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TRIALS FOR IMPROVING REPRODUCTIVE TRAITS OF BALADI BUCKS USING FEED ADDITIVES (With 3 Tables and 1 Figure)

By

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**محاولات لتحسين الخصائص التناسلية لذكور الماعز البلدية
باستخدام الإضافات الغذائية**

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أجرى هذا البحث بهدف تحسين الخواص التناسلية لذكور الماعز البلدية حيث تمت التجارب على عدد ٢٠ رأس من ذكور الماعز البالغة والتي ترعى بمزرعة المركز القومي للبحوث بالجيزة . قسمت الحيوانات إلى ٤ مجموعات تجريبية . المجموعة الأولى غذيت على العليقة الروتينية للمزرعة بينما المجموعات الباقية تم تغذيتها على نفس العليقة بالإضافة إلى أن كل رأس أخذت يومياً: ٥٠ جم من الدهون النباتية الجافة (المجموعة الثانية) ، ٥٠ جم من مخلوط الأحماض الأمينية (المجموعة الثالثة) و ٣ جم /كم عليه من مخلوط الأملاح المعدنية (المجموعة الرابعة) واستمرت التجارب خلال موسم التزاوج (سبتمبر - مارس) مع تجميع عينات من السائل المنوي مرة واحدة أسبوعياً لمدة ٦ أسابيع بعد شهرين من بداية التجربة كما تم تجميع عينات من الدم كل نصف ساعة لمدة ٦ ساعات بعد شهرين أيضاً من بداية التجربة لتحليل مستوى الهرمون الذكري ثم عقب ذلك تم تجميع عينات الدم أسبوعياً لتحليل بعض المكونات الخلوية والبيوكيميائية. أشارت النتائج إلى أن إضافة الدهون للعليقة أعطى أفضل النتائج متمثلة في قصر زمن التفاعل، تحسن صورة السائل المنوي نتيجة زيادة تركيز الحيامن وانخفاض نسبة التشوهات بها وكذلك ارتفاع مستوى الهرمون الذكري ولم تحدث هذه المعاملات التجريبية تغيرات ملحوظة في صورة الدم مع زيادة تركيز الجلوكوز والدهون الكلية والثلاثية وانخفاض الكوليسترول في السائل المنوي وزيادة تركيز الجلوكوز والدهون الكلية في الدم للحيوانات المغذاة على عليه تحتوي على الدهون الجافة كما زاد تركيز البروتينات الكلية الجلوبيولينات في السائل المنوي للحيوانات التي تم تجربتها بالأحماض الأمينية بينما زادت تركيزات عنصر الكالسيوم والفوسفور غير العضوي في السائل المنوي للحيوانات المغذاة على عليه تحتوي على مخلوط الأملاح المعدنية. أوصى الباحثون بضرورة رفع مستوى الطاقة في علائق ذكور الماعز البلدية المستخدمة في التربية لتحسين خواصها التناسلية.

SUMMARY

The present investigations were carried out as a trial to improve the reproductive performance of local Baladi bucks through using feed additives. Twenty mature bucks raised at the National Research Center Experimental farm (Abou-Rawash, Giza, Egypt) were divided into four equal groups. The first group fed merely on the farm ration and was kept as a control. The second group was supplemented with 50 g dry fat (Magnapac)/head/ day. The third group was drenched 5 ml amino acid mixture/head/day. The fourth group was supplemented with 3 g mineral mixture/head/day. Supplementation of all groups was carried out daily during the breeding season (September – March). Semen collection was carried out once weekly for six times two months post supplementation for performing complete seminal analysis. Blood samples were collected after two months (every half an hour for six hours) for evaluation of testosterone, thenafter, weekly samples were collected for six times for cellular and biochemical analysis. Results revealed that bucks supplemented with dry fat showed the shortest reaction time, improved semen characteristics as indicated by increased sperm concentration and decreased sperm abnormalities and acrosomal damage as well as increased testosterone level. Such improvement was more obvious in dry fat followed by amino acid, then mineral groups when compared with the control. Supplementations didn't markedly alter the haemogram in all groups. Supplementation with dry fat increased the concentrations of glucose, total lipids and triglycerides with decreased cholesterol concentration in semen and increased glucose and total lipids in the blood. Supplementation with amino acids obviously increased total protein and globulins in semen. Calcium and inorganic phosphorus values markedly increased in semen of bucks supplemented with mineral mixture. It is recommended that increasing the energy content in the ration of breeding Egyptian Baladi bucks improved their reproductive traits.

Key words: Reproductive traits, bucks, feed additives.

