Prolonged oral cyanide effects on feed intake, growth rate and blood parameters in rabbits

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Abstract: Twelve adult rabbits bred locally were divided into two equal groups of 6; experimental and control groups. Rabbits in the experimental group were orally dosed with KCN at 3mg/kg body weight for 40 consecutive days. Members in control group were given placebo (distilled water) for the same period. Animals in both groups were offered feed at 90gm/kg/day while ample drinking water was available *ad lib*. Feed consumption and body weight of rabbits in both the groups were recorded. Blood samples were also drawn to determine various hematological parameters. Statistical analysis revealed a non-significant difference of total and daily feed intakes in rabbits of experimental and control groups. Whereas the feed efficiency of rabbits in the experimental group were significantly reduced ($P<0.05$) compared to controls. Likewise a significant decrease in body weight gain of rabbits in experimental group ($P<0.05$) was observed. A non-significant difference ($P>0.05$) was observed in leukocyte count, differential leukocyte count and platelets of rabbits in both the groups. Erythrocyte count, hemoglobin concentration, packed cell volume and mean corpuscular hemoglobin were significantly decreased in treated rabbits. It was concluded that chronic cyanide intake had a deleterious effect on feed efficiency, growth rate and blood components of rabbits.

Keywords: Cyanide; feed intake, erythrocytes, growth rate.

INTRODUCTION

Cyanide is widely distributed in the ecosystem and has been associated with toxic effects in humans and animals. Cyanide toxicity may be the result of intake from food sources, environmental pollution, intentional ingestion, chemical warfare, occupational exposure, homicide, and sometimes through the use of drugs such as nitroprusside and laetrile (Way *et al*., 1984; Watts, 1998).

Cyanide is quickly absorbed from the alimentary tract after ingestion and exerts its toxicity by inhibiting cytochrome oxidase enzyme of electron transport chain. Resultant disruption of electron transport chain and oxidative phosphorylation causes decreased ATP synthesis due to anaerobic metabolism and elevation in lactic acid which lead to tissue anoxia and metabolic acidosis (Solomonson, 1981; Isom *et al*., 1975; Isom and Way, 1974).

Many food plants which are important in economic view point contain high concentration of cyanogenic glycosides and have produced toxic effects of acute cyanide poisoning in animals as well as man. Moreover, cyanide toxicity and its pathologiccal effects still exist in areas where major item of the diet is cyanogenic plants such as cassava and lima beans (Poulton, 1983).

Cyanide intake has been associated with syndromes of the central nervous system (CNS) and thyroid in both animals and humans (Soto-Blanco *et al.,* 2005). Prolonged cyanide exposure has also been associated with reduced growth rate in animals (Tewe *et al.,* 1984; Okolie and Osagie, 1999; Soto-Blanco *et al.,* 2001), low feed efficiency (Okolie and Osagie, 1999), disturbance in thyroid metabolism (Philbrick *et al.,* 1979; Okolie and Osagie, 1999), lesions in liver, kidneys, lungs (Okolie and Osagie, 1999, 2000) and also CNS pathology (Soto-Blanco *et al.,* 2002a; Soto-Blanco *et al.*, 2002b). This paper describes the effect of prolonged cyanide intake on feed efficiency, weight gain and various blood parameters in a rabbit model.

MATERIALS AND METHODS

Twelve local breed adult male rabbits of approximately same age and body weight supplied by University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan were used. Rabbits were housed singly in separate clean metal cages under standard farming practices and were provided with feed (table 1) for two weeks prior to the commencement of the experiment for acclimatization purpose. Animal care and the experimental protocol applied were approved by the Ethical Committee of the University of Veterinary and Animal Sciences, Lahore.

Rabbits were then divided into two groups of 6 viz. experimental and control. Rabbits in control group were

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given feed and distilled water while experimental group were given feed and KCN solution orally at 3 mg/kg/day. The KCN solution was prepared fresh daily. The treatments were given for a period of 40 days.

