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وحماية المستهلك

THE EFFECT OF ADDING BLACK MACA BY ORAL METHOD TO IMPROVE SOME CHARACTERISTICS OF FRESH SEMEN IN AWASSI RAMS

Ahmed B. Al-ani[1]*, Firas A. Mahmood[2], Ahmood M. Ajeel[3]

[1]Ministry of Agriculture, Office of Agricultural Research, Baghdad, Iraq. ahmedbasembasem88@gmail.com

[2]Professor, Department of Animal Production, Collage of Agricultural Engineering Sciences, University of Baghdad. firas.a@coagri.uobaghdad.edu.iq

[3]Ministry of Agriculture, Office of Agricultural Research, Baghdad, Iraq, xxx@gmail.com

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ABSTRACT

This study was conducted with the aim of demonstrating the effect of adding black maca to improve semen quality at different concentrations by oral for Awassi rams. The study included tests of fresh semen. 12 rams of the Awassi breed were used, with three rams. adults aged 2.5-3.5 years for each group, The semen was collected using an artificial vagina for sheep and goats at the rate of one ejaculate/ram/week, for a period of 12 weeks. The semen of the rams (pooled semen) was mixed in order to eliminate individual differences between the rams. The sample was divided equally among the experimental groups using Tris diluent with a dilution ratio of 1:20, and the rams were dosed with different concentrations, which amounted to (10g, 20g, 40g Maca Black\Ram\Week), which are represented by (T1, T2, T3) treatments, respectively, compared with the control group without the addition of black maca (0 g). The necessary tests were performed to evaluate the semen in terms of ejaculate volume, individual and mass activity, concentration, and testosterone. The results of the experiment showed that adding and dosing 20 grams of black maca powder to Awassi rams had a significant effect on some characteristics of fresh semen, as individual sperm motility increased, accompanied by a significant increase ($p<0.05$) of ejaculate volume, concentration, mass activity, testosterone in all the different treatments. Compared with the control group. It can be concluded that adding black maca at a concentration of (20 g) to the treatment (T2) led to improving the semen characteristics of Awassi rams.

Keywords: Awassi rams, fresh semen, maca black, Testosterone.

*This article is taken from the doctoral dissertation of the first researcher.

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تأثير أضافة الماكا السوداء بطريقة التجريع في تحسين بعض صفات السائل المنوي الطازج لدى الكباش العواسية

أحمد باسم العاني [1]، فراس احمد محمود [2]، حمود مظهور عجيل [3]

[1] باحث، وزارة الزراعة، دائرة البحوث الزراعية، بغداد، العراق، ahmedbasembasem88@gmail.com.

[2] أستاذ، قسم الأنتاج الحيواني، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق، firas.a@coagri.uobaghdad.edu.iq.

[3] باحث، وزارة الزراعة، دائرة البحوث الزراعية، بغداد، العراق، xxx@gmail.com

الخلاصة

أجريت هذه الدراسة بهدف بيان تاثير أضافة نبات الماكا السوداء لتحسين نوعية السائل المنوي وبتراكيز مختلفة عن طريق عملية التجريع للكباش العواسية، وتضمنت الدراسة فحوصات السائل المنوي الطازج ،تم أستخدم 12 كبشاً بواقع ثلاثة كباش بالغة بعمر 2.5 – 3.5 سنة لكل مجموعة ، وجمع السائل المنوي بوساطة المهبل الاصطناعي الخاص بالاغنام والماعز بمعدل قذفة واحدة/ كبش/ أسبوع، ولمدة 12 اسبوع . تم مزج السائل المنوي للكباش من أجل إزالة الفروق الفردية بين الكباش . وتم تقسيم العينة بالتساوي على مجاميع التجربة باستخدام مخفف Tris وبنسبة تخفيف 1: 20 ، وتم تجريع الكباش بالتراكيزالمختلفة اذ بلغت (10غم و 20غم و 40غم ماكا سوداء/كبش/أسبوع) والتي تتمثل معاملات(T1وT2 وT3) وعلى التوالي مقارنة مع مجموعة السيطرة بدون أي تجريع الماكا السوداء للكباش (0 غم) . وتم إجراء الفحوص اللازمة لتقييم السائل المنوي من حيث حجم القذفة والحركة الفردية والجماعية والتستستيرون. بينت نتائج التجربة أن أضافة وتجريع 20 غم مسحوق ماكا سوداء لدى الكباش العواسي تاثير معنوي في بعض صفات السائل المنوي الطازج أذ ازدادت الحركة الفردية للنطف، رافقتها زيادة معنوية ($p<0.05$) حجم القذفة والحركة الجماعية وهرمون التستستيرون في جميع المعاملات المختلفة مقارنة مع مجموعة السيطرة. ويمكن الاستنتاج بان اضافة االماكا السوداء بتركيز 20غم للمعاملة(T2) أدى الى تحسين صفات السائل المنوي لدى الكباش العواسي.

