Studies on Barki Goat experimental exposed to hypercholesterolemia and a trial of treatment

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Abstract: Fifteen goats with average body weight (15-25 kg) were used to determine the effect of cholesterol level on goat. They were divided into 3 equal groups; group 1(control) and groups "2&3" (tested group). The effect of administration of high level of corn and corn with titanium ascorbate to groups "2&3" respectively. After 21 days hematological changes monocytosis and lymphocytosis in goat blood were detected, as well as highly increases of cholesterol, triglycerides, phospholipids and calcium level in goat blood. These hematological and biochemical parameters were corrected by administration of titanium ascorbate (5g/head) as shown in-group 3. The animals of each group had been penned individually to carry out the digestion trials which lasted for 21 days. The goats raised on diet contain corn (group 2) and supplemented with titanium, consumed high amount of ration and nutrient compared with control group. It was observed that the digestibility of all nutrients in group 2 and 3 was increased significantly in comparable with control group. Highest values of nitrogen balance were recorded in group 2 and group 3 compared with the control group. Microbiological examination showed that, Bacillus species, Proteus sp., Enterococcus, and Micrococcus, Streptococcus pyogenes, and Staphylococcus aureus micro-organisms were isolated from cholestermic goat (group2) isolation before and after administration of *Nigella sativa* and titanium ascorbate as a trial to detect their antibacterial activity in vivo. Bacterial count was noticed to be decreased after this administration. Moreover, there were changes in the protozoa of the rumen, which increase acetate level, decrease propionate, and butyrate levels. Post mortem examination of internal organs revealed deposition of fats in internal organs, moreover, bones of goat was fragile due to deposition of calcium in internal organs.
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1. Introduction

In many countries all over the world particularly, goat & sheep were considered as a source of wool, meat and milk production. The importance of the meat production from goat & sheep is increased with the increased demand due to highest increase rate of the population (1). The sheep affected when fed diets with high fat contents or highly carbohydrate diet where previous reports have indicated that the incidence and degree of accumulation of free fatty acid were enhanced with age and were correlated to an increase in blood level of lipids (14, 15). In blood most of lipids combine with apolipoproteins to form lipoproteins. The water soluble titanium ascorbate in animal nutrition is very important, that uptake of this element of physiological importance, as the water-soluble T-ascorbate is able to cross the cell wall as demonstrated by Ti-isotope compounds (2, 19, 20).

Our interest in the black seed (*Nigella sativa*) was initiated by the Prophetic Saying "In the black seed there is healing for every illness except death". Although *N. sativa* has been in use in many middle eastern and far eastern countries as a natural remedy for over 2000 years and the Prophetic Saying was declared 140 years1400 ago, it was only in 1959 that the crystalline active principle (Nigellone) was isolated from the oil of *N. sativa* seeds (10, 15, 18). An *N. sativa* seed is a member of family Ranunuclaceae and is native to some parts of Mediterranean region. The seeds are widely many medicinal purposes such as carminative and stomachic. The Egyptians as diuretic, carminative and flavoring agent use the seeds. The expressed oil had been used for treatment of bronchial asthenia (5, 8, 10). Recent clinical and animal studies have shown that the extracts of the black seed have an antibacterial and anti-fungal effect (10), anti-helmintic effect (5, 8), improved the natural killer cell activity by an average of 74% (5).

The aim of the present study was to investigate the effect of titanium ascorbate in ration contain highly concentrate on some clinicpathological changes, and if titamin ascorbate can correct the over cholesterol level in blood of goat as well as its effect on the number and kind of rumen protozoa.

2. Materials and Methods

This study was carried out at Abd El-Moneam Reyade village, El-Bostan, West of Nubaria, Animal Production Department and Parsitology Department, National Research Center, Dokki, Giza.

Three digestibility trials were designed on 3 groups of animals fifteen goats were used. The animals were kept in metabolic cages a preliminary period of 15 days followed by 7 days for faeces and urine collection. The animals were fed on concentrate mixture on basis of 3% of their live body weight (15-25 kg). The animals were usually offered their diets at 8.00 am. and 2.00pm. Animals were allowed to drink fresh water.

Proximate analysis of feeds and faeces were carried out according to A.O.A.0 (1984).