Typical Rabbit diet was prepared as per formula described by Singh (2005) with slight modification. The feed ingredients used were as follows:

Table 1: Feed ingredients and their percentages used in rabbit feed

Ingredients	Percentage
Maize	30
Wheat bran	53
Cotton seed meal	10
Fish meal	5
Mineral mixture	1.5
Salt	0.5

In addition, vitamin B2, and D3 were also added at 10mg/100 kg mixture.

Feed was given at 90 g/kg/day while clean drinking water was provided *ad lib*. The decayed feed leftovers were removed and discarded on regular basis. Feed intake and body weight of each rabbit of both groups were recorded at day 0, 10, 20, 30 and 40.

Table 2: Total feed intake, daily feed intake and feed efficiency of rabbits in both groups during experiment[†]

Parameter	Group	
	Control (n = 6)	Experimental (n=6)
Total feed consumption (gm)	2353.7±119.504[a]	2544.5±83.354[ab]
Daily feed consumption (gm/day)	58.84±2.987[a]	63.61±2.084[ab]
Feed efficiency	0.08±0.004[a]	0.04±0.001[b]

[†]Values are mean±SE
n = number of rabbits used
Means in the same row bearing different superscript letters (a,b) are statistically significantly different (P<0.05)

Blood samples from rabbits in each group were collected at day 0, 10, 20, 30 and 40. For this purpose fur over the jugular vein was clipped and area was swabbed with methylated spirit. A 24G x 1" needle attached to disposable syringe was quickly inserted into jugular vein and 5 mL blood was drawn. To avoid clotting blood samples were poured into heparinized (green topped) vacutainer and refrigerator until further analysis within the same day.

Leukocytes count, differential leukocytes count, erythrocytes count, hemoglobin concentration, packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined using automated hematology analyzer at University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Table 3: Body weight gain (gm) of rabbits in experimental and control groups at various days of the experiment[†]

Day	Group	
	Control (n=6)	Experimental (n = 6)
10	48.500±2.141[a]	32.166±2.024[b]
20	47.000±1.693[a]	30.500±1.875[b]
30	42.833±2.056[a]	26.000±1.291[b]
40	39.333±1.585[a]	19.666±0.882[b]

[†]Values are mean±SE
n = number of rabbits used
Means in the same row bearing different superscript letters (a,b)are statistically significantly different (P<0.05)

Table 4: Daily weight gain (gm) and net weight gain (gm) of rabbits in experimental and control groups[†]

Group	Daily weight gain	Net weight gain
Control (n = 6)	4.44±0.159[a]	177.67±6.359[a]
Experimental (n = 6)	2.71±0.111[b]	108.33±4.439[b]

Data were analyzed by ANOVA with LSD post hoc to compare means. A *P*-value of <0.05 was considered statistically significant. A statistical software package "SPSS13.00" was used for statistical analysis.

RESULTS

The difference in total and daily feed consumption between CN^- and control group rabbits was non-significant (P>0.05), whereas the feed efficiency of rabbits in the control group was significantly higher (P<0.05) than for rabbits in experimental group (table 2). It was found that body weight gain of rabbits in experimental group was significantly lower (P>0.05) compared to control group at 10, 20, 30 and 40 days (table 3). Daily weight gain and net weight gain of rabbits in the control group was significantly higher (P<0.05) than in the experimental group (table 4).

The difference in erythrocytes count within control group was non-significant (P>0.05) at 0, 10, 20, 30 and 40 days. On the other hand, a significant decrease (P<0.05) in erythrocytes count of experimental group rabbits was observed at day 40 compared to day 0. On comparison between experimental and control group at day 40, the erythrocytes count of experimental group was significantly lower (P<0.05) compared to control group

