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Study Purslane Portulaca oleracea plant and its alcoholic extract on the fertility of male Ross-308 broilers and hatching characteristics.

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ABSTRACT

The study was carried out on the fruit of the local apple cultivar Barwary (*Malus* x *domestica*) cultivated within a private orchard located in Dohuk Governorate, Ekmalla village, Barwary bala, Iraq. The primary objective was to assess the impact of immersing fruits for 3 minutes in varying concentrations (0, 15, 30, 45, and 60%) of *Aloe Vera* gel solution on the quality of apples during storage periods of 1.5 and 3 months at cold storage conditions maintained at 1+1°C with relative humidity (RH) ranging between 85-90%. In general, it was observed that the fruit maintained its quality across all levels of *Aloe Vera* gel solution. Consequently, enhancements in firmness, acidity, and vitamin C content were noted in the apple fruit. Furthermore, the fruit weight loss reduced significantly compared to untreated fruits, also TSS and juice (%) but not reached significant. Concerning the storage duration, it was found that prolonged storage period from 1.5 to 3 months resulted in a significant increase in both total soluble solid % and fruit weight loss %. Despite these variations, no discernible impact was observed on total soluble solid %, juice %, and weight loss% across the experimental processes. Moreover, all levels of *Aloe Vera* gel maintained firmness, vitamin C concentration (mg.100 ml-1 juice), and acidity percentage significantly compared to the control group following cold storage for 3 months plus an additional 25 days under ambient temperature conditions (shelf life).

***Keywords*:** Fruit, Aloe Vera, cold storage, shelf life, postharvest.

INTRODUCTION

Fertility is considered one of the important economic characteristics that influence the success or failure of the project in the production of hatching eggs, and because of its impact on the final economic return of the project in the production of meat chickens or eggs. Many factors affect the characteristics of semen and reduce the fertility of male poultry, including genetic factors such as breed and type. And others among them are environmental factors, such as nutrition, lighting, mycotoxins, and age, as it was found that after 45 weeks in males, all semen characteristics begin to decline due to a decrease in the level of the hormone testosterone, which is responsible for male fertility [1,2]. As age increases, the aromatase enzyme works to convert Testosterone to estrogen and lacks other sex hormones [3].

Intensive genetic selection to improve and increase the efficiency of broiler production is also considered one of the reasons for the deterioration of the reproductive characteristics of maternal flocks because of its negative impact on secondary sexual characteristics and the ability of males to mate, given that most fields depend on natural mating, which has led to a decline in the fertility of the flocks [4]. It reduces the quality of semen and the activity of antioxidant enzymes in sperm and thus increases the production of free radicals, leading to cell death [5].

This prompted the use of safe and cheap medicinal plants and the active substances they contain that act as natural antioxidants and reduce the side effects of the chemical drugs used [6]. One of these medicinal plants is the Purslane plant, *Portulaca oleracea*, as it is considered a good source of flavonoids, terpenes, alkaloids, phenolic acids, saponins, vitamins and minerals [7]. It contains beta-carotene compounds, glutathione, melatonin, and fatty acids represented by omega-3 [8]. It also acts as an anti-inflammatory [9,10], antioxidant [11,12], and strengthens the body's immunity [13]. And due to the lack of studies on the effect of the Purslane plant on fertility and hatching characteristics, this study aimed to investigate the effect of using powder and alcoholic extract of Purslane seeds on Ross-308 broiler mother roosters in improving fertility and hatching qualities.

Materials and methods

This study was conducted in the poultry field of the Department of Animal Production – College of Agriculture – University of Diyala from ½/2023 to 30/5/2023. Sixteen Ross-308 broiler breeder roosters, 54 weeks of age, were used in this study, preceded by a two-week preparatory period to accustom the birds and acclimate them to the atmosphere of the hall and the broiler. During that period, the Roosters were also trained to respond to semen collection. The roosters were randomly

distributed into four treatments (Four roosters per one) and four replicates (1 cock/replicate). The roosters of the 1st treatment (negative control group) were fed a standard ration without any addition. The 2nd treatment (positive control group) was fed with the addition of 0.250 grams of vitamin E/kg of feed, the 3rd treatment added 10 g of Purslane seed powder/kg feed, and the 4th treatment added the alcoholic extract of Purslane seeds at a concentration of 10 ml/L of drinking water. The roosters were placed in individual cages with dimensions of 40 x 40 x 50 cm, and feed and water were provided individually. The birds were fed the diet shown in Table 1, which contained a representative energy of 2,800 kcal/kg feed and 14% crude protein. After completing the evaluation of the semen characteristics of the roosters and comparing them with Control Treatments, Artificial insemination was performed after withdrawing semen from the roosters, diluting it, and then injecting it into the hens for insemination and collecting the resulting eggs

Table 1: The production diet for Ross308 broiler breeders and the percentage of components and their calculated chemical composition.

