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(Original Article)

Impact of Antioxidant (Astaxanthin) Supplementation on Farafra Ewes Reproductive Performance and Growth Performance of Their Lambs Exposed to Heat Stress

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Abstract

This study was carried out to investigate the effect oral administration of astaxanthin (Keto antioxidants) on the reproductive performance of Farafra ewes and the growth performances of their lambs exposed to heat stress. Sixty- seven apparently healthy, pluripara Farafra ewes were divided randomly into two similar groups and they were similar in initial body weight (36.18 $\pm$ 0.56 kg) and age. The ewes in control (CG33 ewes) were fed on control diet (concentrate feed mixture without any supplement). The treated group (ASXG, n= 34 ewes), ewes were fed control diets plus 0.25 mg, orally administration, astaxanthin /kg/ body wt/d. during pre-mating, mating and pregnancy. Animals of both groups exposed to solar radiation for two hours daily as a heat stress. Reproductive traits were recorded and data were statistically analyzed using SPSS program. The results revealed that, the oral administration of astaxanthin increased the percentage of ewes in estrus and the conception rate of all ewes by about 20% and 12%, respectively. Also, no of lambs born and lambs born alive increased by about 53% and 33% in favor of ASXG. Oral ASX improved ($P< 0.05$) twining rate. Moreover, the group that received ASX had higher ($P< 0.05$) lambs weaning weight by about 25% than the control one. However, gestation length and average birth weight did not differ between the CG and ASXG.

In conclusion, supplementation of ASX around mating and during pregnancy could improve the reproductive performance of ewes exposed to heat stress.

Keywords: *Antioxidants, Farafra Ewes, Astaxanthin, Reproductive performance.*

Introduction

Heat stress during the summer disturbs reproductive processes in farm animals as it affects the physiology of the reproductive tract by several means like hormonal imbalance, decreased oocyte and semen quality, and decreased embryo development and survival (Wolfenson *et al*., 2000). Heat causes

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decreased secretion of the luteinizing hormone and oestradiol which causes reduced length and intensity of estrus expression, increased incidence of anestrus and silent heat in farm animals (Singh *et al*., 2021). In addition, based on temperatures-controlled studies, pregnant ewes exposed to high temperature (≥ 32° C) reduced lamb birth weight and lamb survival (Wettere *et al*., 2021). Such negative effects may be related to the produced reactive oxygen species, particularly during the stress of pregnancy and hot weather. At the same time using antioxidants may counteract such hazardous effects (Al-Gubory *et al*., 2010).

Astaxanthin (ASX) is a xanthophyll carotenoid which is found in various microorganisms and marine animals (Ambati *et al*., 2014). Supplementation of ASX improved the ability of maturation, fertilization, and development of porcine oocytes exposed to heat stress (Kim Do *et al*., 2015). A fundamental role of ASX in reproductive performance, including egg production and quality, has been evidenced in many aquatic animals (Tizkar *et al*., 2013; Palma *et al*. 2015). On The other hand, ASX prevented ovarian aging in laying hens and promoting the production of reproductive hormones hence the declining in reproductive performance in late laying period could be improved using astaxanthin (He *et al*., 2023). Supplementation of ASX may improve semen quality by increasing antioxidant enzyme activities and the ability to scavenge hydroxyl radicals aging layer breeder roosters (Gao *et al*., 2021).

In the current work it was hypothesized that astaxanthin (ASX) supplementation around mating and during pregnancy in Farafra ewes exposed to heat stress could improves the reproductive performance in ewes. Therefore this study aimed to investigate the ASX supplementation effect on Farafra ewes reproduction during heat stress and their lambs' performance until weaning.

Materials and Methods

Animals and dietary treatment

The present study was carried out at Mallawi Animal Production Research Station (location, 13° 30' S and 34° 00' E), Animal Production Research Institute (APRI), Agriculture Research Center (ARC), Ministry of Agriculture, Minia Governorate, Egypt; the experiment was from April 2022 to January 2023.

