

Investigation Of Protective Role Of Ascorbic Acid On Ochratoxin Toxicity In Growth Performance And Some Biochemical Parameters In Growing Lambs

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ABSTRACT

Twenty 5 months old healthy balady lambs 20 kgm body were divided equally into four groups to elucidate the deleterious effect of ochratoxin on growth performance thyroid hormones and some biochemical parameters. Group (Gp. 1) was the control, Gp. (2) was fed on a ration containing 1 mg ochratoxin/kg ration for 30 successive days. Gp. (3) received 50 mg ascorbic acid /kg b.wt. daily by I/M injection for 30 successive days. Gp. (4) was supplemented with ochratoxin and ascorbic acid by same dose and period. Lambs of each group were weighed individually at the beginning of the experiment and at the end of 1st, 4th and 6th week post ochratoxin supplementation. Body weight gain were recorded, at same periods, blood samples were collected for biochemical analysis.

The present work revealed that, ochratoxin induced a significant reduction in body weight, weight gain, triiodothyronine(T₃), thyroxin(T₄), total protein, albumin and globulin while a significant increase in the serum levels of AST, ALT, alkaline phosphatase, urea and creatinine was reported.

Vitamin C treatment was able to improve body weight, thyroid hormone and biochemical parameters and overcome the toxic effect of ochratoxin

INTRODUCTION

Mycotoxins are structurally diverse group of mostly small molecular weight compounds which are produced by the secondary metabolism of filamentous fungi and are toxic to mammals and poultry (1). The impact of mycotoxins on human and animal health is well recognized. Mycotoxins have a wide range of adverse biological effects (mutagenic, carcinogenic, teratogenic and / or estrogenic) (2). Mycotoxins have an adverse effects on the health status, production and reproduction in ruminants (3).

Ochratoxin was firstly detected by *Merwe et al.* (4). It is secondary metabolites synthesized mainly by some toxigenic species of *Aspergillus* and *Penicillium* (5) that contaminate various raw agricultural commodities. The chemical structure of ochratoxin consists of a dihydroisocoumarin part coupled, via its 7-carboxy group; with L-beta-phenylalanine part (6). Exposure to ochratoxins through diet can produce an acute toxicity to mammalian kidneys, and may be carcinogenic (7). Ochratoxin A affects the liver

through a reduction in the ability to counterbalance oxidative stress which leads to altered gap junction intercellular communication and loss of cell adhesion and polarity. This mild oxidative damage in combination with cytotoxic effects enhance the promotion of liver tumors (8). The target organ for ochratoxin is kidney, as accounted by the selective action on the proximal tubules of the renal cortex (9). The toxin induced kidney damage in rats at a dietary level of 0.2 ppm, (10).

Vitamins are organic micro food materials that are needed for optimal cell, tissue, organ and body functions. Vitamins activate some enzymes which have important roles in protein, carbohydrate and fat metabolisms (11). An important water-soluble vitamin is vitamin C (L-ascorbic acid or L-ascorbate) is an essential nutrient for human and some animal species. It has diverse functions including being an antioxidant (12), exerting positive effects on lipid and iron metabolism (13), and promoting improved immune function (14). Vitamin C makes up an antioxidant system for mammalian

cells. It (ascorbic acid) is considered the most important antioxidant in plasma and forms the first line of defense against plasma lipid peroxidation (15).

The present study was designed to evaluate prophylactic efficacy of vitamin C, to modulate the toxic effect of ochratoxin on growth performance, thyroid hormones and some biochemical parameters in growing lambs.

MATERIAL AND METHODS

Drugs: Vitamin C (Cevaryl®):- Memphis Co. I/M. injection. Each ml of solution contains 100 mg Ascorbic acid (15).

Animals and experimental design

The present investigation was carried out on twenty healthy growing balady lambs aging about 5 months old and about 20 kg body weight. Lambs were obtained from a private farms in Sharkia Province and housed under hygienic conditions. Lambs were divided into four equal groups, 5 lambs each. Gp. (1) was the control group, Gp. (2) was fed on a ration containing 1 mg ochratoxin/kg ration for 30 successive days, Gp. (3) was received 50 mg ascorbic acid /kg b.wt. daily by I/M injection for 30 successive days and Gp. (4) was fed on a ration containing 1 mg ochratoxin /kg ration and 50 mg ascorbic acid /kg b.wt. daily by I/M injection for 30 successive days.