INTRODUCTION

Small ruminants breeding has spread in Egypt especially in the new reclaimed areas due to socioeconomic factors. Goats are considered as an important cheap source of animal protein. However, investigations

revealed inefficient reproductive potential of goats under the Egyptian conditions (Soliman, 1991; Ahmed *et al.*, 1998 and Zaabal *et al.*, 2001).

In order to improve herd fertility, it is important to take into consideration the male fertility and methods of assessing semen quality. Certainly, there is an ample evidence to show that although the female component is important, due regard must be paid to the influence of the male on the herd fertility (Gordon, 1996).

The pattern of testicular development, endocrine functions and semen traits are mainly influenced by nutrition in cattle (Tegegne *et al.*, 1994). The plane of nutrition greatly influences the onset of puberty and the development of spermatozoa through both GnRH independent and dependent pathways in sheep and goats (Martin and Walkden-Brown, 1995 and Robinson, 1996).

Supplementing a maintenance diet with extra-energy stimulates the hypothalamic reproductive center (Boukhliq and Martin, 1997) and it was found that lipid additives in the diet increased sperm motility, sperm survival and conception rate in sheep (Davidenko *et al.*, 1991).

Extra-protein in the diet improved semen quality in bucks (Abisaab *et al.*, 1997). Buffalo bulls subjected to feeding with bypass protein exhibited improvement in their semen quality (Prajapati *et al.*, 1998). Supplementing the diet with minerals especially zinc and selenium plays a role in sperm production in ovines (Irvine, 1996). In the same time, Kendall *et al.* (2000) found that supplementation of rams with zinc, cobalt and selenium in the ration improved sperm motility and viability.

The present study was a trial to improve semen quality through supplementing Baladi bucks with dry fat, amino acids and minerals.

MATERIALS and METHODS

The present study was carried out on male Baladi bucks reared at National Research Center Experimental Farm (Abou-Rawash, Giza, Egypt) during the breeding season (September – March) after El-Sharabassy *et al.* (1990).

I-Experimental animals:

Twenty mature bucks weighing 30 – 35 kg were kept in a covered shelter under the prevailed natural environmental condition. Bucks were kept freely and given rice straw and water *ad libitum*. Each animal was fed on 1 kg commercial concentrate mixture in Summer. Barseem was provided *ad libitum* during the green season.

II-Additives:

Dry Fat:

Protected dry fat (Magnapac®, Norel Co., Spain) derived from palm oil was used. It is a fatty acid calcium salt for inclusion in ruminants diet at a rate of 5 – 10%. It contains brute fat (84%) and ash (16%).

Amino acids mixture:

Concentrated solution (From Ibex Int.) containing D-methionine (150.0 g/L), L-lysine (120.0 g/L) and choline (200 g/L). It is used at a rate of 1 – 2 ml/L drinking water.

Minerals mixture:

A minerals mixture was obtained from Agrivet, each kg of the mixture contained 33.33 g manganese, 26.67 g zinc, 200 g Iron, 66.67 g copper, 0.93 g iodine, 0.1 g selenium, 0.17 g cobalt and 672.16 g calcium carbonate. It is used at a rate of 3 kg/Ton.

III-Experimental design:

Bucks were divided into four groups:

- 1-Control group: include five animals received nothing other than the routine farm ration.
- 2-Dry fat group: include five animals given 50 g Magnapac / head/day in the ration.
- 3-Amino acids group: include five animals drenched 5 ml amino acids mixture/head/day.
- 4-Mineral mixture group: include five animals, which were fed on a ration containing the mineral mixture (3 g/kg ration) and subjected to thorough mixing.

Supplementation was carried out daily using the recommended doses of the manufacture companies during the whole experimental period (September – March).

***Semen collection and evaluation:**

Semen collection was carried out once/week for 6 times after 60 days from the start of the experiment. Sex drive was estimated depending upon the reaction time which was estimated as the time from presenting to the teaser until dismount and ejaculation (Singh et al., 2000). Two ejaculates were used for performing complete semen picture including volume, motilities, concentration, alive sperm, abnormal sperm and acrosomal damage (Sansone et al., 2000). Samples of each group were pooled, centrifugated (x1500 g/ 15 min. at 4°C) and the separated plasma was kept at –20°C for chemical analysis.

***Blood sampling:**

After two months post-supplementation, heparinized blood samples were collected every half an hour for 6 hours for evaluation of testosterone profile, thenafter, samples were weekly collected for 6 weeks. Samples were collected early in the morning and after semen collection. Part of the weekly collected samples was utilized for performing complete blood picture (Jain, 1993) and the other part was used for separation of plasma, which was kept at -20°C until biochemical analysis.