Feed material	Percentage %
yellow corn	46.2
Wheat	25
bran	12.4
Soybean meal	10
Premix	2.5
oil	0.5
limestone	2.5
Monocalcium phosphate	0.7
Sodium bicarbonate	0.1
Antimycotoxin	0.1
the total	100
** chemical composition	
Representative energy (kilocalorie/kg feed)	2800
Protein %	14
Lysine %	0.49
methionine %	0.34
Threonine %	0.49
Calcium	1.2
Available phosphorus	0.45
Linolenic acid	1.25
sodium	0.18
potassium	0.6
chloride	0.18
Methionine + cysteine	0.59

Artificial insemination process

80 chickens were used 54-week-old broiler breeder hens were used and placed in cages for laying hens in the field belonging to the Department of Animal Production / University of Diyala. After a preliminary period of two weeks, the females were inseminated using artificial insemination. 20 hens were allocated to each treatment with four replicates (five hens /replicate) and the females were Inseminate once every four days to ensure the fertilization process. In the afternoon to ensure that there is no egg inside the oviduct,

On the second day following the insemination process, eggs were collected daily for five days and stored in the refrigerator at a temperature of 15°C. The eggs resulting from each treatment were numbered separately and then taken to one of the private hatcheries (Al-Safa hatchery) for a first hatching. The eggs were collected again for another five days and taken to the hatchery as a second hatching. Then the eggs were collected for five consecutive days and brought into the hatchery for a third hatching until there were three hatchings. The unhatched eggs were then broken to determine the fertility rate, the total hatchability rate, the hatching rate of fertilized eggs, and the percentage of perished embryos for each treatment. This process was repeated for all hatched eggs. These percentages were calculated according to the equations referred to by [14]

Statistical analysis

Complete Randomize Design (CRD) was used in the statistical analysis of the experimental data, to study the effect of the different parameters of the studied characteristics. [15] multinomial test was also used to test the significance of the differences between the studied means at the probability level (0.05). While the ready-made statistical program [16] was used to analyze the data.

Results and discussion

The results of Table 2 indicated an effect of Purslane grain powder and its alcoholic extract on the fertility and hatchability characteristics of the first hatchling. It is clear from the table that there is no significant difference in the weight of the hatched chicks for the two additional treatments compared to the positive and negative control treatments. Also, there is a significant superiority of $p \leq 0.05$ in favor of the two addition treatments in the fertility rate, as it is noted that the third treatment (10 grams of terpene grains powder) was superior to the fourth treatment (10 ml of terpene grains alcoholic extract) on the second treatment (positive control) and the first treatment (negative control), as there is no significant difference between them.

Regarding the hatching rate of fertilized eggs, it is superior to all treatments compared to the negative control treatment, which recorded the lowest hatching rate 62.69%, although there is no significant difference between the second and third treatments and between the third and fourth. As for the overall hatching rate, we notice that the fourth treatment is superior compared to all treatments, and the third treatment is superior to the second and first treatment, and the second treatment outweighs the positive control over the first treatment, which recorded the lowest hatching rate 49.99%. Also, a significant decrease of $p \leq 0.05$ in the percentage of fetal deaths, as the fourth treatment recorded a significant decrease compared to the two control treatments, even though there was no significant difference between it and the third treatment, followed by the second treatment with a decrease (there was no significant difference between it and the third) to which vitamin E was added compared to the first treatment, which the highest percentage of fetal deaths was recorded 35.37%

Table 2: Effect of adding Purslane powder or extract on the fertility and hatchability characteristics of the first hatching (means ± standard error).

Adjectives *Treatments	Weight of chicks (g)	Fertility rate%	Hatching rate of fertilized eggs%	Total hatching rate%	Percentage of embryos dead %
T1	42.72±0.28 a	79.99±2.72 b **	62.69±3.03 c	49.99±1.92 d	35.37±1.25 a
T2	42.61±0.62 a	86.66±2.72 b	82.78±1.43 b	71.66±1.66 c	17.21±1.43 b
T3	41.88±0.27 a	96.66±1.92 a	86.42±3.70 ab	83.33±1.92 b	13.57±3.71 bc
T4	42.55±0.29 a	98.33±1.66 a	93.33±2.72 a	91.66±1.66 a	6.66±2.72 c

Different letters within one column indicate the presence of significant differences at the probability level ($P \leq 0.05$).

*T1 = negative control without additions. T2= Positive control, addition of vitamin E. T3 = Add 10 g of Purslane grain powder/kg feed. T4 = Adding the alcoholic extract to the Purslane grains at a concentration of 10 ml/liter of drinking water.

