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Full Length Research Paper

Actellic 2% dust as pesticide in feed ingredients: Effects on haematological and serum metabolites in growing rabbits

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The study was designed to evaluate the tolerance, survivability, hematological and biochemical indices of weaner rabbits fed diet contaminated with varying levels of Actellic 2% dust. The effects of the pesticide on the clinical state of the animals were also appraised. A uniform diet was compounded from ingredients certified to be apparently pesticide residue-free. This diet was partitioned into 5 sub-diets. The first partition A had no residue, serving as control, while B, C, D, and E had 0.01, 0.02, 0.03 and 0.04% inclusion of the Actellic 2% dust, respectively. A total of 20 rabbits of mixed breeds and sexes were used in the experiment. The animals were balanced for breed, sex and weight in a completely randomized design experiment. Four animals each were placed on each treatment diets for 45 days which were preceded by 3 weeks of acclimatization. Blood was collected and analysed at the end of the experiment. Haematological parameters (PCV, WBC, RBC, ESR, platelets, MCV, MCH, MCHC) and biochemical parameters (urea, serum protein, ALP, SGOT, SGPT, K^+) were comparable in all groups and found not to be statistically (p >0.o5) influenced. Whereas heamoglobin, monocyte, eosinophil, Na^+ and creatinine were statistically influenced ($P < 0.05$). Only one mortality was recorded in treatment group with the highest dose. The results generally showed that weaner rabbits can tolerate the presence of Actellic dust in feed up to 0.03%. At this dose, the Actellic dust does not have much adverse effect on haematological and biochemical parameters. Higher concentration of 0.04% can result in manifestation of toxicity symptoms and sometimes death, such values are rarely naturally present in feeds. However, animals which are able to tolerate high values hardly manifest poor performances.

Key words*:* Actellic 2% dust, feed-ingredients, haematological, serum, metabolites, rabbits.

INTRODUCTION

A number of studies made in the tropics consisting of spot checks of produce and carried out over a 30 year period arrived at losses ranging from 10 - 80% for cereals and over 50% for legumes (Anonymous, 1978). On the basis of subjective assessments and similar piecemeal observations, Appert (1987) reported that total crop losses (from harvesting to consumption) were as much as 40% of production in the hot, humid regions of the world. The greatest losses are due to ravage of insects and other pests after harvesting when grains and other crops are in storage. The need for storage is increasing and methods of storage are changing. Where a farmer still needs to store for himself and family, the traditional small cribs and barns still suffice but it is now necessary to provide facilities to store larger quantities needed for rapidly growing towns, cities and livestock industry. One of the most effective ways of controlling insects in large storage facilities is the use of chemicals called pesticides.

Actellic 2% dust is one of the most popular pesticides used in the storage of grains in Nigeria and its environs. The active ingredient (a.i) has a chemical name O-(2-

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diethylamino-6-methylpyrimidin-4-yl)-O,O-methyl phosphorothioate. It is generally called pirimiphos-methyl for the short form. Bullock (1974) undertook numerous trials where Actellic 2% dust was admixed with grains. The mean initial residues were frequently in the range of 40 - 60% of the nominal dose. He also estimated the half-life to be of the order of 9 months. These residues are obviously transferred to the diets when these grains are used as livestock feeds.

Haematological values in general are commonly used in disease diagnosis in domestic animal health practice (Ogunsusi, 1978). Haematological constituents usually reflect the physiological responsiveness of the animal to its external and internal environments and this is serving as a veritable tool for monitoring animal health (Wekhe, 2000). On the above background and for further studies, Actellic 2% dust impact on rabbit was investigated. Weaner rabbits were fed diets contaminated with graded doses of Actellic 2% dust with the aim to test their tolerance, clinical symptoms, haematological and biochemical responses to the pesticide toxicity.

MATERIALS AND METHODS

The experiment was conducted at the Teaching and Research Farm of the University of Benin, Nigeria. Benin City is located between latitude 6^o and 6^{o}30'N of the equator and longitude 5^{o}40' and 6^{o}E of the Greenwich Meridian with mean annual rainfall of about 2162 mm and a mean relative humidity of 72.5%. (Google earth, 2006).

