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The acute effect of methyl-2-benzimidazole carbamate on haematological parameters in the Japanese quail (*Coturnix coturnix japonica*)

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SUMMARY

Methyl-2-benzimidazole (carbendazim) is widely used fungicide in the field and post-harvest crops for control of fungal diseases. It inhibits microtubule assembly by binding to the β -tubulin sub-unit of the microtubule. This study was carried out to assess the effect of carbendazim on haematological parameters in the Japanese quails. Carbendazim in sunflower oil was administered orally to mature Japanese quails at dosages of 0 mg/kg (control), 25mg/kg, 100mg/kg, 400mg/kg and 800mg/kg. Blood samples were analysed for total plasma protein, total red blood cell count (RCC), haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), haematocrit (PCV), total white cell count (WCC) and differential white cell count. Carbendazim generally decreased the mean values of total plasma protein, RCC, WCC, as well as differential white cell count. In addition, carbendazim significantly decreased mean values of PCV and MCV ($P \leq 0.05$). The results of the current study indicate that carbendazim has a potential effect on haematological parameters in exposed birds. The use of this chemical should be monitored to reduce environmental contamination which might be the source of exposure to wild animals

Keywords: Carbendazim, haematological parameters, total plasma protein, Japanese quail

INTRODUCTION

Several studies have shown incidences of poisoning in both wild and zoo animals due to environmental contaminants (Anderson, 1968; Feldman and Kruckenberg, 1975). These environmental contaminants include chemicals used as fungicides, pesticides and herbicides. Methyl-2-benzimidazole carbamate (carbendazim[®]) is a derivative of benzimidazole group of fungicides, which are N-substituted esters of carbamic acid (carbamate). Carbendazim can also be produced from benomyl by dissociation when dissolved in water. According to a report by the International Program on Chemical Safety (IPCS) of 1986, benomyl

and carbendazim became commercially available in 1970. Since then, both chemicals have been used as fungicides on ornamental plants, vegetables, fruits and cereals. Studies on the biodegradation of benomyl and carbendazim have shown the presence of metabolite residues in plants (Still and Mansager, 1975). According to Finnish National Board of Health report (1982), the level of benomyl and carbendazim metabolites in food and potable water is increasing. IPCS report (1986) showed presence of carbendazim metabolites in the surface and ground water. Pico *et al.* (2007), reported presence of carbendazim metabolite residues in fruits. Due to the increasing levels of

benomyl and carbendazim metabolites in the environment, it is likely that aquatic and terrestrial organisms are being exposed to these chemicals.

Exposure to carbendazim has been reported to inhibit haematopoiesis in male rats (Selmanoglu *et al.*, 2001). In addition, the effect of carbendazim on haematological parameters were also reported in Wister rats after being fed repeated doses of carbendazim in the diet for 28 days (Jacobsen *et al.*, 2004). Limited studies have been carried out to identify and characterize the effect of carbendazim on the haematological parameters in birds. Therefore, this study was designed to investigate the acute effect of fungicide carbendazim on haematological parameters in the Japanese quail.

MATERIALS AND METHODS

A total of 25 adult Japanese quail were used in this study. The birds were purchased from Irene Improvement Research Farm, Pretoria. After acclimatization for two weeks, birds were randomly divided into five groups i.e. one control and four treatment groups according to the administered dose. Each group contained five birds. In the treatment group, methyl-2-benzimidazole carbamate (97% Sigma Aldrich) was dissolved in sunflower oil and administered once *per os* at a dosage of 25mg/kg, 100mg/kg, 400mg/kg and 800mg/kg body weight. The control birds were given only the sunflower oil orally. After treatment, birds were returned to their respective cages and observed for clinical and/or behavioural changes. Food (growers mash containing maize grain) and water were provided *ad libitum*. Light was controlled at a ratio of 14: 10 hours (light and darkness) throughout the experiment.

After 48 hours post-exposure to carbendazim, 1 ml of blood was collected from wing vein into EDTA coated tubes between 08.00 and 10.00 a.m. Thereafter the blood was analysed for haematological parameters using an automatic counter (Coulter T890, Beckman) in the clinical pathology laboratory of the University of Pretoria, Onderstepoort. The parameters analysed included total protein, total red cell count (RCC), haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), haematocrit (PCV), total white cell count (WCC) and differential white cell count (heterophils, lymphocytes, monocytes, eosinophils and basophils). The protocol for this study was approved by the Animal Use and Care Committee (AUCC) of the University of Pretoria with approval number V031-07.

