

EFFECT OF SELENIUM AND/OR VITAMIN E ADMINISTRATION ON SOME REPRODUCTIVE TRAITS OF BALADI BUCKS

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SUMMARY

Twenty two Baladi bucks were divided into 4 groups; the first group injected (i.m) with saline and kept as control; the second group injected with sodium selenite; the third group injected with vitamin E acetate and the fourth group simultaneously injected with both selenium and vitamin E acetate. Injections were given twice weekly for two months. Semen and blood samples were collected weekly . Additional blood samples were collected every half an hour for 4 hours before and after the experiment. Recording of the reaction time, complete semen picture, electrophoretic and immunogenetic analyses of seminal plasma, and radioimmunoassy of plasma testosterone levels were carried out.

Results showed that administration of selenium

and/or vitamin E improved sexual desire as indicated by reduction of the reaction time. Also, semen characteristics were improved as monitored by increased sperm cell concentration, increased percent of alive sperm and decreased sperm abnormalities, and acrosomal damage. Plasma testosterone levels showed noticeable increase in comparison to pre-injections levels. These changes were more obvious with the advance of time and in bucks injected with selenium and vitamin E.

Seminal plasma proteins of Baladi bucks contained 15 polymorphic genetic markers, which are controlled by $F\alpha 2^A$, $S\alpha 2^A$ and Mc^1 genes and did not change through administration of selenium and/or vitamin E.

In conclusion, administration of selenium and/or vitamin E markedly improved sexual desire, se-

men characteristics and plasma testosterone level in Baladi bucks without inducing marked changes in the genetic markers.

INTRODUCTION

Reproductive performance of farm animals depends mainly on adequate balanced level of vitamins and essential minerals due to their vital roles in cellular metabolism, maintenance and growth. It was reported that administration of vitamins especially A, D, E and /or selenium improved reproductive traits of bovines in both female (Helal et al., 1998; Ahmed et al., 2001) and male (Ibrahim et al., 1996). In small ruminants, reproductive performance was improved following administration of vitamin E and /or selenium in rams (Irvine, 1996; Kendall et al., 2000).

Supplementation with selenium and /or vitamin E improved libido in buffalo-bulls (Khalifa, 1997), improved semen characteristics as indicated by increased sperm concentration, motility and alive sperm percent, and decreased sperm abnormalities in bulls (Rabie, 1992; Ibrahim et al., 1996) and rams (Kendall et al., 2000). Vitamin E and selenium were reported to have an antioxidant protecting effect besides having a stimulatory effect on the immune system (Macpherson and Garnsworthy, 1994; Kolb and Seehawer, 1998).

Goats have a prominent situation in most of the developing countries including Egypt (Ahmed et al., 1998; Zaabal et al., 2001), so the present study was carried out to investigate the impact of administration of selenium and/or vitamin E on semen characteristics and plasma testosterone level and immunogenetic constituents of Baladi bucks as a trial to improve fertility of such breed.

MATERIALS AND METHODS

The present study was carried out at the National Research Center Experimental Farm, Abu-Rawash, Giza, Egypt during the breeding season (September -March).

I-Experimental animals:

Twenty-two mature bucks aged 2-3 years; weighed 30-35 kg were kept freely away from does in covered shelter under the prevailed environmental condition. Each animal was daily fed one kg commercial concentrate mixture, Barseem (December - May), water, rice straw were provided *ad libitum*.

II- Experimental design:

Bucks were trained for semen collection for one month, before the start of the experiment. Bucks were divided into four groups:

1. Control group: included 7 bucks, each buck was injected with 1ml of sterile saline I.m.

2. Selenium group: included 5 bucks, each buck was injected with 0.10 mg/kg live body weight sodium selenite in 1 ml sterile distilled water (Ahmed et al., 2001).

3. Vitamin E group: included 5 bucks, each buck was injected with 1.35 i.u /Kg live body weight vitamin E acetate (Kim et al., 1997).

4. Vitamin E and selenium group: included 5 bucks, each buck was simultaneously injected with the previous doses of vitamin E and selenium. Injections were carried out twice a week for two months.

