

Effect of arginine and selenium with vitamin E in some biochemical properties of Inseminated ewes

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Abstract

The study was conducted in one of the fields of the private sector within Basaaer the village of the district of Hit / Anbar at 180 km west of Baghdad, during the period from 17/4/2018 to 30/5/2018. 28 ewes with an age of 2-4 years with a weight of 40-50 kg with at least one birth. All ewes were tested using ultrasonography to ensure that they were free of pregnancy before the start of the experiment. Synchronization of estrus with intravaginal sponges was achieved in ewes, and inseminated with rams at the end of the hormonal program. The rams remained with the females for five days. The ewes were randomly divided into four equal groups (7 ewes in each group). The first group were injected with 160 micro mol / kg body weight and from the fifth day of the insemination until day 20 of pregnancy. The second group was treated by injecting selenium plus vitamin E with (2 ml/head α -Tocopheryl Acetate 68mg/ml Selenium 1.5mg/ml) intramuscular. The first dose was given on day 5 and the second one on day 19 of insemination the early at pregnancy. The third group were injected the arginine with the selenium plus vitamin E emulsion, The fourth group was injected with a physiological saline solution and considered as control group. Blood samples were collected from jugular vein prior to treatment on day 4 of the insemination as well as on days 10, 15, and 18, to measure the changes in the chemical properties at the early of pregnancy. The results of the study showed a significant difference ($P \leq 0.05$) between the treatments and control groups. The results showed a decrease in the concentration of urea and ALT enzyme as compared with the first group and control. The present study did not show a significant differences at early of pregnancy in the concentration of cholesterol, total protein, albumin, globulin, glucose, and AST enzyme at the early of pregnancy for ewes. The results also showed a significant difference ($P \leq 0.05$) between the different withdrawal periods within the same treatment in the control and other groups the concentration of ALT enzyme, and total protein.

It can be concluded from this study that the use of arginine and selenium with vitamin E improves maternal health by reducing the level of urea and ALT enzyme and maintaining pregnancy after insemination.

تأثير الارجنين والسلينيوم مع فيتامين هـ في بعض الصفات الكيموحيوية للنعاج المسفدة
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الخلاصة

أجريت الدراسة في احد حقول القطاع الخاص ضمن قرية بصائر التابعة لقضاء هيت/الأنبار والتي تبعد 180 كم غرب بغداد ، للفترة من 17/4/2018 ولغاية 30/5/2018. على 28 نعجة مسفدة بعمر يتراوح بين 2-4 سنوات وذات وزن يتراوح بين 40-50 كغم، وذات ولادة واحدة على الأقل وتم فحص جميع النعاج باستخدام جهاز الموجات صوتية (السونار) للتأكد من خلوها من الحمل قبل بداية التجربة. تم توحيد الشبق في النعاج باستخدام الاسفنجيات المهبلية لمدة 12 يوم وعند الازالة حقن هرمون eCG، ثم سفدت النعاج من خلال اطلاق الكباش معها عند نهاية البرنامج الهرموني واستمر وجود الكباش مع الإناث لمدة خمسة ايام. قسمت النعاج عشوائيا الى اربع مجاميع متساوية (7 نعاج في كل مجموعة) عوملت المجموعة الاولى بحقن الأرجنين بالعضل بمقدار 160 مايكرومول/كغم وزن الجسم وحقن من اليوم الخامس من التسفيد لغاية اليوم 20 من التسفيد (بداية الحمل)، و عوملت المجموعة الثانية بحقن بالعضل وكانت الجرعة الاولى في اليوم 5 والثانية

