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Influence of Aluminum Chloride and Ascorbic Acid on Performance, Digestibility, Caecal Microbial Activity and Biochemical Parameters of Rabbits

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Abstract: The effects of aluminum chloride ($AlCl_3$), ascorbic acid (AA) and/or their combination were investigated on the performance, digestibility, nitrogen balance, caecal microbial activity and biochemical parameters in male New Zealand white rabbits. The experimental rabbits were randomly divided into four groups (n=6/ group). The first group was used as a control, the second group was used to study the effect of ascorbic acid, the third group was used to study the effect of aluminum chloride, while the fourth group was given the combination of ascorbic acid and aluminum chloride. The tested doses of aluminum chloride and ascorbic acid were given every other day for 16 weeks. The treatment with $AlCl_3$ resulted in significant ($p<0.05$) decrease in body weight, feed intake, drinking water, nitrogen balance, digestibility coefficients (DM, OM, CP, CF, EE and NFE), TDN and DCP. Also, treatment had significant ($p<0.05$) effects onthe activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AlP), acid phosphatase (AcP) and the concentration of thiobarbituric acid-reactive substances (TBARS) plasma enzymes activity. But, presence of AA alone or combination with $AlCl_3$ could be indication of the improved liver function and protective from the toxicity of aluminum.

Key words: Rabbit, ascorbic acid, aluminum chloride, digestion, biochemical parameters,

INTRODUCTION

Aluminum (Al), the most abundant metal, comprising about 8% of the earth's crust, is found in combination with oxygen, silicon, fluorine and other elements in soil, rocks, clays and gems[1]. Aluminum metal is used as a structural material in the construction, of automotive and aircraft industries, in the production of metal alloys and in the electricity industry in power lines, insulated cables and wiring. Other uses of aluminum metal include cooking utensils, decorations, fencing, highway signs, cans, food packaging, foil and dental crowns and dentures[2]. Aluminum is released to the environment by both natural processes and anthropogenic sources, 5,605,000 Ib of aluminum was released to the environment (air, water and soil) from 264 large processing facilities. Of this total, about 30, 0.9, 69% were released to the air, water and soil, respectively.

Human exposure to large quantity of aluminum in nature and its many uses is made through intake of major sources i.e. drinking water, food residues, cooking utensils of packaging, food and beverage and aluminum-containing medications[3].

Minami *et al*.[4] observed an age-dependent accumulation of aluminum in the aorta and cerebral arteries. Bush *et al*.[5] reported increased concentration of aluminum in serum, bone, liver and spleen and slight increase in the brain. Aluminum has been proposed as an environmental factor that may contribute to some neurodegenerative diseases and affects several enzymes and other biomolecules relevant to Alzheimer's disease. Salts of aluminum may bind to DNA, RNA, inhibit such enzymes as hexokinase, acid and alkaline phosphatase, phosphodiesterase and phosphooxydase[6].

Chemical toxicants may alter nutrients intake, digestion, absorption, transport, or function metabolism. In general, chemicals toxicants can affect nutrition by alteration of the nutrient contents of feed. Toxicants may decrease feed intake by modulation of appetite. In general sense, any substance which disturbs metabolism or physiology sufficiently to cause growth inhibition causes decreased feed intake. These substances also inhibit ATP

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production and therefore inhibit protein and active transport, with a final result of decreased body weight and feed intake[7].

Recent studies were carried out to evaluate the potential role of antioxidant vitamins, such as vitamin C, vitamin E and $-carotene[8,9]. Vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body. Many biochemical, clinical and epidemiologic studies have indicated that vitamin C may be of benefit in chronic diseases such as cardiovascular disease, cancer and cataract, probably through antioxidant mechanisms[10].

Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine and neurotransmitters[11,12]. In addition, vitamin C is used as a cofactor for catecholamine biosynthesis, in particular the conversion of dopamine to norepinephrine catalyzed by dopamine "-monooxygenase[11]. Also,Vitamin C is reducing the negative effects of aflatoxin B_1 on production and reproduction[9]. Vitamin C prevents free radical damage in the lungs and may even help to protect the central nervous system from such damage. It acts against the toxic, mutagenic and carcinogenic effects of environmental pollutants by stimulating liver detoxifying enzymes[13] .

Therefore, this work aimed of studying the effect of aluminum chloride, vitamin C and their combination on rabbits performance, digestion, microbial activity, free radicals and enzyme activities in plasma.

MATERIALS AND METHODS

This research was carried out at Hormone Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University. In this study aluminum chloride ($AlCl_3$) and ascorbic acid (vitamin C, liquid supplement) were used. Aluminum chloride was purchased from Aldrich Chemical Company (Milwaukee wis, USA) and vitamin C (20%) was purchased from Neofarma, Italy (Via Emilia Km 18, No. 1854-47020 Longiano, Fo, Italy).

Twenty four male New Zealand White rabbits (age of 7 months and initial weight of 2920±42 g) were used. Animals were individually housed in cages and weighed weekly throughout 16 weeks experimental period. The chemical analysis of the pellets[14] showed at Table 1. Rabbits were randomly divided into four equal groups (n = 6/group). The groups were control, ascorbic acid (AA, 40 kgG^{1} body weight), aluminum chloride ($AlCl_3$, 34 kgG^{1} body weight , 1/25 LD_{50}) and AA plus $AlCl_3$. The LD_{50} of aluminum chloride when given orally to rabbits was reported to be 400 kgG^{1} BW[15].

Table 1: Approximate chemical analysis of commercial concentrate mixture (% as DM basis)

Items	% DM basis
Organic Matter	92.88
Ash	7.12
Crude Protein	15.77
Crude Fiber	11.23
Ether Extract	3.52
Nitrogen Free Extract	62.36

Blood samples were collected from the ear vein of all animals every week throughout the 16 weeks experimental period. Blood samples were obtained in the morning before accesses to feed and water and placed immediately on ice. Heparin was used as anticoagulant. Plasma was obtained by centrifugation of samples at 860×g for 20 min and was stored at -20°C until used for analysis. Stored plasma samples were analyzed for Thiobarbituric Acid-reactive Substances (TBARS) were measured in the plasma by Tappel and Zalkin[16]. The activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel[17]. While alkaline phosphatase (ALP) and acid phosphatase (ACP) was determined according to Principato *et al*.[18] and Moss[19], respecyively. The Glutathione S-transferase (GST) and Acetylcholinesterase (AchE) was estimated by Habig *et al*.[20] and Ellman *et al*.[21], respectively.

During the last week of the experimental period, the rabbits of each group, fed the same diet, were individually housed in metabolic cages for digestibility and nitrogen balance determinations according to F ekete[22]. These animals were kept for 7 days as a collection period. During this period, faeces and urine were quantitatively collected. Spot samples of fresh urine were stored frozen at -10°C for the analysis of total N. Faeces from each animal was pooled over the 7-d period for determine dry matter and chemical analysis. Caecal contents were removed immediately after slaughter and stored frozen at -10°C until analysis. The complete chemical composition of feed, feed residue, faeces and dry caecal contents were determined according to the AOAC[14]. Nitrogen was determined by micro-kjeldahl method. Total Volatile Fatty Acids (VFA) in the caecal contents were estimated by steam distillation and titration[23]. Ammonia-N was determined in the wet caecal samples by magnesium oxide methods[24].

Data of the experiment for all variables were subjected to ANOVA as a completely randomized design according to Snedecor and Cochran[25]. The significant differences between treatment means were tested by the Duncan, Least Significant Range Test[26].