DATA ANALYSIS

Mean values of different blood parameters were analysed statistically using one way analysis of variance (ANOVA). A probability of 5% was considered to be significant.

RESULTS

Clinical and behavioural changes

In the control group, sunflower oil did not cause any changes on the behaviour of birds. In all treatment groups however, birds showed signs of lethargy, as well as droopy wings following administration of carbendazim. These behavioural signs disappeared within 20 minutes after returning the birds into their cages.

Haematological parameter changes

Table 1 summarizes mean values of haematological parameters of the control and carbendazim-treated birds. The results show a general decrease in the mean values of Hb, RBC, WCC, heterophils and lymphocytes post-exposure to 25 mg/kg, 100 mg/kg, 400 mg/kg and 800 mg/kg bodyweight carbendazim. When compared to the control, this decrease was not statistically significant ($P < 0.05$). Similar changes were recorded on values of MCHC when compared to the control. When comparing between treatment groups,

changes of MCHC values were significant between 100mg/kg and 800mg/kg bodyweight. In addition, significant changes were recorded on PCV values at doses of 25 mg/kg, 100 mg/kg and 400 mg/kg bodyweight carbendazim ($P < 0.05$). Significant changes were also recorded on MCV values at a dose of 100 mg/kg bodyweight carbendazim. Administration of carbendazim increased mean values of circulating monocytes. The increased values of monocytes were statistically significant in all treatment groups ($P < 0.05$). The mean values for eosinophils and basophils were generally unchanged.

Table 1. Mean \pm Standard deviation (SD) values of haematological parameters and plasma protein of Japanese quail in control and carbendazim-treated birds

Parameter	Control	25 mg/kg	100 mg/kg	400 mg/kg	800 mg/kg
HB (g/dl)	210.75 \pm 19.79	188.75 \pm 16.68	190 \pm 12.94	204.5 \pm 23.53	205.75 \pm 3.1
RBC (x 10 ¹² /L)	3.75 \pm 0.26	3.43 \pm 0.21	3.55 \pm 0.14	3.65 \pm 0.39	3.66 \pm 0.07
PCV	49.13 \pm 3.79*	37.5 \pm 3.11*	33.88 \pm 2.02*	47 \pm 6.35*	49.13 \pm 1.93
MCV	150 \pm 3.37*	151.25 \pm 0.96	138.75 \pm 4.99*	145.75 \pm 5.32	148.25 \pm 3.10
MCHC	37.53 \pm 0.75	37.63 \pm 1.26	40.23 \pm 0.17 ^b	38.4 \pm 0.86	37.73 \pm 0.48 ^b
WCC	9.35 \pm 5.28	5.65 \pm 3.34	5.78 \pm 0.69 ^b	7.2 \pm 5.09	9.45 \pm 2.25 ^b
Heterophils	2.96 \pm 1.57	1.94 \pm 0.72 ^a	1.84 \pm 0.36 ^b	2.2 \pm 1.77	2.87 \pm 0.19 ^{ab}
Lymphocytes	6.09 \pm 3.86	3.78 \pm 2.25	3.84 \pm 1.01	4.56 \pm 3.3	5.87 \pm 2.35
Monocytes	0.06 \pm 0.09*	0.24 \pm 0.2*	0.17 \pm 0.04	0.23 \pm 0.37*	0.2 \pm 0.07*
Eosinophils	0.23 \pm 0.13	0.23 \pm 0.09	0.32 \pm 0.02	0.22 \pm 0.17	0.21 \pm 0.1
Basophills	0.02 \pm 0.03	0.08 \pm 0.1	0.07 \pm 0.05	0	0.05 \pm 0.0
Total plasma protein g/100 ml	42.45 \pm 4.99	42.25 \pm 12.66	38.75 \pm 1.71	41.0 \pm 6.8	36.75 \pm 1.71

*Indicate significant difference from the control ($P < 0.05$)

^{a,b} Indicate significant difference between treatment groups ($P < 0.05$)

Total plasma protein (g/100 ml) changes

There was general decrease in values of total plasma protein post-exposure to carbendazim when compared to the control. However, the observed decrease in total plasma protein was not statistically significant ($P < 0.05$). The lowest mean value was recorded at a dose of 800 mg/kg bodyweight carbendazim. In addition, no significant changes in plasma protein levels were recorded between carbendazim treatment groups.