Sixty-Seven clinically and healthy mature, pluripara Farafra ewes, and average body weight of 36.18 ± 0.56 kg aged 2 - 7 years, were used in this study. Ewes were randomly divided into two groups. The first group (33 ewe) served as control group (C); ewes were fed control diet (concentrate feed mixture without any supplement). The second group (34 ewe) was Astaxanthin treated group, ewes were fed control diets plus 0.25 mg orally administration with astaxanthin /kg/ body wt/d (Somagond *et al*., 2019*)*. The duration of astaxanthin supplementation for 4 weeks transitional period, 35 days mating period and 3-month pregnancy period. Animals were kept in semi open pens under the normal environmental conditions at morning and animals were daily exposed to heat stress (solar radiation) from 12.00 pm to 14.00 pm. Air temperature and humidity

were recorded before and after exposure and THI was calculated according Mader *et al*. (2006). All ewes were fed on concentrate and roughage according to NRC (2007). Water was available at all times. The nature of lambing is normal. After lambing, lambs' number was recorded and weighed once weekly until weaning. Lambs body weight gain was calculated.

Table 1. Chemical composition of used feedstuffs (on DM basis)

Item	Dry matter, %	Organic matter, %	Crude protein, %	Crude fiber, %	Ether extract, %	NFE, %	Ash, %
Concentrates	92.3	82.34	13.75	14.28	3.23	51.09	10.01
Rice straw	90.9	84.10	4.0	33.4	1.6	45.1	15.1

In this part, ewes were estrus detected by using a teaser ram three times daily for 35 days and fertile rams were used to breed ewes that showed estrus. Ewes exhibited estruses were bred twice, at the beginning of estrus and later after 12 hrs. Estrus duration (hrs) was recorded and conception rate (%) was determined according to the number of diagnosed pregnant ewes by using the Chison Ultrasonic Scanner (Chison Medical Imagin Co. Ltd, 8300) equipped with a 5-MHz linear transducer at 45 days after mating. The gestational period was calculated. Also, number of lambs born/ewe and birth weight were recorded. Ambient temperature and relative humidity were recorded at all periods of the measurements through the experiment. A mercury centigrade thermometer was used to measure ambient temperature. A hygrometer hanging from the shed's roof at a level of about two meters from the ground was used to measure relative humidity.

Statistical analysis

Statistical analysis was performed by SPSS v. 21.0 for Windows (SPSS Inc., Chicago, IL). Data was analyzed by General Leaner Model (GLM) procedure and analyzed by an independent sample T-test. The following statistical model was used: $Yij= \mu +Ti + Eij$ Where, Yij= The studied trait, μ = The overall mean, Ti= The effect of treatment, Eij= The experimental error.

Results and Discussion

Temperature humidity index

Air temperature, relative humidity and temperature humidity index are presented in Table (2). According to air temperature animals were under extreme heat stress. Quadros (2023) reported that heat stress in small ruminants is moderate (27.75 -<28.86○ C), severe (28.86 - <29.97○ C) or extreme (> 29.97○ C). Also, according to Mader et al. (2006) ewes in our experiment were under mild stress before exposure and sever heat stress after exposure to solar radiation, particularly during the pregnancy period, when the air temperature was from 38 to 42 ○ C during pregnancy.

Table 2. Climatological data during the experimental period

Item		Ambient temperature (ºC)		Relative humidity (%)		Temperature humidity index (THI)	
		BSE	ASE	BSE	ASE	BSE	ASE
Premating	CG	32.13 ± 0.32	34.10 ± 0.30	16.96 ± 0.18	15.27 ± 0.16	63.15 ± 0.28	64.35 ± 0.32
	ASXG	32.07 ± 0.12	33.96 ± 0.24	16.96 ± 0.17	15.64 ± 0.32	63.09 ± 0.12	64.41 ± 0.17
At mating	CG	31.82 ± 0.14	34.40 ± 0.20	30.07 ± 0.23	23.31 ± 0.48	67.03 ± 0.15	67.70 ± 0.17
	ASGX	31.85 ± 0.13	34.66 ± 0.18	30.81 ± 0.33	23.02 ± 0.41	67.15 ± 0.11	68.09 ± 0.16
1 Mo. Pregnant	CG	35.03 ± 0.21	38.25 ± 0.19	32.31 ± 0.17	19.43 ± 0.46	70.29 ± 0.12	71.08 ± 0.16
	ASXG	34.98 ± 0.16	38.60 ± 0.20	32.38 ± 0.32	19.83 ± 0.25	69.94 ± 0.13	71.00 ± 0.21
2 Mo. Pregnant	CG	36.09 ± 0.13	42.09 ± 0.14	31.81 ± 0.14	15.54 ± 0.18	71.43 ± 0.14	72.23 ± 0.13
	ASXG	36.11 ± 0.14	41.94 ± 0.17	31.82 ± 0.15	16.16 ± 0.32	71.33 ± 0.14	72.40 ± 0.16
3 Mo. Pregnant	CG	34.86 ± 0.21	37.18 ± 0.27	39.13 ± 0.16	27.75 ± 0.14	71.23 ± 0.24	72.06 ± 0.28
	ASXG	34.73 ± 0.20	36.77 ± 0.32	39.15 ± 0.15	27.55 ± 0.12	71.00 ± 0.21	71.79 ± 0.24
4 Mo. Pregnant	CG	25.80 ± 0.17	33.13 ± 0.21	34.46 ± 0.23	36.33 ± 0.54	61.53 ± 0.21	70.54 ± 0.30
	ASXG	26.00 ± 0.18	32.57 ± 0.26	34.73 ± 0.18	37.00 ± 0.20	62.25 ± 0.51	70.24 ± 0.33