Samples:

1- Blood samples (5m1) by jugular vein puncture (with and without EDTA) were taken from both control and cholesterimic animals before and after administration of T- ascorbate. These samples were subjected to test hematological and biochemical parameters.
2- Specimens for bacteriological examination were collected under aseptic condition and transferred in sterile containers in ice box. These specimens were swaps from live, lung, kidney and intestine of 2 scarified cholesteremic goat chose at random. After administration of *N. sativa* and T- ascorbate to the rest of the animals, they were all scarified and swaps from such organs were collected.
3- Isolated microorganisms from bacteriological samples were subjected to test the antibacterial activity of *N. sativa*.

Methods:

A) Hematological and Biochemical examinations:

Hemoglobin packed cell volume and R.B.Cs. total and differential leucocytic count l4 were estimated from whole blood samples. Serum was obtained by centrifugation at 3000 r.p.m. for 5 min, (the serum samples were kept in deep freeze at -200^{O}C (till use). The serum tested for total cholesterol, phospholipids and trigylecerides and calcium by using diagnostic Kits from Alkan Company diagnostic kits Bio Merieux, France.

B) Ruminal examination:

The ruminal fluids were examined for the number and kinds of protozoa, pH, volatile fatty acids and ammonia nitrogen (3,4,7).

C) Microbiological examinations:

Specimens of liver, lung, intestine and kidney were collected under aseptic condition and transferred in sterile container in ice box .The inoculums were directly inoculated into 5% defibrinated sheep blood agar (Difco), Columbia CAN (Biomereux), and MacConkey's agar plates and incubated at 37^{O}C under 5-10 % CO_2 for 24 – 48 hrs.

Suspected colonies were picked up for further identification according to (25,26). Cellular morphology, catalase test, haemolysis on blood agar, indol test and other biochemical test according (8,25,26).

In vitro sensitivity test:

Discs of 5mm in diameter of *Nigella sativa* oil extract by concentrations (1/10, 1/20, 1/40, 1/80 and 1/160) were prepared to detect its antibacterial activity by the agar diffusion technique according to (9,26).

Discs with each concentration were fixed on agar surface. All plated were incubated at 37°C for 24 hours and the results were recorded by measuring the diameter of inhibition zone for all isolated strains.

3. Results

The daily mean of feed intake by the experimental male goats from the tested three rations are presented in Table (3). The Three experimental groups showed fluctuating values of the whole dry matter intake (DMI) with the three rations. The DMI expressed as g/kg $w^{0.75}$ showed that control group consumed the lowest ($p<0.05$) value, being 65.60g/kg $w^{0.75}$ than the other two rations which showed comparable values without any significant difference, being 69.49 and 70.07 g/kg $w^{0.75}$.

However when the daily DMI from the three rations was related to body weight almost similar and ranged between 3.02 and 2.27% of the BW (Table3).

The control ration showed the lowest ($P<0.05$) digestion coefficients of all nutrients than the other two rations (Table 4). The latter ration showed comparable digestion coefficients for all nutrients.

Non significant differences were detected between nutrient digestibility's of these two rations.

The low digestibility coefficients is control ration were reflected on TDN values which lower than the other two rations. The latter rations showed comparable ($P<0.05$) energy values.

The DCP value of control ration (9.64%) was lowest when compared with the other two rations, but no significant differences between the three rations were recorded.

Goats in groups (2 and 3) showed higher values in nitrogen intake, while the difference between the three groups was not significant. The mean values as g N /head was (7.88,18,68 and 18.57), respectively. The main pathway of nitrogen excreted by goats was throwing the faeces. The retained nitrogen by different groups 1,2 and 3, respectively. Goat in group 2 and 3 showed higher values in N retention than the control group. The results indicated that goat fed ration supplemented with titanium utilized N better than goat fed control ration.

Table (6) revealed that the obtained result from group `2' goat concentrate 3g/kg B.W. for 21 days (cholestremic goat), there was significant decrease in P.C.V., hemoglobin, RBCs and increase in WBCs with lymphocytosis and monocytosis. Administration of titanium ascorbate 5g/ head caused correction of these parameters (Tables 6-7). There was a notable increase in cholesterol, triglycerides, phospholipids and calcium level .In addition, level of cholesterol, triglycerides and phospholipids and calcium were corrected after treatment.

Concerning rumen protozoa, there was decrease of acetate and pH as well as an increase of propionate shown in Table (8).

Bacteriological findings revealed that there were saprophytic bacteria Proteus species, *Micrococci, Streptococus pyogenes, Staphlococcus aureus*, administration of *Nigella sativa* and ascorbate decrease bacteria count. The antimicrobial activity of Nigella extract oil was detected in dilutions 1/10,1/20 and 1/40, and decreased activity was noticed with the increase of the dilution (Table 9).