Blood and serum samples

Blood samples were taken from jugular vein at the end of 1st, 4th and 6th week post ochratoxin supplementation. These samples were collected and centrifuged at 3000 r.p.m. for 15 min to separate clear serum, for determination of T3 and T4 (16). Serum total protein (17), albumin (18), globulin was calculated by subtraction of serum albumen from total protein and albumin, transaminases (AST-ALT) (19), alkaline phosphatase (20), serum urea was estimated (21) and creatinine (22).

Body weight

Lambs of each group were individually weighed at the beginning of the experiment and at the end of 1st, 4th and 6th week post ochratoxin

supplementation. Body weight gain was recorded.

Statistical analysis

The obtained data were statistically analysed (23).

RESULTS AND DISCUSSION

Mycotoxins are group of toxic chemical compounds produced by certain strains of fungi when they grow under favourable condition on a wide variety of different substrates (24). These compounds are toxic to animals causing diseases collectively known as mycotoxicosis (25). On the basis of their target organ and pathological manifestation, they can be recognized as carcinogens, hepatotoxins, nephrotoxin, etc. from the obtained result of clinical signs and post mortum lesions, it was obvious that the hepatotoxic and nephrotoxic effect of both mycotoxin (aflatoxin and ochratoxin) (25).

The obtained results showed as recorded in Table 1 a significant decrease in the live body weight and weight gain in lambs fed on ration contaminated with ochratoxin when compared with the control. Cchratoxin can induce decrease in the activity of several enzymes needed for the digestion of feed nutrient (26). The decreased activities of these enzymes could contributed to the malabsorption of nutrients. Another explanation for reduction of body weight and weight gain (28) is that ochratoxin induces a reduction in feed intake which reflected in low weight gain (29). Improvement in the body weight and weight gain was recorded post ochratoxicosis treatment with vitamin C when compared with the normal control vitamin C induced a significant improvement in body weight gain in rabbits (30), which may be due to increasing feed intake, digestibility of nutrients which had biological role in digestive enzyme biosynthesis and activation (31).

Table 1. Effect of ochratoxin and vitamin C on weight gain (Kgm) in lambs (n=5).

Parameter	Initial body weight Kgm	1 st week		4 th week		6 th week	
		Body weight Kgm	Weight Gain Kgm	Body weight Kgm	Weight Gain Kgm	Body weight Kgm	Weight gain Kgm
Gp(1)	20.59±0.41	25.79±0.70	5.2±0.35	31.94±0.79	3.15±0.38	37.08±0.96	5.14±0.62
Gp(2)	20.81±0.70	23.50±0.27*	3.32±0.33**	29.28±0.51	2.78±0.26	32.85±0.81	3.57±0.66
Gp(3)	20.27±0.48	27.49±0.56	7.22±0.52**	31.98±0.68	2.49±0.34	38.72±0.74	4.74±0.70
Gp(4)	20.90±0.38	24.83±0.49	3.93±0.45*	30.37±0.81	2.54±0.42	34.38±0.81	4.01±0.44

*Significant at P < 0.05

** Significant P < 0.01

In the present experiment, a significant decrease in the serum thyroid hormones (T3 and T4) at the 4th week post ochratoxin supplementation was recorded as shown in Table 2. Ochratoxicosis in rats induced significant decrease in T3 and T4 (29). Reduction in thyroid hormone may be due to

toxic effect of ochratoxin on thyroid gland (32). Vitamin C treatment was able to improve thyroid hormone. This could be attributed to the ameliorating action of vitamin C, which may detoxify ochratoxin by its transformation or degradation into detoxified products (33).

Table 2. Effect of ochratoxin and vitamin C on thyroid hormone in lambs (n=5).

Parameter	Gp(1)	1 st week			4 th week			6 th week		
		Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)
T3 (ng/dl)	130.2±1.89	116.73±1.84**	1361±1.39	132.18±1.71	124.2±1.55*	133.1±1.42	130.1±1.84	128.5±1.79	131.4±1.81	130.5±1.98
T4 (ng/dl)	3.83±0.48	2.28±0.50*	4.12±0.63	3.90±0.51	2.40±0.43*	3.92±0.49	3.87±0.49	2.72±0.47	3.80±0.80	3.71±0.64