Table 3 showed the effect of adding berbene grain powder and its alcoholic extract on the hatching characteristics of the second hatching, as no significant differences were recorded in the weight of hatched chicks for all study treatments compared to the two control treatments, and in the fertility rate, the third and fourth treatments (there is no significant difference between them) were superior to the second and first treatments, and the treatment was superior. The second was compared to the first, and the hatching rate was higher, as the fourth and third treatments outperformed the two control treatments.

The second treatment also outperformed the first treatment (negative control), and a significant decrease of $p \leq 0.05$ was observed in the percentage of embryonic deaths for the additional treatments. It was noted that the fourth and third treatments decreased (there was no significant difference between them) compared to the two control treatments, and the second treatment decreased compared to the first treatment, which recorded the highest rate 37.81%.

Table 3: Effect of adding Purslane powder or extract on the fertility and hatchability characteristics of the second hatching (means ± standard error).

Adjectives *Treatments	Weight of chicks (g)	Fertility rate%	Hatching rate of fertilized eggs%	Total hatching rate%	Fetal mortality rate%
T1	40.17±0.44 a	83.33±1.92 c**	62.17±3.02 c	51.66±1.66 c	37.81±3.02 a
T2	41.18±0.38 a	89.99±1.92 b	83.37±1.62 b	74.99±1.66 b	16.61±1.62 b
T3	40.42±0.47 a	98.33±1.66 a	91.42±1.90 a	89.99±3.33 a	8.56±1.90 c

T4	40.90±0.62 a	98.33±1.66 a	94.99±3.19 a	93.33±2.72 a	4.99±3.19 c

Different letters within one column indicate the presence of significant differences at the probability level ($P \leq 0.05$).
*T1 = negative control without additions. T2= Positive control, addition of vitamin E. T3 = 10 g of Purslane grain powder/kg feed. T4 = Adding the alcoholic extract to the Purslane grains at a concentration of 10 Ml/L of drinking water.

Table 4 shows the effect of adding Purslane grain powder and its alcoholic extract on the hatchability characteristics of the third hatching, as it is noted that there is no significant difference in the weight of the hatched chicks for all experimental treatments. As for the fertility rate, we note that the third and fourth treatments of adding the powder and alcoholic extract (no significant difference recorded) were superior to the two treatments. Positive and negative controls and the second positive treatment outperformed the first negative treatment, which recorded the lowest percentage 86.66%. Regarding the hatching rate, it is also noted that the third and fourth addition treatments (no significant difference between them) are superior to the second and first control treatments. The second treatment is superior to the first treatment, which recorded the lowest hatching rate and a significant decrease of $p \leq 0.05$ in the percentage of embryonic deaths in favor of the fourth and third treatments (no A significant difference between them) compared to the second and first treatment, and a decrease in the second treatment compared to the first treatment, which recorded the highest percentage 36.53%

Table 4: Effect of adding Purslane powder or extract on the fertility and hatchability characteristics of the third hatching (means ± standard error).

Adjectives *Treatments	Weight of chicks (g)	Fertility rate%	Hatching rate of fertilized eggs%	Total hatching rate%	Fetal mortality rate%
T1	41.64±0.28 a	** 86.66±0.00 c	63.45±1.92 c	54.99±1.66 c	36.53±1.92 a
T2	41.90±0.25 a	93.33±0.00 b	±80.35±1.78 b	74.99±1.66 b	19.63±1.78 b
T3	41.64±0.31 a	98.33±1.66 a	91.54±1.63 a	89.99±1.92 a	8.44±1.63 c
T4	41.48±0.60 a	98.33±1.66 a	93.33±2.72 a	91.66±1.66 a	4.99±1.66 c

Different letters within one column indicate the presence of significant differences at the probability level ($P \leq 0.05$).
*T1 = negative control without additions. T2= Positive control, addition of vitamin E. T3 = Add 10 g of Purslane grain powder/kg feed. T4 = Adding the alcoholic extract to the Purslane grains at a concentration of 10 Ml/L of drinking water.

The improvement in the characteristics of hatching, fertility, and embryonic embryos might be due to the active substances contained in the Purslane grains and their alcoholic extract, such as flavonoids, phenols, terpenes, and omega3, and many nutritional elements, such as vitamins and minerals, which have a significant role in raising the rates of these economically important production traits [17].

Perhaps improving the characteristics of the semen, such as movement and the percentage of dead and deformed sperm shown in the tables of the first experiment, is one of the reasons for increasing fertility and hatching due to the presence of a positive significant correlation coefficient between the characteristics of the semen and the fertility rate [18], as well as increasing the ability of the sperm to penetrate the egg membrane and fertilization occurs. [19] indicated a highly significant positive correlation between the ability to penetrate and the characteristics of fertility and hatching, and [20] indicated that the fertility rate of eggs depends on males to a greater extent than females.

