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Effect of licorice extract (*Glycyrrhiza glabra*) on some semen traits for local black turkey males

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Abstract

This study was conducted at the poultry farm for the department of Allatefya Researches, Agricultural and biological researches directorate, ministry of sciences and technology, during the period from 20/8/2019 to 28/12/2019. This study aimed to investigate the effect add three concentration of licorice with drinking water in some semen's characteristics for local Iraqi black turkey males. A total of 36 local Iraqi turkey males 44 weeks old were used in this study. The turkey males were randomly distributed on 4 treatments which consisted of 9 birds to each. Birds were fed during the experimental period on diet containing 2950 Kcal metabolic energy/kg and 18 % crude protein/ kg. Three levels of licorice extract were added with drinking water 5g, 10g, and 15g to treatments T2, T3 and T4 respectively. While T1 treatment stays without any addition as the control group. The birds were reared in ground pens during this study. Semen collection by using abdominal massage procedure after ganders trained for two weeks to give semen. The investigated characteristics were ejaculate volume, individual motility, mass motility, sperm concentrations, percentage of dead spermatozoa, deformation spermatozoa ratio and spermatocrit. Results revealed that T4 (15 g per litre) recorded highest values in all traits except in deformation spermatozoa ratio as compared with other treatments (T1, T2 and T3) in most of all experimental period. On the other hand T3 (10 g per litre) achieved high significant effect compared with T2 (5 g per litre) and T1 (0 g per litre), whereas T1 recorded lowest values during all experimental period. However, the overall means of all traits achieved the same trend of treatments effect. While deformation spermatozoa ratio trait had no significant effects recorded from licorice extract addition to this study.

Keywords: licorice, black turkey, semen, birds, antioxidants.

تأثير اضافة مستخلص عرق السوس مع ماء الشرب على بعض صفات السائل المنوي لذكور الرومي الاسود المحلي

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المستخلص:

اجريت هذه الدراسه في حقل الطيور الداجنه التابع لقسم ابحاث اللطيفيه / دائرة البحوث الزراعية / وزارة العلوم والتكنلوجيا للمدة من 20/ 8/ 2019 لغاية 2019/12/28 . الهدف من الدراسة لبحث تاثير اضافة ثلاث تراكيز من مستخلص عرق السوس مع ماء الشرب على بعض صفات السائل المنوي لذكور الرومي الاسود المحلي. استعمل 36 ذكر رومي محلي اسود اللون بعمر 44 اسبوع وزعت عشوائيا على 4 معاملات وبواقع 9 طيور لكل معامله. غذيت الطيور اثناء مدة التجربة على عليقة تحتوي على طاقة ممثله مقدارها 2950 كيلو كلري/ كغم علف وبروتين خام مقداره 18% لكل كغم علف، اضيف مستخلص عرق السوس الى ماء الشرب بواقع ثلاث تراكيز 5غم ، 10غم و 15غم لكل لتر ماء لتمثل المعاملات T2 وT3و T4 بينما لم تضاف اي كمية من مستخلص عرق السوس الى المعامله T1 لتمثل معاملة السيطرة ، ربيت الطيور في اقفاص ارضيه (pens) قدم العلف والماء بصورة حره اثناء مدة الدراسة. جمع السائل المنوي بطريقة المساج البطني بعد ان دربت الطيور لمدة اسبوعين قبل الجمع، كانت الصفات المدروسه تتمثل في قياس حجم القذفه والحركه الفرديه للنطف والحركه الجماعية للنطف و تركيز النطف و النسبة المئوية للنطف الميته والمشوهة وحجم خلايا النطف المرصوصه. اشارت النتائج الى تفوق معنوي للمعامله T4 في جيع الصفات المدروسة مقارنة بباقي المعاملات ياستثناء نسبة النطف المشوهة تلتها المعاملة T3 ثم المعاملة T2 بينما سجلت المعاملة T1 اقل القيم خلال مدة هذه الدراسة، ولم يكن هناك تاثير لاضافة مستخلص عرق السوس الى ماء الشرب على نسبة النطف المشوهة لطيور الديك الرومي اذ لم تختلف معاملات الاضافه معنويا مقارنة بمعملة السيطرة اثناء مدة الدراسة.