Semen Collection and Evaluation:

A single semen ejaculate (from each buck) following a false mount was collected weekly using A.V(44-45°C; Vale, 1997) and an estrous female as a teaser. Libido was expressed by estimating the reaction time/ minute as the interval of time between introducing the buck to the estrous female after performing the false mounting till ejaculation (Singh et al., 2000).

Complete semen evaluation including ejaculate volume, motility, concentration, alive sperm, abnormal sperm and acrosomal damage was performed (Sansone et al., 2000). Samples were centrifugated (X 1500 g/ 15 minutes at 4°C) and the

separated plasma was kept at -20°C for chemical analysis.

Blood samples:

Jugular blood samples (5ml) were collected before injection (0-time) as well as weekly throughout the experimental period. Additional blood samples were collected (0-time as well as after two months) every half an hour for four hours to follow up changes in testosterone level. Plasma samples were separated by centrifugation (X 3000g/15 minutes at 4°C) and kept frozen at -20 °C for biochemical analysis.

Radioimmunoassay of plasma testosterone:

Testosterone level was assayed by RIA (Abraham, 1981) in blood plasma using commercial kits from Diagnostic Product Corporation (Los Angles, USA). Assay has sensitivity of 0.04 ng/ml with inter- and intra-assays C.Vs. both being <13%.

Electrophoretic analysis of seminal plasma proteins:

Seminal plasma was subjected to electrophoresis using SDS polyacrylamide electrophoresis (Wolfe et al., 1993). Genotyping and gene frequency of seminal plasma proteins were estimated according to Hardi-Weinberg (Andersson and Davis, 1993).

Statistical analysis:

Data were statistically computed and analyzed

using two ways analysis of variance and Chi Square test according to Snedecor and Cochran (1980).

RESULTS

Effect of selenium and/or vitamin E administration on some reproductive traits of Baladi bucks:

1-Libido:

Table (1.1) reveals that the reaction time significantly ($P<0.05$) improved in all supplemented groups as compared to the control group. Moreover, this improvement was more obvious with the advance of time. The more favorable reaction time was observed in bucks simultaneously injected with selenium and vitamin E with significant ($P<0.05$) time X treatment interaction.

2-Semen characteristics:

It is clear that selenium and/or vitamin E administration significantly ($P<0.05$) improved semen characteristics with advance of time as indicated by increased sperm cell concentration and percent of alive sperm and the decreased incidence of sperm abnormalities and acrosomal damage (Tables 1.2 -1.8). Such improvements were more obvious for those bucks simultaneously injected with selenium and vitamin E. However, the ejaculate volume revealed little non-significant changes due to these treatments.

3-Plasma testosterone level:

The effect of selenium and/or vitamin E administration on plasma testosterone level is shown in table (2) and in figure (2.1-2.4). It is obvious that these treatments improved testosterone levels in all groups with significant ($P<0.05$) time X treatment interaction. Moreover, frequent blood sampling, 60 days post-treatments revealed marked increases in plasma testosterone levels especially, in bucks simultaneously injected with selenium and vitamin E followed by vitamin E group then selenium group as compared with the pre-injection levels. Such increases were peaked during 3.5, 1 and 2 hours in selenium + vitamin E, Vitamin E and selenium groups, respectively as shown in figures (2.1-2.4

4-Electrophoretic pattern and genotyping of seminal plasma proteins:

Electrophoretic patterns of the seminal plasma proteins in Baladi bucks (Table 3) and figures (2 &3) revealed 15 bands represent the different genotypes. These bands ranged in their molecular weight from 30-200 KDa (Fig. 2). The majority of these bands (12 bands) were situated in α and β - globulin loci. Among these bands, 3 major components migrated towards the anode while, a single component migrated towards the cathode. The most predominate genes in the studied bucks were $F\alpha 2^A$, $S\alpha 2^A$, Tf^A and Mc^1 gene markers. Administration of selenium and /or vitamin E induced no characteristic variations in either the electrophoretic pattern or gene frequency in the

Table (1): Effect of selenium and/or vitamin E administration on libido and semen characteristics of Baladi bucks (Mean \pm SE).