في اليوم 19 من التسفيد في بداية الحمل، اما المجموعة الثالثة حققت بالارجنين مع خليط السلينيوم فيتامين هـ، وحققت نجاح المجموعة الرابعة بالمحلول الملحي الفسيولوجي مثل المجموعة الثانية واعتبرت مجموعة سيطرة. سحبت عينات الدم من الوريد الوداجي للنجاح قبل المعاملة بيوم اليوم 4 من التسفيد وكذلك عند الأيام 10، 15، 18. من الحمل وكان هذا لقياس التغيرات في الصفات الكيمائية في بداية الحمل. اظهرت نتائج الدراسة وجود فرق معنوي بين مجاميع المعاملة والسيطرة وسجلت انخفاض في تركيز اليوريا وانزيم ALT مقارنة مع المجموعة الاولى والسيطرة. ولم تظهر نتائج الدراسة الحالية فروق معنوية في بداية الحمل في تركيز كل من الكولسترول والبروتين الكلي والاليومين والكلوبيولين والكلوكوز وانزيم AST في بداية الحمل للاغنام. واطهرت النتائج وجود فرق معنوي بين فترات السحب المختلفه ضمن المعاملة الواحدة في مجاميع المعاملات دون السيطرة في تركيز انزيم ALT والكلوبيولين والبروتين الكلي.

يمكن الاستنتاج من هذه الدراسة بان استخدام الارجنين والسلينيوم مع فيتامين هـ تؤدي الى تحسين الحالة الصحية للام من خلال خفض مستوى انزيم ALT والحفاظ على الحمل بعد التسفيد.

الكلمات المفتاحية: الارجنين، السلينيوم مع فيتامين هـ، بداية حمل، كيموحيوية

Keywords :- : Arginine, Selenium with vitamin E, Early pregnancy, Chemical

Introduction

The livestock sector suffers from a constant deficit in providing the required needs, especially in the Arab world and Iraq in particular, and there is a need to develop this sector to meet the challenges of increasing the demand for it, Sheep suffer from some of the problems in the reproduction leads to cause economical losses. The most important problems are low fertility, fetal loss, fetal weakness and death after birth, which may occur for several reasons, including nutrition, illness or diseases (1, 2). So many researchers in their studies have been interested in increasing production by increase the reproductive efficiency of the proportion of fertility and the proportion of twins and births to good levels, as well as the care of pregnant animals for healthy lambs or through the use hormones (3). It has been reported the importance of vitamins and minerals, including vitamin E and selenium (4), Amino acids, including amino acid arginine (5) and fatty acids (6). Both nutrition, immunology, endocrinology and reproductive studies suggest that the lack of selenium and vitamin E leads to decreased fertility, placental retention, abortion and poor fetuses (7). Arginine has an important role in regulating growth and metabolism (8). Recent studies have shown that arginine plays a role in stimulating the growth and development of the placenta (9), Prolactin and IGF Insulin like Growth Factor 1, IFN-t, Glycogen (10). Treatment of pregnant ewes with arginine or

selenium plus vitamin E increases fetal growth by increasing The arrival of food to the fetus and the maintenance of pregnancy (11), nitrogen uptake and reduction of ammonia toxicity in tissues (12). However the previous studies did not showed significantly the use of arginine with selenium and vitamin E during pregnancy and its impact on the state of health of the dam and fetus and may considered as an indicators of the values of lambs and chemists in addition to measuring concentrations of sex hormones. The goal of the study is to show the effect of arginine or selenium vitamin E combination or both of them on the chemical properties include cholesterol, total protein, albumin, globulin, glucose, and liver enzymes A.S.T and A.L.T at the early of pregnancy.

Material and method

This experiment was conducted in one of the fields of the private sector in the village of Hit / Anbar (Basaaer village) located on the right side of the river Euphrates and 70 km west of Ramadi, during the period from 17/4/2018 to 30/5/2018.

Twenty-eight non-pregnant local ewes were selected with a good physical condition and at least give one birth with an average age of 2-4 years and an average weight of 40-50 kg. ultrasonography was applied to ensure that they are free from pregnancy before the start of the experiment. Synchronization of estrus using vaginal sponges (60 mg MAP, Novormon made

in Argentina). When the sponges removed, eCG dos of 500 IU (Novormon 5000 made in Argentina) was injected and naturally spilled with rams. Six rams were selected for insemination (1 male: 4 females). The rams were kept for five days, then the ewes were divided into four equal groups (7 ewes in each group). Each group was then placed in a 16 m² area with 4 × 4 fenced, in well-ventilated place.

The animals were fed at morning and evening with at 500 g per head feeding concentration, (barley, corn, the bran, the wheat bran) using the fodder in each fold, the coarse feed, which represents straw, green and the dry food, the during the experiment. Mineral licks were hanged at the wall of barns.

