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Effect of vitamin E supplementation on production performances in arsenic exposed goats

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ABSTRACT

The present study was designed to investigate the effect of vitamin E supplementation on production performances in arsenic fed goats. Crossbred lactating goats (24) were distributed randomly into 4 equal groups (control, T_1, T_2 and T_3) of 6 in each, on the basis of average body weight (36.10 ± 0.11 kg) and milk yield (1.61 ± 0.004 kg/day). The animals in T_1, T_2 and T_3 were given 50 mg/kg dry matter arsenic daily, while in T_2 and T_3, vitamin E @100 IU and 150 IU/kg dry matter, respectively, was also supplemented additionally for 12 months. Both the arsenic and arsenic plus vitamin E supplementation, did not show any significant effect on dry matter intake, body weight and milk yield. Milk Cu, Zn and vitamin E level remain unaffected due to dietary treatments. Except control, in all arsenic exposed groups, milk arsenic level increased and vitamin E in higher doses showed comparatively less increase in milk arsenic level. It can be concluded from the present study that arsenic exposure in goats resulted no changes in production performances, milk Cu, Zn and vitamin E level but increases the milk arsenic level and concomitant treatment with vitamin E is helpful in reducing the burden of arsenic in milk.

Key words: Arsenic, Body weight, Copper, Goat, Milk, Vitamin E, Zinc

Chronic exposure of arsenic, a cumulative poison, through contaminated feed and water may produce toxic effect to animals reducing their production, and its excretion through milk poses threat to human health (Bhattacharya *et al*. 2009). Basic information on arsenical poisoning in cattle and small ruminants are meagre, excepting a report of development of anemia, gastrointestinal signs, toxic nephropathy and mortality in goats with induced chronic arsenic toxicity (Biswas *et al*. 1998). For cattle, sheep and swine maximum tolerable dietary arsenic recommended is 50mg/kg (inorganic) and 100 mg/kg (organic). The purpose of this study was to evaluate the cumulative effect of maximum tolerable dose of inorganic arsenic in a long term exposure. Previous studies (Uthus 2001, Schmolke *et al*. 1992) showed that arsenic might interfere the transport and metabolism of zinc and copper. But, very little work has been done in examining the interactions between arsenic exposure and copper and zinc retentions in milk of ruminants.

A number of direct studies on the effects of nutrition on arsenic toxicity have been conducted in laboratory animals but a little work has been done in ruminants. Beneficial role of feeding ascorbic acid as an antioxidant has been reported in rabbit, rat and in rodents (Rana *et al*. 2010). Vitamin E is dietary essential which acts as first line of defence in ruminants. As the vitamin E requirement of all classes goat is 100 IU/kg (Morand-Fehr 1981), therefore 100 and 150 IU/kg doses were decided to see the antioxidant effect. Considering the facts, the present investigation was undertaken in lactating goats to assess the protective effect of different doses of vitamin E against arsenic induced effects on productive performances and retention of arsenic in milk.

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MATERIALS AND METHODS

Twenty-four crossbred lactating goats (second to third stage of lactation) were selected from the Cattle Yard of National Dairy Research Institute, Karnal, India and distributed randomly into 4 groups of 6 each. The distribution of the animals was done on the basis of average milk yield (1.61 ± 0.004 kg/day) and body weight (36.10 ± 0.11 kg). The experimental feeding period was of 12 months. Animals in control (C) group received only basal diet without arsenic supplementation, whereas, animals in group T_1 were given arsenic 50 mg/kg dietary DM as sodium arsenite (minimum assay ≥98% purity), group T_2 animals, simultaneously with arsenic exposure, received vitamin E 100 mg/kg DM as dl-α-tocopheryl acetate (minimum assay 50%) and group T_3 animals simultaneously with arsenic exposure, received vitamin E 150 mg/kg DM. The experiment was performed with approval from Institute (NDRI, India) Animal Ethics Committee (IAEC No. 28/09–21/11/2009).