Values are least square means ± standard error. CG = Animals fed untreated diet. ASXG = Animals fed control diets plus 0.25 mg orally administration with astaxanthin /kg/ body wt/day. BSE = before sun exposure, ASE = after sun exposure

Reproductive traits

Effect of astaxanthin (ASX) on reproductive performance of ewes are presented in Figs. (1 and 2) and Table (3). No of ewes exhibited estrus tended to increase in ASX–supplanted ewes, its percentage increased by about 20%. Conception rate, % from total ewes increased by about 12% in favor of ASX – treated ewes. Also, no of lambs-born and lambs born alive increased by about 53% and 33%, respectively in ASX-treated ewes. Twining rate increased by about 19% in ASX-treated ewes. Ewes treated with ASX tended to give more female than male at birth. However, gestation length and average birth weight were not affected by ASX supplementation. Lambs weaning weight was improved by ASX supplementation, it increased ($P< 0.05$) about 25% (Fig. 3).

Astaxanthin (ASX) supplementation improved reproductive performance, particularly during heat stress. ASX is a free radical's scavengers which protect the body's defense system against excessively produced free radicals particularly during heat stress. Similarly, ASX improved fertility during heat stress (Sivakumar *et al*., 2010). ASX reduced the adverse effect of heat stress on the reproductive performance of sheep. In literature, lower levels of antioxidants are associated with poor fertility and production level of ruminants (Nayyar and Jindal, 2010).

The high conception rate in the present result may be related to change in luteal function in ASX- treated ewes under heat stress. Similarly**,** Sejian *et al*. (2013) found an increase in progesterone concentration in ewes who receive se-vitamin E compared to controls. Also, antioxidants such as melatonin improve follicular development, luteal function, early embryonic development and conception rate (Bauroutzika *et al*., 2020).

Table 3. Some reproductive parameters of ewes as influenced by antioxidant astaxanthin supplementation

Items	CG	ASXG
No. of ewes	33	34
No. of ewes exhibited estrus	24	31
Ewes exhibited estrus, %	70.85	91.17
No. of services /conception	1.70	1.83
No. of lambed ewes	14	18
Conception rate % from mated ewes	58.33	58.06
Conception rate% from total ewes	41.17	52.94
No. of lambs born	15	23
No. of lambs born alive	12	16
Twining rate	1.07 ± 0.07	1.27 ± 0.13
Males No.	10	8
Females No.	5	15
Sex ratio%	M 66.6 F 33.4	M 34.78 F 65.22
No of lambs produced (alive) /ewe lambed (litter size)	0.85	0.88
Gestation period (days)	147.25	146.66
Average birth weight	3.328	3.320
Lamb weaning weight	9.58^{b} ± 0.68	11.93^{a} ± 0.70

No: total number of ewes in each group. Values are least square means ± standard error. a and b means in the same row with different superscripts are significantly different (P <0.05). CG = Animals fed untreated diet. ASXG = Animals fed control diets plus 0.25 mg orally administration with astaxanthin /kg/ body wt/day.

