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UTILIZATION OF *MORINGA OLEIFERA* AS A NEW RABBITS FEED. 3- EFFECT OF DIETARY INCLUSION OF *MORINGA OLEIFERA* HAY ON REPRODUCTIVE PERFORMANCE OF BUCK RABBITS

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ABSTRACT: *A total number of 18 New Zealand White buck rabbits with (2671± 185g average initial body weight and 24 weeks of age were used in this study to evaluate the effect of dietary inclusion of different levels of Moringa oleifera hay (leaves+ twigs) as a partial substitute for alfalfa hay on their reproductive and productive performance. The animals were randomly allotted to three experimental groups (6 bucks in each). The 1st group was served as a control and fed basal pelleted diet (Diet A) contained 18% alfalfa hay, represents about 14.7 % of the total crude protein of the diet. The 2nd and 3rd groups were fed the same basal diet, but 50 and 75% of alfalfa hay (B and C Diets, respectively) were replaced by moringa hay (leaves+ twigs) on the basis of its contents of protein.*

Buck rabbits fed Diet B and C had improving semen quality. Mean values of physical semen characteristics were significantly higher (P < 0.01) for buck rabbits fed Diet B and C as compared to those fed Diet A (control). The correlation coefficients (r) between testosterone and each of ejaculate volume, wave motion, sperm motility, live spermatozoa, sperm concentration and total sperm output were significantly (P < 0.05 and 0.01) positive and ranged between 0.57 to 0.91. However, negative (P < 0.01) correlation coefficients were detected between testosterone concentration and abnormal spermatozoa (- 0.85). The coefficients of determination (r^2) between testosterone and each of ejaculate volume, wave motion, sperm motility, live spermatozoa, sperm concentration, abnormal spermatozoa and total sperm output were significant (P < 0.05 and 0.01) and ranged between 0.33 to 0.83 .

The results of the present study demonstrate that Moringa oleifera hay (leaves+ twigs) is good unconventional source of protein for feeding buck rabbits and could be added in the diet at levels up to 13.5% to replace about 75% of alfalfa hay without any adverse effects on their reproductive traits.

Key word: *Moringa oleifera*, reproductive traits, buck rabbits

INTRODUCTION

Shortage of animal feedstuffs is the main obstacle facing the development of animal production in Egypt. Inadequate nutrition for animals and poultry resulted in low milk, eggs and meat production, in addition that human population is increasing rapidly which led to more decrease in animal products. Many attempts are devoted to solve this problem, among these, cultivation of unconventional new forage crops that tolerate drought and salinity in the newly reclaimed soils in Sinai and north coast of Egypt.

Moringa oleifera plant is one of the most of widely distributed species of family moringaceae. *Moringa oleifera* is a perennial tree grows in most of the tropics and has several industrial, agricultural and medicinal uses, therefore is so called the life tree. It tolerates drought, high environment temperature up to 45^0 (Morton, 1991) and a wide range of soils (pH from 4.5-9, Palada and Changl, 2003). Moringa plants can be harvested several times during the growing season. One hectare cultivated area gives about 240 tons or more green forage resulted from 8 cuts in the year (Foidl *et al*, 2001). Moringa leaves are rich in antioxidants that have high capacity to scavenge free radicals and play a significant role in reducing mortality and morbidity due to cancer, heart diseases and other chronic illness. *Moringa oleifera* leaves meal are good source of high quality protein and can be used to replace soya bean or groundnut cake in livestock diet (Makker and Becker, 1997 and Sarwatt *et al*., 2002). Leaves meal of *Moringa oleifera* are abundant also in many of essential vitamins, and minerals (Fuglie and Lowell, 2001, Anwar and Bhanger, 2003, Siddhuraju and Becker, 2003, Anhwange *et al*., 2004 and Anwar *et al*., 2007).

The phenolic compounds present in moringa leaves, could serve as antioxidants and may effectively scavenge various reactive oxygen species and free radicals under *in vivo* conditions. Moreover the aqueous extract of moringa leaves contains certain biologically active components (selenium, thiocarbamates, glucosinolates, and their hydrolysis products such as glucoraphanin, isothiocyanate sulforaphane, nitriles, Faizi *et al.*, 1994).

