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EFFECT OF SOME PESTICIDE FORMULATIONS ON BLOOD BIOMARKERS, EGG QUALITY AND EGG PRODUCTION OF *COTURNIX COTURNIX* JAPONICA

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ABSTRACT

Pesticide treated seeds may be an important source of food for some birds in times of scarcity and thus a route of pesticide ingestion. The present study was carried out to evaluate the effects of different concentrations of seed dressing pesticide formulations on haematological parameters, egg production and egg quality parameters of Japanese quails. Forty- two adult Japanese quails (*Coturnix coturnix* japonica) were randomly assigned into 7 groups of six quails each. They were fed diets containing equal amounts of millet seeds treated with dress force and apron at 2.0 g/kg, 2.5 g/kg, and 3.0 g/kg of seed for 8 weeks. Results showed significant ($p<0.05$) loss in the weight of the birds treated with dress force while weight gain in the apron star-treated quails was significantly lower than the control. The pesticide formulations induced hemotoxicity in the quails dose-dependently including anaemia and leukocytosis. Dress force treated quails recorded significantly lower egg production rate (18.00 ± 4.06 and 17.11 ± 1.04) compared to apron star (40.67 ± 3.23 and 29.00 ± 2.96) at the 2.0 g/kg and 3.0 g/kg doses respectively. Egg weight, shape index, yolk index, and haugh unit of the treated birds were not significantly different from the control birds. The study revealed that the seed dressing formulations were toxic to *C. coturnix japonica* and affected the growth, hematology and egg production even at recommended doses. Thus, careful attention should be given to their application and use of alternative safe bio-pesticides should be encouraged.

Keywords: Apron star®, Dress force®, Pesticide, Japanese quails, Biomarkers

INTRODUCTION

One of the primary sources of environmental pollution is the overuse of pesticides, which have toxic ecological effects on the surroundings (Poudel et al., 2020). Pesticides are one of those agrochemicals that are widely used in agriculture to control different types of pests e.g. insects, unwanted weeds, parasitic nematodes and fungus (Tudi et al., 2021). Pests are major agents which affect agricultural crops and consequently threaten food security. In order to control or reduce the effect of agricultural pests, these pesticides are used to combat the effects at pre- planting and post-harvest stages. Seeds to be planted are treated with different classes of seed dressers such as insecticides, fungicides etc. to ensure adequate germination and emergence (Adejumo et al., 2015). This pesticide dressed seeds may be an important source of food for some birds in times of scarcity, sometimes they are not properly buried or often spilled, which makes them accessible to birds, representing a risk of pesticide intake (Lopez-Antia et al., 2013).

As a result of lack of information of toxicity of these pesticides, death rate has increased in developing countries and people are continuously coming down with serious diseases like cancer, kidney failure, and infertility (Zaluski et al., 2015). Also, overdosage and extensive misuse of pesticide products in Nigeria due to poor pesticide education can be deleterious to non-target avian species (Ojo, 2016). Birds may be exposed to pesticides via ingestion of granular formulations, seeds or foliage, contaminated water, poisoned invertebrates or vertebrates, via dermal contact and through inhalation (Miladinovic et al., 2018).

In human and animal research, hematological profiles are a crucial measure of an individual's physiological status (Memon et al., 2024). Hematology evaluation was employed, particularly in birds, to evaluate nutrition and overall health, identify diseases, forecast how long they would last, and determine how well a treatment intervention would work (Orakpoghenor et al., 2021)

Potential toxicity of pesticides and its adverse effects have been reported in the Japanese quail even at low concentrations (Arshad et al., 2024; Kalsoum et al., 2024). Pesticides in the feed of quails have been observed to affect feed intake, haematological parameters and egg production (Hussain et al., 2020). Decline in some hematology values are associated with swelling of erythrocytes and subsequent hypoxia (Amaeze et al., 2020). Changes seen in several birds due to pesticide toxicity include immunological response deficit, oxidative stress, neurological problems, endocrine disruption, and lesions in diverse organ tissues (Suliman et al., 2020).