Before the trial, animals were adapted to the new environment and fed arsenic and vitamin E capsule for 7 days. The animals were fed as per NRC (1981) feeding standard to meet their nutrient requirements. As it was a long term study, two types of fodder either berseem or maize depending upon the season/availability was provided. Concentrate mixture (maize 33%, groundnut cake (oiled) 21%, mustard oil cake (oiled) 12%, wheat bran 20%, deoiled rice bran 11%, mineral mixture 2% and common salt 1%) having CP 19.81% and TDN 70% was procured commercially. Content of arsenic in concentrate mixture, berseem and maize were 1.116, 0.10 and 0.09 mg/kg and content of vitamin E were 10.72, 24.86 and 5.99 mg/kg respectively. The gelatin capsules containing weighed quantity of arsenic and vitamin E were prepared and fed daily to animals in group T_1, T_2 and T_3, respectively. Proximate analysis (DM, CP, EE, CF, NFE and ash) of concentrate mixture, green fodder (berseem and maize) was performed according to methods described in AOAC (2005). DM intake was recorded weekly for 2 consecutive days by subtracting the residual DM from the quantity of DM offered. The animals were weighed before feeding and watering in the morning on 2 consecutive days at the start of experimental feeding and thereafter, at monthly intervals during the entire experimental period of 12 months. Milk yield was recorded daily for 112 days. The concentration of Cu and Zn were determined by flame atomic absorption spectrophotometry using acetylene gas as fuel and air as oxidant. A HPLC method for simultaneous estimation of α-tocopherol and retinol in feeds, fodders, plasma samples was adopted (Chawla and Kaur 2001). A specific programme was adopted for the separation of retinol and α-tocopherol at different wavelengths using millennium software method set programme. The data were analyzed using 2-way repeated measures ANOVA (Sigma Plot software, version 11.0) with 2 factors i.e., different treatment groups and periods. All results were compared to control animals, as well as to the arsenic exposed animals, to elucidate the possible protective effect of vitamin E supply on arsenic toxicity. Tukey's all pair-wise multiple comparison tests were used to compare means between the different treatment groups. The results were expressed as mean and standard error. The specific P values were mentioned in the text for where there was significant difference found.

RESULTS AND DISCUSSION

Statistical analysis of data revealed no difference (P>0.05) in dry matter intake between treatments (Table 1). Significant interaction between treatment and period (P=0.964) was not encountered during the experiment. In previous studies, As_2O_3 treatment in crossbred kids fed arsenic showed nonsignificant effect on dry matter intake over time (Patel 2007, Vaswani *et al.* 2010) which is consistent with the present findings, whereas, Henkin (1977) reported reduced feed intake due to arsenic induced stress that might be associated with deficiency of trace minerals specially Zn and Cu (Thompson 1993).

There was no significant effect (P>0.05) of dietary treatments on changes in body weight over a period of 1 year (Table 1). It may be due to lower dose of dietary arsenic exposure (which was equivalent to maximum permissible level) or concentration of vitamin E used, which failed to produce significant differences in body mass loss or gain. Interaction between treatment and period (P = 1.000) was not observed during the study. Our results are corroborated with the findings of Mishra *et al.* (2005), Patel *et al.* (2009) and Vaswani *et al.* (2010) in calves and kids. Contrarily, Biswas *et al.* (1998) reported significant decrease in body weight from ninth week onwards in goats fed sodium arsenite (25mg/kg body weight) for the period of 12 weeks. In that study, they provided arsenic @478 ppm which was 9.56 fold higher than experimental dose used in the present study.

With increase of time, milk yield gradually decreased in all the experimental groups. At the end of the lactation, there was no difference (P>0.05) between the treatment

Table 1. Effect of vitamin E supplementation on dry matter intake and body weight in arsenic exposed goats[a]

	Dry matter intake (kg/day)*													SE
	Months													
	0	1	2	3	4	5	6	7	8	9	10	11	12	
Treatment														
C	1.58	1.45	1.40	1.32	1.44	1.50	1.52	1.53	1.41	1.38	1.45	1.38	1.36	0.02
T_1	1.63	1.38	1.34	1.33	1.37	1.39	1.46	1.49	1.35	1.39	1.39	1.36	1.32	0.02
T_2	1.60	1.38	1.32	1.29	1.43	1.38	1.51	1.54	1.37	1.38	1.48	1.38	1.35	0.02
T_3	1.63	1.40	1.36	1.33	1.36	1.45	1.47	1.51	1.40	1.39	1.44	1.36	1.31	0.02
Body weight (kg)**														
C	36.25	36.17	34.67	34.33	35.40	36.50	37.42	38.58	34.67	34.50	35.17	35.00	35.83	0.57
T_1	36.33	33.67	33.50	33.17	34.00	35.17	35.67	37.83	32.83	34.83	35.60	32.67	33.40	0.76
T_2	35.83	31.92	31.50	33.50	33.20	35.50	36.00	38.42	33.50	34.83	36.33	35.50	36.00	0.35
T_3	36.00	33.25	32.50	32.33	36.50	37.17	36.83	36.17	32.80	34.30	35.90	34.25	34.50	0.70

[a]No differences (P>0.05) among treatments. * The SE for month effect is 0.025. ** The SE for month effect is 1.176.

Table 2. Effect of vitamin E supplementation on milk yield (kg) in arsenic exposed goats[a]

Days	Treatment			
	Control	T_1	T_2	T_3
0	1.62	1.62	1.61	1.60
14	1.50	1.50	1.54	1.53
28	1.29	1.36	1.39	1.32
42	1.20	1.28	1.23	1.27
56	1.23	1.29	1.16	1.31
70	1.16	1.25	1.12	1.29
84	0.97	1.14	1.13	1.23
98	0.93	1.05	1.15	1.15
112	0.82	0.83	0.83	0.90
S.E.	0.05	0.04	0.05	0.04

[a]No differences (P>0.05) among treatments.