Therefore, the present study was carried out to ascertain the effects of inclusion of moringa hay (leaves and twigs) at different levels in the diet (to substitute alfalfa hay) on reproductive and productive performance of bucks NZW rabbits under Egyptian condition.

MATERIALS AND METHODS

The experimental work of the present study was carried out at Rabbits Research Unit, Department of Animal and Poultry Production, Faculty of Technology and Development, Zagazig University, Zagazig, Egypt. The experimental work was initiated in December 2015 and terminated in October, 2016 by using bucks at sexual maturity (24 weeks of age) and till fulfillment of three parities. A total number of 18 bucks of New Zealand White buck rabbits, 24 weeks old and with average initial body weight of 2671± 185g were randomly allotted into three experimental groups (six bucks in each) to evaluate the physical semen quality.The 1st bucks group was served as a control and fed a basal pelleted diet (Diet A) that contained 18% alfalfa hay, represents about 14.7 % of the total crude protein of the diet. The 2nd and 3rd groups were fed Diets B and C, where 50 and 75%, respectively of alfalfa hay in the basal diet were replaced by moringa hay (leaves +twigs). All the experimental diets were iso nitrogenous and iso caloric.

Moringa plants were cultivated at Research Farm of Faculty of Technology and Development, Zagazig University. All plants were harvested at 90 days of growth and each 45 days after there for the following successive cuts. Representative samples of the air- dried moringa leaves and twigs were taken for chemical analysis, also samples of feed ingredients of the experimental diets were taken for chemical analysis to determine crude protein, crude fiber, ether extract, nitrogen free extract, calcium and phosphorus according to the methods of AOAC (1995).Chemical analysis was performed in the Central Lab for Soil, Foods and Feedstuffs (International accredited Lab, since 2012 and has ISO 17025), Faculty of Technology& Development, Zagazig University, Egypt. The diets were formulated to meet the nutrient requirements of rabbits for reproduction according to NRC (1977). The diet were mixed and pelleted at a commercial feed mill of Atmida, Meet Ghamr, Dakahlia Governorate, Egypt

Chemical analysis of alfalafa hay and *Moringa oleifera* is presented in Table 1.

Chemical analysis	**Alfalfa hay**	***Moringa oleifera* hay**	
		Leaves	**Twigs**
Dry matter	10.93±0.11	8.03±0.11	9.14±0.10
Crude protein	14.80±0.58	24.40±0.84	6.20±0.56
Ether extract	2.20±0.03	5.20±0.07	3.20±0.04
Crude fiber	28.20±2.13	19.20±0.82	43.00±2.51
NFE	34.90	34.20	29.10
Ash	8.97±0.06	8.97±0.06	9.36±0.05
Total	100	100	100

Table 1: Chemical analysis of alfalfa hay and *Moringa oleifera* hay (leaves and twigs)

Ingredients and chemical composition of the experimental pelleted diets are shown in Table 2.

Table (2): Ingredients and chemical analysis of the experimental diets

Ingredients	**Diet A**	**Diet B**	**Diet C**
Yellow corn	16.00	16.00	16.00
Barley	18.00	18.00	18.00
Wheat bran	26.00	26.00	26.00
Soybean meal (44%)	19.00	19.00	19.00
Alfalfa hay	18.00	9.00	4.50
Moringa leaves meal	0.00	4.50	7.00
Moringa twigs meal	0.00	4.50	6.50
Limestone	2.00	2.00	2.00
Salt	0.50	0.50	0.50
Vit. & Mineral Premix*	0.30	0.30	0.30
Methionine	0.10	0.10	0.10
Anti Mycotoxins	0.10	0.10	0.10
Total	100.00	100.00	100.00
Chemical analysis:			
Dry matter	14.27	13.32	13.89
Crude protein	18.38	18.70	19.11
Ether extract	2.22	2.26	2.51
Crude fiber	10.64	10.81	11.13
Ash	9.56	9.22	9.13
NFE	44.93	45.69	44.23
Lysine**	0.99	1.21	1.31
Ca	0.88	0.94	0.99
P	0.52	0.56	0.57
Meth+cysteine**	0.65	0.79	0.85