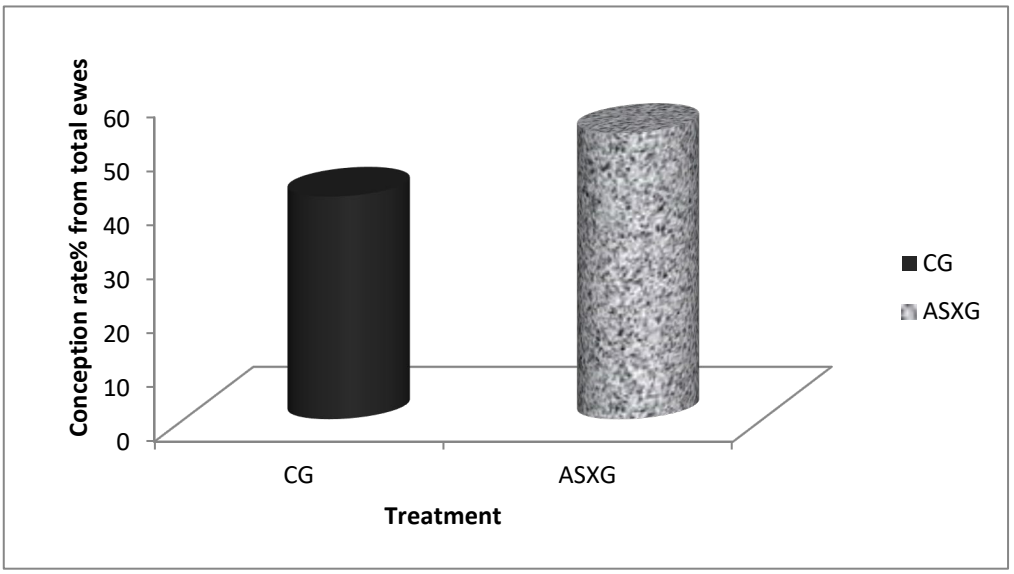

Fig. 1. Influence of astaxanthin supplementation ewes exhibited estrus, %.

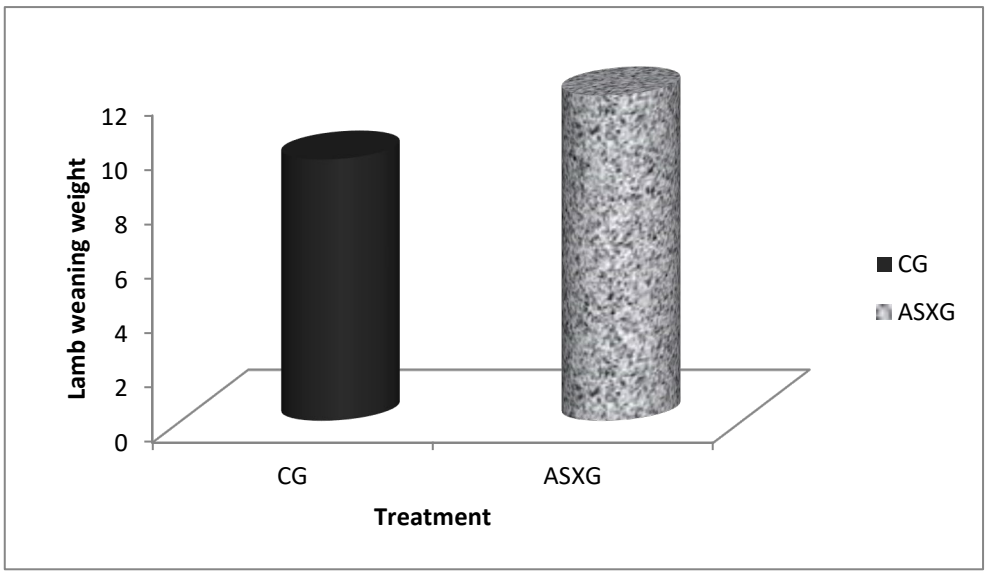

Fig. 2. Effect of astaxanthin supplementation on conception rate from total ewes.

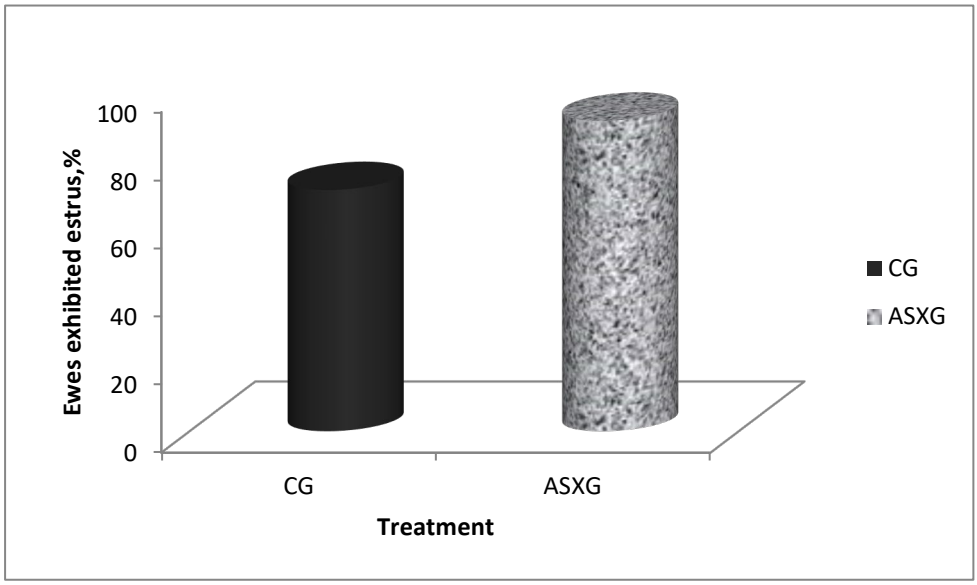

Fig. 3. Lamb weaning weight as influenced by astaxanthin supplementation.