Introduction.

Verity producing poultry protein sources is one of the effective methods to resolve Shortage animal protein especially poultry protein (Aletor et al., 2000). Most important sources of poultry protein are chickens, whereas the other poultry species did not find the same interesting such as turkey, guinea fowl, geese, ducks, quails, pigeons and ostriches (Li and Hsieh, 2004). Turkey has been raised for centuries from the south and north America and most of the European countries(Kotowska et al.,2005). Turkey has not enough interested and is still raised from single farmers, no specific companies to develop this industry in the third world countries, put Many different varieties of turkeys have been developed for productivity, in the United

States and British, the American poultry association (APA) determined eight varieties of turkeys, they are Black, Narragansett, Bronze, White Holland, Slate, Bourbon Red, Beltsville Small White, and Royal Palm (Frank et al., 2007). Licorice grows in well-drained soils, some countries producing licorice include India, Iran, Italy, Afghanistan, China, Pakistan, Iraq and Turkey, licorice extract has been used in traditional medicine for stomach inflammation and upper respiratory problems, today people use it as a dietary supplement for digestive problems, cough, bacterial infection and viral infection (Kriker et al., 2013). The scent of Licorice roots extract comes from its compounds such as anethole, glycyrrhizin and isoflavan glabridin (phytoestrogen) (Belinky et al., 1998). Bernardi et al(1994) stated that licorice root contains glycyrrhizin, 50 times sweeter than sucrose, which encourages the production of hormones such as hydrocortisone and this help to explain its anti-stress and anti-inflammatory action and also its role in stimulating the adrenal cortex after steroid therapy. Several studies of licorice root in poultry have been published, but not enough to support the use for specific poultry health and production (Kriker et al., 2013). This study was carried out influence addition of licorice extract with drinking water to turkey males on their semen's characteristics.

Materials and methods

This study has been conducted at poultry farm of Alatefya Researches department / Agricultural researches directorate / Ministry of sciences and technology, during the period from 20/8/2019 to 20/12/2019. This experiment included a total of 36 local Iraqi black turkey males in 44 weeks olds. The turkey males were distributed into four treatments, each treatment contained 9 birds. Each treatment divided into 3 replicates. All birds housed under the same environmental conditions. Birds were fed during the whole period on diet containing 2950 Kcal metabolic energy/kg and 18% crude protein /kg, water was available for all the period (ad libitum). The flock was reared in-ground cages (pens) during the experiment period. Semen was collected after ganders were trained for two weeks to give semen before the collection began, semen collection by using abdominal massage procedure (Al-Daraji et al., 2012). Licorice extract was added to the drinking water of birds to T2, T3 and T4 in levels 5g per litre, 10g per litre and 15g per litre respectively. Whereas T1used as control treatment without any addition.

Traits measured:

Ejaculate Volume.

Semen samples were immediately evaluated for volume (ml), by graduated (ml) test tube. (Al-Daraji, 2007a).

Mass motility

Mass motilities of spermatozoa (%) were estimated according to the index of motilities, which ranges 0 - 100 (Al-Daraji, 2007b).

Individual motility.

Individual motilities (%) were determined by the index of motilities which ranges 0 - 100 (Al-Daraji, 2007b).

Sperm concentration.

The spermatozoa concentrations were estimated by using hemacytometer chamber (Cecil and Bakst, 1993).

Percentage of dead spermatozoa.

The percentage of dead spermatozoa was estimated by using the procedure described by Al-Daraji, (2007b).

Deformation spermatozoa ratio.

The percentage of abnormal spermatozoa was an evaluation by using the procedure mentioned by Al-Daraji, et al (2002).

Spermatocrit.

The spermatozoa packed cells volume was determined by using the procedure mentioned by Al-Daraji, (2007b).

A completely randomized design (CRD) has been used in this study. Statistical analyses for various variables were using the SAS program (SAS Institute, 2012). Significant differences between treatments mean were determined by using Duncan's multiple range tests (Steel and Torrie, 1980).

