The effect of exogenous melatonin administration on sperm quality and some stress related parameters of broiler breeder males under natural summer conditions

H. KOZANOĞLU*, E. BABACANOĞLU, S. ÖZKAN

Faculty of Agriculture, Department of Animal Science, Ege University, 35100 Izmir, Turkey.
***Corresponding author: kozanoglu@mail.ege.edu.tr**

Twenty-four broiler breeder males (39 weeks old) were randomly distributed in two groups consisting of twelve each and were caged, individually. In the melatonin treated group (M), roosters were orally administrated with 3 mg of melatonin per kg body weight between per day 11.00 and 12.00 a.m. The other group was considered as control (C). During the experimental period of 20 days, duration of hot hours measured (≥32 ºC) per day was ranged between 6 to 10 h with an average of 8 h. Blood samples of birds were collected at the beginning (day 0) and 2 wk (day 14) after the treatment had been started for white blood cells count and heterophil to lymphocyte ratio (H:L). Sperm was collected at 0, 3, 7, 12 and 16 days of experiment to evaluate sperm quality parameters. Melatonin administration significantly increased the percentages of normal live sperm (P<0.05) and decreased abnormal and dead sperm percentages (P<0.05). However, numerical increase in sperm concentration of M males was not statistically significant. At the end of the experimental period, semen was collected from each male and 7 hens per male were artificially inseminated. Thereafter, roosters were killed and organ weights were recorded. Hatching eggs were collected for 7 days and incubated. At hatch, chick weight and organ weights were recorded in sampled chicks. A significant positive effect of melatonin has been observed on hatch weight and relative spleen weight of chicks from males in M (P<0.05). However, percentage of fertile eggs did not differ with melatonin treatment. H:L ratio, as a good indicator of stress, differed significantly between treatments being lower (0.34±0.01) in M males than C (0.44±0.01). However, rectal body temperatures of males were similar in both M and C. As a result, melatonin had a positive effect on stress status of roosters as shown by lower H:L ratios under natural summer conditions. However, improvement in sperm quality parameters did not result in a better fertility rate in M. Higher hatching and relative spleen weight of chicks from M males might be referred to the positive effect of melatonin on growth however this needs further investigation.

Keywords: broiler breeder; male; sperm quality; melatonin; heat stress

Introduction

High ambient temperature is one of the most important problems for poultry production especially in hot climate areas. The detrimental effects of high ambient temperatures on poultry production have been reported by several authors (McDaniel et al., 1995; Karaca et al., 2002 a, b). There are reductions in food consumption, body weight, egg production, semen quality, fertility, and hatchability under high ambient temperature. There is a marked alteration in sperm function of broiler breeder males exposed to high temperatures, which results in poor sperm-egg penetration, low fertility and a lower fertilization rate (Karaca et al., 2002a; Karaca et al., 2002b). Increased circulating heterophil to lymphocyte (H:L) ratio is an accepted indicator of the stress condition in birds (Siegel, 1995). Al-

Murrani et al. (1997) reported that heat stress increased the percentage of heterophil and H:L ratio, decreased the percentage of lymphocytes.

Various methods are available to alleviate the negative effect of high ambient temperature on performance of poultry. Because of the high cost of cooling poultry houses, diet manipulations (Altan et al., 2000a), short term fasting in layers (Altan et al., 2000b) and broilers (Özkan et al., 2003), acclimation to heat during pre and post natal periods (Yalçın et al, 2005) have great interest to reduce heat stress on poultry during the hot summer months. Studies have shown that antioxidant nutrient supplementation such as ascorbic acid and melatonin could be used to decline the negative effects of high ambient temperature on quails (Sahin et al., 2004). However, there are limited numbers of researches regarding the effect of heat stress on male broiler breeders.

The effects of acetylsalicylic acid (ASA) (McDaniel and Parker, 2004) and Vitamin C (McDaniel et al., 2004) on reproductive performance of broiler breeder males were investigated. However they did not report any improvement in the reproductive traits regarding to the supplementations of ASA and Vitamin C. Breque et al. (2003) shown that dietary supplementation of vitamin E was effective in limiting lipid peroxidation of sperm plasma membranes in cockerels.