Table 3. Effect of vitamin E supplementation on monthly milk Cu and Zn concentration (ppm) in arsenic fed goats[a]

Treatment	Milk Cu concentration*					S.E.
	Months of lactation					
	0	1	2	3	4	
C	0.154	0.112	0.122	0.166	0.144	0.012
T_1	0.147	0.095	0.104	0.120	0.162	0.013
T_2	0.156	0.113	0.120	0.144	0.154	0.011
T_3	0.146	0.078	0.090	0.118	0.143	0.013
Milk Zn concentration**						
C	3.97	4.18	3.49	2.66	3.22	0.03
T_1	4.43	3.63	3.13	3.33	2.65	0.04
T_2	4.62	3.58	2.74	2.59	2.75	0.04
T_3	3.81	3.57	3.34	3.20	2.56	0.03

[a]No differences (P>0.05) among treatments

* The SE for month effect is 0.018 ** The SE for month effect is 0.0165

groups which indicates that arsenic at 50 ppm level had no adverse effect on milk yield (Table 2). There was not a statistically significant interaction between treatment and period (P = 0.938). These findings are contrary to the findings of Saha (1995) who reported reduction in milk yield, abortion, still births, nervous disorder and blindness etc.

Concentration of trace minerals (Cu and Zn) in milk of goat under different treatments were presented in Table 3. The present study revealed that there was no significant effect of dietary treatments on milk Cu and Zn concentration. There was not a statistically significant interaction between treatment and period (P = 0.993) of milk Cu during the study. But regarding periodic effect, it was seen that in arsenic and arsenic plus vitamin E supplementation (lower doses) groups, from 2 months onwards there was gradual decline (P<0.05) in milk Zn concentration compared to 0 day. Statistically significant interaction between treatment and period (P = 0.240) was not observed for milk Zn.

Concentration of arsenic in milk as influenced by dietary supplementation of arsenic over 12 months was presented in Table 4. Up to 4 months in all the dietary treatment groups, there was gradual increase (P<0.05) of milk arsenic concentration compared to control. From first month onwards, milk arsenic concentration increased (P<0.05) in only arsenic supplemented group whereas in vitamin E supplemented groups it was observed from 60 days onwards and between the 2 doses of vitamin E there was no significant difference (P>0.05). Statistically significant interaction between treat and period (P = 0.020) for milk arsenic was encountered during the period. Several researchers (Ulman *et al*. 1998, Singh *et al*. 2005, Rana *et al.* 2008)) reported excretion of arsenic through milk which is similar to the present findings. There was a report that the permitted arsenic level in milk should be less than 10 ng/g or 10 ppb (IDF 1986). So, in the present study milk arsenic concentration were slightly higher than the permissible limit which suggested the presence of arsenic in food chain that would be dangerous for human consumption in public health point of view. Increased milk arsenic level in this experiment might be due to the ability of heavy metals to transfer into bovine milk which has been previously reported by Sharma *et al.* (1982). Report of milk arsenic content in vitamin E supplemented animal is not available so results can not be compared.

Table 4. Effect of vitamin E supplementation on monthly milk arsenic and vitamin E concentration in arsenic fed goats

Treatment	Milk arsenic concentration*(ppb)					S.E.
	Months of Lactation					
	0	1	2	3	4	
C	5.67	8.00^{a}	8.67^{a}	10.33^{a}	11.00^{a}	1.33
T_1	8.33	49.00^{b}	69.00^{b}	79.67^{b}	119.67^{b}	11.18
T_2	7.33	33.00^{ab}	50.33^{b}	62.33^{b}	100.33^{b}	9.83
T_3	6.33	30.33^{ab}	54.33^{b}	55.00^{b}	88.33^{b}	9.39
Milk vitamin E concentration**(μg/ml)						
C	0.90	1.32	1.22	0.95	1.00	0.05
T_1	0.87	1.27	1.15	0.89	0.85	0.05
T_2	0.83	1.26	1.15	0.90	0.94	0.05
T_3	0.91	1.31	1.20	0.95	0.96	0.05

*Mean within the column bearing different superscript (a, b) are significantly different (P<0.05); The SE for month effect is 4.69 *Mean within the column bearing different superscript (a, b) are significantly different (P<0.05); The SE for month effect is 0.052

Effects of dietary supplementation on milk vitamin E concentration (μg/ml) are presented in Table 4. It was observed that there was no significant effect (P>0.05) of dietary treatments. The concentration of vitamin E in milk reported in this study was similar to that of Kondyli *et al.* (2007). Significant interaction between treatment and period (P=0.999) was not observed during the experiment.

Critical perusal of the results suggested that arsenic exposure unaffected production performances in lactating goats but it affect the milk arsenic level and concomitant treatment with vitamins such as α-tocopherol reduced arsenic burden in milk as compared to untreated group but the reduction was not sufficient enough to be comparable to that of unexposed controls, thus revealed specific protection of α-tocopherol and may be helpful to combat arsenic retention in animal.

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