Results and discussion

Ejaculate volume

In table 1, as seen, there is a significant effect ($P \leq 0.05$) of Licorice extract on semen ejaculate volume. Table 1, refer to T4 (15 g per litre) recorded highest values in this trait as compared with other treatments (T1, T2 and T3), on the other hand, T3 (10 g per litre) achieved significant effect compared with T2 (5 g per litre) and T1 (0 g per litre), whereas T1 recorded lowest in ejaculate volume during the all experimental period. However, the overall means of ejaculate volume were 0.30, 0.27, 0.22 and 0.17 for T4, T3, T2 and T1, respectively.

Table 1: Effect addition three level of licorice extract with drinking water on ejaculate volume (ml) (Mean ± SE) for local black turkey males.

Periods	Treatments				Significance Level
	T4	T3	T2	T1	
1	0.25 ±0.012 a	0.20 ±0.011 b	0.17 ±0.013 c	0.14 ±0.01 d	*
2	0.28 ±0.01 a	0.25± 0.08 b	0.22 ± 0.01 c	0.15 ±0.06 d	*
3	0.30 ± 0.01 a	0.26 ± 0.06 b	0.22 ± 0.01 c	0.15 ±0.01 d	*
4	0.33± 0.03 a	0.30 ± 0.01 b	0.23 ± 0.04 c	0.18 ±0.02 d	*
5	0.33 ±0.08 a	0.31± 0.03 b	0.20 ±0.02 c	0.18 ±0.03 d	*
6	0.35 ±0.01 a	0.32 ± 0.01 b	0.25 ±0.06 c	0.21 ±0.09 d	*
7	0.33 ± 0.03 a	0.28 ± 0.08 b	0.24 ± 0.01 c	0.20± 0.07 d	*
8	0.30 ±0.01 a	0.27 ±0.02 b	0.23 ±0.02 c	0.18 ±0.03 d	*
Overall means	0.30± 0.01 a	0.27 ±0.02 b	0.22± 0.01 c	0.17± 0.02 d	*

T1: without addition, T2: addition 5g per litre, T3: addition 10g per litre, T_4: addition 15g per litre, means in same rows with the same superscript were not significantly different, with different superscript were significantly different. *(P≤0.05). Each period presented two weeks.

The mass activity of spermatozoa

As seen in Table 2, the data regarding significant effect (P≤0.05) for an additional three levels of Licorice extract with drinking water on the mass activity of sperms refer to T4 (15g licorice) recorded the highest Mass activity as compared with other treatments (T1, T2 and T3). Also, T3 (10g licorice) achieved significant effect compared with groups T2 (5g licorice) and T1(0g licorice) whereas T1 recorded the lowest value in these traits during the all experimental period. However, the overall means of mass activity was 79.25, 75.75, 73.00 and 69.37 for T4, T3, T2 and T1, respectively.

Table 2: Effect addition three levels of licorice extract with drinking water on the mass activity of spermatozoa (%) (Mean ± SE) for local black turkey males.

Periods	Treatments				Significance Level
	T4	T3	T2	T1	
1	75 ± 1.0 a	72 ± 1.20 b	70 ± 1.31 c	67 ± 0.80 d	
2	80 ± 1.10 a	75 ± 1.20 b	73 ±0.79 c	70 ±1.16 d	*
3	83 ± 1.23 a	80 ± 1.20 b	78 ±0.89 c	72 ±1.21 d	*
4	82 ±0.89 a	79 ±1.10 b	75 ±0.29 c	73 ±1.14 d	*
5	82 ± 2.11 a	80 ± 1.11 b	77 ± 1.20 c	74 ± 0.97 d	*
6	80 ± 0.62 a	76 ± 0.68 b	73 ±1.12 c	68 ±1.11 d	*
7	78 ±1.30 a	74 ± 0.82 b	70 ±1.04 c	66 ±1.22 d	*
8	74 ± 1.23 a	70 ±1.21 b	68 ±1.20 c	65 ±0.63 d	*
Overall means	79.25 ±0.77 a	75.75 ±0.60 b	73.00 ±0.61 c	69.37 ±0.62 d	*

T1: without addition, T2: addition 5g per litre, T3: addition 10g per litre, T_4: addition 15g per litre, Means in same rows with the same superscript were not significantly different, with different superscript were significantly different. *(P≤0.05). Each period presented two weeks.