The average litter size per ewe, twining rate, gestation period and average birth weight were not significantly affected by oral administrated of ASX group. Similar results were reported by Özar *et al*. (2022) who showed that litter size was not changed between Awassi ewes supplemented with β-carotene and vitamin E (dl-.-tocopherol acetate) and control group. Also, Hayder *et al*. (2016) showed that number of ewes exhibited estrus was not differed between Saidi and Farafra sheep exposed to heat stress for 2 hours and ewes supplemented with selenium yeast and sodium selenate (100 gm starch powder and 0.3 mg/kg diet) groups. In addition, Tang *et al*. (2022) found that the gestation period did not differ between cows during the summer season and cows during the winter season.

Lamb weaning weight was significantly higher ($P < 0.05$) in the ASX group than in the control group. The significant decrease in lamb weaning weight of the

control group is a normal response to the heat stress of growing animals. Ewes exposed to the heat stress reduces feed intake and increases water intake and there are changes in the endocrine status, which in turn increase the maintenance requirements leading to reduced body weight, average daily gain and body conditions of ewes. On the other hand, the higher lamb weaning weight of the treated group may be related to oral administration of astaxanthin supplementation which played major role in ameliorating the negative impact of environmental stressors on ewes (Kumar *et al*., 2019). Astaxanthin is a potent antioxidant that helps in protection of mammary tissue from free radicals that are produced in heat stress conditions and improves milking performance of dams **(**Somagond *et al*., 2019), consequently daily gain of their lambs.

The number of lambs born tended to be higher in the ASX group than control group. The results are similar to those obtained by DaghighKia *et al*. (2019) reported that Ghezel ewes fed diets containing antioxidant (vitamin E) had a higher ($P<0.05$) number of lambs born than those of the control group.

The average daily gain of suckling lambs was not significantly affected by ASX administration to ewes, however ASX treatment tended to increase the growth rate by about 12% compared to the control group (Fig 3). Similar results were reported by Awawdeh *et al.* (2015) found that the body weight gain of lambs was not affected between lambs born from ewes injected with vitamin E and control group. Also, Pilarczyk *et al*. (2013) reported that the average daily gain of lambs was not significantly affected between lambs born from that received orally selenized yeast oral supplementation of selenized yeast and the control group.

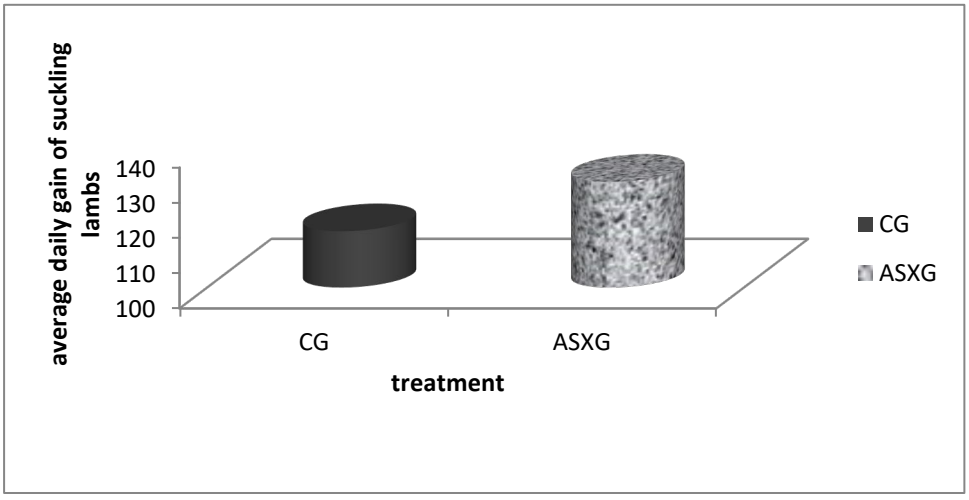

Fig. 4. Effect of astaxanthin supplementation on average daily gain of suckling lambs.