Twenty rabbits, aged between 6 and 7 weeks of age, of mixed breeds were obtained from farms in Benin City. The average initial weight of the rabbits was 670 g. The animals were housed in individual hutches raised inside a house with concrete floor and that allowed cross ventilation. Each rabbit was provided with metallic feeder hung at a reasonable height in the cage to prevent feed wastage and weighted clay bowls of water.

On arrival, the rabbits were given neo-terramycin in drinking water, a combination of vitamin and antibiotics. This served as anti-stress and prophylaxis. The experiment which lasted for 45 days was preceded by a 3 week adaptation period. A uniform diet was formulated from ingredients certified to be pesticide-free to meet the nutrient requirement of weaner rabbits (NRC, 1977). After compounding the diet, the Actellic 2% dust was admixed to obtain the following concentration: 0 g Actellic dust/kg feed (control); 0.1 g Actellic dust /kg feed (0.01%); 0.2 g Actellic dust /kg feed (0.02%), 0.3 g Actellic dust /kg feed (0.03%) and 0.4 g Actellic dust /kg feed (0.04%) for treatments A, B, C, D and E, respectively.

The twenty rabbits were balanced for weight and randomly allocated to the five experimental treatments. Thus, four rabbits were allocated to each of the five treatments with each rabbit serving as a replicate in a completely randomised design.

At the end of the experiment, blood samples were collected from the marginal ear vein of each rabbit into bottles containing EDTA for haematological analysis and in ice-cooled centrifuge tubes for the analysis of the serum metabolites and enzymes at the University of Benin Teaching Hospital Haematology Laboratory. The haemological parameters; red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and packed cell volume (PCV) were determined by the method of Dacie and Lewis (1977). Serum total protein determination was by the method of Wootton (1964) while serum albumin was assayed by Doumas and Biggs (1972) method. Erythrocyte sedimentation rate (ESR) was determined as described by Jain (1986). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from PCV, Hb and RBC values (Jain, 1986).

Sodium and potassium contents were analyzed by flame photometry (Hawkin, et al., 1954). Blood urea was determined by diacetylmonoxide method of Varley et al. (1980) and alkaline phosphatase was as described by Reitman and Frankel (1957).

All data were subjected to the analysis of variance procedure (SAS, 1995) using randomized complete block design. Mean separation was done where there were indications of significance using Ducan's Multiple Range test (Alika, 2006)

Table 1. Ingredient (gross) composition (%) of experimental diet.

Ingredients	Composition (%)
Maize	35.00
Maize cob	7.00
Palm kernel cake	40.75
Soybean meal	14.00
Bone meal	1.00
Oyster shell	1.50
Salt	0.50
*Premix	0.25

*Supplied per kg diet as indicated by the manufacturer: Composition of the mineral and vitamins mix per kg of diet: vitamin A, 7,000 IU; vitamin D_3, 1,400 IU; vitamin E, 5IU; vitamin K, 2.0 mg; vitamin B1, 1.5 mg; vitamin B2, 4.0 mg; vitamin B6, 1.5 mg; vitamin B12, 0.1mg; Niacin, 15 mg; pantothenic acid, 5.0 mg; folic acid 0.5mg; Mn, 75 mg; Zinc, 45 mg; Fe, 20 mg; Cu, 5 mg; I, 1mg; Se, 0.1mg; Co, 0.2 mg; Choline chloride, 100 mg.

Table 2. Proximate composition (%) of the experimental diet before the admixture of Actellic 2% dust.

Nutrient	Amount (%)
Dry matter	91.10
Crude protein	18.12
Crude fibre	10.32
Ash	5.64
Ether extract	5.70
Nitrogen free extract	60.22
***Calculated composition**	
DE (kcal/kg)	2848.73
ME (kcal/kg)	2591.65

*Olomu (1995).

RESULTS

The gross composition of the basal experimental diet is shown in Table 1. The chemical composition of the diet (Table 2) showed that it was able to adequately meet the nutrient requirement of the growing rabbits. Table 3 gives

Table 3. Haematological response of Rabbits fed different levels of Actellic 2% dust.