***Vitamin and minerals premix at level of 0.3% of diet supplies the following per Kg of diet:** Vit. A 12000 IU, Vit. D_3 2000 IU, Vit E 10 mg, Vit. K_3 2mg, Vit B_1 1mg, Vit B_2 5mg, Vit.B_6 1.5 mg, Vit. B_{12} 10 mg; Niacin 30 mg, Pantothenic acid 10 mg; Folic acid 1mg, Choine 250 mg, Biotin 50 mg, Copper 5mg, Manganese 60 mg, Zinc 50mg, Iron 30mg, Iodine 0.3 mg Selenium 0.1mg and Cobalt 0.1mg .

**Calculated according to NRC (1977)

All bucks were fed pelleted diets and drinking water *ad libitum* throughout the experimental period. All bucks were kept under the same managerial and hygienic conditions.

Semen samples were collected once a week for eight consecutive weeks using an artificial vagina device as described by Walton (1958)**.** Each ejaculate was taken to measure physical semen characteristics (ejaculate volume, wave motion, sperm motility, sperm concentration, live and abnormal spermatozoa and total sperm output)**.**

Statistical analysis:

Data were statistically analyzed using Least Squares Analysis of Variance according to Snedecor and Cochran (1982) using the General Linear Model Program of SPSS (2004) using the following fixed model for bucks:

$$Y_{ij} = \mu + Ti + e_{ij}$$

Where, Y_{ij} = The observed value of a given dependent variable, μ = Overall adjusted mean, Ti = Fixed effect of the treatments (*Moringa olifera* hay substitution), i = 1, 2 and 3,e_{ij} = Error of the model.

The differences between LSM (least square means) were analyzed by Duncan's New Multiple Range test (Duncan, 1955). Data in percentage values were transformed with the arcsine square root procedure to normalize variance before analysis.

RESULTS AND DISCUSSION

Effects of dietary inclusion *Moringa oleifera* hay on physical semen characteristics of NZW buck rabbits.

Table 3 shows that ejaculate volume (ml) was significantly higher ($P<0.01$) in buck rabbits fed Diets B and C than those fed Diet A. These results are in agreement with those obtained by EL Deeb *et al*. (2015) who showed that supplementation of *Moringa oleifera* leaves at levels 4 and 8 % increased semen volume in rabbits due to its high nutritive value which subsequently enhanced fertility performance. Improving ejaculate volume may reveal beneficial effect of *Moringa oleifera* leaves as antioxidant on accessory sex glands and testicular tissues (spermatocytes) within the seminiferous tubules as well as on epididymal spermatozoa (Awoniyi, 2010).

Wave motion (score) and sperm motility (%) was significantly higher ($P < 0.01$) in buck rabbits fed Diet B and C than those fed Diet A. These results agreed with those reported by EL Deeb *et al*. (2015**)** and Khalifa *et al*. (2016) who observed that sperm motility percentages were significantly increased in rabbits fed *Moringa oleifera* leaves meal. Yusuf (2014) showed that sperm motility was significantly higher in Turkey toms treated with Moringa *oleifera* leaves extract. The same author suggested that *Moringa*

oleifera leaves meal might enhanced the development and activity of the seminiferous tubules and the interstitial cells of turkey toms as well as possible role in enhancing hormonal functions.

Table 3. Means and standard error for physical semen characteristics of NZW buck rabbits fed different levels of *Moringa oleifera* hay (leaves + twigs) as a substitute for alfalfa hay in the diet.