DISCUSSION

Studies on avian haematology are increasingly becoming an important diagnostic tool for domestic, as well as wild birds. However, much of the haematological information concentrates largely on the normal values of blood parameters (Atwal *et al.*, 1964; Cooper, 1975; Ferrer 1990; Giacomo and Roberta, 1996). According to Ferrer (1990), haematological parameters in birds can be affected by several factors including physiological state, nutrition and seasonal changes. The present study investigated the effect of different doses of methyl-2-benzimidazole carbamate (carbendazim) on haematological parameters in the sexually mature Japanese quails. This report appears to be the first attempt in establishing the effect of cytoskeletal disrupting fungicide on haematological parameters in birds.

In the present study, haematological parameters of control birds were in agreement with findings by Faqi *et al.* (1997). Carbendazim exposure generally decreased mean values of haemoglobin, RBC and MCHC. These observations are in agreement with a report by Selmanoglu *et al.* (2001). According to Selmanoglu *et al.* (2001) carbendazim exposure decreased red blood cell counts in the rat. Contrary to this observation, the effect of carbendazim

on RCC counts in rats was not dose dependent.

It has been shown that carbendazim inhibit haematopoiesis in male rats by interfering with mitotic division in the bone marrow and spleen (Carpenter *et al.*, 1961; Freidman and Platzer, 1978). This could not be the case in the present study. However, the decrease in RCC values observed in this study could be as a result of increased destruction of RBC by carbendazim toxicity. Carbendazim disrupts cellular skeleton by binding to the β -tubulin sub-unit of the microtubules (Burlard and Gull, 1984). A consequence of cytoskeleton disruption is an interference with cell development, division and the maintenance of cell shape (Olmsted and Borisy, 1973). The lowered RCC, PCV and MCV values observed in carbendazim-treated birds could be an indication of anaemia. This hypothesis is supported by Jacobsen *et al.* (2004) that reported the occurrence of anaemia in rats fed carbendazim. In deed, decreased PCV values are normally observed when there is anaemia or haemorrhages (Amand, 1986).

The result of this study shows a general decrease in both total and differential white blood cell counts in treated birds. However, this decrease was not statistically significant. Similar observation was also made in the rat (Selmanoglu *et al.*, 2001). In the rat carbendazim reduced the number of WCC, as well as lymphocyte at a dose of 600mg/kg per day for 15 weeks. In sub-chronic study, carbendazim decreased leukocyte count and lymphocyte after being fed into rat for 30 days (Janardhan *et al.*, 1987). Carbendazim also decreased leukocyte count in the dog after been administered for 90 days (Janrdhan *et al.*, 1988). The increase in the mean values of monocytes observed in the present study is not surprising, similar findings have also been reported in rats following exposure to

low levels of carbendazim (Janardhan *et al.*, 1987).

The results of this study show that administration of carbendazim did not influence significantly the total plasma protein in the Japanese quail when compared to the control. The observed decrease of total plasma protein could be physiologic. This can be explained by the fact that carbendazim possesses indirect effect on plasma protein. It affects DNA synthesis by interfering with metabolic activities involving purine. Consequently, the impact of DNA synthesis inhibition could result into disruption of mRNA transcription, as well as protein translation. In addition, the time period from ingestion of the drug to the sample collection was probably short for the effect to be detectable. According to a research report by Selmanaglu *et al.* (2001), the effect of carbendazim on albumin levels were detected in the rat after being administered daily for 15 weeks.

In conclusion, this study has highlighted the acute effect of methyl-2-benzimidazole carbamate on the haematological parameters of the Japanese quail. Based on the fact that carbendazim is still used in the field, it is probably that wild birds are also affected. Therefore, further study is needed to assess sub-chronic exposure to carbendazim on haematology using repeated doses.

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