***Biochemical analysis:**

Testosterone levels were assayed in both blood and seminal plasma (RIA, Abraham, 1981) using kits from diagnostic product corporation (Los Angeles, USA). Assay had sensitivity of 0.04 ng/ml with inter and intra assay C.V. S, both $<13\%$.

Concentrations of glucose, proteins, total lipids, cholesterol, triglycerides, calcium and inorganic phosphorus were colorimetrically determined in both blood and seminal plasma using chemical kits from Stanbio, Texas, USA.

***Statistical analysis:**

Data were computed (SAS, 1998) and pooled. The mean \pm S.E. values were presented in the tables after Snedecor and Cochran (1980).

RESULTS

The effect of supplementing Baladi bucks with dry fat, amino acid and mineral mixture on sex drive and semen characteristics of Baladi bucks was recorded in Table (1). Bucks supplemented with dry fat exhibited the best sex drive and semen characteristics as indicated by the shortest reaction time, the highest sperm concentration and the least abnormal sperm and acrosomal damage ($P<0.05$).

Table (2) showed the changes in haemogram of Baladi bucks supplemented with the previous feed additives without any significant changes among groups.

The effect of supplementation on testosterone level in 13 consecutive samples with half an hour intervals was depicted in Fig. (1). The highest testosterone level was obtained in the dry fat group and the same result was clear in the overall mean reported in both semen and blood plasma (Table 3).

The effect of supplementation on some selected biochemical values in seminal and blood plasma was reported in Table (3).

Glucose concentration significantly ($P<0.05$) increased in seminal and blood plasma in bucks supplemented with dry fat as compared with other groups. Total protein and globulins significantly ($P<0.05$) increased in seminal plasma of amino acids group. Total lipids and triglycerides significantly ($P<0.05$) increased and cholesterol concentration decreased in seminal plasma while only total lipids significantly increased in the blood of the dry fat group. Plasma calcium and seminal inorganic phosphorus concentrations significantly ($P<0.05$) increased following supplementation with mineral mixture (Table 3).

DISCUSSION

Great attention should be paid to improve our local livestock owing to their fair adaptation to the prevailing socioeconomic circumstances as well as their high resistance to endemic diseases (Ahmed, 1993).

Nutrition is one of the most commonly used approaches for improving reproduction including attainment of sexual maturity, both in terms of spermatogenesis and libido (Robinson, 1996 and Carpenter *et al.*, 1997).

It was previously mentioned that most of the Baladi bucks have non-satisfactory semen characteristics in comparison with the standard breeds (El-Sharabassy *et al.*, 1990; El-Sisy, 1997 and Hanafi *et al.*, 2001). In the present study trials were carried out for improving sex drive and semen quality of this breed using feed additives such as dry fat, amino acids and mineral mixture. It was found that, rising the energy level in the ration was the most beneficial method for improving these parameters. Similar results were reported in sheep and goat (Davidenko *et al.*, 1991 and Martin and Walkden, 1995) and in bulls (Alvarez *et al.*, 1995 and Coulter *et al.*, 1996). It was reported that the increased energy status is associated with increased energy availability to the central nervous system.. This event is consequently associated with change in the endocrine patterns with increased GnRH production and /or receptor numbers (McCaughery *et al.*, 1985; Rubio *et al.*, 1997 and Shalaby *et al.*, 1998) or through the effect on the hypothalamo-pituitary axis which may be mediated by circulating IGF-1 and consequently increased FSH and LH secretion (Adam and Findlay, 1997). In the same time, it was found that bucks supplemented with amino acids revealed improvement in libido and semen quality next to the dry fat. In this respect, it was

previously reported that superior semen quality and mating behaviors were attributed to dietary protein supplementation in bucks (Abisaab *et al.*, 1997), in buffalo bulls (Prajapati *et al.*, 1998), in rabbit bucks (Hemid and Tharwat, 1995) and in drakes (Yutsai *et al.*, 1996). This improvement may be related to improvement of body condition and consequently enhancing the production of neuropeptide-Y as well as FSH and LH (McShane *et al.*, 1992 and Tjondronegoro *et al.*, 1996).