*Significant at P < 0.05

** Significant P < 0.01

Table 3 showed that serum total proteins, albumin and globulins were significantly decreased in lambs received ration contaminated with ochratoxin. These results came in the same line with that recorded in lambs (34). Ochratoxin induced a significant decrease in the serum total proteins, albumin and globulin in calves (35). Decline in total protein level in lambs fed on ration contaminated with ochratoxin could be due to a decline in the protein biosynthesis as ochratoxin molecule appears to be causes disruption in serum proteins levels due to inhibition of hepatic protein synthesis (36). Ochratoxin is biotransformed into genotoxic derivative damaging DNA molecule. Another factor possibly contributing to the observed hypoproteinemia may be attributed to the renal

leakage of albumin resulting from kidney lesions produced by ochratoxin (37). In addition, it has been reported that ochratoxin may compete with phenylalanine for binding on phenylalanine transfer RNA synthetase enzyme, thus inhibiting protein synthesis (38). Treatment of lambs suffering from ochratoxicosis with vitamin C induce improves in protein profile towards the normal level. Male albino rats treatment with vitamin C showed a significant elevation in protein profile (39, 40). Vitamin C induced an improvement in protein profile in animals suffering from mycotoxicosis which was attributed to the effect of vitamin C on improving feed intake and digestibility of nutrients, resulted in increasing the amino acids% in the blood which are highly important for the protein biosynthesis (31).

Table 3. Effect of ochratoxin and vitamin C on protein profile in lambs (n=5).

Parameter	Gp(1)	1 st week			4 th week			6 th week		
		Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)
T.Protein (mg/dl)	8.06± 0.38	5.47± 0.53**	9.86± 0.62*	7.89± 0.87	6.10± 0.55*	8.65± 0.73	7.97± 0.51	7.89± 0.67	8.20± 0.72	8.10± 0.69
Albumin (mg/dl)	4.90± 0.41	2.99± 0.58*	5.89± 0.13*	4.80± 0.41	3.27± 0.39*	5.05± 0.41	4.85± 0.59	4.72± 0.58	4.95± 0.41	4.98± 0.71
Globulin (mg/dl)	3.16± 0.20	2.48± 0.16*	3.97± 0.22*	3.09± 0.26	2.83± 0.21	3.60± 0.23	3.12± 0.32	3.17± 0.23	3.25± 0.18	3.12± 0.12
A/G Ratio	1.55± 0.30	1.22± 0.17	1.48± 0.25	1.55± 0.29	1.16± 0.24	1.40± 0.28	1.55± 0.31	1.49± 0.34	1.52± 0.33	1.60± 0.35

*Significant at P < 0.05

** Significant P < 0.01

It is evident from the present study that ochratoxicosis in lambs induced a significant increase in the activity of liver enzymes (AST, ALT and alkaline phosphatase), urea and creatinin. Similar results were recorded in earlier study (41) which showed that ochratoxin induced significant increase in liver enzymes activity, urea and creatinin. The higher activity of AST, ALT and alkaline phosphatase associated with ochratoxicosis in the present study. Increased serum urea and creatinine in our study may be due to nephrotoxic effect of ochratoxin due to severe damage in the kidney represented by severe degenerative changes in the proximal and distal convoluted tubules, atrophy in a few areas of glomeruli and hypertrophy of glomerular tuft as well as the presence of hyaline casts in collecting tubules (2,34). On the other hand, our study demonstrated that treatment of lambs ochratoxicosis with vitamin C caused marked amelioration of liver enzymes activity

(ALT, AST and alkaline phosphates), urea and creatinin. Vitamin C play a role in hepatoprotection (42) the important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome p-450, thereby favoring liver regeneration (43). It is suggested that liver cytochrome p-450 is significantly reduced in ascorbic acid deficiency (44). Vitamin C prevent the generation of superoxide anion and hydroxyl radicals which may reduce the oxidative stress and prevent consuming the antioxidant defense system (45). Vitamin C also considered nephroprotective agents based on its antioxidant properties (46).

It could be concluded from the results of this work that vitamin C may provide a safe and practical method to alleviation of ochratoxin toxicity.

Table 4. Effect of ochratoxin and vitamin C on liver enzymes in lambs (n=5).