Due to the usefulness of quail meat and eggs for humans (Ali & Abd El-Aziz, 2019) and widespread use of pesticide seed dressers (Nuyttens & Verboven, 2015), it is imperative to assess the hazards arising from their exposure to these toxicants at environmentally relevant concentrations to establish toxicity parameters. Dress force and Apron star are among the most commonly used pesticide formulations used to treat seeds before planting and despite several researches on insecticide and fungicide toxicity, there is little information on the impact of their combination for seed dressing on gallinaceous birds.

MATERIALS AND METHODS

Experimental Design

The study was approved by the ethical committee of Ahmadu Bello University, Zaria with approval number of ABUCAUC/2018/058. Forty-two (42) sexually mature (males and females) Japanese quails (Coturnix coturnix japonica) were purchased from Kundilla Veterinary Clinic Kano. The quails were fed commercial poultry feeds (Vital feed brand) during acclimatization and throughout the period of the research. They were randomly divided into seven groups of 6 birds each comprising of 3 males and 3 females and allowed to acclimatize for two weeks. The quails were kept in wired cages of which the litter changed every week throughout the experimental period, at the Zoology laboratory, ABU Zaria.

Dress force and Apron star formulations were obtained from an agrochemical outlet in Zaria.

i. Dress force®: An insecticide-fungicide mixture which comprises Imidacloprid 20%, metalaxyl-M 20% and Tebuconazole 2%
ii. Apron star®: Insecticide-fungicide formulation with active constituent Thiamethoxam 20%, metalaxyl-M 20% and Difenoconazole 2%

Three application doses of both pesticide formulations were used to give six treatment groups; equal amount of millet seeds were treated with the pesticides at the following concentrations, 2.0 g/kg, 2.5 g/kg and 3.0 g/kg of seeds. The first dose (2.0 g/kg) represents a dose below the recommended dose, the second dose (2.5 g/kg) is the recommended dose for cereal coating by the manufacturers and the third dose (3.0 g/kg) is a dose higher than the recommended dose to assess the effects of potential abuse in the pesticides application. Treated seeds and water were provided ad libitum to birds in the treatment groups while the control was fed untreated millet seeds throughout the period of study which lasted 8 weeks.

At the end of the experimental period, blood was collected from the jugular vein of each bird with a 2ml disposable syringe into labeled sample bottles containing ethylene diamine-tetra acetic acid (EDTA) as anticoagulant. Blood indices including hemoglobin (Hb), packed cell volume (PCV), total red blood cell count (TRBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leucocyte count (TLC) and absolute leucocyte count were analyzed using the analyzing machine (Sysmex Hematology Systems, Coagulation Systems).

Statistical Analysis

All values were expressed as mean ± SEM, One-way Analysis of Variance (ANOVA) was used to analyze the different parameters using SPSS version 20.0. Duncan's multiple ranged test (DMRT) was used to separate the treatment means and differences were considered significant when p< 0.05. Student T-test was used to compare the mean effect of both toxicants at different dose levels.

RESULTS AND DISCUSSION

Results

Effect of seed Dressing Pesticides on Weekly Body Weight of C. Coturnix Japonica

There was a significant difference (p<0.05) in the mean body weight of the control and treated birds, the birds fed Dress force® treated seeds recorded significant weight loss from week 1 to 8 as shown in (Table 1); significant difference in the mean body weight of the control and Apron star® treated birds was observed from the 5th to the 8th week. Birds treated with the recommended and highest doses recorded significant differences in their body weight compared to the control (Table 2).

Table 1: Effect of Dress Force on Weekly body weight of c. Coturnix Japonica

Weeks	Treatment			
	Control	**TDF1**	**TDF2**	**TDF3**
W0	140.47±4.19^{a}	142.98±6.59^{a}	142.22±5.45^{a}	146.07±6.34^{a}
W1	144.72±4.08^{a}	119.47±4.39^{b}	118.73±1.46^{b}	119.55±1.85^{b}
W2	152.43±3.97^{a}	118.35±4.84^{b}	118.47±2.99^{b}	119.03±1.94^{b}
W3	159.97±4.62^{a}	120.03±4.13^{b}	120.55±2.73^{b}	120.57±4.50^{b}
W4	163.98±4.72^{a}	122.22±3.56^{b}	119.87±2.14^{b}	118.87±4.31^{b}
W5	167.92±4.72^{a}	124.47±3.07^{b}	121.10±2.61^{b}	119.12±3.83^{b}
W6	172.67±4.47^{a}	126.58±4.17^{b}	122.32±2.97^{b}	121.08±3.80^{b}
W7	177.57±4.73^{a}	127.18±3.83^{b}	124.02±2.87^{b}	119.52±3.44^{b}
W8	179.93±12.01^{a}	127.42±3.90^{b}	123.85±3.17^{b}	119.57±3.48^{b}