RESULTS AND DISCUSSION

Body weight, feed intake and drinking water: The results of body weight, feed intake and drinking water (DW) are presented in Table 2. Mean values indicated that treatment with $AlCl_3$ caused significant ($p<0.05$) decrease in Live Body Weight (LBW), Feed Intake (FI) and drinking water as compared to control. Ascorbic acid alone had significant ($p<0.05$) increase in LBW, FI and drinking water. The combination of AA with $AlCl_3$ caused increase in the reduction of LBW, FI and drinking water due to treatment with $AlCl_3$. This suggested that AA had protective effect against aluminum toxicity.

The decrease in LBW, FI and DW of animals treated with $AlCl_3$ are in agreement with the finding of Pettersen *et al*.[27], Llobet *et al*.[28] and Bataineh *et al*.[29].

Also, Albina *et al*.[30] found that rabbits treated with doses of aluminium 25, 100 and 400 kgG^1 showed decrease in body weight gain, especially with 400 mmol. Also, Gomez *et al*.[31] found that treatment with aluminium in drinking water at doses (50, 100 kgG^1 dayG^1) for 6.5 month, caused decreased in body weight of rats. Cherroret *et al*.[32] suggested that the reduction in body weight of treated young rats with aluminium chloride (100 kgG^1 dayG^1) for 5 to 14 days could be attributed to the decrease in feed consumption. Ascorbic acid caused significant increase in body weight, feed intake and drinking water compared to the control group (Table 3). Also, previous studies showed that ascorbic acid supplementation stimulated weight gain in rabbits[9]. Yousef *et al*.[33] found that vitamin C caused significant ($p<0.05$) increase in FI, while the increase in BWG was insignificant of rabbits, the beneficial effects of ascorbic acid which was noted in the present study can be attributed to the antioxidant effects of this vitamin; it is scavenge of oxygen free radicals which are toxic by-products of many metabolic processes[34-36].

Table 2: Effect of ascorbic acid (AA), aluminum chloride ($AlCl_3$) and their combination on live body weight, dry matter intake (DMI) and water intake of male rabbits (means±SE)

	Groups			
Items	Control	AA	$AlCl_3$	AA+$AlCl_3$
No. of animals	6	6	6	6
Initial body weight (g)	2905±186.0	3023±128.0	2933±142.0	2820±61.0
Final body weight (g)	3400^a±113.0	3532^a±107.0	1961^b±067.0	2916^a±60.0
DMI (g dG^1)	137.3^a±001.2	142.8^a±001.4	108.7^b±003.9	129.2^a±02.5
Water intake (mL dG^1)	184^a±001.9	194^a±002.0	145^b±003.2	172^a±01.8

Table 3: Digestion coefficients and nutritive values of male rabbits treated with ascorbic acid (AA), aluminum chloride ($AlCl_3$) and their combination (means±SE)

	Groups			
Items	Control	AA	$AlCl_3$	AA+$AlCl_3$
Digestion coefficients:				
DM	67.95±3.00	70.06±2.85	59.09±4.17	71.32±1.60
OM	68.85±2.98	70.46±2.72	60.10±3.97	71.51±1.47
CP	72.83±2.17	76.39±2.45	66.06±5.12	74.15±1.48
EE	87.14±1.03^a	89.72±0.99^a	83.96±2.02^b	90.27±0.73^a
CF	37.00±6.32	40.90±5.30	25.53±1.69	43.23±3.91
NFE	72.41±2.80	73.17±2.41	65.10±3.00	74.87±1.15
Nutritive value:				
TDN	67.73±2.87	69.34±2.52	59.61±3.75	70.39±1.39
DCP	11.49±0.42	12.05±0.63	10.41±1.15	11.66±0.30
NR	3.28±0.10^a	3.03±0.05^a	4.29±0.24^b	3.66±0.08^a

Means within rows with different superscript letters different Significantly ($p<0.05$)

