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Omar M. Tabour;

Ahmed T. Taha*

Animal Production Dept.,
College of Agriculture,
University of Tikrit, Tikrit,
Iraq

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Effect of Adding Different Levels from Melatonin in Some Characteristics Semen of Broiler Breeder Male Ross308

ABSTRACT

The aim of the present study was to evaluate the effect of adding different levels of melatonin in some of semen Characteristics and antioxidant status in male broiler breeder Ross308, from 16/11/2017 to 30/1/2018. A total of 25 birds of Ross 308 broiler breeder males, 30 weeks' old were used in this study. The birds were randomly distributed into five groups with five replicates each. Each treatment group constituted of 5 birds (1 bird each replicate). The broiler breeder males administered orally with capsules containing melatonin. Treatment groups were as following: **T1**: Birds fed the basal diet without any addition (control), **T2**: Birds fed diet supplemented with 15 mg/kg of diet, **T3**: Birds fed diet supplemented with 30 mg/kg of diet, **T4**: Birds fed diet supplemented with 40 mg/kg of diet and **T5**: Birds fed diet supplemented with 60 mg/kg of diet. The result of the study revealed a significant decrease in the ejaculated volume, sperm's mass and individual motility and sperm concentration, With a significant increase in dead and abnormal sperm ratio. However, administration with melatonin at (60) mg/kg diet resulted in significant increase ($p < 0.05$) in glucose and total protein concentration of seminal plasma. Also adding melatonin with (15) mg/kg diet had significantly improved in the antioxidant status.

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INTRODUCTION

Low-Fertility is a serious problem that facing farm breeders in general and poultry breeders in particular, about 30% of these problems are associated with low male fertility (Barkhordari *et al.*, 2013). Which is result from problems in sperm synthesis, a decrease in the sperm quality production or a nutritional deficiency in the bird's diet as a deficiency of vitamins, especially vitamin E (Barreto *et al.*, 1997). Several studies have confirmed the important role of antioxidants in protecting sperm from injury caused by oxidative stress, the imbalance between active oxygen species and free radicals and natural antioxidants in the sperm has a negative impact on sperm fertility rate (Lundsberg *et al.*, 2014). Rooster's semen had a low levels of antioxidants compared to other birds and mammalian within high percentage of polyunsaturated fatty acids (PUFA) in the sperm membrane which make it an easy target to oxidants. Melatonin is a potent antioxidant that exists in most organisms and contribute in many sperm functions such as increased sperm motility, maturation and adaptability (Medrano *et al.*, 2017). Many studies have shown that melatonin is significantly better than the classic antioxidants in resisting free-radical-based molecular destruction. In these in vivo studies, melatonin was more effective than vitamin E and C (Ahmed *et al.*, 2011; Montilla *et al.*, 2001). Melatonin is synthesized and secreted from the pineal gland as well as many other organs such as kidneys and

* Corresponding author: E-mail: dr.att76@gmail.com

retinal in response to darkness, hence the name hormone of darkness (Jahanban-Esfahlan *et al*, 2018). Melatonin plays a series of biological functions such as regulation of circadian cycles, signaling for seasonal reproduction, antioxidant, and immunomodulation (Reiter *et al.*, 2016). As well as anti-inflammatory, anti-cancer and circadian rhythm also its effects to organization endocrine rhythm (Lacoste *et al*, 2015). Peschke *et al.*, (2006) noticed that melatonin plays an active role in increasing the immune system's capacity. Rocha *et al.*, (2015) had shown that melatonin plays an important role to regulating blood sugar level and its metabolism. In addition, Melatonin protect the testicular function and the sperm from the harmful effects that occur in the body (Chabra *et al.*, 2014). The study aimed to:

Determination the effect of adding different levels of melatonin in some of semen characteristics and antioxidant status in male broiler breeder Ross308.