Melatonin is a lipophilic hormone, mainly produced and secreted at night by the pineal gland and is a powerful antioxidant with its high free radical scavenging activity (Barrenetxe et al., 2004). Barriga et al. (2002) suggest the possibility that there exists a connection between the circulating levels of melatonin and increased corticosterone in stress conditions over the 24 h of the day. In addition to its ability to scavenge free radicals, melatonin also is involved in many physiological functions such as immune response, energy metabolism and temperature regulation (Sahin et al., 2004). It has been demonstrated that feeding melatonin to 2 and 3-week-old chickens resulted in a highly significant decline in heat production (Zeman et al., 2001).

Therefore, the present study was undertaken to determine the effect of oral melatonin administration on sperm quality parameters in male broiler breeders under natural high summer conditions. Some growth characteristics of progeny from melatonin treated and control males were also considered in this study.

Materials and Methods

Twenty-four broiler breeder males, 39 week of age, were kept in individual cages. The males were fed with commercial broiler breeder diets including 15 % crude protein and 2800 kcal ME / kg. The males were exposed to 16 h of light/day during the experimental period. At the beginning and at the end of the experiment weights of the roosters were recorded. At the beginning and at the end of the experiment mean body weight of roosters were similar being 5447±60 g, 5384±60 g and 5327±58 g, 5281±58 g for C and M, respectively. Twelve broiler breeder males administrated orally with 5-ml water dissolved melatonin (3 mg per kg body weight, Nature's Bounty, Inc., Illinois, USA) in midday (11.00-12.00) during the experiment. Other twelve males were considered as control. Semen samples were collected from each male at the beginning of the experimental period and thereafter, repeated 4 to 5 days intervals during the experimental period. Sperm quality criterions (sperm concentration with hemocytometer, sperm viability using nigrosin/eosin stain, motility by hanging drop method) were determined according to Bakst and Cecil (1997). The experiment was lasted for 20 day in summer season. Temperatures and humidity were recorded using a data-logger at the cage levels for every hours of each day during the experiment. During the experimental period, house temperature and humidity are shown on Figure 1, respectively. Duration of hot hours measured (≥32 ºC) per day was ranged between 6 to 10 h with an average of 8 h during the experimental period.

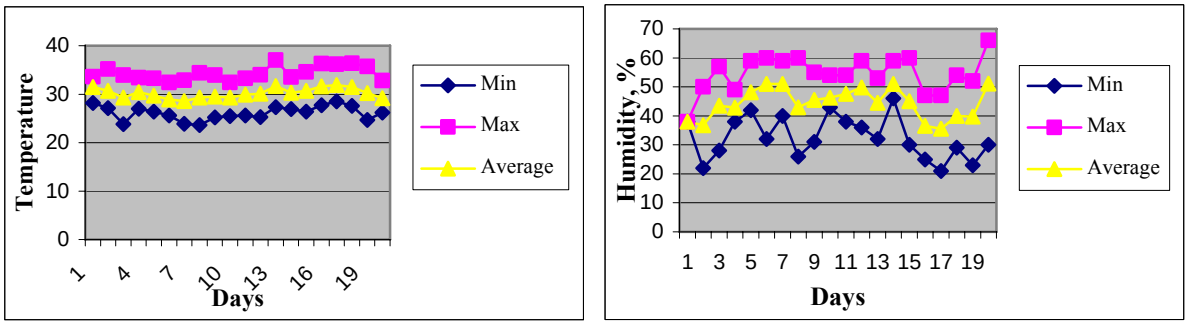

Figure 1. Daily minimum, maximum and mean temperature and humidity values during the experiment