1.1-reaction time (Minutes)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	1.94 \pm 0.18	2.93 \pm 0.22	2.63 \pm 0.24	2.67 \pm 0.33	2.17 \pm 0.44	2.93 \pm 0.23	Weeks 25.92*
Selenium	1.83 \pm 0.28	2.10 \pm 0.50	1.42 \pm 0.08	1.17 \pm 0.17	1.00 \pm 0.29	1.48 \pm 0.21	Groups 10.29*
Vitamin E	2.10 \pm 0.24	2.50 \pm 0.41	1.50 \pm 0.20	1.50 \pm 0.29	1.33 \pm 0.17	1.75 \pm 0.19	Week X group 3.30*
Sel. + E	2.13 \pm 0.14	2.40 \pm 0.20	1.17 \pm 0.19	1.17 \pm 0.17	0.80 \pm 0.09	1.50 \pm 0.18	

1.2-Semen Volume (ml)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	0.80 \pm 0.12	0.70 \pm 0.08	0.63 \pm 0.13	0.75 \pm 0.20	0.68 \pm 0.12	0.67 \pm 0.05	Weeks 0.92
Selenium	0.78 \pm 0.09	0.95 \pm 0.05	0.73 \pm 0.15	0.57 \pm 0.07	0.65 \pm 0.07	0.74 \pm 0.06	Groups 0.25
Vitamin E	0.74 \pm 0.17	0.63 \pm 0.07	0.73 \pm 0.15	0.67 \pm 0.17	0.67 \pm 0.17	0.66 \pm 0.05	Week X group 0.39
Sel. + E	0.70 \pm 0.12	0.74 \pm 0.09	0.74 \pm 0.11	0.75 \pm 0.20	0.65 \pm 0.06	0.72 \pm 0.05	

1.3-Sperm concentration ($\times 10^9$ /ml)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	2.11 \pm 0.07	2.30 \pm 0.06	2.60 \pm 0.07	2.70 \pm 0.10	2.60 \pm 0.09	2.50 \pm 0.05	Weeks 67.73*
Selenium	2.23 \pm 0.09	2.30 \pm 0.01	2.80 \pm 0.01	2.70 \pm 0.10	2.90 \pm 0.02	2.60 \pm 0.09	Groups 10.31*
Vitamin E	2.20 \pm 0.04	2.30 \pm 0.09	2.80 \pm 0.05	2.67 \pm 0.08	3.00 \pm 0.09	2.60 \pm 0.08	Week X group 3.79*
Sel. + E	2.90 \pm 0.07	2.20 \pm 0.05	2.90 \pm 0.05	3.17 \pm 0.09	3.30 \pm 0.09	2.80 \pm 0.10	

* Significant at P<0.05

1.4- Mass activity (Score 1-5)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	1.88 \pm 0.26	1.86 \pm 0.40	1.75 \pm 0.48	2.67 \pm 0.33	2.25 \pm 0.25	2.06 \pm 0.21	Weeks 11.09*
Selenium	2.00 \pm 0.58	2.00 \pm 0.70	3.33 \pm 0.33	3.67 \pm 0.33	3.67 \pm 0.67	3.08 \pm 0.33	Groups 8.73*
Vitamin E	2.00 \pm 0.44	3.00 \pm 0.50	3.33 \pm 0.33	3.67 \pm 0.67	4.00 \pm 0.58	3.38 \pm 0.27	Week X group 1.42*
Sel. + E	2.25 \pm 0.48	2.00 \pm 0.30	4.00 \pm 0.31	4.00 \pm 0.58	4.60 \pm 0.24	3.50 \pm 0.28	

* Significant at P<0.05

1.5-Individual sperm motility (%)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	55.00 ± 3.22	55.00 ± 3.62	56.25 ± 4.73	66.67 ± 3.33	65.00 ± 2.89	59.44 ± 2.17	Weeks 29.58*
Selenium	51.67 ± 6.00	65.00 ± 6.45	81.67 ± 1.67	81.70 ± 1.67	83.33 ± 1.67	76.92 ± 2.97	Groups 14.92*
Vitamin E	53.33 ± 4.01	60.00 ± 5.77	78.33 ± 1.67	76.70 ± 1.68	81.67 ± 1.67	74.17 ± 2.88	Week X group 2.02*
Sel. + E	57.00 ± 3.74	63.57 ± 4.72	83.33 ± 1.66	83.33 ± 1.68	87.00 ± 1.22	77.62 ± 2.75	