Digestibility coefficients and nutritive value: Treatment with $AlCl_3$ caused a significant ($p<0.05$) decrease in Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE), Nitrogen Free Extract (NFE), Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP) and caused a significant ($p<0.05$) increase in nutritive ratio (NR) (Table 3). While, treatment with AA alone caused insignificant increase in DM, OM, CP, CF, EE, NFE, TDN and DCP and caused significant ($p<0.05$) decrease in NR. The presence of AA with $AlCl_3$ caused insignificant increase in DM, OM, CP, CF, EE, NFE, TDN and DCP and caused insignificant ($p<0.05$) decrease in NR. The mean values of nitrogen balance (NB), microbial caecal activity (ammonia (NH_3-N) and volatile fatty acids (VFA) of male rabbits (Table 4). Treatment with $AlCl_3$ caused significant ($p<0.05$) decrease in NB and caused insignificant decrease in NH_3-N and VFA. While, treatment with AA alone caused insignificant increase in NB, NH_3-N and VFA. The presence of AA with $AlCl_3$ caused insignificant increase in NB, NH_3-N and VFA. Analysis of variance showed that nitrogen (intake, digested and balance), NR and DCP were significantly affected by treatment ($p<0.01$). Also, EE was significantly affected by treatment ($p<0.05$), while the other parameters were not affected.

The present results agreed well with Pettersen *et al*.[27], Llobet *et al*.[28], Bataineh *et al*.[29] and Albina *et al*.[30] who reported that aluminum caused significant

Table 4: Nitrogen balance and microbial caecal activity of male rabbits treated with ascorbic acid (AA), aluminium chloride ($AlCl_3$) and their combination (means±SE)

	Groups			
Items	Control	AA	$AlCl_3$	AA+$AlCl_3$
Intake	3.46±0.06	3.60±0.06	2.74±0.07	3.25±0.03
Faeces	0.94±0.09	0.85±0.09	0.93±0.11	0.84±0.05
Urine	0.28±0.05	0.28±0.03	0.34±0.02	0.24±0.03
Digested	2.52±0.07	2.75±0.10	1.81±0.18	2.41±0.05
Balance	2.24±0.12^a	2.47±0.09^a	1.47±0.18^b	2.17±0.06^a
Microbial activity:				
NH_3-N (mg/100 mL)	15.12±0.13	15.47±0.35	14.59±0.21	15.72±0.53
VFA (meq/100 mL)	8.83±0.29	9.09±0.09	8.02±0.05	8.74±0.15

Means within rows with different superscript letters different Significantly ($p<0.05$)

decrease in body weight, feed intake, weight gain and drinking water.

Blood biochemical parameters:

Plasma transaminases: Treatment with $AlCl_3$ resulted in (Table 5) a significant ($p<0.05$) increase in the activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT). While, AA caused significant ($p<0.05$) decrease in these enzymes as compared with control. The presence of AA with $AlCl_3$ significantly ($p<0.05$) decreased the induction of AST and ALT activities due to treatment with $AlCl_3$ and this means that AA alleviated its toxicity. Transaminases (AST and ALT) are important and critical enzymes in the biological processes[37]. The increase in plasma AST and ALT of animals treated with $AlCl_3$ (Table 5) are agreement with the finding of Hassoun and Stohs[38], Chinoy and Memon[39] and in El-Demerdash[40]. They found that exposure to $AlCl_3$ caused necrosis to the liver, AST is significantly increase in such cases. It escapes to the plasma from the injured hepatic cells. Also, ALT level indicates the existence of liver diseases, as this enzyme is present in large quantities in the liver. It increases in serum when cellular degeneration or destruction occurs in this organ. Therefore, the increase of these enzymes in plasma are indicative of liver damage and thus alterations in liver function. The decrease in plasma AST and ALT activities of animals treated with AA alone or in combination with $AlCl_3$ could indicate improvement of liver function and protection from the toxicity of aluminium.