Parameter	A (0.00%)	B (0.01%)	C (0.02%)	D (0.03%)	E (0.04%)
Packed cell volume (PCV, %)	36.33	37.00	40.67	37.00	39.67
Haemoglobin (g/dl)	11.17^{b}	11.67^{ab}	11.87^{ab}	11.60^{ab}	13.03^{a}
Total white blood cell ($X10^{12}$/L)	6.50	9.97	6.83	6.33	7.07
Neutrophil (%)	5.33	11.33	9.00	11.67	10.67
Lymphocyte (%)	87.33	88.00	88.00	88.33	85.00
Monocyte (%)	2.67^{a}	0.00^{b}	0.67^{ab}	0.00^{b}	1.33^{ab}
Eosinophil (%)	4.67^{a}	0.67^{bc}	2.33^{abc}	0.00^{c}	3.00^{ab}
Red Blood cell count ($X10^{12}$/L)	5.10	5.17	5.6	5.20	4.97
Erythrocyte Sedimentation Rate (mm/hr)	3.07^{a}	3.00^{b}	4.00^{a}	3.67^{b}	4.33^{a}
Platelets ($X10^{12}$/L)	252.00	305.67	270.67	244.67	209.33
Mean corpuscular volume (fl)	71.33	72.00	72.0	70.00	83.00
Mean corpuscular Haemoglobin (pg)	22.41	22.59	20.94	22.36	27.36
Mean corpuscular Haemoglobin concentration (g/dl)	30.72	31.42	29.71	31.3	32.8

Means within the same row with different superscripts are significantly differently ($P < 0.05$).

the analytical results of the haemological indices. The appraisal of the results as shown in the table indicated that values for the packed cell volume increased with the presence of Actellic 2% dust up to 0.02% level, thereafter the increase was not consistent with corresponding increase of the pesticide. The lowest PCV value (36.33%) resulted from the control treatment and the highest value (40.67%) in the treatment having 0.02% of Actellic 2% dust. There was however no significant difference ($P > 0.05$) among the treatment means. The haemoglobin also increased with the inclusion of pesticide and peaked with the highest dose of the Actellic 2% dust. Treatment E had 13.03 g/dl which was not significantly higher ($P > 0.05$) than treatments B, C and D but significant ($P < 0.05$) from the control treatment (A). The mean values for the white blood cells for treatment A, B, C, D and E respectively were 6.50, 9.97, 6.83, 6.33 and 7.07 (X 10^{9}/L), respectively. There was no significant difference ($P > 0.05$) among the means. The values of percent neutrophils and lymphocytes were highest in treatment D. The lowest percent neutrophil was seen in control while treatment E recorded the lowest lymphocytes. There was no significant difference ($P > 0.05$) among the treatments in both cases. Higher percent values of monocytes were recorded between the extreme treatments: A (2.67%) and E (1.33%). Treatment A was significantly higher ($P < 0.05$) than treatments B and D. Values of eosinophils also followed the order of monocytes. There was a progressive decrease of the eosinophils from the control to absence at treatment D. There was significant difference ($P < 0.05$) among the treatment means.

Although the red blood cells (RBC) increased with increase in Actellic 2% dust in the diet, there was no significant difference ($P > 0.05$) observed among the treatments. The erythrocyte sedimentation rate (ESR) was highest in treatment E (4.33 mm/hr) and least in B (3.00 mm/hr) with the platelets having the highest value in treatment B (305.67 x 10^{12}/L) while treatment E had 209.33 x 10^{12}/L as the least mean value. There was however no significant difference ($P > 0.05$) among the means in both variables.

The results obtained from the computation of the mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) indicated that dosing the rabbit with Actellic 2% dust up to 0.04% did not have any significant difference ($p > 0.05$) on the parameters.