* Significant at P<0.05

1.6- Alive sperm (%)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	55.19 ± 2.57	65.36 ± 2.69	66.25 ± 5.91	65.00 ± 2.89	71.67 ± 6.00	66.62 ± 1.99	Weeks 42.87*
Selenium	57.97 ± 3.25	63.33 ± 4.41	81.67 ± 1.67	83.33 ± 3.33	90.00 ± 2.89	79.58 ± 3.28	Groups 12.51*
Vitamin E	58.14 ± 3.33	64.33 ± 5.20	81.67 ± 1.67	78.33 ± 1.67	83.30 ± 3.30	76.82 ± 2.67	Week X group 1.86*
Sel. + E	56.56 ± 3.81	68.92 ± 3.36	85.83 ± 1.54	86.67 ± 3.33	90.00 ± 1.58	80.83 ± 2.31	

* Significant at P<0.05

1.7- Total sperm abnormalities (%)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	11.01 ± 1.08	11.39 ± 1.19	11.63 ± 0.94	10.00 ± 1.73	9.67 ± 1.45	10.89 ± 0.64	Weeks 7.77*
Selenium	10.26 ± 1.25	10.93 ± 0.41	8.73 ± 0.93	7.67 ± 0.88	7.00 ± 1.15	8.76 ± 0.57	Groups 3.89*
Vitamin E	11.10 ± 1.21	12.14 ± 0.44	9.33 ± 1.76	7.33 ± 1.45	6.67 ± 1.20	9.34 ± 0.80	Week X group 0.72*
Sel. + E	10.42 ± 1.41	11.08 ± 1.18	6.50 ± 0.50	6.67 ± 0.88	6.40 ± 0.05	8.02 ± 0.64	

* Significant at P<0.05

1.8- Acrosomal damage (%)

Control	Before injection	Time post injection (week)				Overall mean	ANOVA
		2	4	6	8		
Control	5.59 ± 0.50	5.33 ± 0.43	6.63 ± 0.24	5.67 ± 0.33	6.38 ± 0.63	5.90 ± 0.25	Weeks 1.27*
Selenium	5.30 ± 0.35	4.96 ± 0.16	4.50 ± 0.65	4.67 ± 0.33	4.67 ± 0.89	4.96 ± 0.30	Groups 10.84*
Vitamin E	5.00 ± 0.67	4.90 ± 0.43	4.25 ± 0.63	4.67 ± 0.33	4.33 ± 0.33	4.40 ± 0.24	Week X group 1.67*
Sel. + E	5.53 ± 0.35	5.07 ± 0.07	4.58 ± 0.37	4.90 ± 0.07	4.60 ± 0.10	4.73 ± 0.14	

* Significant at P<0.05

Table (2): Effect of selenium and/or vitamin E administration on plasma testosterone level (ng/ml) of Baladi bucks (Mean ± SE).

Control	Before injection	Time post injection (Week)		Overall mean	ANOVA
		First month	Second month		
Control	0.16 ± 0.04	0.17 ± 0.001	0.19 ± 0.01	0.22 ± 0.06	Weeks 0.05
Selenium	0.17 ± 0.04	0.18 ± 0.009	0.54 ± 0.02	0.36 ± 0.13	Groups 0.26
Vitamin E	0.44 ± 0.14	0.68 ± 0.06	0.89 ± 0.08	0.79 ± 0.13	Week X group 1.12*
Sel. + E	0.59 ± 0.12	0.65 ± 0.13	0.82 ± 0.08	0.78 ± 0.05	

Table (3): Effect of selenium and/or vitamin E administration on fractionation and genotypes of seminal plasma protein of Baladi bucks .

