

Effect of N-Acetyl Cysteine, Selenium and Zinc as Oral Nutritional Supplement on Reproductive Performance in Local Iraqi Ewes.

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Abstract

The study aimed to investigate the effect of the administration N-acetyl cysteine to ewes with or without selenium and zinc on reproductive performance and the level of estrogen and progesterone hormones for local ewes. 24 local ewes were used, 3-5 of age and weighing 45-55 kg. They were randomly distributed to 4 group, 6 ewes for each one. All ewes were treated six days before pushing the vaginal sponges and continued for 45 days. The Group 1 (T1) was treated with (10 mg N-acetyl cysteine/kg body weight), Group 2 (T2) was treated with (10 mg N-acetyl cysteine/kg body weight + 0.03 mg selenium/kg body weight), Group 3 (T3) was treated with (10 mg N-acetyl cysteine/ kg of body weight + 0.03 mg zinc / kg of body weight) while the Group 4 (T4) was left without treatment (control group). The results showed a significant increased ($p \leq 0.05$) of treatment (T1) at the time of first estrus, followed by the control treatment, then treatment (T2, T3). Results recorded a significant increased ($p \leq 0.05$) of T1, T3 and T2 respectively in estrus rate. Furthermore, the treated group showed a significant increased ($p \leq 0.05$) compared with control group in the fertilization %pregnancy, the percentage of twins, births and the number of births. The supplement N-acetyl cysteine with selenium or zinc led to an improvement in the reproductive performance.

Keywords: N-acetyl cysteine, Selenium, Zinc, Reproductive performance, Iraqi ewes

تأثير التجريب الفموي N-acetyl cysteine والسيلينيوم والزنك كمكمل غذائي في الأداء التناسلي للنعاج العراقية

المحلية

الخلاصة

هدفت الدراسة الى معرفة تأثير تجريب النعاج N-acetyl cysteine مع او بدون السيلينيوم والزنك في الاداء التناسلي ومستوى هرموني الاستروجين والبروجستيرون للنعاج المحلية. اذ تم استخدام 24 نعجة محلية تولدت اعمرها من 3-5 سنوات ووزن 45-55 كغم تم توزيعها بشكل عشوائي الى 4 معاملات بواقع 6 نعاج لكل معاملة، وعوملت النعاج قبل دفع الاسفنجات المهبلية بستة ايام واستمر لمدة 45 يوما، اذ عوملت المجموعة الاولى (T1) بواقع 10 (ملغم/ N-acetyl cysteine كغم من وزن الجسم المجموعة الثانية (T2) (10 ملغم / N-acetyl cysteine كغم من وزن الجسم + 0.03 ملغم سيلينيوم/ كغم من وزن الجسم) والمجموعة الثالثة (T3) (10 ملغم- N-acetyl cysteine/ كغم من وزن الجسم + 0.03 ملغم زنك/ كغم من وزن الجسم) اما المجموعة الرابعة (T4) تركت دون معاملة (مجموعة سيطرة). أظهرت النتائج زيادة معنوية ($p \leq 0.05$) في المعاملة (T1) في وقت ظهور الشبق الأول ، تليها معاملة السيطرة ، ثم المعاملة (T2 ، T3). سجلت النتائج زيادة معنوية ($p \leq 0.05$) في T1 و T3 و T2 على التوالي في معدل الشبق. كما أظهرت المجموعة المعالجة زيادة معنوية ($p \leq 0.05$) مقارنة بالمجموعة السيطرة في نسبة الإخصاب % ، الحمل ، نسبة التوائم والمواليد وعدد المواليد. أدى تجريب المكمل الغذائي N-acetyl cysteine بالسيلينيوم أو الزنك إلى تحسين الأداء التناسلي.