The average estrus period length is shown in Table (4). In general, about 85% of total ewes had an estrus period of less than 32 hours, while the rest (15%) had an estrus period 32 hours. or more. The result is a normal estrus duration period of ewes (Jainudeen *et al*., 2000; Bearden and Fuquay, 1984). It could be observed that the ewes's estrus received orally Asaxanthin were not significantly

affected by treatment. However, the percentage of treated ewes that extended their estrus until 16 hours was 25.8%, while that reached 32 hours was 48.39%, and the percentage of ewes that recorded 40 hours or more were 19.36 and 6.45%, respectively. The long estrus duration period of ASX-ewes may improve the chance of fertilization and reproductive performance of heat stressed-ewes. However, Hayder *et al*. (2016) showed that estrus duration did not differ in Saidi and Farafra sheep exposed to heat stress for 2 hours and ewes supplemented with selenium yeast and sodium selenate (100 gm starch powder and 0.3 mg/kg diet) groups. In addition, Saleh and Gomaa (2016) showed that estrus duration was not affected in Farafra and Saidi ewes exposed to heat stress for 2 hours and ewes supplemented with probiotic 3 Kg/ton (rich source of vitamins) as compared with control group.

Table 4. Influence of antioxidant astaxanthin supplementation on estrus duration length (hrs)

Item	No of ewes	Estrus duration length (hours)							
		8 to 16 hrs		16 to 32 hrs		32 to 40 hrs		40 hrs and more	
		No	%	No	%	No	%	No	%
CG	24	13	54.16	11	45.84	-	-	-	-
ASXG	31	8	25.80	15	48.39	6	19.36	2	6.45

No: total number of ewes in each group. Values are least square means ± standard error. CG = Animals fed untreated diet. ASXG = Animals fed control diets plus 0.25 mg orally administration with astaxanthin /kg/ body wt/day.

In conclusion, supplementation of ASX around mating and during pregnancy could improve the reproductive performance of ewes exposed to heat stress.

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تـأثير أضـافة الأسـتازانثين كمضـاد للأكسـدة علـى الأداء التناسـلي لنعـاج الفرافـرة وأداء النمـو لحملانـها المعرضة للإجهاد الحراري

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الملخص

أجريت هذه الدراسة لتحديد تأثير التجريع بالأستازانثين كمضاد للأكسدة على الأداء التناسلي لنعاج الفرافرة وأداء النمو لحملانها حتى الفطام والمعرضة للإجهاد الحراري. 67 نعجة فرافرة قسمت الى مجموعتين متساويتين في وزن الجسم 0.56 ±36.18 كجم والعمر. المجموعة الضابطة (33 نعجة) تم تغذيتها على مخلوط العلف المركز بدون أي إضافة. المجموعة المعاملة (34 نعجة) تم تغذيتها على العلف المركز بالإضافة الى التجريع بالأستازانثين بمعدل 0.25 مجم من وزن الجسم لكل كجم وزن حي خلال فترة ما قبل التلقيح والتلقيح والحمل. الحيوانات في كلتا المجموعتين عرضت لأشعة الشمس لمدة ساعتين يوميا كإجهاد حراري. القياسات التناسلية سجلت وحللت إحصائيا باستخدام برنامج SPSS. أظهرت النتائج أن التجريع بالأستازانثين زود عدد النعاج التي أظهرت شياع كنسبة مئوية ومعدل الإخصاب كنسبة مئوية من عدد النعاج الكلى بحوالي 20 بالمائة و 12 بالمائة على التوالي. بالإضافة الى عدد الحملان المولودة وعدد الحملان التى بقت على قيد الحياة زادت بنسبة حوالي 53 بالمائة و 33 بالمائة لصالح معاملة الأستازانثين. التجريع بالأستازانثين حسن من معدل التؤأمية ووزن الفطام للحملان ($p< 0.05$) بصورة معنوية. التجريع بالأستازانثين زود وزن الفطام للحملان بنسبة 25 بالمائة. ولكن مدة الحمل ومتوسط وزن الميلاد لم يختلف بين المعاملتين. ويستخلص من ذلك أن التجريع بالأستازانثين أثناء التلقيح وأثناء فترة الحمل قد تحسن من الأداء التناسلي خلال التعرض للإجهاد الحراري.

الكلمات المفتاحية: *مضادات الأكسدة، النعاج الفرافرة ، الأستازانثين ، الأداء التناسلي.*