Plasma phosphatases: The overall means of the activities of plasma alkaline phosphatase (ALP) and acid phosphatase (ACP) were listed in (Table 5). Data indicated that treatment with $AlCl_3$ caused a significant ($p<0.05$) increase in the activities of plasma ALP and ACP. While, AA caused significant ($p<0.05$) decrease in these enzymes compared to control animals. The presence of AA with $AlCl_3$ caused significant ($p<0.05$) decrease in the induction of ALP and ACP activities due to treatment with $AlCl_3$ and this means that AA had protective effect against the toxicity of $AlCl_3$.

Table 5: Effect of ascorbic acid (AA), aluminium chloride ($AlCl_3$) and their combination on some plasma enzymes of male rabbits (means±SE)

	Groups			
Items	Control	AA	$AlCl_3$	AA+$AlCl_3$
ALP (U L^{-1})	61.5 ± 0.40^{c}	58.4 ± 0.72^{d}	82.4 ± 1.54^{a}	65.4 ± 0.60^{b}
GST (µmol h^{-1})	0.66 ± 0.006^{b}	0.80 ± 0.004^{a}	0.50 ± 0.004^{d}	0.62±0.004^{c}
AST (U L^{-1})	51.8 ± 0.42^{c}	48.4 ± 0.59^{d}	65.9 ± 1.22^{a}	57.3 ± 0.82^{b}
ALT (U L^{-1})	55.7 ± 0.61^{c}	52.7 ± 0.87^{d}	73.1 ± 1.85^{a}	59.9 ± 0.94^{b}
ACP (U L^{-1})	10.9 ± 0.17^{c}	10.1 ± 0.16^{d}	14.5 ± 0.29^{a}	12.4 ± 0.21^{b}
AChE (µmol min^{-1})	2.97 ± 0.07^{a}	2.91 ± 0.09^{a}	1.90 ± 0.07^{c}	2.63 ± 0.05^{b}
TBARS (µmol min^{-1})	2.12 ± 0.034^{c}	1.57 ± 0.04^{d}	2.94 ± 0.08^{a}	2.33 ± 0.10^{b}

^{abcd}Within row overall mean with different superscript letter differ significantly ($p<0.05$)

Alkaline phosphatase (ALP) enzyme is a sensitive biomarker to metallic salts since it is a membrane bound enzyme related to the transport of various metabolites[41]. The activity of ALP is concerned with energy metabolic activities and processes in the body and the decrease in its activity may indicate impaired energy processing of the cells[42]. The increase in the activities of ALP and ACP of animals treated with $AlCl_3$ (Table 5) are in accordance with the findings of Szilagyi *et al*.[43], El-Sebae *et al*.[44] and Ochmanski and Barabasz[6]. Also, El-Demerdash[40] found that the activities of these enzymes were increased in mice fed on wheat containing aluminium residue of 0.2 kg^{-1} BW. Szilagyi *et al*.[42] found that exposure to aluminium caused increase the levels of ALP due to increased osteoblastic activity, provoked by the disturbance of bone formation. Ochmanski and Barabasz[6] reported that aluminium may bind to DNA, RNA and inhibit the activities of acid and alkaline phosphatases. In addition, they reported that the increase in the activity of ALP or ACP in blood might be due to the necrosis of liver, kidney and lung. The decrease in plasma activities of ALP and ACP due to treatment with AA alone or in combination with $AlCl_3$ could be indication to improved liver function and protection from the toxicity of aluminium.

Plasma acetylcholinesterase: The effects of AA, $AlCl_3$ and/or their combination on plasma acetylcholinesterase (AChE) activity during the 16 weeks experimental period are shown in (Table 5). Data indicated that $AlCl_3$ caused significant ($p<0.05$) decrease in plasma and activity compared to control animals. While, AA alone did not change the activity of AChE. The presence of AA with $AlCl_3$ caused significant ($p<0.05$) increase in the reduction of plasma and brain AChE due to treatment with $AlCl_3$ and this means that AA alleviated its toxic effect on the activity of AChE.