Introduction

Vaginal sponges are one of an important techniques to increase the fertility sheep (1), However, despite of these advantages, it has disadvantages, including collection of mucus, obstruction of sperms movement, and occurrence of infections (2), this would reduce fertility rate from this important technology, in addition to producing immature eggs with a low ability to fertilization (3), therefore, researchers worked to reduce these disadvantages and develop solutions by use of antibiotics (4) Modern science has turned to use of nutritional supplements that reduce negative effects of vaginal sponges and improve reproductive performance (5), The N-Acetyl Cysteine (NAC) is nutritional supplement had antioxidant and immune-stimulating properties while it must be reduces the negative effects of vaginal fluid and bacterial accumulation (6), its works as a mucolytic agent by changes its viscosity to watery consistency by breaking sulfide bonds due to presence of a sulfhydryl group, thus facilitate mucus discharge (7). In addition, it helps in accelerating eggs formation by increasing blood supply and increasing follicles growth through increasing nitric oxide (NO) concentration, which positively affects growth and eggs development with a high fertilization capacity. in other hand, the nutritional supplements (selenium (Se) and zinc (Zn)) have many advantages, such as they have synergistic effects for the action of NAC, being antioxidants, and decreased oxidative stress. There is a lack that also has a role in blood supply and increases reproductive health, which may have a positive role in increasing the effectiveness of female genitalia. the lack of scientific studies on using the nutritional supplement NAC alone or with selenium and zinc, the study aimed to treat estrus-uniform ewes using vaginal sponges with nutritional supplement NAC supplemented with selenium and zinc to discover its effect on improving reproductive performance and level of estrogen and progesterone hormones.

Material and methods

This study was conducted in the College of Agriculture \University of Anbar from 10/24/2021 to 04/24/2022, (24) local Iraqi ewes were used, aged (3-5) years, with an average weight of 45-55 kg. Ewes were randomly divided into four treatments group, with 6 ewes per treatment, Group1 (T1) treated with N-acetyl cysteine (10 mg/kg of body weight), Group2 (T2) treated (10 mg N-acetyl cysteine +0.03 mg selenium/kg of body weight), Group3 (T3) was treated (10 mg N-acetyl cysteine +0.03 mg zinc/kg of body weight) while the group4 (T4) serve as control group. oral dosing was started 6 days before vaginal sponges placed and continued for 45 days. Blood samples were collected from jugular vein six times, at the first day of oral dose, the second of time at sixth day of placing vaginal sponges, third at the vaginal sponges withdrawal, the fourth is at estrus time, fifth period is to measure progesterone concentration level to diagnose pregnancy. Finally, the sixth period, on the 45th day of oral dose, to determine the level of estrogen and progesterone concentrations. hormones level was measured using a ready-made kit for sheep produced by Korain Biotech Co. The measurement was carried out using ELISA device manufactured by Bio-tek company. Statistical analysis was conducted (One Way Analysis), included effect of experimental treatments on studied traits, by following the General Linear Model and using the ready-made SAS statistical program 9.6th version (8). The significant differences between means were tested using Duncan's polynomial test (9) at the level of significance ($P \leq 0.05$), and the significant differences between percentages of reproductive efficiency indicators were compared with the Chi-Square test.

Results and discussion

The effect of treatment on the time and percentage of estrus:

The results Table (1) showed a significant increased ($p \leq 0.05$) of T1 at the time of the first estrus appearance, followed by control group, then the T2 and T3 at a rate of 30, 45, 48, 48 hours, respectively. The reason for that may be attributed to effectiveness of NAC in restoring ovarian activity and increasing growth and development of mature follicles thus increases the level of estrogen concentration (10).

The results showed that the T1 and T2 were highly significant in incidence of estrus, followed by the T3 and T4, which recorded (100%, 100%, 66.66%, 66.66%), respectively. The reason may be attributed to combined effectiveness of NAC and selenium as antioxidants and reduce oxidative stress inside ovary and increase activity of glutathione inside mature follicle, which reflects positively on increase in the level of estrogen secretion responsible for appearance of signs of estrus. (11, 12, 13), As well as the role of NAC nutritional supplement in raising levels of zinc oxide, as it works to expand blood vessels and increase supply of nutrients to reproductive system, which leads to the growth of mature follicles and improve the secretion of estrogen (14). The results match with results of (6) when local ewes were orally supplemented with NAC at different levels for 30 days, as it was noted that the treatments were significantly superior in rate of estrus emergence.