الكلمات المفتاحية: الكباش العواسي، السائل المنوي الطازج، الماكا السوداء، التستستيرون.

INTRODUCTION

Medicinal plants and herbal medicines are one of the current areas of focus, as a number of medicinal plants have been identified that have a positive role on fertility **(Lee *et al.*, 2021).** The effect of *Lepidium meyenii*, known as maca, is its ability to improve semen quality, concentration, sperm motility, plasma membrane integrity, and acrosome and genetic material integrity **(Leiva-Revilla *et al.*, 2022)** and reduce the production of reactive oxygen species **(Gloria *et al.*, 2023).** On the other hand, black maca plays a role in treating infertility, as it contains antioxidants that are important for semen **(Hye *et al.*, 2022)** in humans, and in general, antioxidants act as scavengers that prevent the formation of (ROS) Reactive Oxygen Speies **(Petruska *et al.*, 2014; Eehab & Firas.,2022).** Sperm preservation is a well-established technique in animal production technology and is widely used to preserve the genetic quality of male However, there are many factors during the preservation process that can affect the vitality, function, and quality of sperm, which Reduces the possibility of their fertility, and one of these factors is the synthesis of high levels of oxidative stress (OS) during semen preservation, which can have adverse effects on sperm health and function ,Therefore, interest has increased recently in searching for natural alternatives such as plant extracts, and plants that have an ethnopharmacological reputation have been extensively investigated to determine their potential biological activities **(Banana *et al.*, 2023; Kanyar & Karadas, 2023; Almallah, 2022; Athraa, 2022; Fayyad &Mahmood, 2019; Saieed *et al.*, 2019; Mahdi *et al.*, 2019; Saieed *et al.*, 2018).** On the other hand, Maca has been used to improve semen

quality characteristics **(Alcalde & Rabasa, 2020).** Many studies have shown the effects of Maca on semen quality, spermatogenesis, sperm count and sperm motility in different species of animals **(Aoki *et al*., 2019).** These effects have been observed in both healthy animals and subfertility problems **(Inoue *et al*., 2016; Valdivia *et al*., 2016; Al-Obaedi *et al*., 2017; Onaolapo *et al*., 2018; Huda,2023).** In addition, a recent study showed that maca has a beneficial effect in rams on fresh semen quantity and quality after storage at 5°C for 72 hours **(Del Prete *et al*., 2018).** On the other hand, there are a variety of compounds of medicinal and nutritional importance in the black maca plant, including sugars, phenols, maca, macamides, glucosinolates, vitamins and minerals **(Khalifa *et al*., 2023).** Alone or in combination, these components in maca have shown a range of bioactive activities, including enhancing fertility **(Aoki *et al*.,2019).**

MATERIALS AND METHODS

Experimental animals and design

In this experiment, 12 rams of the local Iraqi Awassi breed were used. Their ages ranged between (2.5-3.5) years and body weight ranged between (45-55) kg. This study was conducted at the Ruminant Research Station Under the Livestock Department / Agricultural Research Department/Ministry of Agriculture in the Akarkov area (25 km west of Baghdad) during the period from December 2022 to June 2023.All the rams included in the experiment were in good health, free of diseases, and subject to continuous veterinary supervision.The animals are housed in a semi-closed barn with dimensions of 10 x 10 m and an area of 100 m², while the open part of the barns consists of a comfortable space for the animal.

STUDIED ATTRIBUTES

Semen collection and evaluation

Semen was collected from 12 local Awassi rams using an artificial vagina at the rate of one ejaculation/ram/week. The semen was mixed with some (pooled semen) for each group, with three rams per group in order to eliminate individual differences between Rams, and then split the semen using Tris diluent, which was previously prepared according to the method of The diluent was placed in a water bath 37°C before the dilution process so that it is at the same temperature as the semen, as indicated by **(Evans &Maxwell ,1987).** The necessary tests were performed to evaluate the semen in terms of ejaculate volume, individual, mass activity, and testosterone.

Ejaculate Volume

The ejaculate volume was measured immediately after collection in collection tubes and then transported to the laboratory to complete the rest of the other tests.

Mass Activity

Collective motion was estimated according to the method of (**Evans &Maxwell 1987)** by taking a drop of semen immediately after collection and placing it on a clean glass slide at a temperature of 37°C. Then the sample was examined under a microscope with magnification

(10X). Collective motion was estimated based on the speed and intensity of the moving waves. Then convert it to a percentage for ease of conducting statistical analyses.