(table 5). In control group, the hemoglobin concentration was not significantly different (P>0.05) at different days. Contrary to these, hemoglobin concentration of experimental group were significantly decreased (P<0.05) at 20, 30 and 40 days as compare to day 0. Furthermore, the hemoglobin concentration of experimental group was also significantly lower (P<0.05) compared to control group (table 6). Packed cell volume of control group was not significantly difference at 0, 10, 20, 30 and 40 days. Whereas, Packed cell volume of experimental group was decreased significantly (P<0.05) at 30 and 40 days as compare to 0 and 10 days. Likewise, experimental group showed significantly lower PCV than control group (table 7). A non-significant difference (P>0.05) was observed in mean corpuscular volume of control group throughout the experiment period of 40 days. On the other hand, experimental group exhibited significant decrease in MCV at day 40. Mean corpuscular volume of experimental group was also significantly lower (P<0.05) compared to control group (table 8). It was found that mean corpuscular hemoglobin values were non-significantly different (P>0.05) in experimental and control group throughout the experiment period (table 9). No significant difference (P>0.05) was observed in mean corpuscular hemoglobin concentration values of control group during the whole span of the experiment. However, MCHC values of experimental group were significantly increased at day 40 compared to control group (table 10). The difference in leukocyte count, differential leukocyte count and platelets count was non-significant (P>0.05) between experimental and control group. Therefore this data is not presented over here.

Table 5: Erythrocytes count (x10^6/ul) of rabbits in both groups at different days of the experiment†

Group	Values at day				
	0	10	20	30	40
Control (*n*=6)	**5.98±0.314^{a}	**5.88±0.269^{ab}	**5.88±0.256^{abc}	**5.89±0.177^{abcd}	*5.90±0.234^{abcde}
Experimental (*n*=6)	**5.90±0.201^{a}	**5.90±0.161^{ab}	**5.71±0.151^{abc}	**5.44±0.299^{abcd}	*4.90±0.303^{de}

Table 6: Hemoglobin concentration (gm/dl) of rabbits in both groups at different days of the experiment†

Group	Values at day				
	0	10	20	30	40
Control (*n* = 6)	**12.7±0.303^{a}	**12.7±0.307^{ab}	*12.9±0.272abc	*12.8±0.365^{abcd}	*12.9±0.306^{abcde}
Experimental (*n*=6)	**13.0±0.345^{a}	**12.5±0.269^{ab}	*11.8±0.245^{bc}	*11.1±0.303^{cd}	*10.8±0.183^{de}

†Values are mean±SE
n = number of rabbits used
Means in the same row bearing different superscript letters (a,b,c,d,e) are statistically significantly different (P<0.05)
* Means for each day in the same column are statistically significantly different (P<0.05)
** Means for each day in the same column are statistically non-significantly different (P>0.05)

Table 7: Packed cell volume (PCV) (%) of rabbits in both groups at different days of the experiment†

Group	Values at day				
	0	10	20	30	40
Control (*n* = 6)	**38.9±1.262^{a}	**38.4±1.014^{ab}	**38.5±1.327^{abc}	*38.5±1.262^{abcd}	*38.4±1.837^{abcde}
Experimental (*n*=6)	**38.5±1.071^{a}	**38.4±0.715^{ab}	**35.21±0.939^{abc}	*31.9±1.743^{cd}	*27.8±1.902^{e}

Table 8: Mean corpuscular volume (MCV) (fl) of rabbits in both groups at different days of the experiment†

Group	Values at day				
	0	10	20	30	40
Control (*n* = 6)	**65.4±1.620^{a}	**65.5±1.673^{ab}	**65.4±1.064^{abc}	*65.0±0.743^{abcd}	*64.9±0.774^{abcde}
Experimental (*n* =6)	**65.3±1.637^{a}	**66.2±2.972^{ab}	**61.7±1.545^{abc}	*59.2±1.132^{cd}	*57.6±1.269^{ce}

Table 9: Mean corpuscular hemoglobin (MCH) (pg) levels of rabbits in both groups at different days of the experiment†

Group	Values at day				
	0	10	20	30	40
Control (*n*=6)	**21.7±0.862^{a}	**21.9±0.760ab	**22.0±0.779^{abc}	**21.7±0.823^{abcd}	**22.0±1.137^{abcde}
Experimental (*n*=6)	**22.2±1.149^{a}	**21.5±0.585^{ab}	**20.8±0.914^{abe}	**20.7±0.816^{abcd}	**20.1±1.266^{abcde}