Data = Mean ± SEM; Statistical tool: one way ANOVA followed by DMRT; means with different superscripts along rows are significantly different (p<0.05).
TDF1= Treatment Dress force at 2.0g/kg, TDF2= Treatment Dress force at 2.5g/kg,
TDF3= Treatment Dress force at 3.0g/kg, n-6.

Table 2: Effect of Apron Star on Weekly Body Weight of C. Coturnix Japonica

Weeks	Treatment			
	Control	**TAS1**	**TAS2**	**TAS3**
W0	140.47±4.19	145.38±7.85	144.36±3.78	144.08±6.52
W1	144.72±4.08	143.30±7.09	140.78±2.71	139.78±5.30
W2	152.43±3.97	147.57±8.52	144.92±6.32	143.82±6.73
W3	159.97±4.62	153.00±8.56	149.58±6.23	147.03±7.84
W4	163.98±4.72	153.57±8.32	151.88±5.02	146.88±6.89
W5	167.92±4.73^{a}	153.78±7.19^{ab}	148.60±4.15^{b}	146.03±4.90^{b}
W6	172.67±4.72^{a}	158.05±7.47^{ab}	151.28±4.81^{b}	149.50±4.87^{b}
W7	177.57±4.73^{a}	160.72±7.29^{b}	155.58±3.78^{b}	150.97±3.77^{b}
W8	179.93±12.01^{a}	161.50±5.84^{ab}	157.08±3.43^{ab}	152.82±3.56^{b}

Data = Mean ± SEM; Statistical tool: one way ANOVA followed by DMRT; means with different superscripts along rows are significantly different ($p<0.05$).
TAS1= Treatment Apron star at 2.0g/kg, TAS2= Treatment Apron star at 2.5g/kg, TAS3= Treatment Apron star at 3.0g/kg, n-6.

Effect of Pesticides Treated Seeds on Hematological Parameters of C. coturnix japonica

There was significant difference in the mean PCV, Hb, TRBC, TWBC, LYM and HET counts of the control and treated birds. With increase in dose, the treated birds showed a significant decrease ($p<0.05$) in the PCV, Hb and TRBC counts as compared to the control. The WBCs count was increased significantly ($p<0.05$) at the dose of 2.5g/kg and 3.0g/kg in the dress force treated birds and at the dose of 3.0g/kg in the Apron star treated birds. Significant increase in the LYM count was recorded in the dress force treated birds at the dose of 2.g/kg and 3.0 g/kg. HET count reduced significantly in the dress force treated birds, but was increased in the apron star treated birds. There were no significant changes ($p>0.05$) in the mean MON, MCV, MCH and MCHC values of the control and treated birds (Tables 3 and 4).

Table 3: Hematological Parameters of Japanese Quails Fed Dress Force Treated Seeds

GROUP	PCV	Hb	TRBC	TWBC	LYM	HET	MON	MCV	MCH	MCHC
Control	39.20±	13.02±	6.50±	12.16±	82.40±	16.60±	0.80±	60.51±	20.10±	33.21±
	1.99^{a}	0.66^{a}	0.39^{a}	0.52^{b}	1.12^{b}	1.69^{a}	0.37	1.37	0.44	0.04
TDF1	28.20±	9.38±	4.66±	12.60±	85.20±	13.20±	0.83±	60.44±	20.12±	33.27±
	2.31^{b}	0.77^{b}	0.37^{b}	0.44^{ab}	1.07^{ab}	0.80^{b}	0.65	0.43	0.14	0.03
TDF2	26.80±	8.92±	4.56±	13.86±	85.80±	12.00±	1.33±	58.78±	19.70±	33.28±
	2.18^{b}	0.72^{b}	0.35^{b}	0.55^{a}	0.86^{a}	0.84^{b}	0.56	1.28	0.4	0.03
TDF3	26.00±	8.66±	4.48±	13.94±	87.00±	12.40±	1.17±	57.79±	19.26±	33.28±
	2.81^{b}	0.94^{b}	0.42^{b}	0.61^{a}	1.10^{a}	0.93^{b}	0.75	1.38	0.46	0.03