Experimental design: The ewes were randomly assigned to four equal groups (7 ewes / group), taking into account the rate of weights when the animals were divided into groups. During the study, the first group I (T1) was injected with arginine by 160 µMol / kg body weight and 3 times daily by the day 5 of the insemination to 20 days at early pregnancy. The second group (T2) were injected with a selenium vitamin E mixture (Selenium 1.5mg/ml, vitamin E 68 mg/ml, made in United Kingdom Company Norbrook) with 2 ml of head / day 5 and day 19 of the early pregnancy. The third group (T3) was injected with the combination of selenium vitamin E 2 ml with arginine 160 µMol early pregnancy. fourth group (T4) was injected with the normal saline.

Blood samples: (5 ml) were collected with vacuotainer tube from the jugular vein of ewes. placed in a tube and left for two hours at room temperature, then centrifuged (3000 cycles / min) for 20 minutes. The serum was separated from the rest of the ingredients and kept at -20 °C until the total protein, albumin, cholesterol, cholesterol, glucose, AST and ALT. Blood was collected before the initiation of treatments Arginine and Selenium vitamin E at the early pregnancy on day 4 of the insemination as well as on days 10, 15, 18. Using kits of Linear chemicals made in Spanish.

Statistical analysis type Duncan test were done using SAS program 9.1 (13).

Results and Discussion

Effect of arginine 160 µmol, selenium and vitamin E 2 ml on chemical

1-Cholesterol concentration(Table-1).

The results of the present study showed no significant differences in the level of ($P \leq 0.05$) in the concentration of cholesterol at the early pregnancy between the three experimental groups and control group. The results were concurred agreed with Kaminski et al., (14) that giving arginine and did not showed affect on the level of serum cholesterol in ewes.

The results of the current study agreed with Kamada (15) who reported that cholesterol in blood plasma was not significantly affected when giving selenium at the late pregnancy. The resorts also agreed with Ahmed (16) who reported that the concentration of cholesterol in eugenic serum was not significantly affected between the arginine injected group and the control group at the early pregnancy. The results disagreed with Moeini et al., (17) where he observed that injecting selenium vitamin E before birth four weeks to two weeks lead to an increase in the level of cholesterol. Soliman (18) indicated that the superiority of the group selenium vitamin E group as compared with the control group. The non-significant effect of cholesterol in the experimental groups mi the be attributed to the fact that cholesterol enters the cycle of formation of steroid hormones (19).

2-Total protein concentration(Table2). The results of the present study showed no significant differences between the three experimental groups and the control group in the total protein concentration and were concentrated within the normal level.

The results of the study were consistent with those found by Alhidary et al., (4) that giving selenium or vitamin E or both

did not significantly affect the value of total protein in ewes. Results showed that the dietary supplements used in this study did not affect the total protein level during the different periods of withdrawal, but the high concentration of total protein were observed in the second group (selenium vitamin E) After the injection of selenium vitamin E on the fifth day of pregnancy and increased its concentration on the 10 day, 15 and 18 with the progress of pregnancy and injection of the second dose of selenium vitamin E.

The results were agreed with Soliman (18), which found an increase in the total

Table – 1: The effect of different treatments in different days on Cholesterol (mg/dl)

Days	Treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 2.66 ± 70.0 a	A 2.71 ± 66.6 a	A 6.18 ± 67.4 a	A ± 64.2 * 4.18 a	N.S.**
Ten day	A 2.29 ± 69.6	A 2.18 ± 68.6	A 6.05 ± 68.0	A 3.30 ± 65.0	N.S.
Fifteenth day	A 2.78 ± 69.2	A 2.48 ± 66.4	A 4.69 ± 67.4	A 1.07 ± 63.4	N.S.
Eighteen Day	A 2.60 ± 66.6	A 1.76 ± 69.0	A 6.76 ± 68.2	A 3.46 ± 65.2	N.S.
Significant Level	N.S.	N.S.	N.S.	N.S.	

* Values=Means ± SE.