In the current study, it was found that bucks supplemented with mineral and vitamin mixture showed improved semen quality as compared to the control bucks. In this respect, Bedo *et al.* (1990) and Kendall *et al.* (2000) in rams, Castillo *et al.* (1991) and Udala *et al.* (1995) in bulls and El-Sheltawi *et al.* (1999) in buffalo reported marked improvement in semen quality as a result of mineral and / or vitamin mixture supplementation. Such improvement was related to improved appetite, general health condition, blood constituents, LH pulse frequency (Ahmed *et al.*, 1998) and the antioxidant effect (Oteiza *et al.*, 1996) or their protective action on spermatozoa (El-Sheltawi *et al.*, 1999). Moreover, Shalash (1986) recommended addition of mineral mixture especially copper and zinc due to deficiency of these elements in Egyptian soil and plants. In this study, feed supplementations induced no marked changes in the blood picture of supplemented bucks and were more or less similar to those reported in goats (Jain, 1993 and Hanafi *et al.*, 2001).

In the present investigation, supplementations especially with dry fat improved testosterone levels in both seminal and blood plasma. Similar results were reported by Schallenberger *et al.* (1996). This condition could be attributed to improved body condition (Ahmed *et al.*, 1998), positive energy balance (Shalaby *et al.*, 1998) and subsequently increased frequently of LH pulses (Tjondronegoro *et al.*, 1996).

Bucks supplemented with dry fat revealed significant increases in the concentrations of glucose and total lipids in both blood and seminal plasma as well as triglycerides in their semen. These results were in accordance with those reported by Boukhliq and Martin (1997) and Carpenter *et al.* (1997). Such improvements are due to increased energy level in the ration. Moreover, cholesterol level was significantly decreased as it is utilized in the biosynthesis of steroid hormones (Stryer, 1996). In the same time, the recorded improvement in seminal proteins, in bucks supplemented with amino acids as well as blood calcium and seminal phosphorus in mineral mixture supplemented bucks could be attributed to increased dietary intakes from these components following supplementation.

It could be concluded that, rising the energy components of the ration, rather than the protein or mineral contents, seems to be responsible for improving libido and semen quality in Baladi bucks.

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Table 1. Effect of feed supplementation on libido and semen characteristics of Baladi bucks (Mean \pm S.E.).

Parameter	Control	Dry fat	Amino acids	Minerals
Reaction time (sec)	127.80 \pm 8.0 ^b	78.00 \pm 5.57 ^a	111.00 \pm 6.27 ^b	118.50 \pm 12.42 ^b
Semen volume (ml)	0.62 \pm 0.04	0.61 \pm 0.05	0.71 \pm 0.07	0.66 \pm 0.04
Mass activity (0-5)	3.53 \pm 0.13	3.89 \pm 0.14	3.50 \pm 0.11	3.55 \pm 0.14
Individual motility (%)	77.87 \pm 1.13	76.15 \pm 1.47	75.16 \pm 0.99	73.50 \pm 1.10 ^a
Sperm concentration ($\times 10^6$ /ml)	2646.67 \pm 84.36 ^b	3345.00 \pm 43.69 ^a	2955.00 \pm 24.32 ^b	3091.25 \pm 41.82 ^b
Alive sperm (%)	81.69 \pm 1.48	79.90 \pm 1.83	77.00 \pm 1.20	76.44 \pm 1.14
Abnormal sperm (%)	13.24 \pm 0.99 ^b	6.11 \pm 0.17 ^a	6.75 \pm 0.18	6.98 \pm 0.13 ^a
Acrosomal damage (%)	9.85 \pm 0.42 ^b	5.67 \pm 0.20 ^a	5.65 \pm 0.13 ^a	6.33 \pm 0.24 ^a

Means (in the rows) with different superscripts significantly differ at least at P<0.05

Table 2. Effect of feed supplementations on haemogram in Baladi bucks (Mean \pm S.E.).

Parameter	Control group	Dry fat group	Amino acids group	Minerals group
RBCs ($\times 10^6$ /ml)	13.05 \pm 0.27	12.29 \pm 0.16	13.04 \pm 0.45	12.37 \pm 0.14
Hb (g/dl)	10.16 \pm 0.40	10.01 \pm 0.22	10.91 \pm 0.30	10.09 \pm 0.25
PCV (%)	31.60 \pm 0.59	30.40 \pm 0.51	31.60 \pm 0.93	31.20 \pm 0.37
MCV (fl)	24.23 \pm 0.47	24.74 \pm 0.34	24.26 \pm 0.29	25.22 \pm 0.14
MCH (pg)	7.79 \pm 0.29	8.14 \pm 0.17	8.37 \pm 0.86	8.15 \pm 0.14
MCHC (g/dl)	32.12 \pm 0.89	32.91 \pm 0.49	34.54 \pm 0.52	32.31 \pm 0.48
WBCs ($\times 10^3$ /ml)	4.79 \pm 0.37	5.24 \pm 0.16	5.26 \pm 0.02	5.47 \pm 0.35
Lymphocytes (%)	60.6 \pm 1.16	61.4 \pm 0.68	59.4 \pm 0.81	61.4 \pm 0.81
Neutrophils (%)	34.0 \pm 1.09	34.2 \pm 0.86	36.6 \pm 1.03	34.6 \pm 0.68
Monocytes (%)	3.5 \pm 0.84	2.2 \pm 0.37	2.4 \pm 0.24	2.2 \pm 0.37
Eosinophils (%)	1.4 \pm 0.51	2.2 \pm 0.37	1.6 \pm 0.39	1.8 \pm 0.49
Basophils (%)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Thrombocytes ($\times 10^3$ /ml)	3.40 \pm 0.87	4.5 \pm 0.26	4.6 \pm 0.39	2.4 \pm 0.39