Data = Mean ± SEM; Statistical tool: One way ANOVA followed by DMRT; means with different superscripts along columns are significantly different ($p<0.05$).
TDF1= Treatment Dress force at 2.0g/kg, TDF2= Treatment Dress force at 2.5g/kg, TDF3= Treatment Dress force at 3.0g/kg; PCV = packed cell volume, Hb = hemoglobin, TRBC = total red blood cell count, TWBC= total white blood cell count, LYM= lymphocyte, HET= heterophil, MON= monocyte MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration

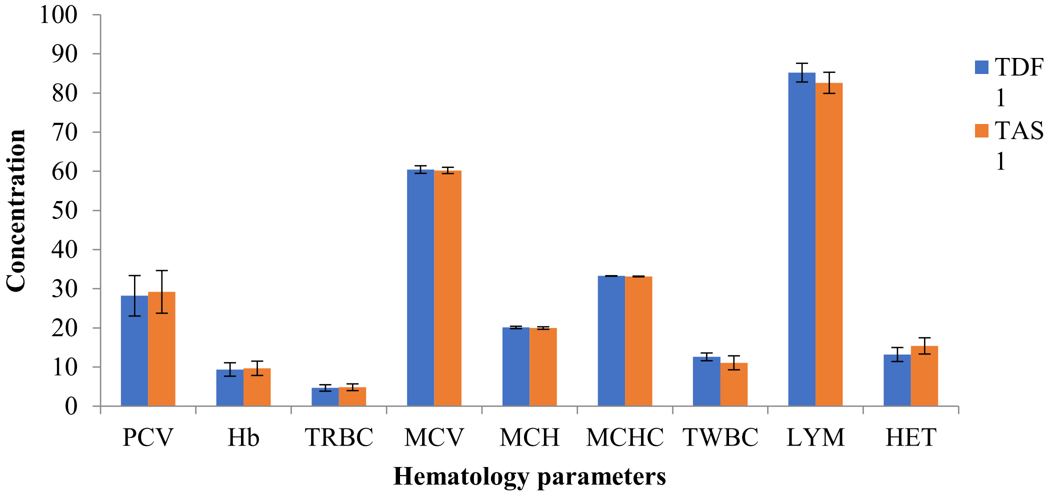
Table 4: Hematological Parameters of Japanese Quails Fed Apron Star Treated Seeds

GROUP	PCV	Hb	TRBC	TWBC	LYM	HET	MON	MCV	MCH	MCHC
Control	39.20±	13.02±	6.50±	12.16±	82.40±	16.60±	0.80±	60.51±	20.10±	33.21±
	1.99^{a}	0.66^{a}	0.39^{a}	0.52^{b}	1.12	1.69^{b}	0.37	1.37	0.44	0.04
TAS1	29.20±	9.68±	4.84±	11.08±	82.60±	15.40±	0.83±	60.22±	19.96±	33.14±
	2.44^{b}	0.82^{b}	0.38^{b}	0.80^{b}	1.21	0.93^{b}	0.65	0.36	0.14	0.04
TAS2	27.60±	9.16±	4.62±	11.60±	83.00±	17.20±	1.33±	59.88±	19.86±	33.16±
	3.10^{b}	1.05^{b}	0.54^{b}	0.90^{b}	1.48	0.86^{ab}	0.56	0.69	0.22	0.08
TAS3	21.40±	7.08±	3.62±	14.56±	84.80±	20.20±	1.17±	58.90±	19.49±	33.09±
	1.66^{b}	0.55^{b}	0.23^{b}	0.74^{a}	1.99	0.58^{a}	0.75	1.44	0.47	0.03