Table 10: Mean corpuscular hemoglobin concentration (MCHC) (gm/dl) of rabbits in both groups at different days[†]

Group	Values at day				
	0	10	20	30	40
Control (*n*=6)	$^{**}32.8\pm0.742^{a}$	$^{**}33.3\pm0.734^{ab}$	$^{**}33.5\pm0.789^{abc}$	$^{**}33.4\pm1.380^{abcd}$	$^{**}34.1\pm2.062^{abcde}$
Experimental (*n*=6)	$^{**}33.9\pm1.261^{a}$	$^{**}32.6\pm0.820^{ab}$	$^{**}33.7\pm0.951^{abc}$	$^{**}34.9\pm1.307^{abcd}$	$^{**}39.7\pm2.365^{e}$

[†]Values are mean ±SE, n = number of rabbits used
Means in the same row bearing different superscript letters (a,b,c,d,e) are statistically significantly different (P<0.05)
** Means for each day in the same column are statistically non-significantly different (P>0.05)

DISCUSSION

The reduced feed efficiency of CN^- treated rabbits is in agreement with the findings of Okolie and Osagie (1999) who reported increased feed intake in CN^- fed rabbits with reduced feed efficiency. According to Ibebunjo *et al*., (1992) CN^- retarded muscle development in dogs. Reduction in weight gain with prolonged CN^- exposure has been described in numerous animal species such as dogs (Ibebunjo *et al*., 1992), broilers (Panigrahi *et al*., 1992), sheep (Onwuka *et al*., 1992), pigs (Tewe *et al*., 1984), goats (Soto-Blanco *et al*., 2001), and rabbits (Okolie and Osagie, 1999), as well as rats (Philbrick *et al.*, 1979; Tor-Agbidge *et al*., 1999; Sousa *et al*., 2002). According to Soto-Blanco *et al*., (2001b) goats treated with KCN exhibited reduce growth could be due to reduced T3 levels, since hypothyroidism may lead to decreased growth hormone release (Lloyd *et al*., 1990) as well as decreased number of growth hormone receptors (Koenig *et al*., 1987). Ibebunjo *et al*., (1992) have proposed that CN^- induced reduced growth rate might be the result of methionine reduction by CN^- clearing system, resulting into reduced protein turnover. Furthermore, the SCN (detoxification product) affect the thyroid function by reducing iodine uptake (Bourdoux *et al*., 1978), an effect that has been associated with reduced protein turnover in tissue of rats (Hayase *et al*., 1987). The metabolic effects of organic CN^- are similar to those of inorganic CN^- (Ibebunjo *et al*., 1992; Padmaja and Panikker, 1989). Another hypothesis is that KCN stimulates hypoxia, constraining mitochondrial oxidative phosphorylation, so could affect growth through diminished cellular metabolism (Sousa *et al*., 2002). Nevertheless, the possibility that disturbances of hepatic and renal metabolism could affect growth should not be disregarded (Sousa *et al*., 2002).

Hematological studies may be an important and relatively easy parameter by which to monitor chronic CN^- intoxication (Soto-Blanco *et al*., 2001). Increased hemoglobin concentration, lymphocytosis, and basophilia have been reported in humans (El Ghawabi *et al*., 1975) and goats (Soto-Blanco *et al*., 2001) exposed to CN^-. The lower hemoglobin concentration, RBC and PCV in goats from an experimental group receiving KCN for five months are characteristic of normocytic normochromic anemia (Soto-Blanco *et al*., 2001). The mechanism of this hematological disorder is unclear; however, a possible explanation is a secondary effect of decreased T3 levels, since the same pattern of anemia in goats is produced by hypothyroidism (Reddi and Rajan, 1984). However, a direct action of CN^- on erythorpoiesis should not be ruled out (Soto-Blanco *et al*., 2001).

In conclusion, chronic cyanide intake had a deleterious effect on feed efficiency, growth rate and blood components of rabbits.

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