (NP) and Low Protein-energy (LP) diets (Tables 1 and 2). Each dietary group was further divided into two groups, each containing 10 quails, as follows: (i) an untreated control group that received vehicle, corn oil, only (NPO, LPO) and (ii) a treated group that received Carbendazim (NPC, LPC). All birds were placed on the appropriate diet one week before commencement of the experiment.

Table 1
Composition of normal and low protein diets used

Component	Normal Protein %	Low Protein %
Maize	47.9	67
Soya	21.6	-
Bone Meal	1.6	8
Full Fat Soya	17.5	-
Soya oil	3.8	-
Limestone	0.8	-
Fish Meal 65%	8.03	-
DL Methionine	0.2	-
Broiler premix	0.75	0.75
Salt	0.25	0.25
Wheat Offal	-	22
Maize Offal	-	1
Wheat Bran	-	1
TOTAL	**100.00**	**100.00**

Table 2
Proximate analysis of diets used

Component	Normal	Low
Crude Protein	24.60%	4%
Metabolizable	3151.60 MCal	1026 MCal
Energy	128.1	2.57
Energy-Protein	1.22%	1.06%
Ratio	0.49%	0.42%
Ca	1.56%	0.64%
P	0.66%	0.37%
Lysine		
Methionine		

Preparation of chemical and dosage

Ninety-seven percent pure Carbendazim (Aldrich Chemical Company Inc., Milwaukee, USA) was used for the experiment. This was suspended in 100% pure corn oil to constitute 80mg of active agent per ml of corn oil.

After seven days of stabilizing the birds on the appropriate diet, the NPC and LPC groups were weighed and administered by oral gavage with Carbendazim (400 mg/kg body weight) suspended in corn oil. The control groups (NPO and LPO) were also weighed and received only corn oil (1ml/200g body weight) by oral gavage.

Blood collection, sacrifice and harvest of organs.

On Day 08 post-administration, all the birds were weighed using a digital balance (Scout Pro. SPU 402, OHAUS Corporation, Pine Brook, New Jersey, USA). Each quail was deeply anaesthetized with a

combination of Ketamine and Diazepam. Blood samples were collected by cardiac puncture into Lithium heparinized tubes, centrifuged at 3000rpm for 10 minutes using a centrifuge (CF-405 Gallenhamp, England). The supernatant was collected for plasma testosterone assay using commercially available kits.

The testes were removed from each bird, wiped dry of fluid, using a filter paper, and weighed on a digital balance. The relative weight of the testes was calculated as a percentage of the body weight. Each testis was also examined for the presence of gross lesions. Testicular tissues were obtained from birds in each group and fixed in Bouin's fluid for 48 hours. These tissues were processed for histopathological examination using the routine paraffin-wax embedding method. Sections, 5 µm thick, were stained with Haematoxylin and Eosin and observed under the light microscope for histopathological changes.

Statistical Analysis.

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

RESULTS

Behavioural alterations

Within few minutes of Carbendazim administration, quails of the LPC and NPC 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 (LPO and NPO groups) but they were very mild. In both cases the signs returned to normal within a day after administration.

Relative testes weights

The relative testes weights of the quails in the various groups are presented in Figure 1. The relative testes weights of the Carbendazim-treated (LPC and NPC) groups were lower than those of their corresponding diet control (LPO and NPO) groups. However, only the reduced relative testicular weights observed in the LPC and the NPC groups were significantly different ($p<0.05$) from the normal protein-energy diet control (NPO) group as shown in Figure 1.

Plasma testosterone levels

The plasma testosterone levels of the quails that were fed with either Low Protein-energy or Normal Protein-energy diets and dosed with Carbendazim are as presented in Figure 2. Generally, the plasma testosterone concentrations of the Carbendazim-treated (LPC and NPC) groups were lower than those of the oil-treated (LPO and NPO) groups, irrespective of the dietary status. There was a significant decrease ($p<0.05$) in the plasma testosterone level of the LPC and the NPC groups relative to that of the NPO group. However, there was no significant difference ($p>0.05$) between the plasma testosterone levels of the LPO, LPC and NPC groups as well as between the LPC and the NPC groups.