MATERIALS AND METHODS

Birds and diet

Twenty-five broiler breeder males, thirty weeks of old individually caged. Fed with broiler breeder diets containing (2700) kcal/kg and (11.50 %) crude protein. This birds exposed to 16 L : 8 D. They were randomly divided to five group each one included 5 replicate (one per each), the males administrated orally with capsules contain melatonin as the following: (supplemented with 15,30,45,60 melatonin mg/kg diet for 2nd, 3rd, 4th and 5th treatment respectively). While the first group consider as control. After a 2-week adaptation period to the basal diet and training to abdominal massage for semen collection (Burrows and Quinn, 1937).

Semen Processing and Evaluation

The birds were subjected to experimental treatments and seminal characteristics were determined weekly for 4 and 8 weeks. Ejaculates obtained from the birds individually in each replicate evaluated. Seminal volume was measured in graded collecting tubes. Sperm's mass and individual motility was assessed by using an Olympus compound light microscope (Shinjuku, Tokyo) by method of (parker *et al* ,1942) , Sperm live/dead ratio and abnormality were evaluated, using a portion of ejaculate stained with warmed eosin-nigrosin solution. The stained diluted seminal smear was prepared, and at less 250 spermatozoa in each slide were evaluated and unstained spermatozoa were considered as live (Lack and Stewart,1978). By the method of Allen and Champion (1955) sperms concentration were estimated.

Seminal plasma Processing and Evaluation

After 4 and 8 weeks on treatment, semen was collection as pooled sample with three times for all replicates. And put in cold centrifuge 5000 r/m (30 mints) to obtaining the seminal plasma which used to determined glucose, total protein concentration this estimated by using kit (Bio- Labo French company). Glutathione (GSH) was measuring by method of (Al- Zamely *et al*,2001), malondialdehyde (MDA) was estimated as method of (Guidet and Shah,1989).

Statistical analysis.

The data obtained were subjected to analysis of variance (ANOVA) at $P \leq 0.05$. Significant means were separated using (Duncan multiple range test,1955) using SAS (2012) software package. vectorial design According to the following mathematical model:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + e_{ijk}$$

where Y_{ijk} is the individual observation, μ is the overall mean, T_i is the treatment effects ($i=1, 2, \dots, 5$), S_j is the period effects ($j=1, 2$), TS_{ij} Interaction effect between treatment and period, and e_{ijk} is the error term.

RESULTS AND DISCUSSION

Results

The results in table (1) and (2) showed a significantly decrease in ejaculate volume for the groups that treated with melatonin (T2, T3 and T5), a significant increase of dead and abnormal sperm ratio compared with control, while adding melatonin at 45 mg/kg (T4) diet for four weeks has resulted in a significant decrease in the dead and abnormal sperm ratio, compared to all other study treatments. Also showed lower percentages of mass and individual progressive motility, the impact of the Treatment duration in the semen Characteristics find a significant decrease in the second period compared to the first period. It is noted from the same table that there is no significant differences between melatonin addition treatments T2, T3 and T4 compared to the control treatment. While T5 significantly reduce sperm concentration compared with melatonin treatment and control.

The results in the table (3) showed that T5 result in a significant increase in glucose concentration compared to T1 and other treatment. The effect of period showed a significant decrease in glucose concentration in the first period compared with the second. The addition of melatonin at the concentration of (60) mg/kg feed led to a significant increase in the concentration of total protein compared to control. second treatment records the lowest values compared to the third, fourth and fifth treatments. As showed in the same table MDA level were significantly higher in the fifth group compared to control group, on the other hand, GSH level were significantly increase in the second group compared to melatonin treatments and control.