Parameter	Gp(1)	1 st week			4 th week			6 th week		
		Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)
AST (U/L)	41.34± 1.47	52.08± 1.89**	40.15± 1.34	45.47± 0.77	47.20± 0.86*	41.84± 1.51	43.15± 0.84	43.11± 0.59	41.05± 1.38	42.05± 0.93
ALT (U/L)	30.27± 1.24	39.18± 1.62**	28.21± 0.86	33.55± 0.93	35.21± 0.62*	30.45± 0.71	32.09± 0.65	32.72± 0.48	31.10± 0.38	31.13± 0.93
Alk.Ph. (I.U/ml)	75.28± 1.63	83.74± 1.56**	73.37± 0.93	76.49± 0.72	80.02± 0.38*	76.59± 0.89	75.14± 0.80	77.11± 0.45	74.39± 0.79	75.10± 0.69

*Significant at P < 0.05

** Significant P < 0.01

Table 5. Effect of ochratoxin and vitamin C on kidney function in lambs (n=5).

Parameter	Gp(1)	1 st week			4 th week			6 th week		
		Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)	Gp(2)	Gp(3)	Gp(4)
Urea (mg/dl)	15.64± 1.04	20.55± 0.91**	13.26± 0.67	17.16± 0.79	17.99± 0.30*	15.10± 0.94	15.71± 0.63	16.09± 0.57	15.85± 0.48	15.63± 0.96
Creatinine (mg/dl)	1.46± 0.22	3.10± 0.50**	1.30± 0.24	1.92± 0.29	2.42± 0.37*	1.42± 0.28	1.64± 0.23	2.02± 0.39	1.46± 0.38	1.50± 0.29

*Significant at P < 0.05

** Significant P < 0.01

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الملخص العربي

استبيان الدور الوقائي لحمض الاسكوربيك على سمية الاوكراتوكسين على كفاءة النمو وبعض الوظائف البيوكيميائية فى الحملان النامية

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أجريت هذه الدراسة على عدد 20 من الحملان النامية وبتراوح عمر كل منها حوالى 5 شهور يزن كل منها 20 كجم بهدف دراسة تأثير إضافة الاوكراتوكسين وحامض الاسكوربيك على كفاءة النمو وبعض الوظائف البيوكيميائية فى الحملان النامية، وقد تم عمل البحث على اربع مجموعات متساوية كلا منها يتكون من 5 حملان) المجموعة الاولى غذيت على عليقة خالية من الاوكراتوكسين (مجموعة ضابطة)، المجموعة الثانية غذيت على عليقة مضاف اليها الاوكراتوكسين بجرعة (1 مجم لكل كجم عليقة) لمدة 30 يوم متتاليه. المجموعة الثالثة تم حقنها بالعضل بحامض الاسكوربيك بجرعه 50 مجم/كجم لمدة 30 يوم متتاليه. المجموعة الرابعة غذيت على عليقة مضاف اليها الاوكراتوكسين بجرعة (1 مجم لكل كجم عليقة) بالاضافة إلى ذلك حقنها بالعضل بحامض الاسكوربيك بجرعه 50 مجم/كجم لمدة 30 يوم متتاليه. تم وزن الحملان فى كل مجموعته عند نهاية الأسبوع الأول، الرابع والسادس من نهاية استخدام الاوكراتوكسين وحامض الاسكوربيك وتم أخذ عينه دم من كل حيوان عند نفس المدد السابقه لفصل مصل الدم وذلك لقياس هرمونى التريايودوثيرونين والثيرونكسين بالاضافه الى بعض المؤشرات البيوكيميائية . تشير نتائج الدراسة أن الاوكراتوكسين ادى الى وجود نقص معنوى فى وزن الجسم المكتسب هرمونى التريايودوثيرونين، الثيرونكسين، البروتين الكلى الزلال والجلوبولين ونقص غير معنوى فى النسبة بين الالبومين والجلوبولين وزيادة معنوية فى معدل كلا من الترانس امينيزسس. والفوسفاتيز القاعدى، اليوريا والكرياتين فى مصل الدم ولكن حامض الاسكوربيك قلل وعادل من التأثيرات العكسيه للاوكراتوكسين . نستخلص من هذه الدراسة أن الاوكراتوكسين أحدث تأثيرات عكسية على هرمونات الغده الدرقيه، وزن الجسم وبعض القياسات البيوكيميائية فى الحملان النامية ولكن استخدام حمض الاسكوربيك ادى الى تلافى تلك الاثار العكسيه. لذلك ينصح باستخدام حمض الاسكوربيك لتلافى الاثار العكسيه للاوكراتوكسين فى علائق الحيوانات.