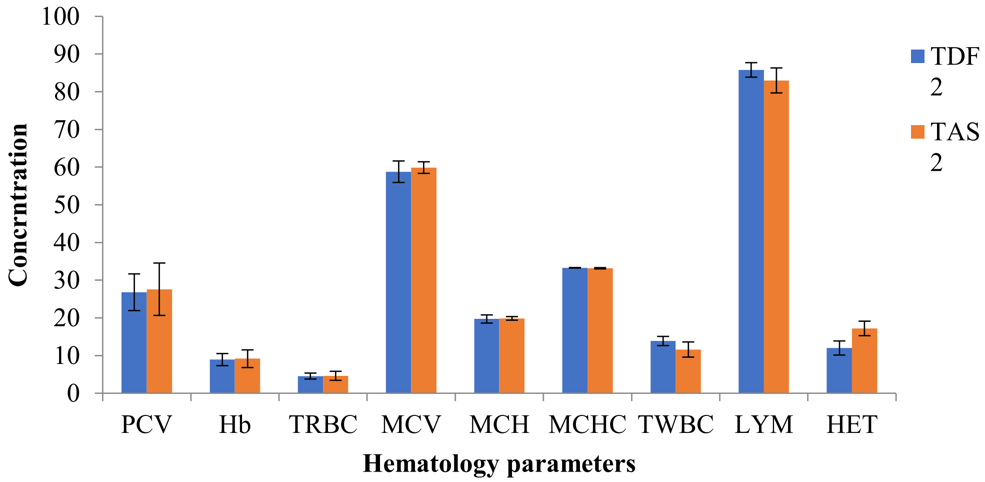
Data = Mean ± SEM; Statistical tool: One way ANOVA followed by DMRT; means with different superscripts along columns are significantly different ($p<0.05$).
TAS1= Treatment Apron star at 2.0g/kg, TAS2= Treatment Apron star at 2.5g/kg, TAS3= Treatment Apron star at 3.0g/kg, PCV = packed cell volume, Hb = hemoglobin, TRBC = total red blood cell count, TWBC= total white blood cell count, LYM= lymphocyte, HET= heterophil, MON= monocyte MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration

Comparison of Hematological Parameters of C. Coturnix Japonica Fed Dress Force® and Apron Star® Treated Seeds

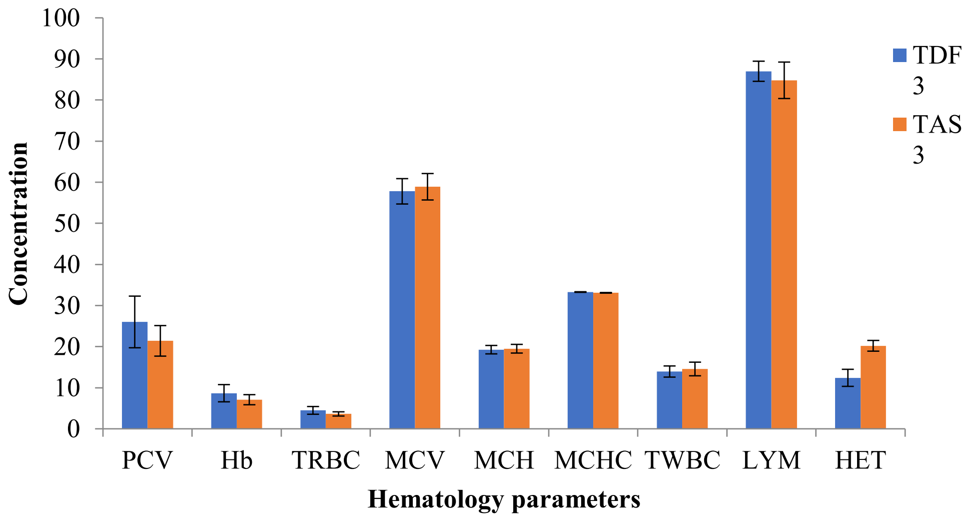
At the lowest dose (2.0g/kg), the hematological parameters did not differ significantly ($p>0.05$) between the toxicants except for MCHC which was significantly ($p<0.05$) higher in dress force than apron star treated quails (Fig 1a). At the recommended dose (2.5g/kg), there was no significant ($p>0.05$) difference in PCV, Hb, TRBC, TWBC, MCV, MCH, MCHC and LYM counts of dress force and apron star treated quails, but HET count differed significantly with dress force recording lower HET value compared to apron star (Fig 1b). The highest dose of 3.0 g/kg showed no significant difference ($p>0.05$) in PCV, Hb, TRBC, TWBC, LYM, MCV and MCH values of the pesticides. MCHC was significantly ($p<0.05$) higher in dress force treated quails. While quails in the apron star group recorded significantly ($p<0.05$) higher value of HET count (Fig 1c).