Semen was diluted (1:1) and 0.05 ml diluted semen was used for insemination. Eggs were collected for 7 d, labeled and stored at 20 ºC for one week. The eggs were incubated in an incubator with usual temperature and humidity controls. On day 10 of incubation, fertility was determined by light control and all un-hatched eggs were inspected after hatch for true fertility. Before and two weeks after melatonin treatment, roosters were bled and 100 white blood cells were counted, and heterophil/lymphocyte ratios were estimated for measuring stress levels of roosters (Gross and Siegel, 1983). Rectal body temperature was measured in the morning (09.00 a.m.) and afternoon (16.00 p.m.) by using a digital thermometer on day 2 and day 14 after melatonin administration. At the end of the experiment, 6 roosters from each group were decapitated and testis, liver and spleen were removed. Relative weights of organs to body weight were calculated. Testis narrow diameter, wide diameter, testis volume and testis shape index (narrow diameter/wide diameter x 100) was determined.

Sperm quality parameters, white blood cell counts and H:L ratios were analyzed using GLM procedure with a model contains treatment and observation day and interaction effect. Before analyze, logarithmic transformation was used for sperm concentration and abnormal live sperm data to get a normal distribution. However, actual values were presented on the tables. All the other data were analyzed using Oneway ANOVA to test the effect of treatment. Means were compared using t-test. JMP statistical software was used to analyze data (SAS, 2000).

Results and Discussion

Sperm quality parameters are presented in Table 1. Although a numerical increase in sperm concentration has been observed due to melatonin administration, the difference was not significant ($P=0.076$). The percentage of normal live sperm significantly increased, while percentage of abnormal sperm and dead sperm significantly decreased in the melatonin treated males ($P\leq0.05$). Observation day was a significant effect on sperm concentration however treatment by observation day interaction was not significant for any of the parameters. Karaca et al. (2002a) reported that sperm concentration (packed cell volume) was not influenced by ambient temperature (heat stress or control) and the percentage of dead sperm decreased for heat stressed males. In our results, the ratio of dead sperm and abnormal sperm in semen from M males were lower than C group. Furthermore, the percentage of normal sperm increased in M males.

Relative testis, liver and spleen weight, testis volume and testis shape index of roosters were presented in Table 2. Any of the parameters were not affected by melatonin administration. Although relative liver and spleen weight of male breeders were numerically higher in M compared with C, difference between treatments was not statistically significant. Hatching weight and relative spleen weight increased in chicks that progeny of melatonin administrated roosters compared to control group roosters (Table 3). It is well known that maternal diets directly affect embryo development (Surai, 2002). However, paternal diet effect does not seem probable. In the study of Kovacikova et al. (2003) no improvement in embryo growth due to in-ovo melatonin administration in different doses has been reported.

Table 1. The effect of melatonin and day of observation on sperm quality parameters of male broiler breeders

	Sperm Concentration ($x10^9$/ml)	Normal Live Sperm (%)	Abnormal Live Sperm (%)	Dead Sperm (%)
Treatment				
Control	5.23±0.39[a]	74.64±1.01[a]	9.75±0.51[a]	15.61±0.80[a]
Melatonin	6.20±0.38[b]	80.05±0.89[b]	7.85±0.45[b]	12.10±0.70[b]
Observation Day				
***Day0**	6.49±0.63	74.27±1.73	10.04±0.87	15.69±1.36
Day 3	5.19±0.61	79.79±1.47	8.22±0.74	11.99±1.16
Day 7	4.18±0.58	78.02±1.38	7.97±0.70	14.00±1.09
Day 12	6.86±0.64	76.16±1.46	10.11±0.73	13.74±1.15
Day 16	6.29±0.60	79.00±1.44	7.61±0.72	13.39±1.37
P values				
Treatment	0.076	<.0001	0.008	0.001
Observation Day	0.015	0.080	0.056	0.182
Treat.*Observ.	0.541	0.986	0.414	0.736

[a, b] : means with different superscripts in the columns significantly differ (P≤0.05) ; * Before melatonin administration

Table 2. Effects of melatonin on testis volume, testis shape index, relative weight of testis, liver and spleen of male broiler breeders