Physical semen characteristics	*Buck rabbit groups*			Sig
	A(Control)	B	C	
Ejaculate volume(ml)	0.6± 0.02^{b}	0.9±0.04^{a}	0.9±0.04^{a}	**
Wave motion(score)	2.8±0.11^{b}	3.4±0.11^{a}	3.5±0.10^{a}	**
Sperm motility (%)	70.0±1,16^{b}	78.2±1.3^{a}	81.4±1,2^{a}	**
Live spermatozoa (%)	73.7±1.13^{b}	79.7±0.99^{a}	83.5±1.3^{a}	**
Abnormal spermatozoa (%)	21.5±1.3^{a}	16.2±0.9^{b}	12.7±0.6^{b}	**
Sperm concentration (x10^{6})	147.5±7.5^{c}	212.5±7.7^{b}	266.6±11.6^{a}	**
Total sperm output (x10^{6})	90.1±6.2^{b}	194.5±11.3^{a}	228.1±15.3^{a}	**

** =P < 0.01,

a, b, c Means with different superscript in the same row differ significantly(P < 0.05).

Group A (control): was fed a basal pelleted diet contained zero% *Moringa oleifera* hay (leaves+ twigs).

Group B: was fed a diet contained 9% *Moringa oleifera* hay as a substitute for 50% of alfalfa hay in basal diet.

Group C: was fed a diet contained 13.5% *Moringa oleifera* hay as a substitute for 75% of alfalfa hay in basal diet.

Live spermatozoa percentages were significantly higher (P < 0.01) in buck rabbits fed Diets B and C(83.5±1.3% and 79.7±0.9%, respectively) compared with buck rabbits fed Diet A (73.7±1.1%).These results are in agreement with the findings of Fatoba *et al*. (2013) who treated rats with moringa root extract. The same authors recorded increased mass activity and sperm cell livability than the control. Also, the same author observed a persistent dose dependent with moderate increase in sperm viability percentage for rabbit bucks fed diets contained 50% and 75% *Moringa oleifera*. Consequently, rabbit bucks under the control diet have higher percentages of nonviable spermatozoa than the treated rabbit bucks, leading to that sperm viability in rabbits is at least partly influenced by the amount of supplementation of natural antioxidant source. Amin and Hamza (2005) suggested that the protective effects of *Moringa* leaves may be attributed to the

presence of phytoconstituents (polyphenols, tannins, anthocyanin, glycosides, thiocarbamates) that scavenge free radicals, activate the antioxidant enzymes, and inhibit oxidases.

The differences in abnormal spermatozoa percentages among the experimental groups were highly significant (P<0.01). The mean percentages of abnormal spermatozoa of buck rabbits fed Diet A (control group) was significantly higher (P < 0.01) than those fed Diets B or C. These results are in agreement with those obtained by Kabbashi and Allah (2016) who reported that dietary moringa leaves extract supplementation decreased abnormal sperm percentage in albino rats. Priyadarshani and Varma (2014) found that hyperglycaemia induced sperm morphological defects in mice and a subsequent repair mechanism with moringa leaves meal was achieved by decreasing in sperm headless, banana head, amorphous head, round head and coiled tail sperm in large quantity. *Moringa oleifera* leaves contain fundamental antioxidants and phenolic compounds that helps in protecting the testis against morphologic, spermatogenic and oxidative changes brought about by toxic materials and certain antineoplastic agents (Siddhuraju and Becker, 2003 and Saalu *et al.,* 2011).

Mean values of sperm concentrations were significantly higher (*P* <0.01) in buck rabbits fed Diet C (266.6 $x10^6$) followed by those fed Diet B (212.5 $x10^6$) and Diet A (147.5 $x10^6$). These results are in agreement with those reported by Yusuf (2014) who found a significant increase in sperm concentration in Turkey toms treated with *Moringa oleifera* leaves meal. The high sperm concentrations recorded in *Moringa oleifera* treated toms suggest that testicular development and proper hormone balance were triggered by treatment with *Moringa oleifera* leave meal. Also Ghodaia (2016) reported an increase in sperm count in buck rabbits.

Mean values of total sperm output were significantly higher (P < 0.01) for buck rabbits fed Diets B and C (194.5±11.3 and 228.6±15.3, respectively) as compared with those fed Diet A (90.1±6.2). These results are in agreement with those obtained by Fatoba *et al*. (2013) who reported that dietary supplementation with Moringa root extract supplementation significantly increased the total sperm output of albino rats**.** The improvement in semen quality may be due to that *Moringa oleifera* leaves have excellent source of protein , micronutrients such as vitamins (B-carotene , ascorbic acid and tochoferols) an minerals (calcium, potassium, iron, zinc and selenium). *Moringa oleifera* leaves contain also, many of antioxidants such as Kaempferol, Quercetin, Rutin and Caffeoylquinic acids (Fuglie, 1999).