a) Mean hematological parameters of dress force and apron star treated quails at 2.0 g/kg



b) Mean hematological parameters of dress force and apron star treated quails at 2.5 g/kg



c) Mean hematological parameters of dress force and apron star treated quails at 3.0 g/kg
Figure 1 (a-c): Comparison of the hematological parameters of Dress force and Apron star treated Japanese quails at the different dose levels

Egg production and Egg Parameters of C. Coturnix japonica Fed Pesticides Treated Seeds-Based Diets

There was no significant difference (p>0.05) in the egg quality parameters of birds fed dress force an apron star treated seeds compared to the control. While there was a significant (p<0.05) dose dependent decrease in the percentage egg production of birds fed Dress force and Apron star treated seeds, with the lowest egg production being recorded at the highest dose of 3.0 g/kg (Table 5 and 6).

Table 5: Effect of Different Concentration of Dress Force on Egg Production and Egg Parameters of Japanese Quails

Variables	Egg Weight (g)	Haugh unit	Shape index	Yolk index	Egg Production (%)
Control	8.91±0.13	76.97±1.92	0.78±0.02	0.49±0.05	52.33^{a}
TDF1	8.23±0.76	73.52±0.28	0.74±0.01	0.42±0.01	18.00^{b}
TDF2	8.60±0.19	72.92±0.17	0.80±0.03	0.45±0.05	19.99^{b}
TDF3	8.83±0.24	72.81±0.51	0.74±0.03	0.43±0.02	17.11^{b}

Data = Mean ± SEM; Statistical tool: One way ANOVA followed by DMRT; means with different superscripts along columns are significantly different (p<0.05).

TDF1= Treatment Dress force at 2.0g/kg, TDF2= Treatment Dress force at 2.5g/kg, TDF3= Treatment Dress force at 3.0g/kg

Table 6: Effect of Different Concentration of APRON Star on Egg Production and egg Parameters of Japanese Quails

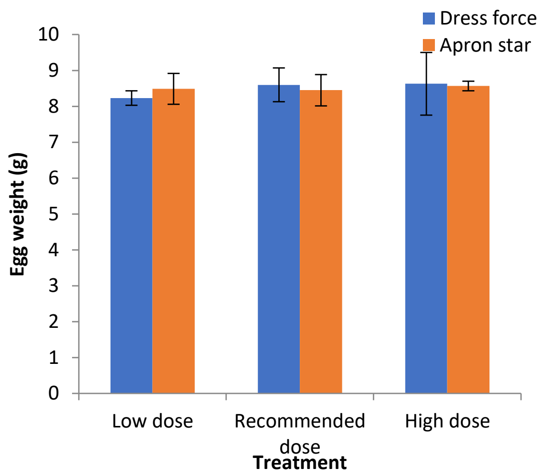
Variables	Egg Weight (g)	Haugh Unit	Shape Index	Yolk Index	Egg Production (%)
Control	8.91±0.13	76.97±1.92	0.78±0.02	0.49±0.05	52.33^{a}
TAS1	8.49±0.16	73.39±0.25	0.76±0.02	0.42±0.03	40.67^{ab}
TAS2	8.45±0.15	73.66±0.26	0.77±0.02	0.46±0.04	31.00^{b}
TAS3	8.57±0.05	73.58±0.61	0.79±0.04	0.41±0.04	29.00^{b}

Data = Mean ± SEM; Statistical tool: One way ANOVA followed by DMRT; means with different superscripts along columns are significantly different (p<0.05).