Table (1): Formula of feed mixture.

	Control	Maize ration	Maize + Titanium
Sunflower meal	25	25	25
Wheat bran	17	17	17
Barley	25	-	-
Corn	-	40	40
Berseem hay	30	15	15
Limestone	2	2	2
Sodium chloride	1	1	1
Titanium	-	-	0.005

Table (2): Chemical composition of rations.

	Control	Maize ration
Dry matter	88.35	86.73
Organic matter	83.88	85.25
Crude protein	16.56	16.92
Crude fiber	15.13	13.64
Ether extract	5.89	6.49
Nitrogen free extract	46.30	48.20
Ash	16.12	14.75

Table (3): Body weight and nutrient intake in goat.

	Control	Maize	Maize +titanium
Av. body wt, kg	22.38	21.33	20.95
Body weight w0.75	10.29	9.93	9.97
DM intake g/head/d	675[b]	690[a]	686[a]
DM intake kg/100kg/BW	3.02	3.23	3.27
DM intake g/kg w 0.75	65.60[b]	69.49[a]	70.07[a]
Water intake ml/head	1120[b]	1230[a]	1235[a]
Water intake ml/g DMI	1.66[b]	1.78[a]	1.80[a]
Nutrients intake g/head			
TDN	56.69[b]	60.89[a]	62.25[a]
DCP	9.64	10.19	10.39

a, b: Means with different superscripts in the row differ significantly (P<0.05).

Table (4): Nutrient digestibility and nutritive values by goats.

	Control	Maize	Maize +titanium
Dry matter	63.29[b]	67.59[a]	69.43[a]
Organic matter	63.47[b]	68.34[a]	70.27[a]
Crude protein	58.23[b]	60.25[a]	61.38[a]
Ether extract	60.21	60.35	61.22[a]
Crude fiber	55.49[b]	58.48[a]	59.39[a]
Nitrogen free extract	66.21[b]	70.29[a]	72.25[a]
Nutritive values			
TDN	56.69b	60.89[a]	62.25[a]
DCP	9.64	10.19	10.39

a, b: Means with different superscripts in the row differ significantly (P<0.05).

Table (5): Nitrogen balance.

	Control	Maize	Maize +titanium
N.intake g/head	17.88	18.68	18.57
Fecal N	7.38	6.99	6.82
Urinary N	5.73	6.31	5.82
N. balance	4.77	5.38	5.93

Table (6): Some hematological changes in goat suffering from hypercholesterolemia and treated with titanium ascorbate.

	P.C.V. %	R.B.C.s 106/mm3	Hemoglobin	W.B.C.s 103/nm3	Neutrophils %	Lymphocytes %	Monocytes %
Control	36.2±1.23	8.95±0.24	9.2±0.72*	10.27±0.14	25.2±0.27	66.2±0.72	3.2±0 64
Group 2	133±0.27*	7.2±0.16*	8.0±0.18*	11.23±0.94	30.2±0.67*	71.3±0.72*	5.8±0.28*
Group 3	37.2±0.27	8.00±0.09	9.0±0.16*	10.00±1.42	24.3±013	76±0.78	3.68±0.24

*Significant at 5% level of probability.

Table (7): Some hematological changes in goat suffering from hypercholesterolemia.

	Cholesterol mg/dl	Phospholipid mg/dl	Triglycerides mg/dl	Calcium mg/dl
Control	66.0 ± 0.72	98.00± 1.23	68 ± 0.23	12.00 ± 0.75
Group 2	104 ± 1.27*	150 ± 0.27*	99 ± 1.24*	13.95± 1.30*
Group 3	60 ± 0.16	90 ± 1.67	72 ± 0.14	12.08 ± 1.24

*Significant at 5% level of probability.

Table (8): Changes in characteristics ruminal fluid protozoa before and after administration titanium ascorbate (5gm).

Parameters%	Pretreatment	1st week	2nd week	3rd week
pH	7.2	7.0	6.6	6.6
No of protozoa 1.5/m	2.33	2.36	2.23	7.62
Protozoa %	82	84.2	83.2	68.3**
Diplodinum%	4.2	4.2	2.6	12.6*
Entodinum%	3.8	1.5	2.2	4.8**
Isotricha%	2.0	3.4	3.6	6.7*
Other protozoa %	2.3	0.5	5.3	2.1*
Volatile fatty acids:				
Acetate%	65.2	53.3**	53.3**	64.2
Propionate %	21.2	26.0	25.2	17.2**
Butyrate	11.0	15.3	16.03	13.00

*Significant at 5% level of probability.
**Significant at 1% level of probability

Table (9A): Bacteriological finding in internal organ of cholestremic goats after administration of *Nigella sativa* + titanium ascorbate.