Individual motility of spermatozoa

Results in Table 3 revealed that treatments T4 (15g licorice), T3 (10g licorice) and T2 (5g licorice) resulted in significant (P≤0.05) increase in the percentage of individual motility of spermatozoa as compared with T1 (0g licorice) during the all experimental period. It is clear that T4 had higher individual motility of spermatozoa, also T3 had achieved significant effect compared with T2 and T1 during the all experimental periods, except in one and two periods were not significant differences betweenT2and T3. While T1 had the lowest individual motility of spermatozoa during all experimental periods. Similarly, the overall means of spermatozoa individual motility (%) were higher in the experimental treatments (T4, T3 and T2) (79.87, 76.62 and 74.25, respectively) than that in the T1 (69.36).

Table 3: Effect addition three levels of Licorice extract with drinking water on individual motility of spermatozoa (%) (Mean ± SE) for local black turkey males

Periods	Treatments				Significance Level
	T4	T3	T2	T1	
1	77 ±1.11 a	73 ± 0.99 b	73 ± 1.10 b	70 ±2.79 c	*
2	81 ±0.99 a	77 ±1.31 b	75 ±1.11 b	72 ± 1.10 c	*
3	86 ±0.98 a	83 ±1.11 b	80 ±1.10 c	73 ±0.82 d	*
4	84 ±1.13 a	81 ±1.11 b	78 ±1.10 c	73 ±0.89 d	*
5	81 ±1.00 a	79 ±0.97 b	77 ±0.91 c	70 ±0.96 d	*
6	77 ± 0.82 a	74 ± 1.15 b	72 ±1.11 c	67 ±0.92 d	*
7	78 ± 1.03 a	74 ±1.03 b	70 ±1.01 c	65 ±0.87 d	*
8	75 ±1.10 a	72 ± 1.10 b	69 ±1.08 c	65 ± 0.91 d	*
Overall means	79.87 ±0.89 a	76.62 ±0.93 b	74.25 ±0.88 c	69.36 ±0.98 d	*

T1: without addition, T2: addition 5g per litre, T3: addition 10g per litre, T_4: addition 15g per litre, Means in same rows with the same superscript were not significantly different, with different superscript were significantly different. *(P≤0.05). Each period presented two weeks.

Spermatozoa concentration

As were given in table 4. The parameter of spermatozoa concentration refer that T4 was detected a significant difference (P≤0.05) from the others groups (T3, T2 and T1) as well as the treatment T3 had achieved a significant effect compared with T2and T1,while T1recorded lower sperm concentration during all experimental periods. Significant differences (P≤0.05) were observed among overall means of treatments, the overall means of sperm concentration were higher in the experimental treatments (T4, T3 and T2) (8.33x109 cell /ml, 8.00x10^9 cell /ml and 7.65 x10^9 cell/ ml, respectively) than that in the T1 (7.21 x10^9 cell/ ml).

Table1: Effect addition three levels of licorice extract with drinking water on spermatozoa concentration (x 10^9/ml) (Mean ± Se) for local black turkey males

Periods	Treatments				Significance Level
	T4	T3	T2	T1	
1	8.3 ±0.020 a	7.9 ± 0.050 b	7.5 ±0.13 c	7.0 ±0.022 d	*
2	8.4 ± 0.020 a	8.0 ± 0.121 b	7.8 ±0.070 c	7.5 ±0.202 d D	*
3	8.8 ±0.120 a	8.5 ± 0.130 b	8.2 ±0.172 c	7.6 ± 0.120 d	*
4	8.9 ±0.028 a	8.6 ± 0.136 b	8.3 ±0.053 c	7.6 ± 0.018 d	*
5	8.5 ± 0.35 a	8.1 ± 0.080 b	7.8 ±0.022 c	7.4 ± 0.013 d	*
6	8.0 ±0.020 a	7.6 ± 0.108 b	7.4 ±0.69 c	6. 8 ±0.29 d	*
7	7.8 ±0.016 a	7.4 ± 0.031 b	7.2 ±0.080 c	6.98 ±0.201 d	*
8	8.0 ± 0.038 a	7.9 ± b 0.201	7.2 ± 0.090 c	6.85 ±0.207 d	*
Overall means	8.33 ± 0.070 a	8.00 ± 0.078 b	7.65 ±0.073 c	7.21 ±0.041 d	*

T1: without addition, T2: addition 5g per litre, T3: addition 10g per litre, T_4: addition 15g per litre, Means in same rows with the same superscript were not significantly different, with different superscript were significantly different. *(P≤0.05). Periods: each period presented two weeks.