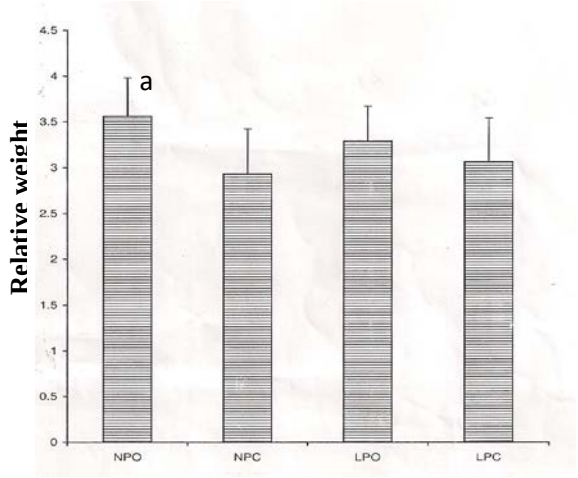

Figure 1:
Mean ± SEM relative testis weights (%) of Quails treated with Carbendazim and fed with either Low Protein-energy or Normal Protein-energy diets. Means with different superscripts are significantly different ($p < 0.05$)

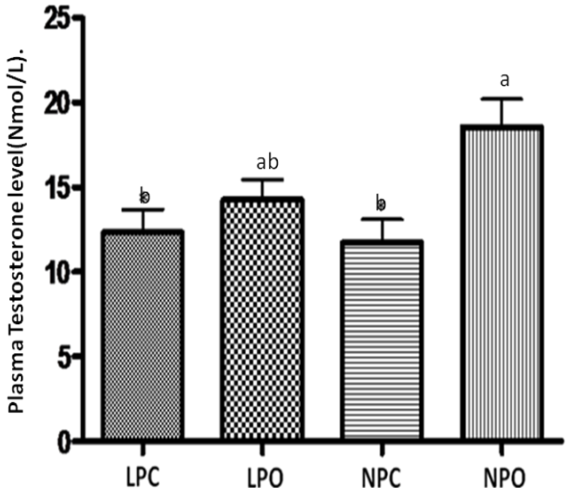

Figure 2:
Mean ± SEM plasma testosterone (Nmol/L) concentrations of male Quails treated with Carbendazim and fed with either Low Protein-energy or Normal Protein-energy diets. Means with different superscripts are significantly different ($p< 0.05$)