The effect of treatments on fertility, fertilization and pregnancy rate:

The results Table (1) showed the T2 (10 mg NAC + 0.03 Se) was a significant increased ($p \leq 0.05$) to all treatments in percentage of fertilization, fertility and pregnancy, which amounted to (100%), followed by T1 (10 mg NAC / kg of live weight). It reached 83.33%, and then T3 (10 mg NAC + 0.03 mg Zn), which

recorded to 66.66% compared with control group 50%.

Selenium is effective in stimulating growth and development of follicles, increasing the DNA activity of cumulus cells that have a role in transferring glutathione directly to egg and reducing oxidative stress, which results in high quality eggs capable of fertilization and pregnancy (11). On the other hand, its important role in raising the rate of implantable blastocyst formation (11) and improving function of corpus luteum through its secretion of progesterone, thus making uterine environment more conducive to sustaining pregnancy (15) as well as the effectiveness of NAC in Inhibition of IL2, IL6, IL8 and Tumor Necrosis Factor, which leads to scavenging of free radicals (ROS) and remove the effect of oxidative stress in ovarian epithelium and fertilized eggs (13). It also has a role in preparing the lining of uterus for pregnancy by increasing the thickness of uterus lining suitable for formation and implantation of blastocyst and raising rate of pregnancy (16). The zinc element also has a role in activating and preparing egg for fertilization in addition to its effectiveness in converting fertilized egg into blastocyst through a spark Zinc (17).

The effect of treatments on birth rate and the percentage of twins:

The results Table (1) showed that the T2 (10 mg NAC + 0.03 Se) was a significant increased ($p \leq 0.05$) in birth rate, as it reached 150%, followed by (T1) (10 mg NAC / kg of live weight), which amounted to 116.66%, then (T3) (10 mg NAC + 0.03 mg Zn) was superior to control by 83.33%, and control group was least with rate 66.66%. As for percentage of twins, (T2) was a significant increased ($p \leq 0.05$), reaching 50%, followed (T1) group, which recorded 40%, while (T3) was equal (T4) (control group) in percentage of twins, which recorded 25%.

The reason may be attributed to effectiveness of nutritional supplement NAC in raising the level of zinc oxide, which enhances supply of nutrients to body's organs, including the reproductive system, through dilation of blood vessels, improving blood flow and accelerating its transmission to the genitals, thus developing the growth of mature follicles and obtaining a number of eggs of quality and quality. Highly fertile, thus increasing the proportion of twins (14). As well as the combined effectiveness of treatments used (NAC, Se, Zn) as antioxidants and their ability to remove effect of oxidative stress inside the ovary (12), in addition the role of NAC, Se in preparing uterus for pregnancy by increasing thickness of endometrium and promoting blastocyst implantation and promoting Luteal corpus luteum secretion of progesterone to maintain pregnancy (18)

The results of T1 matched with (14) and (6) explained that use of NAC leads to an increase in the birth rate in sheep and goats compared to the control group.

Effect of treatments on the number of single and fertile births

The results of study showed Table (1) the a significant increased ($p \leq 0.05$) of T2 in number of births, which amounted to 1.5, followed by T1, T4 and T3 which recorded (1.4, 1.33 and 1.25), respectively. As for fertility (Fecundity, T2 group) was significantly superior, as it reached 1.5, followed by T1, with 1.16, and T3 group with 0.83 compared control group which recorded 0.66. The reason may be attributed to effectiveness of treatments used (NAC, Se, Zn) as antioxidants and their ability to remove effect of oxidative stress inside the ovary, which is reflected positively on improving the quality and number of fertilized eggs as well as their role in preparing uterus for pregnancy by increasing the thickness of endometrium and promoting implantation of cyst Blast blastoma also has a role in enhancing corpus luteum function by increasing progesterone to sustain pregnancies.

(19, 16, 15).