Spermatocritc

As shown in (Table 5). There were significant increases (P≤0.05) in the average of spermatocrit for treatments T4, T3 and T2 compared with the T1 during the all experimental periods. However, T4 recorded the highest values of this trait during all periods and as regards the overall means of this trait. The overall means for spermatocritc were 35.39%, 31.43% and 27.013% for T4, T3, and T2, respectively as compared to T1 which was 24.07%.

Table 5: Effect addition three levels of licorice extract with drinking water on spermatocritc (%) (Mean ± SE) for local black turkey males.

Periods	Treatments				Significance Level
	T4	T3	T2	T1	
1	35.34 ± 0. 12 a	30.23 ± 0. 38 b	25.11 ±0.82 c	20.72 ±0.92 d	*
2	35.20 ±0. 21 a	32.00 ± 0. 53 b	28.10 ± 0. 28 c	25. 15 ±0.14 d	*
3	38.60 ± 0. 78 a	34.50 ±0.81 b	30.40 ±0. 22 c	28.20 ±0.92 d	*
4	42.80 ±0. 23 a	38.10 ±0. 78 b	34.00 ±0.72 c	32.00 ±0.62 d	*
5	38.10 ±0.53 a	34.70 ±0.71 b	30.80 ±0.90 c	26.80 ± 0.71 d	*
6	35.80 ±0.51 a	31.60 ±0.71 b	25.20 ±0.81 c	21.86 ±0.60 d	*
7	31.50 ±0.61 a	28.10± 0. 23 b	21.86 ± 0. 75 c	19.88 ±0. 39 d	*
8	25.80 ±0. 46 a	22.10 ±0. 96 b	20.65 ±0.70 c	18.00 ±0.043 d	*
Overall means	39.35 ±0.55 a	34.31 ±0.51 b	27.013 ±0.46 c	24.07 ±0.29 d	*

T1: without addition, T2: addition 5g per litre, T3: addition 10g per litre, T_4: addition 15g per litre, Means in same rows with the same superscript were not significantly different, with different superscript were significantly different. *(P≤0.05). Each period presented two weeks.

Percentages of dead spermatozoa.

As were given in table 6 percentages of dead spermatozoa refer to a significant decrease (P≤0.05) of the treatments (T4, T3, and T2) throughout the experimental period, as compared with (T1). The T4 had achieved the lowest percentages of dead spermatozoa except in two, seven and eight periods were not significant differences betweenT4and T3. Also, significant differences were found between T3 and T2 during periods of the experiment, T3 was recorded low percentage of dead sperm. While T1 had recorded the highest percentage of dead sperm. The overall means were 12.83, 12. 86, 15.21 and 18.07% for the treatments T4, T3, T2 and T1, respectively in the percentage of dead sperm and that refer to T4 and T3 treatments had lowest mean and then the mean of T2, while the mean of T1 had recorded the highest percentage of dead sperm.

Table 6: Effect addition three levels of licorice extract with drinking water on Percentages of dead spermatozoa (%)(Mean ± SE) for local black turkey males.

Periods	Treatments				Significance level
	T4	T3	T2	T1	
1	10.25 ±0.81 d	11.30 ±0.67 c	15.20 ±0.89 b	18.11± 1.20 a	*
2	10.00 ±0.78 c	10.22 ±0.62 c	14.88 ±0.82 b	16.90± 0.67 a A	*
3	10.30 ±0.92 d	11.00 ±0.86 c	14.50 ±1.33 b	18.25 ±0.51 a A	*
4	13.34 ±0.77 d	13.35 ±0.91 c	14.25 ±1.22 b	20.30 ±0.87 a A	*
5	12.50 ±0.88 d	13.60 ±0.73 c	15.80 ±0.44 b	20.00 ±1.10 a	*
6	13.80 ± d 0.73	14.90 ±0.77 c	16.10 ±0.91 b B	22.10 ±0.85 a	*
7	14.00 ±0.66 c	14.55 ±0.85 c	15.80 ±0.71 b	22.70 ±0.65 a	*
8	13.80 ±0.73 c	14.00 ±0.71 c	15.15 ±0.83 b	23.10 ±0.88 a	*
Overall means	12.38 ±0.79 **c**	12.86 ±0.86 **c**	15.21 ±0.93 **b**	18.07 ±0.85 **a**	*