** N.S = Mean No significant difference (P≤0.05).

a. b. c. small letters within one row indicate significant differences between the treatments. The capitals letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

Table – 2: The effect of different treatments in different days on Total Protein (mg/dl)

Days	Treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A ± 6.44 0.355 a	A 0.177 ± 6.42 a	B 0.087 ± 6.04 a	A * 0.165 ± 6.18 a	N.S.**
Ten day	A ± 6.80 0.366 a	A 0.374 ± 7.18 a	A 0.228 ± 7.20 a	A 0.234 ± 6.70 a	N.S.
Fifteenth day	A ± 6.76 0.390	A 0.344 ± 6.92 a	A 0.381 ± 6.78 a	A 0.263 ± 6.92 a	N.S.
Eighteen Day	A ± 6.42 0.432 a	A 0.321 ± 6.86 a	A 0.169 ± 7.16 a	A 0.326 ± 7.14 a	N.S.
Significant Level	N.S.	N.S.	0.0128	N.S.	

* Values=Means ± SE.

** N.S = Mean No significant difference (P≤0.05).

a. b. c. small letters within one row indicate significant differences between the treatments. The capitals letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

Table – 3: The effect of different treatments in different days on Albumin (mg/dl)

Days	Treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A ± 3.60 0.151 a	A ± 3.84 0.081 a	A ± 3.98 0.135 a	A ± 3.88 *0.193 a	N.S.**
Ten day	A ± 3.64 0.201 a	A ± 3.84 0.128 a	A ± 3.80 0.126 a	A ± 3.62 0.222 a	N.S.
Fifteenth day	A ± 3.66 0.169 a	A ± 3.92 0.080 a	A ± 3.80 0.122 a	A ± 3.62 0.159 a	N.S.
Eighteen Day	A ± 3.64 0.215 a	A ± 3.94 0.102 a	A ± 3.74 0.092 a	A ± 3.62 0.190 a	N.S.
Significant Level	N.S.	N.S.	N.S.	N.S.	

*Values=Means ± SE.

** N.S = Mean No significant difference (P≤0.05).

a. b. c. small letters within one row indicate significant differences between the treatments. The capitals letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

Table – 4: The effect of different treatments in different days on Globulin (mg/dl)

Days	Treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 0.347 ± 2.84 a	A 0.165 ± 2.58 a	B 0.166 ± 2.06 a	B *0.089 ± 2.20 a	N.S.**
Ten day	A 0.180 ± 3.16 a	A 0.393 ± 3.34 a	A 0.223 ± 3.40 a	AB 0.272 ± 3.08 a	N.S.
Fifteenth day	A 0.288 ± 3.10 a	A 0.296 ± 3.20 a	A 0.373 ± 3.22 a	A 0.379 ± 3.30 a	N.S.
Eighteen Day	A 0.345 ± 2.78 a	A 0.333 ± 3.12 a	A 0.195 ± 3.42 a	A 0.369 ± 3.52 a	N.S.
Significant Level	N.S.	N.S.	0.0041	0.0342	

*Values=Means ± SE.

** N.S = Mean No significant difference (P≤0.05).

a. b. c. small letters within one row indicate significant differences between the treatments. The capitals letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

protein level significantly in the group of Selenium. This increase in the total protein level may be due to the role of selenium, which is included in the protein synthesis, and vitamin E, which is considered as an antioxidant.

3-Albumin concentration (Table – 3).

The results explained there was no significant differences between the experimental and control groups or during the different withdrawal periods at the early pregnancy. This indicates that Arginine or Selenium E or both did affect albumin concentration at the early pregnancy. The results were consisted with Soliman (18). There was no significant difference between selenium vitamin E and albumin concentration and was 3.2 in both groups. Kamada (15) observed that albumin in blood plasma was not significantly affected when selenium was given at the late pregnancy. Li et al., (20) claimed there were no significant differences between the Arginine group and control in albumin concentration. Zaho et al., (21) confirmed that there were no significant differences between the Arginine group and the control group in albumin concentration.

4- Globulins concentration (Table – 4).