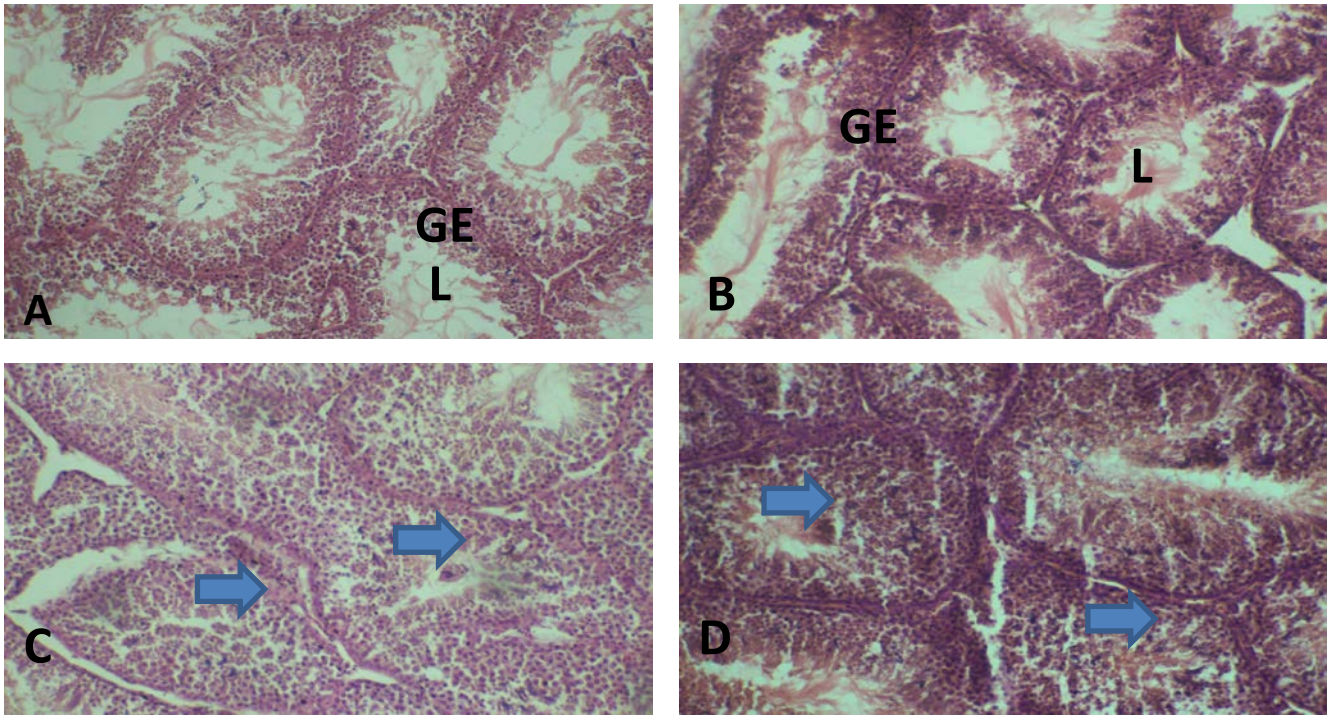

Figure 3:
Photomicrographs showing seminiferous epithelium of the testes of quails treated with Carbendazim and fed with either low protein-energy or normal protein-energy diets. A**:** Testis of the Normal Protein-energy diet-Oil (NPO) / positive control group showing normal germinal epithelium (GE) and seminiferous tubular lumen(L); B: Normal germinal epithelium (GE) and seminiferous tubular lumen (L) as seen in the Low Protein-energy diet-Oil (LPO) / negative control group; C: Seminiferous epithelium of the Normal Protein-energy diet-Carbendazim- treated (NPC) group showing multiple foci of germinal epithelial erosions and sloughing(arrows)**;** D: Focally extensive areas of tubular disruption and germinal epithelial sloughing (arrows) observed in the Low Protein-energy diet-Carbendazim-treated (LPC) group (Haematoxylin & Eosin).

Histopathology of the testis

All histological features described (Fig. 3) were seen in about 60% of animals in each group. The testis of the NPC group had multiple foci of seminiferous tubular degeneration characterized by defoliation of germinal epithelium into the lumen with presence of immature spermatic stages in the seminiferous tubular lumen.

The testis of the LPC group showed lesions that were very similar to those of the NPC group with focally extensive and greater disruptions of the seminiferous tubular structure. The seminiferous tubules of the control groups (NPO and LPO) did not show any observable lesions.

DISCUSSION

Quails and other food birds get exposed to Carbendazim through consumption of post-harvest dressed seeds. The behavioural alterations characterized by depression, reduced crowing, feeding and drinking**,** which occurred after a few minutes of Carbendazim administration had been reported previously (Aire, 2005; Khan *et al.*, 2008). However, some of these alterations could be attributed to the stress of handling and the oil-based oral infusion, as the control groups given corn oil also manifested some slight alterations after dosing. There were no diet-related differences in these behavioural alterations

Reduction in relative testicular weight in Japanese quails on Carbendazim administration has been reported previously (Aire, 2005; Khan *et al.*, 2008). And the current work confirms these earlier reports. The dietary status of the quails, however, did not play a very significant role in this regard.

The significantly lower mean testosterone values observed in the Carbendazim-treated groups (LPC and NPC), compared with the Normal Protein-energy diet-Oil (NPO) control group lends credence to the relationship of Carbendazim treatment with testicular weight and function. Carbendazim disrupts the production of spermatozoa and damages testicular structure and function in adult rats and Japanese quails (Hess and Nakai, 2000; Selmanoglu *et al.*, 2001; Aire, 2005; Khan *et al.*, 2008). Decrease in plasma testosterone concentration could be attributed to the toxic effect of Carbendazim, a reported endocrine

disruptor that acts through androgen and androgen receptor-receptor mechanisms (Lu *et al.*, 2004).

Sloughing of the germinal epithelium and occlusion of the lumen of the seminiferous tubules, seen in both treated Carbendazim-groups, have been reported previously (Aire, 2005). Within the scope of this study, in about 60% of test animals, the lesions observed were similar in both LPC and NPC groups. However, the lesions were more severe in the LPC than the NPC groups; an indication of a dietary effect on the reproductive toxicity of Carbendazim in the Japanese quails.

In conclusion, protein-energy malnutrition potentiates the deleterious effects of reproductive toxicity of Carbendazim at 400mg/kg body weight in the Japanese quail. Quite a lot of attention has been focused on the reproductive toxicity of Carbendazim. This study has, therefore, dealt with an important factor on this focus because Carbendazim exposure and its effects might vary across geographical zones based on either availability or non-availability of adequate sources of protein rich diets.

Acknowledgement

The authors thank Dr T. A. Aire, School of Veterinary Medicine, St George's University, Grenada, for the donation of the pesticide, Carbendazim, used in this study

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