The results of biochemical analysis are presented in Table 4. The values (mg/dl) obtained for urea were 8.40, 17.30, 11.40, 12.00 and 10.80 for treatments A, B, C, D and E, respectively. The lowest urea content was from the control. The serum sodium ion showed a marked increase with increasing inclusion of pesticide. Treatments B and E were outrageously larger (154.10 mmol/L and 110.97 mmol/L) respectively than the value for the control (69.00 mmol/L). The control was however significantly different ($P < 0.05$) from treatment B but not significantly different ($P > 0.05$) from others. Potassium manifested an inconsistent increase with increase Actellic 2% dust. There was however a sharp drop in the value of K^{+} at the highest dose of the pesticide.

The mean values of creatinine (mg/dl) obtained were 0.46, 0.35, 0.34, .39 and 0.39 for treatments A, B, C, D and E, respectively. This showed that the highest value was observed in the control treatment and reduced gradually as the dosage increases to treatment C and then a rise with additional inclusion of the pesticide. Treatment A was significantly higher ($P < 0.05$) than treatments B and C. The values of enzymes, glutamic pyruvic transaminase (GPT) decrease as the contamination of Actellic 2% dust increases. The control treatment had a value of 31.73 IU/L which drastically reduced to

Table 4. Biochemical characteristics of rabbits exposed to Actellic 2% dust.

Parameters	A (0.00%)	B (0.01%)	C (0.02%)	D (0.03%)	E (0.04%)
Blood Urea (mg/dl)	10.80	17.30	11.40	12.0	10.80
Sodium (mmol/l)	69.00[b]	154.10[a]	99.73[ab]	75.60[b]	110.97[ab]
Potassium (mmo/dl)	5.30	6.03	6.00	7.67	5.17
Creatinine (mg/dl)	0.46[a]	0.35[b]	0.34[b]	0.39[ab]	0.39[ab]
Alkaline phosphatase (IU/L)	41.97	35.23	65.80	37.87	49.80
Glutamic-oxaloacetic Transaminase (IU/L)	26.47	19.20	13.70	40.67	22.43
Glutamic-pyruvic Transaminase (IU/L)	31.73	21.47	28.87	15.50	15.33
Total protein (g/dl)	6.10	6.13	5.83	7.57	6.00
Globulin (g/dl)	2.00	2.30	1.87	3.47	2.20
Albumin (g/dl)	4.10	3.83	3.97	4.10	3.80

Means within the same row with different superscripts are significantly different (P < .0.05).

15.33 IU/L for treatment E receiving the highest quantity of the pesticide. This was nonetheless significantly different (P > 0.05) from the others. Other enzymes such as alkaline phosphate (ALP) and glutamic-oxaloacetic transminase (GOT) did not vary significantly among the treatments. The concentration of the serum metabolites was not significantly influenced by the pesticide inclusion.

DISCUSSION

The proximate chemical composition of the diet used in this study is similar to the values reported by Pond et al. (1995) and Bamikole et al. (2000). The nutrient composition of the diet could ensure adequate availability of nutrients for growing rabbits according to the standard of NRC (1977).

No appreciable or consistent effects were noticed in any of the dosage group with respect to packed cell volume (PCV). Although, there was no significant difference (P > 0.05) between the control and the experimental animals, there was increase in PCV with increasing dosage. The highest PCV was from animals in mid-dose which had a borderline with the upper limit of 41% recommended by Sanderson and Christie (1981) for normal values. Turgeon (1993) reported that high PCV results indicate polycethemia or haemoconcentration. There may be a total number of red cells in the body increased or there may be a decrease of fluid caused by excess loss of water by vomiting, diarrhea and other losses. The haemoglobin levels also increased and the highest dose was significantly higher (P < 0.05) than the control. There was an inconsistent rise in the red blood cells with increasing dosage but a sharp fall with the highest contamination. There was however no significant difference (P > 0.05) relating to treatment effects. Results were similar to those reported by Bamikole et al. (2000) and fall within the normal limits documented by Sanderson and Christine (1981).

There was an approximate 53% increase in white blood cells at a slight inclusion of Actellic 2% dust of 0.01% dosage which could be accounted for by the response of the lymphocyte due to the presence of toxic substances. This “stress response” by lymphocyte has been duly acknowledged by Sanderson and Christie (1981). No significant difference (P > 0.05) among the means was however observed. Significant differences (P < 0.05) occurred in monocyte and eosinophil counts which were not related to treatment effects. These were common phenomena observed by several investigators (Gage, 1971; Rajini and Krishnakumari, 1988a).