Sperm Cell Individual Motility

It was estimated according to the method of **(Chemineau *et al* ., 1991)**, where diluting the semen by 20:1 using gear diluent, a drop of semen was placed on a glass slide at a temperature of 37°C, then a cover slide was placed on the sample, and it was examined under a powerful microscope. Magnification (40X). Individual movement was calculated based on the percentage of sperm moving forward, the strength of their movement, and their speed. After estimating the movement, it was converted into a percentage to facilitate statistical analyses.

Testosterone Measurement

The hormone was measured at the end of each month of the experiment. Blood was collected from the experimental animals from jugular vein, and the blood plasma was isolated using a centrifugation device at a speed of (2000 rpm) and a temperature of 20°C. Blood plasma was kept frozen for the purpose of measuring testosterone concentration

Statistical analyses

The program **SAS (2012)** was used to analyze the data according to a Complete Random Design (CRD), to study the effect of various factors on the studied characteristics according to the mathematical model below, and the significant differences between the means were compared with the **Duncan (1955)** multinomial test. The statistical model for analysis of variance was as follows:Yij = μ + Ti + + eij

Where:

Yij :The value of the view(j) returned to the transaction.

μ= Overall mean.

Ti :Treatment effect.

Eij= error term.

RESULTS AND DISCUSSION

Ejaculate Volume

Table (1) showed the effect of black maca with a significant increase (P<0.01) in the volume of ejaculate after the third month of dosing. Treatment T3 recorded a highly significant superiority (P<0.01) over the rest of the different experimental treatments and the control group, reaching T3 (1.16±0.03), then followed by T2 (1.05±0.01), T1 (0.99±0.01), and finally the control group (0.97±0.0). Table (1). The improvement in volume may be due to several reasons, as semen volume is affected by age, season, frequency of collection, and testicle size. Semen volume increases with an increase in testosterone concentration during the reproductive season, and the activation of Sertoli cells that support and nourish sperm **(Sarkar *et al*., 2008; Al-Saedi &Talal, 2022).** The increase in semen volume occurs mainly due to the increase in seminal plasma produced by the accessory glands **(Gündoğan, 2007; Al-Obaedi *et al*., 2017).** An increase in the level of Testosterone in the blood plasma during the reproductive season also leads to an increase in the volume of the ejaculate and the density and vitality of the semen **(Muthanna *et al*., 2022).**

Table (1); The effect of dosing different concentrations of black maca on ejaculate volume (ml) for different groups of Awassi rams for three months period (mean ± standard error).

Period Group	Period (month)		
	Month (1)	Month (2)	Month (3)
Control	0.95 ± 0.06 a	0.98 ± 0.03 b	0.97 ± 0.00 b
T1	0.90 ± 0.05 a	0.98 ± 0.03 b	0.99 ± 0.01 b
T2	0.95 ± 0.03 a	0.96 ± 0.02 b	1.05 ± 0.01 b
T3	0.96 ± 0.00 a	1.06 ± 0.03 a	1.16 ± 0.03 a
Level of significance	N.S	*	**

Small superscripts within a similar column indicate differences, Control=0 maca ; T1=10g maca ; T2=20g maca ; T3=40g maca.

Mass Activity

The results of the current study showed that there were no significant differences in the collective movement of sperm between the treatments and the control group after the first month of dosing (Table 2). Within the same context, it was noted that there were significant differences ($P<0.05$) in the percentage of mass movement after the second month of dosing, as all treatments were superior compared to the control group (Table 2). It was also noted that there were significant differences ($P<0.05$) between the treatments in the percentage of collective sperm motility after the third month of dosing, as the highest value was recorded in the T2 treatment (82.40 + 2.61%) and the lowest value was recorded in the control group (78.34 + 2.24%) Table (2) .The improvement in the percentage of motility and viability seen in this study may be attributed to the widely known antioxidant compounds contained in black maca including carotenoids, polyphenols, and flavonoids **(Yang *et al*., 2016; Lin *et al*., 2018; Fu *et al*., 2021).** Also, the presence of ascorbic acid, flavonoids, polyphenols, and carotene in maca powder **(Khalifa *et al*., 2023).** It may be one of the reasons for improved sperm motility, as indicated by **(Jayawardana *et al*., 2015).**

Table (2): The effect of dosing different concentrations of black maca on mass activity in fresh semen for different groups of Awassi rams for three months period (mean ± standard error).

Period Group	Period (month)		
	Month(1)	Month(2)	Month(3)
Control	76.67 ± 2.11 a	78.21 ± 2.77 b	78.34 ± 2.24 c
T1	77.68 ± 2.10 a	80.32 ± 2.06 a	82.00 ± 2.55 ab
T2	79.00 ± 2.19 a	80.41 ± 2.62 a	82.40 ± 2.61 a
T3	78.33 ± 2.10 a	79.15 ± 2.54 a	80.16 ± 2.20 bc
Level of significance	N.S	*	*

Small superscripts within a similar column indicate differences. Control=0 maca ; T1=10g maca ; T2=20g maca ; T3=40g maca.