Table 3. Effect of feed supplementations on some semen and blood biochemical constituents in Baladi bucks (Mean \pm S.E.).

Parameters	Semen				Blood			
	Control group	Dry fat group	Amino acids group	Mineral group	Control group	Dry fat group	Amino acids group	Mineral group
Testosterone (ng/ml)	0.39 \pm 0.01 ^a	0.54 \pm 0.04 ^b	0.47 \pm 0.03 ^a	0.38 \pm 0.01 ^a	0.53 \pm 0.06 ^c	2.96 \pm 0.59 ^d	1.74 \pm 0.28 ^c	2.02 \pm 0.29 ^c
Glucose (mg/dl)	12.04 \pm 0.17 ^a	14.68 \pm 0.12 ^b	13.00 \pm 0.38 ^b	13.96 \pm 0.43 ^b	112.61 \pm 0.20 ^c	129.61 \pm 3.21 ^d	114.59 \pm 0.87 ^a	113.50 \pm 1.42 ^a
Total protein (g/dl)	102.40 \pm 0.13 ^a	103.63 \pm 0.98 ^a	192.85 \pm 0.93 ^b	181.88 \pm 2.17 ^c	6.87 \pm 0.27	6.67 \pm 0.14	6.42 \pm 0.11	6.49 \pm 0.11
Albumin (g/dl)	19.97 \pm 0.51	21.50 \pm 0.06	21.21 \pm 0.14	18.48 \pm 0.45	2.47 \pm 0.12	2.49 \pm 0.14	2.45 \pm 0.15	2.20 \pm 0.09
Globulins (g/dl)	82.43 \pm 0.38 ^a	82.13 \pm 0.92 ^a	170.40 \pm 0.79 ^b	162.40 \pm 1.72 ^c	4.40 \pm 0.15	4.18 \pm 0.01	3.97 \pm 0.04	4.29 \pm 0.02
Total lipids (mg/dl)	1088.66 \pm 10.34 ^a	2591.40 \pm 51.35 ^b	2086.66 \pm 139.33 ^f	2295.33 \pm 31.20 ^e	207.20 \pm 2.67 ^c	379.00 \pm 2.00 ^d	200.00 \pm 17.69 ^c	248.53 \pm 16.98 ^c
Cholesterol (mg/dl)	730.00 \pm 50.00 ^a	380.00 \pm 29.40 ^b	880.00 \pm 70.00 ^a	720.00 \pm 110.00 ^a	90.00 \pm 10.00	100.00 \pm 10.00	110.00 \pm 10.00	110.00 \pm 20.00
Triglycerides (mg/dl)	980.00 \pm 79.05 ^a	1386.42 \pm 24.70 ^b	976.50 \pm 34.06 ^a	958.00 \pm 51.92 ^a	83.33 \pm 1.64	81.69 \pm 1.62	89.36 \pm 2.50	78.90 \pm 3.05
Calcium (mg/dl)	6.30 \pm 0.38	6.37 \pm 0.04	6.43 \pm 0.12	6.21 \pm 0.52	7.45 \pm 0.16 ^c	6.82 \pm 0.39 ^c	7.98 \pm 0.31 ^c	9.14 \pm 0.13 ^d
Inorganic Phosphorus (mg/dl)	16.35 \pm 0.66	15.00 \pm 1.87	15.80 \pm 0.83	17.93 \pm 1.62	3.33 \pm 0.33 ^c	4.00 \pm 0.08 ^c	4.57 \pm 0.07 ^c	6.10 \pm 0.44 ^c

Means (in the rows) with different superscripts for semen or blood significantly differ at least at $P < 0.05$

Fig.(1):Effect of feed supplementation on plasma testosterone level in Baladi bucks(ng/ml)