The results showed that there were no significant differences between the different treated groups and control and one the results disagreed with Soliman (18), who indicated significant differences between the group of selenium vitamin E and the control one in the level of globulin in lambs. There were also no significant differences in the different periods of withdrawal in the third group and control one. However, there was a significant difference in globulin (T1) in days 15 and 18 and the group of selenium vitamin E (T2) in days 10, 15, 18. This increase in the level of clopiolin indicates that dietary supplements of Arginine and selenium vitamin E To raise the globulins in the animal's body, where Zhu et al., (22) stated that treatment with Arginine leads to an increase immunity of the body and its resistance against diseases. The mechanism of action of arginine on immunity is the presence of arginine receptors on the surface of epithelial cells of the thymus gland (23). Moeini and Jalilian (24) observed that selenium with vitamin E increases immunity in

the body. Erdoğan et al (25) noted that selenium supplementation was important for maintaining the concentration of globulins, and Zhao et al., (21) found that there was a significant increase ($P \leq 0.05$) in the concentration of globulin when injected arginine.

5- Aspartate amino transferase (AST) (Table- 5).

The results of the present study did not show any significant differences in the concentration of AST enzyme at the early pregnancy. This indicates that there was no tissue breakdown, but the growth and growth of the placenta and the fetus and the increase in the size of the uterine horn in which the pregnancy occurred. The results of the study were consist with Soliman (18), where there was no significant difference between selenium vitamin E and control in AST concentration. In addition, Tian et al (26) showed no significant differences in AST concentration when injecting arginine. Zhao et al., (21) showed no significant differences in AST concentration when intravenous injection of arginine in cattle. Haenlein and Anke (27) suggest that selenium vitamin E plays a role in removing the free hydrogen peroxide root, which leads to tissue breakdown by the enzyme glutathione peroxidase, which is accompanied by a decrease in the level of serum AST enzymes (28). While the results disagreed with Hassan et al., (29) who observed that giving arginine showed significant differences in the concentration of AST as compared with control group. Alhidary et al., (4) claimed that giving selenium or vitamin E or both together increased AST concentration in the serum from 95.6 to 109 units / l in ewes. As for the different withdrawal times, the results showed no significant differences between the experimental groups and the control group.

6 –Alanin Amino Transferase (ALT) (Table-6).

The enzyme ALT is an enzyme of the cells that make up the body's tissues and enters the enzyme into the blood when cells break down due to causes or an apoptosis (30). Elevation of ALT in the serum is evidence of the breakdown of tissue-forming cells and occurs when birth problems such as obstetrics dystocia, or placental retention, etc. (27)

Table – 5: The effect of different treatments in different days on AST ($\mu\text{mol/L}$)

Days	Treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 3.68 \pm 38.4 a	A 7.80 \pm 39.3 a	A 9.90 \pm 35.4 a	A *5.84 \pm 37.7 a	N.S.**
Ten day	A 5.44 \pm 30.2 a	A 13.1 \pm 22.6 a	A 17.1 \pm 39.4 a	A 5.64 \pm 35.3 a	N.S.
Fifteenth day	A 5.31 \pm 33.8 a	A 11.1 \pm 28.3 a	A 16.1 \pm 26.0 a	A 3.59 \pm 41.3 a	N.S.
Eighteen Day	A 5.85 \pm 38.1 a	A 7.87 \pm 44.6 a	A 15.5 \pm 47.5 a	A 12.5 \pm 49.8 a	N.S.
Significant Level	N.S.	N.S.	N.S.	N.S.	

* Values=Means \pm SE.** N.S = Mean No significant difference ($P \leq 0.05$).

a. b. c. small letters within one row indicate significant differences between the treatments. The capitals letters within column indicate that significant differences between the sampling days within the ones treatment at significant level ($P \leq 0.05$).

Table – 6: The effect of different treatments in different days on ALT ($\mu\text{mol/L}$)

Days	Treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 2.16 \pm 10.6 a	A 1.00 \pm 10.6 a	A 2.15 \pm 9.90 a	A *2.18 \pm 10.0 a	N.S.**
Ten day	A 1.96 \pm 9.96 a	A 0.523 \pm 8.58 a	B 0.949 \pm 5.64 a	A 0.971 \pm 9.20 a	N.S.
Fifteenth day	A 1.71 \pm 10.7 a	A 2.04 \pm 6.86 ab	BC 0.176 \pm 3.10 b	A 0.760 \pm 11.1 a	0.0026
Eighteen Day	A 2.14 \pm 10.5 a	A 3.08 \pm 7.42 ab	C 0.111 \pm 1.18 b	A 2.00 \pm 9.42 a	0.0306
Significant Level	N.S.	N.S.	0.0005	N.S.	