Erythrocyte sedimentation rate (ESR) showed a rise in values with increase in dosage. Ganti (1998) reported that ESR may be accelerated in certain destructive and inflammatory conditions which may be due to some alteration in the physio-chemical properties of blood. Here, the surface charge of the erythrocytes is altered, rendering them to aggregate together and form rouleaux. The platelets counts or clotting functions inconsistently fluctuated with increasing levels of Actellic 2% dust. They were however not statistically different (P > 0.05) and the respective values were within the recommended limits.

Red cell indices (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) examination revealed no significant effects. The three indices recorded highest values with the highest dosage of Actellic 2% dust (Treatment E) which were larger than recommended maximum limits for rabbits (Sanderson and Christie, 1981). Although, there were no significant difference (P > 0.05) in agreement with Clapp and Conning (1970), Rajini and Krishnakumari (1988a, 1988b), outrageous values of the largest dosage of Actellic 2% dust suggest a possible threat of macrocytic aneamia (Vit. B_{12} and Folate deficiencies),megaloblastic and haemolytic aneamia (Turgeon, 1993).

Blood urea levels were inconsistently eleveated above values from control treatment. Dose-effect relationships do not appear to be present as there was no significant difference (P > 0.05) among the means. This agreed with Rajini and Krishnakumari (1988b). Similarly, data on blood sodium and potassium sporadically increased while only means of sodium were statistically different (P < 0.05) among the treatments. Dose-effect relationships were virtually non-existent despite statistically significant (P < 0.05) decrease in the mean values of the creatinine with increasing dosage. These general inconsistencies in blood metabolites were noticed by several investigators (ICI, 1988; Rajini and Krishnakumari, 1988b).

Investigation of plasma alkaline phosphate (ALP), serum glutamic-oxaloacetic transminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) showed variations which though were not statistically different (P > 0.05) except for slight consistent decrease in SGPT means with increasing dosage which could be correlated with histopathology. Though the values obtained were comparable to the observations and records of Onifade and Tewe (1993) and Bamikole et al. (2000), a wide variation even within the control treatment values, however, renders this interpretation difficult. The results of the serum protein, albumin and globulin were comparable in all the group means. They did not appear to be toxicologically significant as they are consistent with the controls and values reported by Bamikole et al. (2000).

Pathologically, signs of poisoning appeared the second week of dosing and increased in severity as the experiment progressed. Severity was also dependent on quantity of Actellic 2% dust inclusion. These included occasional episode of vomiting, watery stools, abnormal gaits, loss of appetite, dullness, rough fur and loss of hair. Some of these clinical signs were acute and persistent throughout the experimental period. Several of these persistence were however intermittent. Many authors have reported from a complete absence of clinical signs (Onifade and Tewe, 1993), to mild (Roberts et al., 1983; Chester and Hart, 1986) and acute toxicity (Lock and Johnson, 1990; WHO, 1992). Water consumption and urination could also be said to have increased due to the quantity of water normally left at the end of the day and the level of wetness of feaces on the floor since the animal tend to excrete the residue from the body. Bratt and Jones (1973) observed that rats and dogs had 73 to 83% of the dose of pirimphos-methyl excreted in the urine during the 24 h after dosing. This may also account for the low toxicity.

However, high Actellic 2% dust contamination of 0.04% resulted in 25% mortality of the treatment animals which is comparable to report of Clark (1970) who recorded a cumulative mortality of 3 of 10 in rats when there was a daily dose of 200 mg/kg bw of pirimiphos-methyl.

Conclusion

Actellic 2% dust has protective properties against pests. This study has shown that there is little or no possibility of acute or chronic toxicity. Although the presence of Actellic 2% dust may distort some haematological and biochemical indices, this does not negatively or adversely affect the performance of the animal. High concentration values of about 0.04% in feed can result in manifestation of toxicity symptoms and sometimes death, but such values are rarely naturally present in feed. Actellic 2% dust can therefore be recommended for the storage of feed ingredients as far as it is used as recommended by the manufacturer.

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