Discussion

Semen characteristics

Ejaculated volume was significantly decrease during melatonin administration especially in the second period of the study compared to the first. That's due to the negative impact of melatonin on testosterone secretion from leydig cell in the testes. Taha, (2008) noticed a positive correlation between ejaculated volume and testosterone levels in male broiler breeders. McGuire *et al*, (2011) observed that melatonin enters into the gene expression of GnIH in male gonadal, as well as observed in *a in Vetro* studied that both of Melatonin and GnIH are significantly decrease the secretion of testosterone from the quail testes. the effect of testosterone reduction may extend in its negative effect on sperm motility and viability, since the decrease of this hormone may give signals to the male reproductive system to stop or reduce its activity, and led to increase in dead/abnormal sperm ratio. Al-Darraj (1998) observed a highly negative correlation between dead and abnormal sperm and it mass and individual motility. Ortiz *et al.*, (2011) showed that adding melatonin with high concentration led to significant increase in dead and abnormal sperm ratio because of loses the flexibility and liquidity necessary for the movement. It may be due to reduce in sperm concentration as the mass and individual movement of the sperm increases with concentration (Taha, 2008).

Characteristics of seminal plasma

The reduction of glucose concentration in the seminal plasma when treated with melatonin may be due to the fact that glucose is the primary source of energy production in sperm birds, meaning that increased concentration and vitality of the sperm is matched by a decrease in the concentration of plasma glucose (Taha, 2008). When increase in total protein concentration may be attributed to the high percentage of dead and abnormal sperm table(1). The level of MDA and GSH is one of the most important indicator for the antioxidant status. Increasing in the MDA level reflects the amount of

oxidative damage suffered by sperm, which is characterized by its high content of long-chain unsaturated fatty acids (Surai *et al*, 1998).

Table (1) Effect of melatonin in some of semen characteristics in broiler breeder males Ross308 during different periods.

Ejaculate volume (ml)			
Treatments	First period	Second period	Ejaculate volume(ml)
T1	0.35 ± 0.04 a	0.31 ± 0.02 a	0.33 ± 0.02 A
T2	0.33 ± 0.01 a	0.27 ± 0.02 a b	0.30 ± 0.01 A B
T3	0.33 ± 0.02 a	0.22 ± 0.02 b c	0.28 ± 0.02 B C
T4	0.31 ± 0.02 a	0.18 ± 0.02 c d	0.25 ± 0.02 C
T5	0.22 ± 0.02 b c	0.12 ± 0.01 d	0.16 ± 0.02 D
Effect of period	0.32 ± 0.01 A	0.22 ± 0.01 B	
Mass motility %			
Treatments	First period	Second period	Mass motility %
T1	85.10 ± 0.94 c	86.76 ± 2.31 b c	85.93 ± 1.20 A B
T2	87.68 ± 0.95 b c	88.04 ± 0.65 a b c	87.86 ± 0.54 A
T3	90.32 ± 1.12 a b	79.46 ± 1.23 d	84.98 ± 1.97 A B
T4	91.98 ± 1.13 a	68.40 ± 1.41 e	80.19 ± 4.02 C
T5	79.18 ± 1.36 d	59.62 ± 1.53 f	69.40 ± 3.40 D
Effect of period	86.58 ± 1.02 A	76.45 ± 2.31 B	
Individual motility %			
Treatments	First period	Second period	Individual motility %
T1	83.30 ± 1.49 c d	84.32 ± 1.86 b c d	83.81 ± 1.14 A
T2	88.12 ± 0.84 a b	80.65 ± 1.44 e d	84.34 ± 1.48 A
T3	87.26 ± 1.07 a b c	73.63 ± 1.23 f	80.56 ± 2.36 B
T4	89.30 ± 0.68 a	66.74 ± 1.63 g	78.02 ± 3.85 B
T5	78.92 ± 1.39 e	53.42 ± 2 h	66.17 ± 4.44 C
Effect of period	85.38 ± 0.90 A	71.78 ± 2.33 B	

T1:control treatment .**T2:** supplemented with melatonin 15 mg/kg diet.**T3:** supplemented with melatonin 30 mg/kg diet .**T4:** supplemented with melatonin 45mg/kg diet.**T5:** supplemented with melatonin 60mg/kg diet.

Table (2) Effect of melatonin in some of semen characteristics in broiler breeder males Ross308 during different periods.