Table 4 shows that the mean values of cholesterol (mg/dl), alanine amino transferase (ALT) and testosterone concentration were significantly ($P<0.05$ or $P < 0.01$) increased in rabbits fed Diets B and C than those fed the control. However, total protein, albumin, globulin, glucose; aspartate amino transferase (AST) levels were not significantly affected by *Moringa oleifera* hay feeding. These results are agree with those obtained by Afolabi *et al*. (2013) who reported that treatment of cryptorchid rats with *Moringa oleifera* leaves extract in seminal plasma increased the total protein level. Ghodaia (2016) reported that seminal plasma proved to be of great biochemical interest as it contains many organic compounds. These substances produced by various accessory glands in response to testosterone hormone (El-Sherbieny, 2004).

The biochemical components in seminal plasma play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism during the process of fertilization and in the maintenance of constant osmotic pressure during semen preservation (Dhami and Kodagali, 1987). Estimation of this biochemical in the ejaculated semen or directly in the glands can be used as an index of accessory glands function (White, 1976).

Table 4. Means and standard error for seminal plasma of NZW buck rabbits fed different levels of *Moringa oleifera* hay (leaves + twigs) as a substitute for alfalfa hay in the diet.

Seminal plasma components	***Buck rabbit groups***			**Sig**
	A(Control)	***B***	***C***	
Total protein (g/dl)	3.8±0,4	3.9±0.7	4.1±0.1	NS
Albumin(g/dl)	1.9±0.1	1.9±0.2	2.1±0.1	NS
Globulin (g/dl)	1.9±0.2	2.0±0.2	2.1±0.1	NS
Glucose(mg/dl)	49.3±0.6	49.2±0.4	49.5±0.5	NS
Cholesterol (mg/dl)	41.0 ± 1.6^{a}	24.3 ± 1.8^{c}	32.0 ± 1.2^{b}	**
Aspartate amino transferase (IU)	41.1±0.8	41.2±1.1	40.5±1.1	NS
Alanine amino transferase(IU)	18.7 ± 0.5^{a}	16.8 ± 0.5^{b}	16.9 ± 0.2^{b}	*
Testosterone (ng/ml)	2.6 ± 0.0^{b}	2.9 ± 0.1^{a}	3.1 ± 0.0^{a}	**

NS= Not-significant, *= $P < 0.05$ and* *= $P < 0.01$,

a, b, c Means with different superscript on the same row differ significantly($P < 0.05$).

Group A (Control): was fed a basal pelleted diet contained zero% *Moringa oleifera* hay (leaves+ twigs).

Group B: was fed a diet contained 9% *Moringa oleifera* hay as a substitute for 50% of alfalfa hay in basal diet.

Group C: was fed a diet contained 13.5% *Moringa oleifera* hay as a substitute for 75% of alfalfa hay in basal diet

Interrelationship between testosterone and semen characteristics traits:

Table 5 shows that the correlation coefficients (r) between testosterone and each of volume, wave motion, sperm motility, live spermatozoa, sperm concentration and total sperm output were significantly ($P < 0.05$ or $P < 0.01$) positive and ranged between 0.57 to 0.91. However, negative ($P < 0.01$) correlation coefficients between sperm abnormalities (- 0.85) were detected.

The coefficients of determination (r^2) between testosterone and each of volume, wave motion, sperm motility, live spermatozoa, sperm concentration, sperm abnormalities and total sperm output were significant ($P < 0.05$ or $P < 0.01$) and ranged between 0.33to 0.83.

Table 5. Correlation coefficients (r) and coefficients of determination (r^2) between testosterone and semen characteristics of NZW buck rabbits fed different levels of *moringa oleifera* hay as a substitution for alfalfa hay.