Plasma protein loci	alleles	Geontype	phenotypes	genes frequency
α -globulin (F α 2 and S α 2)	A-B	(AA-BB) (AA-Bb) (Aa-BB) (Aa-Bb)	A	F α_2^A = 0.75
		(aa-BB) (aa-Bb)	B	F α_2^B = 0.25 S α_2^A = 0.75 S α_2^B = 0.25
β -globulin	A-B-C	(AA-BB) (AA-Bb) (Aa-BB) (Aa-Bb)	A	T Γ^A = 0.60
		(aa-BB) (aa-Bb) (BB-cc) (Bb-cc)	B	T Γ^B = 0.30
		(aa-CC) (aa-Cc) (bb-CC) (bb-Cc)	C	T Γ^C = 0.10
Major globulins	Mc ¹ Mc ⁰	(Mc ¹ Mc ¹ -Mc ⁰ Mc ⁰) (Mc ¹ Mc ¹ Mc ⁰ mc ⁰)	(Mc ¹)	Mc ¹ = 0.833
		(Mc ¹ mc ¹ Mc ⁰ Mc ⁰) (mc ¹ mc ¹ Mc ¹ mc ⁰)		
		(mc ¹ mc ¹ Mc ⁰ Mc ⁰) (mc ¹ mc ¹ Mc ⁰ mc ⁰)	Mc ⁰	Mc ⁰ = 0.167

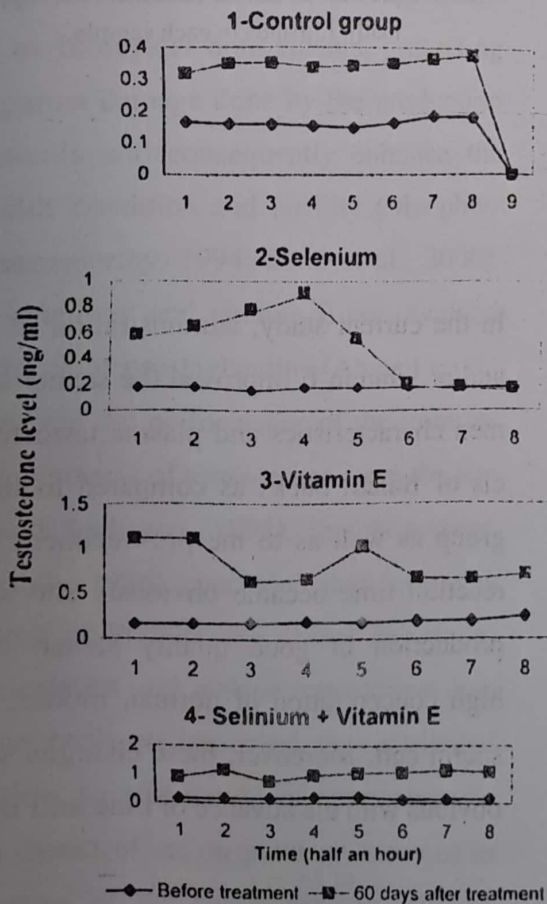


Fig. (1): Effect of administration of selenium and/or vitamin E on plasmatestosterone levels(ng/ml)in Baladi buck

Fig.(2):Diagram of electrophoretic fractions showed the mollecular weight of seminal plasma protein (30-200 KDa)

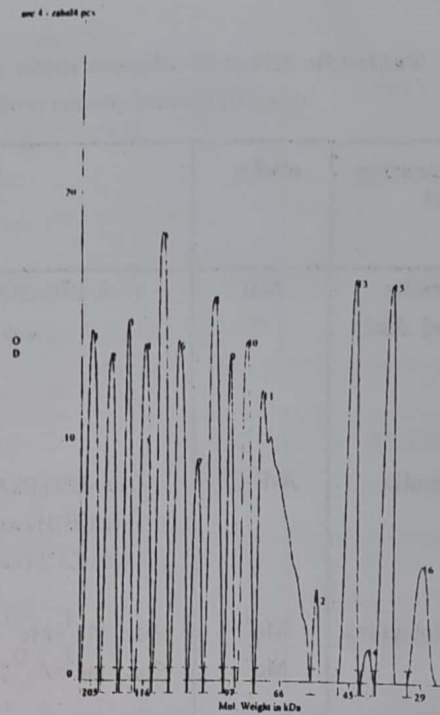
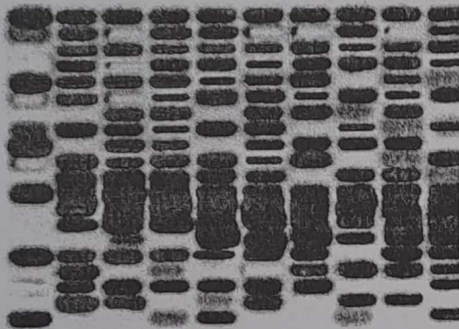