T1: without addition, T2: addition 5g per litre, T3: addition 10g per litre, T_4: addition 15g per litre, Means in same rows with the same superscript were not significantly different, with different superscript were significantly different. *(P≤0.05). Each period presented two weeks.

Percentages of abnormal spermatozoa

As shown in Table 7. There were no significant differences among treatments T4, T3, T2 and T1 in all experimental periods in the percentages of deformation of spermatozoa ratio. Also, evaluations overall mean values of the percentages of abnormal spermatozoa were not significant differences among all treatments.

Table7: Effect addition three levels of licorice extract with drinking water on abnormal spermatozoa (%)(Mean ± SE) for local black turkey males

Periods	treatments				significance Level
	T4	T3	T2	T1	
1	8.6± 0.41 a	8.5 ± 0.31 a	8.7 ± 0.22 a	8.9 0.12± a	NS
2	9.7 ± 0.32 a	9.8 ±0.110 a	9.7 ±0.011 a	9.8 ± 0.121 a	NS
3	9.8 ±0.415 a	9.9 ±0.335 a	9.8 ± 0.41 a	10.0 ± 0.61 a	NS
4	11.5 ±0.73 a	11.5± 0.72 a	11.7 ±0.83 a	11.5 ± 0.88 a	NS
5	11.9 ± 0.66 a	11.9 ±0.51 a	12.0 ±0.71 a	12.2 ±0.61 a	NS
6	13.5 ±0.71 a	13.7 ± 0.42 a	13.6 ±0.63 a	13.5± 0.42 a	NS
7	13.5 ±0.615 a	13.8 ±0.719 a	13.9 ±0.729 a	13.8 ±0.821 a	NS
8	13.9 ± 0.52 a	14.0 ±0.42 a	14.0 ± 0.41 a	14.2 ±0.52 a	NS
Overall means	11.55 ±0.61 a	11.63 ±0.41 a	11.67 ±0.68 a	11.73 ±0.41 a	NS

T1: without addition, T2: addition 5g per litre, T3: addition 10g per litre, T_4: addition 15g per litre, Means in same rows with the same superscript were not significantly different, with different superscript were significantly different. *($P \leq 0.05$). Each period presented two weeks.

Results refer to Significant effected regarding semen traits like ejaculate volume, mass motility, Individual motility, spermatozoa concentration, spermatocrit, and percentage of dead spermatozoa for black local turkey males were dependent on the level of licorice addition in drinking water of birds (tables 1,2,3,4,5 and 6). While deformation spermatozoa ratio had not effected by licorice addition (table7). These results indicated that the licorice addition had better quantitative and qualitative semen parameters compared with the control group in this study. Differences in semen quantity and quality in relation with licorice addition were also reported by AL- Daraji, (2013) he had been proofed that semen diluent supplementation with licorice extract led to enhance of quality and storage ability. The positive effect of adding to licorice extract with drinking water on semen characteristics may come from biological antioxidant of licorice extract to protect sperms from damage effects of free radicals and reactive oxygen species (ROS), furthermore, the mechanism responsible for licorice protection of low-density lipoprotein (LDL) and Polly-unsaturated fatty acid (PUFA) against oxidation is due its ability to bind LDL, scavenge free radicals, and protect other oxidants associated with LDL. Some dietary nutrients such as licorice and its components such as isoflavanes play important role as antioxidants against LDL and

(PUFA) oxidation (Vaya et al., 1997). Flavonoids are components of licorice root extract(glabridin , glabrene) shown to have anti-inflammatory, antimicrobial and anti-oxidative activity as well as its major flavonoid, the isoflavin glabridin, are powerful antioxidants against lipid peroxidation, so it protects certain vital organs from being harmed by oxidants (Niki et al.,1993).

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