Dead sperm %			
Treatments	First period	Second period	Dead sperm %
T1	13.72± 1.50 e f	12.68 ± 0.84 f g	13.20 ± 1.40 C
T2	8.88 ± 1.86 g h	17.24 ± 1.62 e d	13.06 ± 1.63 C
T3	10.76 ± 1.08 f g h	24.14 ± 1.23 c	17.45 ± 2.36 B
T4	7.72 ± 0.68 h	30.28 ± 1.62 b	19 ± 3.85 B
T5	20.08 ± 1.39 c d	45.58 ± 2 a	32.83 ± 4.40 A
Effect of period	12.23 ± 1.01 B	25.98 ± 2.44 A	
Abnormal sperm %			
Treatments	First period	Second period	Abnormal sperm %
T1	15.52 ± 1.50 f	11.12 ± 1.86 f	15 ± 1.14 D
T2	14.48 ± 0.84 e f	18.38 ± 1.44 e d	14.75 ± 1.14 D
T3	13.46 ± 1.08 f	25.40 ± 1.36 c	19.43 ± 2.14 C
T4	12.22 ± 0.68 f	33.96 ± 1.63 b	23.09 ± 3.71 B
T5	22.38 ± 1.39 c d	47.00 ± 2 a	35.13 ± 4.40 A
Effect of period	28.02 ± 0.94 A	14.94 ± 2.53 B	
Sperm concentration (10 ⁶ /ml)			
Treatments	First period	Second period	Sperm concentration(10 ⁶ /ml)
T1	4.05 ± 0.15 a b	3.98 ± 0.22 a b	4.01 ± 0.12 A
T2	3.58 ± 0.25 b c	3.32 ± 0.12 c	3.75 ± 0.15 A
T3	4.05 ± 0.19 a b	2.60 ± 0.06 d	3.32 ± 0.26 B
T4	4.18 ± 0.07 a	1.97 ± 0.11 e	2.77 ± 0.29 C
T5	3.13 ± 0.32 c	1.48 ± 0.06 e	2.31 ± 0.31 D
Effect of period	3.80 ± 0.11 A	2.67 ± 0.19 B	

T1:control treatment .**T2:** supplemented with melatonin 15 mg/kg diet.**T3:** supplemented with melatonin 30 mg/kg diet .**T4:** supplemented with melatonin 45mg/kg diet.**T5:** supplemented with melatonin 60mg/kg diet.

Table (3) Effect of melatonin in the concentration of glucose, total protein, GSH and MDA levels in the seminal plasma of the broiler breeder males Ross308 during different periods.

Glucose (mg/dl)			
Treatments	First period	Second period	Glucose concentration (mg/dl)
T1	15.90 ± 0.73 d e	16.90 ± 0.73 c d	16.40 ± 0.51 B
T2	13.70 ± 0.25 e f	12.93 ± 1.01 f	13.31 ± 0.49 D
T3	13.66 ± 0.58 e f	15.43 ± 0.40 d e f	14.44 ± 0.50 C D
T4	13.76 ± 1.04 e f	18.50 ± 0.50 b c	16.13 ± 1.17 B C
T5	19.50 ± 1.45 b	25.03 ± 0.87 a	22.26 ± 1.45 A
Effect of period	15.30 ± 0.69 B	17.76 ± 1.12 A	
Total protein (gmL/100ml)			
Treatments	First period	Second period	Total protein (gmL/100ml)
T1	1.36 ± 0.02 e f	1.38 ± 0.02 e f	1.37 ± 0.01 C
T2	1.32 ± 0.04 e f	1.12 ± 0.03 f	1.22 ± 0.05 C
T3	1.34 ± 0.17 e f	1.52 ± 0.02 e d	1.43 ± 0.08 C
T4	1.84 ± 0.06 d	2.28 ± 0.03 c	2.06 ± 0.10 B
T5	2.62 ± 0.29 b	3.57 ± 0.05 a	3.10 ± 0.25 A
Effect of period	1.69 ± 0.14 B	1.97 ± 0.23 A	
glutathione(mm/L)			
Treatments	first period	second period	Glutathione(mm/L)
T1	1.17 ± 0.03 c	1.13 ± 0.02 b	1.24 ± 0.03 B
T2	1.32 ± 0.05 b	1.58 ± 0.04 a	1.45 ± 0.06 A
T3	1.15 ± 0.01 c	1.38 ± 0.01 b	1.27 ± 0.05 B
T4	1.07 ± 0.02 c	0.83 ± 0.04 d	0.95 ± 0.05 C
T5	0.09 ± 0.05 d	0.62 ± 0.03 e	0.77 ± 0.07 D
Effect of period	4.50 ± 0.08 A	4.45 ± 0.21 A	
Malondialdehyde (mm/ml)			
Treatments	First period	Second period	Malondialdehyde(mm/ml)
T1	4.76 ± 0.23 c d	4.28 ± 0.41 d c	4.52 ± 0.23 B
T2	4.35 ± 0.08 c d	3.38 ± 0.23 e	3.87 ± 0.24 C
T3	4.11 ± 0.06 d	4.04 ± 0.05 d	4.07 ± 0.04 C
T4	4.43 ± 0.08 d e	5.06 ± 0.08 a b	4.75 ± 0.15 A B
T5	4.84 ± 0.05 b c	5.47 ± 0.28 a	5.15 ± 0.18 A
Effect of period	4.50 ± 0.08 A	4.45 ± 0.21 A	