Effect of treatments on barrenness rate

The results Table (1) showed a decrease in barrenness rate in T2, was recorded 0%, followed by T1 16.66%, T3 33.33%, and T4 (control group) 50% . the reason may be attributed to role of supplements food as antioxidants that reduce oxidative stress and create an appropriate healthy uterine environment to increase efficiency of follicles and improve quality of fertilized eggs, which is positively reflected in a decrease in rate of missed. The viscosity of vaginal fluids and purulent infections being a foreign body, which impedes timely transfer of sperm to fertilization area and thus failure of fertilization process. Results are in agreement with previous studies (14, 6).

Effect of treatments on the level of progesterone concentration

Table (2) shows there were no significant differences between treatments in progesterone concentration level except for fifth period (pregnancy diagnosis). first and second groups were a significant increased ($p \leq 0.05$) compared with control group, the reason for superiority may be attributed to effectiveness of NAC by increasing level of nitric oxide (ON), which act to increase blood flow through dilation of blood vessels, thus promoting corpus luteum growth development and increasing activity of progesterone secretion responsible for survival and placental growth development to secrete progesterone to perpetuate pregnancy (14). In addition to selenium role with regard to progesterone level within same treatment, a significant difference was observed between periods within first, second and third treatment as a result of treatment with vaginal sponges saturated with progesterone, then it decreases when sponges are withdrawn during fourth period, then continues with superiority in sixth period and this may be attributed to selenium role synergistic with NAC by raising implantation

rate and pregnancy blastocyst formation (11), improving corpus luteum function through progesterone secretion and thus making uterine environment more conducive to sustaining pregnancy (15).

Effect of treatments on estrogen concentration level.

Table (3) indicates that there were no significant differences in estrogen concentration level between treatments, except fourth period (period of estrus), where first and second groups were significantly superior to control group, this may be attributed to the effectiveness of NAC in improving the estradiol due to level, also leads to increase in nitric oxide level, as it act to dilate blood vessels and increase blood flow to genitals, thus developing growth of mature follicles, which positively affects estrogen secretion. On the other hand, selenium and zinc stimulate reproductive system and participate in mature follicles growth and development as well as regulate secretion of Estrogen hormone (20) As for estrogen level within one treatment, a significant difference at ($p \leq 0.05$) was observed between periods within all treatments, this treatment might be due to the vaginal sponges saturated with progesterone, then it rises when sponges are withdrawn from fourth period (estrus) to return to decrease in sixth period as a result for pregnancy.

results matched with the result of (14) when Iraqi local sheep were fed with (NAC) with different doses. Who noticed significant differences in reproductive performance compared to control group, and significant differences in estrogen and progesterone concentration, as the results of study matched with (23) observed significant differences in performance reproductive system in female goats The study results disagree with (22), there were no significant differences observed in level of estrogen and progesterone

concentrations. The study results also disagree with (14) when added 7% of NAC with feed to female Nubian goats, as it was found that there were no significant differences in level of estrogen and progesterone concentrations, and this may be due to difference in used dose or the animal species.

Conclusion

The supplement ration by N-acetyl cysteine with selenium or zinc led to an improvement in the reproductive performance, in addition improving in the level of the sex hormones estrogen and progesterone.

Conflict of interest

The authors haven't conflict.