Bacteria isolated	Before treatment with N.S.+T.A.	After treatment with N.S. + T.A.
Staph isolated	+++++	++
S. pyogenes	++++	++
Bacillus. sp.	++	+
Proteus sp.	++	+

N.S= *Nigella sativa*; T.A. = titanium ascorbate

Table (9B): *In vitro* antibacterial activity of *Nigella sativa* oil extract.

Bacteria	Dilution				
	1/10	1/20	1/40	1/80	1/160
S. aureus+ *Micrococci*	++	++	+++	-	-
Steph. Pyogenes	+++	++	-	-	-
Bacillus sp	+++	-	-	-	-
Proteus sp.	++	++	-	-	-

+= 0.16mm; ++ = 0.29; +++ 0.49mm =zone diameter

4. Discussion

The present study tried to investigate the efficiency of combined supplement of concentrate and titanium ascorbate against hypercholesterol level as well as its effect on ruminal fluid from Tables (1-4).

Regarding the effect of hypercholesterolemia, there was a significant decrease in PCV, RBCs and hemoglobin and significant increase, WBCs (Table 2).

Administration of *Nigella sativa* and titanium ascorbate (according to method of Pais (21) and El-Khanawy (6) caused correction of the blood picture of goat suffering of highly cholesterolemia. These results were supported by finding of (16, 18), who observed that Titanum corrected blood picture in mice (13,19,20,27), also recorded that T-ascorbate has very promotive effects in piglets suffering from highly cholesterol level cause suppression of cholesterol and has an accelerating effect on development of the serial organs of young saws (19,20,24) and the blood triglyceride value was significantly lower which agreed with the obtained results. Concerning differential leucocytic count the obtained results goats. These results agreed with (13,15,18) who observed a monocytosis in rate orally intake 100 mg cholesterol /kg/daily for 15 days.

Treatment with titanium ascorbate may exert positive effect on leukocyte neutrophils, eosinophils, lymphocytes and monocytes count if compared with control (Table 6).

The increase of cholesterol and triglycerides cause arteriosclerosis, this increase of the triglycerides noticed may be 'due to the removal of the inhabitation of fat mobilization from adipose tissue (10,11,12,13,14,27). Resulting in increased release of lipoproteins, triglycerides from the liver (13,15,27).

Administration concentrate + titanium ascorbate for 21 days may correct the biochemical parameters (Table 7).

Concerning ruminal protozoa changes, there is a reduction in the rumen protozoa, decrease of acetate, and decrease in pH, and an increase of propionate, the present results are in agreement with these reports by (3,4,7,17,24) (Table 8).

The major constituents of concentrate diet are rapidly digested and volatile fatty acid increased the secretion of saliva to neutralize the rumen fluid was in sufficient and results decrease in rumen pH as seen by (3,4) low pH is known to decrease the number of bacteria and protozoa perhaps entodinium since protozoa increase the acetate level, decrease population of protozoa causes reduction of acetate level (3,4,7). The ruminal protozoa by finding this concentrate such as titanium ascorbate does not return to normal level.

Concerning bacteriological examinations, Tables (9A and 9B) indicated that pyogenic micro-organism *Staphylococcus aureus* and *Streptococcus pyogens* were isolated from internal organs and a similar findings reported by (24) who stated that *Staphylococcus aureus* and *Streptococcus pyogenes* were present in animals suffering from highly stress factors e.g. hypercholesterol level which leads to immulogical suppression (22,26,27).

The results indicated that the black seed (*Nigella sativa*) extract has good antibacterial activity beside a therapeutic potential for the treatment of some bacterial infection. Our results agree with (8, 10, 23, and 25).

Conclusion

Highly cholesterol administration in goat is very dangerous, it cause increase in serum lipid, monocytes, lymphocytes and calcium level in blood,

as well as deposition of calcium in internal organ and the bone become fragile.

The results also indicate that, titanium improve the performance of goat as well as feed intake, digestion coefficient and nitrogen balance.

Titanium ascorbate may correct all these parameters, and may affect protozoa in rumen fluid, acetate, propionate and butyrate level.

Warning from addition a high carbohydrate concentration in the ration since high carbohydrate level transform to fat and cause immunological suppression that leads to bacterial disease.

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This paper considered the first record in Egypt as there was no previous report in this subject (tinanium ascorbate).

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