Sperm Cell Individual Motility

The results of the current study showed that there was a significant difference (P<0.05) in individual movement between all the treatments and the control group in the first month of dosing the Awassi rams, as the percentage of individual movement ranged between 80.33 + 2.35 and 78.68 + 2.88% for the treatments compared to 76.33 + 2.67. % for the control group, during the first month of dosing (Table 3). The results also showed that there were significant differences (P<0.05) in the percentage of individual movement after the second month between all treatments, as it was highest in the T2 (maca black) group (81.17 + 2.92%), then followed by the T1 (maca black) group (80.02 + 2.18). %), T3(maca black)(79.46 + 2.17%), and finally the control group (77.00 + 2.21%), Table (3). On the other hand, the results showed that there were significant differences (P<0.05) in the rate of individual sperm motility in Awassi rams after the third month of dosing with black maca powder, as the T2 treatment was superior to all treatments. Antioxidant compounds contained in black maca, such as carotenoids, polyphenols, flavonoids, and ascorbic acid **(Fu *et al*., 2021; Khalifa *et al*., 2023).** These components reduce free radical levels and the risk of cell death, prevent oxidative damage to key biomolecules, and provide significant protection against oxidative stress **(Wang *et al*., 2017; Ghoora *et al*., 2020).** It improves motility, reduces damage to the cell membrane, and protects sperm from oxidative stress, thus prolonging the period of sperm storage **(Allai *et al*., 2015).** The improvement in the percentage of motile sperm may be due to the presence of iron among the components of black maca powder, as its percentage reaches (16.6 mg) **Khalifa *et al*. (2023).**Which also plays a role in energy metabolism because it facilitates the transfer of electrons in the electron transport chain to form ATP and thus provides sperm with energy and increases sperm motility **(Kozat, 2007).**

Table (3); The effect of dosing different concentrations of black maca on percentage of individual sperm motility in fresh semen for different groups of Awassi ram for three months period (mean ± standard error).

Period Group	Period (month)		
	Month (1)	**Month (2)**	**Month (3)**
Control	76.33 ± 2.67 c	77.00 ± 2.21 b	77.56 ± 2.37 b
T1	79.67 ± 2.98 ab	80.02 ± 2.18 ab	81.66 ± 2.89 ab
T2	80.33 ± 2.35 a	81.17 ± 2.92 a	82.35 ± 2.90 a
T3	78.68 ± 2.88 a	79.46 ± 2.17 a	80.95 ± 2.30 bc
Level of significance	*	*	*

Small superscripts within a similar column indicate differences. Control=0 maca ; T1=10g maca ; T2=20g maca ; T3=40g maca.

Testosterone

The results of the first month highlighted the absence of significant differences in measuring the concentration of testosterone in blood plasma (ng/ml) in Awassi rams for all study treatments, Table (4). The results also showed a highly significant difference ($P<0.01$) after the second month of dosing with black maca compared to the control group, as their values reached 1.46 + 0.06, 1.80 + 0.002, 1.55 + 0.03, and 1.27 + 0.03 (ng/ml) for the treatments (T1 and T2). And T3 and control), respectively, Table (4). The increase in the concentration of testosterone in plasma may be due to an increase in the cytoplasmic area of Leydig cells with the addition of black maca. This is a stimulating effect on the growth of Leydig cells directly or indirectly, as well as an increase in the ability to form steroid hormones with the increase in cell size during The period of puberty increases the metabolism of cholesterol, and this leads to an increase in the concentration of the hormone in the blood **(Ariyaraten & Chamindrani, 2020).**

Table (4); The effect of dosing different concentrations of black maca on concentration of testosterone in blood plasma (ng/ml) for different groups of Awassi rams for three months period (mean ± standard error).

Period Group	Period (month)		
	Month (1)	Month (2)	Month (3)
Control	1.03 ± 0.01 a	1.27 ± 0.03 b	1.42 ± 0.01 a
T1	1.06 ± 1.03 a	1.46 ± 0.06 ab	2.93 ± 0.03 ab
T2	1.10 ± 0.03 a	1.80 ± 0.002 a	3.20 ± 0.01 b
T3	1.05 ± 0.02 a	1.55 ± 0.03 ab	2.90 ± 0.03 b
Level of significance	N.S	**	**

Small superscripts within a similar column indicate differences. Control=0 maca ; T1=10g maca ; T2=20g maca ; T3=40g maca.

CONCLUSION

Based on the data, the black maca plant contains large amounts of flavonoid and phenolic compounds, which gives it high efficiency as a natural antioxidant so we recommend using a dose of 20 grams of maca improve some characteristics of the semen of Awassi rams.

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