**Table 1: Effect of N-acetyl cysteine, selenium and zinc
on reproductive performance**

Adjective	T1	T2	T3	T4	Chi-Square (χ^2)
Total number of females	6	6	6	6	--
Time of first appearance of estrus / hour	30 \pm 1.26 b	48 \pm 2.18 a	48 \pm 2.18 a	45 \pm 1.73 a	**
Average estrus time/hour	50 \pm 2.07	47 \pm 1.85	52 \pm 2.16	51 \pm 2.04	NS
The number of females who showed eroticism	6	6	4	4	--
Occurrence of estrus (%) rate	100%	100%	66.66%	66.66%	9.42 **
Number of pregnant females	5	6	4	3	--
Number of females giving birth	5	6	4	3	--
(%) Fertility percentage	83.33%	100%	66.66%	50%	12.39 **
(%)Fertility rate	83.3%	100%	66.66%	50%	12.39 **
(%)pregnancy rate	83.33%	100%	66.66%	50%	12.39 **
Ttotal number of births	7	9	5	4	--
Number of twin births	2	3	1	1	--
(%) Birth rate	116.66%	150%	83.33%	66.66%	26.02 **
(%)Twins ratio	40%	50%	25%	25%	9.61 **
The number of newborns in one womb	1.4 \pm 0.07 ab	1.5 \pm 0.08 a	1.25 \pm 0.05 c	1.33 \pm 0.05 b	*
Fertility	1.16 \pm 0.06 bc	1.5 \pm 0.09 a	0.83 \pm 0.04 cd	0.66 \pm 0.4 d	**
(%) barrenness rate percentage	16.66%	0%	33.33%	50%	13.47 **

.non-significant :NS ,(P<0.01) **, (P<0.05) *

Table 2. Effect of N-acetyl cysteine, selenium and zinc On progesterone concentration

sampling days	Treatments				morale level
	T1	T2	T3	T4	
First period	B 0.15±1.42 a	C 0.26±1.52 a	B 0.13±1.73 a	A 0.28±1.49 A	N. S
Second period	A 0.23±2.65 a	B 0.31±2.48 a	AB 0.28±2.81 a	A 0.20±2.42 a	N. S
Third period	A 0.34±3.05 a	AB 0.19±2.95 a	A 0.47±2.95 a	A 0.34±2.47 a	N. S
Fourth period	AB 0.24±1.84 a	C 0.15±1.60 a	AB 0.27±1.91 a	A 0.27±1.88 a	N. S
Fifth period	A 0.49±3.08 a	AB 0.35±3.60 a	AB 0.31±2.40 a b	A 0.49±1.70 b	* 0.0252
Sixth period	A 0.66±3.10 a	A 0.35±3.79 a	AB 0.54±2.55 a	A 0.47±2.56 a	N. S
Sign. level	* 0.0203	** 0.0001	* 0.0448	N. S	

* The values represent the mean ± standard error.

** N. S: It means that there are no significant differences between the treatments or the days of sampling at the level of significance ($P \leq 0.05$), ($P \leq 0.01$).

a, b, c: The different small letters within the same row indicate the presence of significant differences between the treatments, while the different capital letters within the same column indicate the presence of significant differences between the days of sampling within the same treatment at the level of significance ($P \leq 0.05$), ($P \leq 0.01$).

Table 3. Effect of N-acetyl cysteine, selenium and zinc On the estrogen concentration

sampling days	Treatments				morale level
	T1	T2	T3	T4	
First period	BC 1.14± 11.70 a	B 0.68± 12.02 a	BC 0.77± 10.81 A	B 1.99± 11.19 a	N. S
Second period	BC 1.06± 8.79 a	B 1.38± 8.98 a	C 1.11± 9.31 a	B 0.88± 9.18 a	N. S
Third period	C 0.96± 7.58 a	B 1.49± 10.27 a	BC 1.36± 10.75 a	B 0.99± 8.78 a	N. S
Fourth period	A 1.83± 19.85 a	A 1.16± 24.95 a	A 3.30± 19.55 a b	A 1.26± 16.61 b	* 0.0462
Fifth period	B 2.62± 13.37 a	B 2.33± 11.57 a	AB 1.74± 16.44 a	A 2.46± 18.39 a	N. S
Morale level	** 0.0002	** 0.0001	** 0.0026	** 0.0005	

* The values represent the mean ± standard error.

** N. S: It means that there are no significant differences between the treatments or the days of sampling at the level of significance ($P \leq 0.05$), ($P \leq 0.01$).

a, b, c: The different small letters within the same row indicate the presence of significant differences between the treatments, while the different capital letters within the same column indicate the presence of significant differences between the days of sampling within the same treatment at the level of significance ($P \leq 0.05$), ($P \leq 0.01$).

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