The inhibition of AChE activity decreased the cellular metabolism, including deformities of cell membrane and disturbance of metabolic and nervous activity[45,46]. Also, the decreased AChE activity could lead to ionic refluxes and differential membrane permeability[47]. Suresh *et al*.[48] reported that the decrease in AChE activity could be due to the decrease of the enzyme synthesis by the inhibitory nature of toxicant. Obviously, an impaired neurochemical mechanism could limit the muscular activity of the animal, including such vital functions as respiration, with lethal consequences[45]. The decrease (Table 5) in AChE of animals treated with $AlCl_3$ is in agreement with Sarin *et al*.[48], Dava *et al*.[49] and El-Demerdash[40]. Moshtaghie *et al*.[50] reported that Al may interfere with either synthesis of acetylcholinesterase or inhibits choline uptake by synaptosomes. They

reported that the neurotoxicity of aluminum may be as a result of lipid peroxidation. Nayak and Chatterjee[51] reported that aluminum induced accumulation of glutamate or other alterations in enzymes of the glutamate-GABA system may be one of the causes of aluminum induced neurotoxicity. Moreover, Chinoy and Memon[38] referred the inhibition in acetylcholinesterase activity to the effect of $AlCl_3$ on the synaptic transmission.

Plasma glutathione S-transferase: The mean values of plasma glutathione S-transferase (GST) activity as affected by treatment with AA, $AlCl_3$ and/or their combination throughout the 16 weeks experimental period are presented in Table 5. Treatment with $AlCl_3$ resulted in a significant ($p<0.05$) decrease in the activity of GST. While, AA caused a significant ($p<0.05$) increase in plasma GST compared to control. The presence of AA with $AlCl_3$ caused significant ($p<0.05$) increase in the reduction of GST activity due to treatment with $AlCl_3$ and this means that AA alleviated its toxic effect.

Glutathione S-transferases (GSTs) are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotice substrates which have electrophilic functional groups. They play an important role in the detoxication and metabolism of many xenobiotic and endobiotic compounds. The decrease in GST (Table 5) of animals treated with $AlCl_3$ is in agreement with Katyal *et al*.[52], Dua and Gill[53] and El-Demerdash[40].

Thiobarbituric acid-reactive substances in plasma: The overall means of plasma thiobarbituric acid-reactive substances (TBARS) concentrations (Table 5). The data indicated that plasma TBARS significantly ($p<0.05$) increased by $AlCl_3$ treatment. While, AA caused significant ($p<0.05$) decrease in plasma TBARS as compared with control. The presence of AA with $AlCl_3$ caused significant ($p<0.05$) decrease the induction of TBARS in plasma due to treatment with $AlCl_3$ and this means that the presence of AA minimized the hazardous effect of $AlCl_3$.

Free radicals attack cell structures within the body, causing cell membrane, enzyme and DNA damage. As a result, free radicals have been implicated in numerous diseases, examples of which are: cancer, conditions associated with premature birth, atherosclerosis, motor neuron disease, rheumatoid arthritis, liver damage, diabetes, respiratory disease, cataracts and central nervous system disorders[54]. Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics[55]. Also, oxidative damage to biomolecules, such as lipids, DNA and proteins, has been implicated in many chronic diseases, in particular, cardiovascular disease, cancer and cataract[10]. The increase in TBARS of animals treated (Table 6) with $AlCl_3$ is in agreement with Fraga *et al*.[56], Katyal *et al*.[52] and Lall *et al*.[57]. It was found that lipid peroxidation induced by aluminium at sub-lethal levels, alter physiological and biochemical characteristics of biological systems[58]. The decrease in TBARS of animals treated with AA is in agreement with Huang *et al*.[59] who reported that ascorbic acid (500 mg ascorbate dG^{1}) supplementation in nonsmokers reduced lipid peroxidation. Herbaczynska-Cedro *et al*.[60] reported that supplementation with ascorbic acid suppresses leukocyte oxygen free radical production in patients with myocardial infarction. Additionally, AA can protect biomembranes against peroxidative damage.

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