Correlated traits	**R**	**R^2**	**Sig**
Testosterone:			
Volume (ml)	0.57	0.33	NS
Wave motion (score)	0.82**	0.67	**
Sperm motility (%)	0.89**	0.79	**
Live Spermatozoa (%)	0.78*	0.61	**
Abnormal spermatozoa (%)	- 0.85**	0.72	**
Sperm concentration($x10^6$)	0.91**	0.83	**
Total sperm output ($x10^6$)	0.90**	0.81	**

NS= Not-significant and **= $P < 0.01$.

Conclusively, the results of the present study demonstrate that *Moringa oleifera* hay (leaves+ twigs) is good source of protein for feeding buck rabbits and could be added in their diet at levels up to 13.5% to replace about 75% of alfalfa hay without any adverse effects on their reproductive traits.

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الاستفادة من المورينجا اوليفييرا كعلف جديد للأرانب.

3- تأثير الدمج الغذائي لحيوان المورينجا على الأداء التناسلى لأرانب بوك

نورا حسن جودة ؛ حسن محمود الكيلاوى – محمد ناجى الجعفرى – حسن إبراهيم
قسم الإنتاج الحيواني والداجني ، كلية التكنولوجيا والتنمية ، جامعة الزقازيق ، الزقازيق ، مصر.

استخدم في هذه الدراسة عدد 18 أرنب باك أبيض نيوزيلندا بمتوسط (2671 ± 185 جم متوسط وزن الجسم الأولي وعمر 24 أسبوعًا لتقييم تأثير التضمين الغذائي لمستويات مختلفة من قش المورينجا (الأوراق + الأغصان). بديل جزئي لدريس البرسيم في أدائها الإنجابي والإنتاجي. تم تخصيص الحيوانات عشوائياً لثلاث

مجموعات تجريبية (6 ذكور في كل مجموعة) ، المجموعة الأولى كانت بمثابة ضابطة وتغذية الحبيبات القاعدية (عليقة أ) احتوت على 18٪ من البرسيم يمثل التبن حوالي 14.7٪ من إجمالي البروتين الخام للنظام الغذائي.تم تغذية المجموعتين الثانية والثالثة على نفس النظام الغذائي الأساسي ، ولكن تم استبدال 50 و 75٪ من دريس البرسيم (عليقة B و C على التوالي) بدريس المورينجا (الأوراق + الأغصان) على أساس محتواها من البروتين

كانت ذكور أرانب التي تغذت على النظام الغذائي B و C قد حسنت جودة السائل المنوي. كانت القيم المتوسطة لخصائص السائل المنوي الجسدية أعلى معنوياً ($P < 0.01$) لذكور الأرانب التي تغذت على النظام الغذائي B و C مقارنة بتلك التي تغذت على النظام الغذائي A (المقارن). كانت معاملات الارتباط (r) بين هرمون التستوستيرون وكل من حجم السائل المنوي وحركة الموجة وحركة الحيوانات المنوية والحيوانات المنوية الحية وتركيز الحيوانات المنوية وإجمالي ناتج الحيوانات المنوية موجبة معنوياً ($P < 0.05$ و $P < 0.01$) وتراوحت بين 0.57 و 0.91. مع ذلك ، تم الكشف عن معاملات الارتباط السلبية ($P < 0.01$) بين تركيز هرمون التستوستيرون والحيوانات المنوية غير الطبيعية (- 0.85).

كانت معاملات التحديد (r2) بين التستوستيرون وكل من حجم السائل المنوي ، وحركة الموجة ، وحركة الحيوانات المنوية ، والحيوانات المنوية الحية ، وتركيز الحيوانات المنوية ، والحيوانات المنوية غير الطبيعية ، وإجمالي ناتج الحيوانات المنوية معنوية ($P < 0.05$ و $P < 0.01$) وتراوحت بين 0.33 و 0.83.

التوصية: تظهر نتائج الدراسة الحالية أن قش المورينجا أوليفيرا (أوراق + أغصان) هو مصدر جيد غير تقليدي للبروتين لتغذية ذكور الأرانب ويمكن إضافته في النظام الغذائي بمستويات تصل إلى 13.5٪ ليحل محل حوالي 75٪ من قش البرسيم دون أي آثار ضارة. على صفاتهم التناسلية.