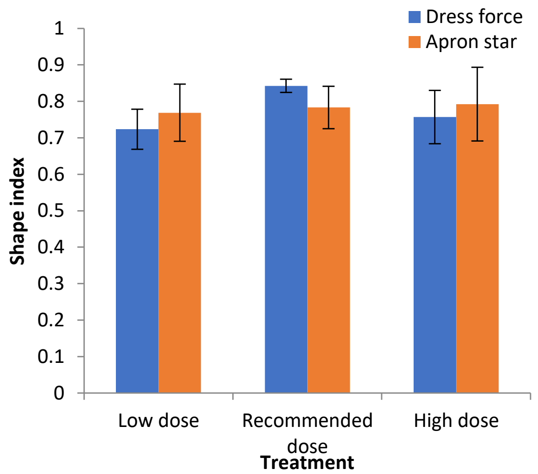
TAS1= Treatment Apron star at 2.0g/kg, TAS2= Treatment Apron star at 2.5g/kg,
TAS3= Treatment Apron star at 3.0g/kg

Comparison of Egg Parameters of C. Coturnix Japonica Fed Dress Force and Apron star Treated Seeds-Based Diets

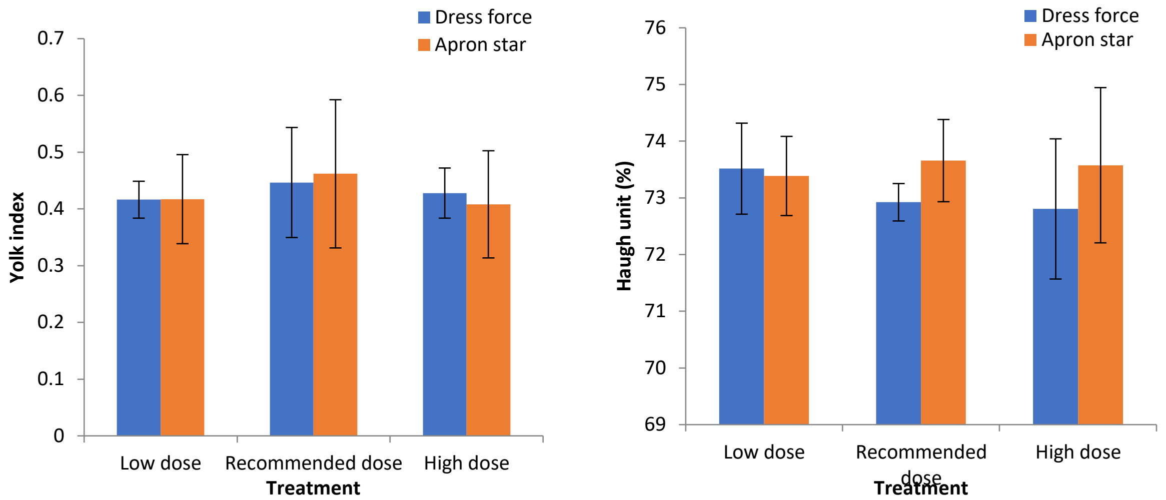
Mean egg weight was not significantly different (p>0.05) in dress force and apron star treated birds at the three doses tested (Fig 2a). There was significant difference in the mean shape index value at a dose of 2.5 g/kg with dress force recording higher value compared to the apron star group. There was no significant difference in shape index at 2.0 g/kg and 3.0 g/kg (Fig 2b). There was no significant difference (p>0.05) in the mean yolk index (Fig 2c) and haugh unit (Fig 2d) of dress force and apron star treated quails. Egg production was significantly higher in apron star treated birds compared to dress force at doses of 2.0 g/kg and 3.0 g/kg, while no significant difference was recorded at 2.5 g/kg (Fig 3).



(a) Comparison of Mean egg Weight of Dress Force and apron Star Treated Quails



(b) Comparison of Mean Shape Index of Dress Force and Apron Star Treated Quails



(c) Comparison of Mean Yolk Index of Dress Force and Apron Star Treated Quails

(d) Comparison of Mean Haugh Unit of Dress Force and Apron Star Treated Quails

Figure 2 (a-d): Comparison of Egg Indices of Japanese Quails Fed Dress Force and Apron Star Treated Seeds at Different Doses