Fig.(3):Electrophoretic patterns of seminal plasma proteins of Baladi bucks, showed approximately 15 bands in each sample.

seminal plasma of Baladi bucks.

DISCUSSION

Adequate balanced nutrition could encourage farm animals to express their biological genetic potentials especially when bred under harsh prevailing environmental conditions (Ahmed et al., 2001).

In the current study, administration of selenium and/or vitamin E improved the sexual desire, semen characteristics and plasma testosterone levels of Baladi bucks as compared to the control group as well as to the pre-treatment data. The reaction time became obviously shortened with production of good quality semen containing high concentration of normal, motile, and alive sperm cell. Moreover, these changes were more obvious with the advance of time and in bucks si-

multaneously injected with selenium and vitamin E followed by those groups injected with vitamin E then selenium injected bucks. In this respect, Khalifa (1997) reported a decreased reaction time in buffalo-bulls supplemented with vitamin E and he attributed such improvements to increased testosterone levels. Also, Tumen and Ozkoca (1994) in rams, Udala et al. (1995) in bulls, Khalifa (1997) in buffalo-bulls and Castellini et al. (1999) in rabbits recorded similar improvements in semen characteristics. Also, the present improvement in testosterone levels was in line with the results recorded by Kolb and Seehawer (1998) who attributed these changes to the direct effect of these elements on the testicular tissue.

Selenium and/or vitamin E have a complementary effect as biological antioxidants, protecting the body against damage done by the production of free radicals and consequently enhance the general health condition and fertility (Macpherson and Garnsworthy, 1994; Surai et al., 2000). Moreover, selenium and vitamin E are involved in the synthesis of prostaglandins (Ahmed et al., 2001), improved the performance of the immune system and synthesis of testosterone from the testis (Kolb and Seehawer, 1998). In this respect, EL-Azab et al. (1996) concluded that following administration of PGF₂ α , the sexual desire, semen characteristics and plasma testosterone levels of Frisian bulls get improved, they attributed such condition to stimulation of spermatogenic activity via direct effect on pituitary tissue or to its acceleration of the sperm passage into the

ejaculate.

From the immunogenetic point of view, the present study showed that seminal plasma proteins of Baladi bucks are polymorphic, whereas 15 genotypes were obtained which were α, β and major globulins. Moreover, these genotypes were distributed according to their molecular weight from 30-200 KDa. No available literature could be traced in this respect in bucks. However, in rams (Lavon, 1972), bulls (Wolfe et al., 1993) and in buffalo-bulls (Zaabal et al., 1996) obtained 11, 25-30 and 8 fractions in the seminal plasma, respectively. However, all these authors agree that these fractions are polymorphic in nature and some of genotypes of major globulins are associated with fertility.

Concerning gene frequency of seminal protein loci, it is evident that F α^A , S $\alpha 2^A$ and Mc^I genes were the most predominate genes which may act through controlling the secretory function of the accessory glands. Also, tight relationships were reported between gonadotrophin, fertility and major protein genotypes of seminal plasma in bovine (Manjunath and Sairan, 1987). Such relationships indicated the possibilities of genetic control of reproduction in farm animals.

In the present study administration of selenium and/ or vitamin E induced non-detectable variations in either the electrophoretic patterns or gene frequency of seminal plasma proteins of Baladi

bucks and this condition could be attributed to interaction of multiple environmental and genetic factors to induce gene expression.

In conclusion, selenium and/ or vitamin E administration improved sexual desire and semen characteristics of Baladi bucks especially if given in combination and in animals raised under harsh managemental conditions. These treatments alert the immune system, improved health status and fertility without affecting the genetic constitution. However, further investigations on a large number of animals, using different doses and routes still needed.

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