T1:control treatment .**T2:** supplemented with melatonin 15 mg/kg diet.**T3:** supplemented with melatonin 30 mg/kg diet .**T4:** supplemented with melatonin 45mg/kg diet. **T5:** supplemented with melatonin 60mg/kg diet.

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تأثير أضافة مستويات مختلفة من الميلاتونين في بعض صفات السائل المنوي لديكة أمهات فروج اللحم Ross 308

عمر مزاحم طاבור واحمد طائيس طه

جامعة تكريت/ كلية الزراعة / قسم الإنتاج الحيواني

المستخلص

أجريت هذه الدراسة في إحدى قاعات الطيور الداجنة التابعة لقسم الإنتاج الحيواني في كلية الزراعة جامعة تكريت للمدة من 16/11/2017 لغاية 30/1/2018. هدفت التجربة إلى دراسة تأثير إضافة الميلاتونين بتركيزات مختلفة ومدد زمنية مختلفة في بعض صفات السائل المنوي والبلازما المنوية لديكة أمهات فروج اللحم. أستخدم في هذه الدراسة (25) ديك من أباء فروج اللحم نوع Ross 308 ويعمر 30 أسبوع. تم توزيع الديكة بصورة عشوائية إلى خمس معاملات بواقع (خمس ديك/معاملة) وبخمس مكررات (1 ديك/مكرر) وفقاً لما يأتي: معاملة السيطرة تناولت علفاً قياسياً بدون أي إضافة أما المعاملات الثانية والثالثة والرابعة والخامسة فقد جرعت بالميلاتونين بتركيز 15 و 30 و 45 و 60 ملغم /كغم علف على التوالي. أدت إضافة الميلاتونين بالتركيز المرتفعة (30، 45، 60) ملغم/كغم علف إلى انخفاض معنوي في حجم القذفة خلال مدتي الدراسة والمدة الكلية رافقه انخفاض معنوي في معدلات الحركة الجماعية والفردية للنطف وتركيز النطف مع وجود ارتفاع معنوي في نسبة النطف المبتة والمشوهة. وفيما يخص صفات البلازما المنوية فقد أدت إضافة الميلاتونين إلى ارتفاع معنوي في تركيز الكلوكوز والبروتين الكلي لصالح المعاملة الخامسة في حين سجلت معاملات الإضافة الأخرى انخفاضاً مقارنة بمعاملة السيطرة، وفيما يخص تركيز GSH سجلت المعاملة الثانية تفوقاً معنوياً في مستوى GSH قابله انخفاض معنوي في مستوى MDA.

الكلمات المفتاحية: الميلاتونين، السائل المنوي، ديك، فروج اللحم، Ross 308