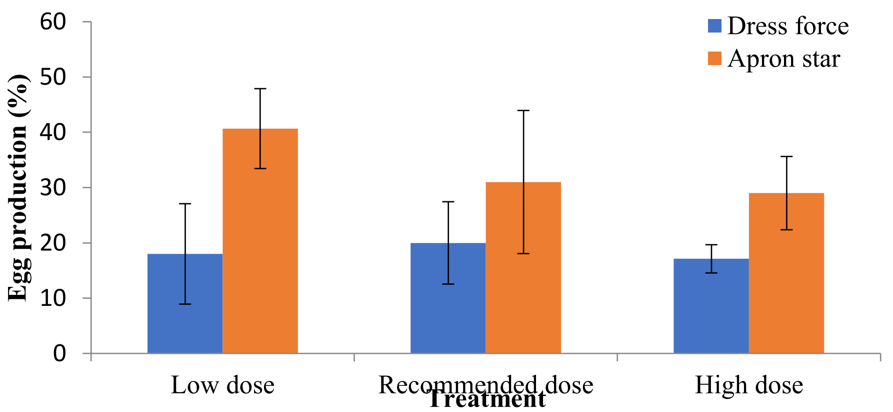


Figure 3: Comparison of Egg Production of Japanese Quails Fed Dress Force and Apron Star Treated Seeds at Different Doses

Discussion

Reduction in body weights of the treated birds was dose related, this might be as a result of reduced feed intake by the quails, rejection of treated seeds, toxic effect of the pesticides and taste aversion. Moreover, previous research has shown that reduction in body weights maybe due to pesticide toxicity or impairment of activation and utilization of nutrients as a result of mal-digestion or mal-absorption caused by gastrointestinal disturbances (Weber, 2003). Similar to this finding, reduction in body weights were also reported in Japanese quails (Arshad et al., 2024; Souda and Abdennour, 2017) and doves (Addy-Orduna et al., 2018) due to pesticide toxicity. Haematological parameters are sensitive index of the physiological changes of an animal to any environmental pollutant as toxic stress shows significant changes in the blood (Lone et al., 2013). Dose dependent reduction in the packed cell volume (PCV), Haemoglobin (Hb) and total red blood cell (TRBC) values of quails treated with the pesticide formulations might be due to the pesticides-induced destruction of the erythrocytes resulting in anaemia. Comparable outcomes have also been shown in the Japanese quails (Kalsoom et al., 2024; Arshad et al., 2024), chickens (Gul et al., 2017; Adejumo et al., 2015) and pigeons (Memon et al., 2024) due to pesticide toxicity.

In the present study, the observed leukocytosis in the quails treated with the highest dose of dress force associated with lymphocytosis and heteropenia could be as a result of its adverse toxic effect and response to the toxicant. Significant increase in leukocyte is an indication of activation of the immune system which could be a positive response for survival. In line with this study, Adejumo et al. (2015) reported that insecticide-treated maize significantly raised WBC counts of layer chickens.

Leukocytosis recorded at the highest dose of apron star treated quails may be due to its direct effect on the number of leukocytes. Significant Increase in heterophil count at the highest dose might be due to acute degenerative changes in various organs and increase in heterophil population while the lack of significant effect in lymphocyte counts even at the highest dose used might be due to tolerance or as a result of a threshold to induce an untoward response.

Similarly, in the findings of Arshad et al. (2024), birds exposed to higher concentrations of acetochlor experienced a significant decrease in RBC, Hb concentration, hematocrit, MCV and MCHC, along with a significant increase in white blood cell count compared to the control group.

Quails that consumed treated seeds containing diet had significantly lower percentage egg production. These adverse effects might be due to anorexia and lack of nutrient utilization, or inhibition of protein synthesis. Lack of significant effect of the formulations on egg quality characteristic might be as a result of dosages and route of administration. The unfavorable effects of the seed dressing pesticides on egg production conforms with the study of Lopez-Antia et al. (2015) where a seed coating fungicide thiram, delayed egg laying in partridges and Elzun et al. (2016) in Japanese quails due to Pirimiphos-methyl toxicity. Adejumo et al. (2015) also reported that seed dressing insecticides did not have adverse effects on egg parameters in chickens; this was attributed to the dose used and the short period of exposure of the birds to the insecticides. Ezeji et al. (2016) reported similar finding in chickens fed diets contaminated with different levels of dichlorvos.

CONCLUSION

Dress force and Apron star induced hemotoxicity including anemia, Leukocytosis in the dress force treated quails was associated with lymphocytosis and heteropenia while leukocytosis in the apron star treated quails was associated with heterophilia. Therefore, the study revealed that the seed dressing formulations were toxic to C. coturnix japonica and affected the growth and hematology of the birds even at recommended doses. Thus, careful attention should be given to their application and use of alternative safe bio-pesticides should be encouraged.

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