The significant improvement in the characteristics of fertility, hatching, and perishing embryos may be through the role of the active substances present in the Purslane grains and their ability to preserve the outer membrane of the sperm from oxidation because they are very sensitive to oxidative stress due to the presence of large numbers of unsaturated fatty acids in their membranes and because of their importance in the fertilization process and this. It leads to sperm damage, infertility, decreased fertility and hatchability, and free radicals damage the DNA inside the sperm, thus affecting fertilization and the growth of the fetus and its early destruction [21,22] Also, the action of the Purslane plant as an antioxidant lies in preserving the sperm mitochondria from damage by free radicals and increasing their numbers, as many studies have attributed the sperm movement to the activity of the mitochondria. The occurrence of any damage in them leads to a defect in the sperm movement. Motility is considered one of the most important standards approved in evaluating the quality of semen in terms of Its concentration and sperm vitality determine the ability to fertilize, meaning that the sperm must be mobile in order to be able to fertilize [23, 24, 25]. The flavonoid compounds in the seeds and extract of the Purslane plant also have important biological roles in their ability to strengthen health and immunity and act as anti-inflammatory, anti-bacterial and anti-virus agents. This works to maintain the growth of the fetus and reduce the incidence of diseases, which leads to an increase in the hatching rate and a reduction in the number of perishing fetuses [13,26,10]

Active substances also work to improve the properties of semen, such as the integrity of the membrane and DNA, and the ability to survive and move, thus increasing its activity, movement, entry and storage in sperm nests within the female reproductive system. These factors increase the rate of hatching and fertilization and reduce the rate of perished embryos [27].

Perhaps the increase in testosterone concentration and the decrease in corticosterone in our current study is one of the reasons for the increase in fertility and hatching rates and the decrease in embryonic mortality due to its high ability to maintain semen characteristics. The increase in the percentage of hatched eggs from fertilized eggs is due to the decrease in the percentage of perished embryos due to the presence of a highly significant negative correlation coefficient between the percentage of perished embryos and the percentage of hatched eggs from the fertilized ones. Regarding the superiority of the treatment of adding vitamin E (positive control) over the first treatment (negative) in terms of hatching and fertility characteristics, as treating the roosters with the vitamin led to combating the negative effects of oxidative stress as it is a powerful antioxidant and is considered the basic component of the antioxidant system in semen [28] and its ability to reduce lipid peroxidation by improving semen quality and increasing its volume, sperm concentration, motility, vitality, and safety in all types of poultry birds [29]. It works to protect and enhance the functions of mitochondria in sperm. It increases the integrity of their membrane, movement and vitality, which increases their ability to reach the egg, attach and fertilize [30]. It also preserves the DNA of the sperm from the effects of free radicals and oxidation, and these abnormalities increase the rate of early fetal deaths as well as congenital malformations of the next generation [31, 32].

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دراسة نبات الرجلة Portulaca oleracea ومستخلصه الكحولي في خصوبة ذكور فروج اللحم Ross-308 وصفات الفقس.

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الخلاصة

أجريت هذه الدراسة في حقل الطيور الداجنة التابع الى قسم الانتاج الحيواني –كلية الزراعة –جامعة ديالى للمدة من *1*/2/2023 ولغاية *30*/5/2023. لبيان تأثير إضافة مسحوق حبوب نبات البربين ومستخلصها الكحولي الى العلف وماء الشرب في دراسة تأثيره على صفات الخصوبة والفقس باستعمال 16 ديك من امهات فروج اللحم *Ross 308* بعمر 54 أسبوع بواقع (اربعة ديكة / معاملة) وبأربع مكررات (1 ديك / مكرر) اذ تم تقسيمها الى أربع معاملات هي المعاملة الأولى السيطرة السالبة بدون إضافات والمعاملة الثانية السيطرة الموجبة إضافة 0.250 غم فيتامين *E*/كغم علف والمعاملة الثالثة أضافة 10 غم من مسحوق حبوب نبات البربين/كغم علف والمعاملة الرابعة اضافة المستخلص الكحولي لحبوب نبات البربين بتركيز 10 مل/ لتر ماء شرب. اظهرت النتائج ارتفاع معنوي *($p\leq 0.05$)* في نسبة الخصوبة والفقس وتحسن معنوي في نسبة الهلاكات الجنينية للمعاملة الثالثة والرابعة مقارنة بمعاملتا السيطرة في حين لم تظهر أي فروق معنوية في اوزان الافراخ الفاقسة بين المعاملات خلال الفقسات الثلاثة.

الكلمات المفتاحية : حبوب البربين، خصوبة، فقس، هلاكات جنينية، أمهات فروج لحم