* Values=Means \pm SE.** N.S = Mean No significant difference ($P \leq 0.05$).

a. b. c. small letters within one row indicate significant differences between the treatments. The capitals letters within column indicate that significant differences between the sampling days within the ones treatment at significant level ($P \leq 0.05$).

Table – 7: The effect of different treatments in different days on Glucose (g/dl)

Days	Treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A ± 62.8 2.13 a	A ± 62.0 1.34 a	A ± 61.2 2.55 a	A *4.62 ± 60.6 a	N.S.**
Ten day	A ± 54.6 1.74 a	A ± 57.2 1.52 a	A ± 56.0 2.34 a	A 4.18 ± 58.8 a	N.S.
Fifteenth day	A ± 57.0 3.11 a	A ± 58.6 1.43 a	A ± 59.0 3.06 a	A 2.65 ± 58.4 a	N.S.
Eighteen Day	A ± 56.0 3.03 a	A ± 61.6 2.58 a	A ± 60.4 3.04 a	A 3.61 ± 59.0 a	N.S.
Significant Level	N.S.	N.S.	N.S.	N.S.	

* Values=Means ± SE.

** N.S = Mean No significant difference (P≤0.05).

a. b. c. small letters within one row indicate significant differences between the treatments. The capitals letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

The results of the present study showed that there were no significant differences between the fourth day (before injection) and the tenth after the injection of and selenium E from the dose, but significant difference was observed in the day 15 and 18 of insemination as the concentration of ALT enzyme in the second treatment when injecting Selenium vitamin E. ALT is therefore an indication of the physical status of the body's in various tissues. Selenium vitamin E plays a role in removing the free radicals by the enzyme glutathione peroxidase, which leads to a decrease in the level of AST enzymes, ALT in the serum because the hydrogen peroxide causes the cells break down Tissues (27). The results of the study were agreed with Yuan et al., (31), indicating that the reasons that may reduce the level of ALT in the serum might be due to the role of selenium in stimulating the production of thyroid hormones and growth factors that repair tissues and that in

this period of pregnancy gets For the placenta and embryo and the growth of uterine tissue to contain the embryo, as well as their role as antioxidants (32). The results also agreed with Tian et al., (26), that between selenium, vitamin E, or both together significantly superior to the ALT group in the serum of ewes (4, 21). These findings suggest that the combination of selenium vitamin E given after five days of gestation during fetal.

7-Blood glucose (Table – 7).

The results of the present study showed no significant differences between the different experimental groups and control group in the concentration of glucose. This may be due to the stability of the level of blood glucose in pregnant ewes because of the injection of dietary supplements of selenium with vitamin E and Arginine, that plays a role in the secretion of the insulin, which regulates the level of sugar in the

blood. The results of the current study coincided with Alhidary et al., (4) that giving selenium, vitamin E, or both did not significantly affect the level of glucose in the blood in the marine ewes. Soliman (18) found that there were no significant differences between selenium vitamin E and control in glucose level. Sterndale et al. (19) noted that there was no significant difference between selenium vitamin E and control in glucose level in ewes during pregnancy (33). Yunta et al (31) noted that giving arginine during pregnancy did not significantly affect glucose (12, 16, 21, 26). While the results of the current study with Jarad and Al-Kaisei (34) showed that the Arginine had an effective effect in reducing the blood glucose level. The results also disagreed with Kaminski et al (14) that giving the Arginine led to lowering the level of glucose in ewes serum increased the level of insulin, Silva et al., (35) confirmed that giving arginine to ewes at 105 and 120 days of pregnancy led to differences in glucose concentration in the serum during pregnancy. The results of the current study showed that there were no significant differences in the different within the different experimental groups.

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