	Testis Volume (ml)	Testis Shape Index, (%)	Testis (%)	Liver (%)	Spleen (%)
Control	24.60±2.82	61.05±4.49	0.44±0.045	0.82±0.032	0.07±0.01
Melatonin	25.83±2.57	61.22±3.67	0.49±0.045	0.87±0.032	0.09±0.01
P values					
Treatment	0.754	0.976	0.431	0.301	0.065

Table 3. Hatching weight and relative spleen weight of progeny from melatonin treated and control males

	Chick Hatching Weight, g.	Relative Spleen Weight, %
Control	40.36±0.84	0.021±0.01
Melatonin	43.27±0.82	0.033±0.01
	P Values	
Treatment	0.018	0.004

Melatonin did not influence fertility in the present study. Although a numerical decrease has been observed in fertility percentage in the M (74.43 %) as compared to that of C (80.30 %), melatonin treatment did not affect fertility, significantly (data not tabulated). A positive effect on sperm quality and fertility would be expected. However, two recent studies are consistent to our findings that fertility was not improved by antioxidant i.e. acetylsalicylic acid (McDaniel and Parker, 2004) and ascorbic acid supplementations (McDaniel et al., 2004).

The effects of melatonin administration on rectal body temperatures are presented in Table 4. Rectal temperatures did not differ significantly between treatments. McDaniel et al. (1995) showed that fertility was negatively correlated with increased rectal temperatures as a result of heat stress. It could also be expected a lowered body temperature due to reduced heat production in M males. However, rectal temperatures of melatonin administrated roosters under high ambient temperature conditions were not decreased significantly. The present data are not consistent with recent papers of Apeldoorn et al. (1999) and Zeman et al. (2001) reporting that melatonin supplementation to a broiler diet reduced heat production. In our study, this contradictory result could be due to administrated melatonin dose or half-life. The half-life of melatonin is quite short, i.e.30-60 min. (Barrenetxe et al., 2004). We measured the body temperatures 4 to 5 h after melatonin treatment and this might be the reason why we could not find a reduced body temperature in M males.

Table 4. Mean rectal temperatures (ºC) of roosters control and melatonin treated groups

Age	Day 2		Day 14	
	08.00	**16.00**	**08.00**	**16.00**
Control	42.85±0.11	43.02±0.09	42.84±010	43.08±0.07
Melatonin	42.88±0.10	43.16±0.09	42.85±0.99	43.04±0.07
P Values				
Treatment	0.809	0.316	0.920	0.692

The effects of melatonin administration on percentage of white blood cells and H:L ratio are shown in Table 5. There were significant differences in percentage of heterophils and H:L ratio and treatment*observation interaction was significant for H:L ratio, percentage of heterophils, and lymphocytes. This may refer to that melatonin reduced the physiological stress level in roosters under natural summer conditions. However, H:L ratios measured in the present study are considered as optimal stress level for poultry (Gross and Siegel, 1993). This may relate to that cockerels developed an adaptation to natural high temperature conditions as we performed the study in mid of the summer. Our results in agreement with Brennan et al. (2002), who found that supplementing melatonin to water significantly increased lymphocyte percentage, decreased heterophil percentage, and H:L ratio of Japanese quail. Melatonin treatment did not affect the percentage of eosinophils, basophiles and monocytes.

Table 5. Effects of melatonin administration on white blood cells in roosters reared under natural summer season

Treatment	Time	H:L Ratio	Heterophil (%)	Lymphocyte (%)	Eosinophil (%)	Basophil (%)	Monocyte (%)
Control	Day 0	0.41±0.01[a]	25.90±0.89[a]	62.82±0.95[a]	6.90±0.66	2.72±0.3	1.72±0.3
	Day14	0.44±0.01[a]	28.66±0.99[a]	64.66±1.05[a]	4.22±0.73	1.44±0.3	0.88±0.3
Melatonin	Day 0	0.43±0.01[a]	26.33±0.99[a]	61.11±1.05[a]	7.66±0.73	2.88±0.3	1.88±0.3
	Day14	034±0.01[b]	23.33±0.99[b]	68.88±1.05[b]	4.22±0.73	1.55±0.3	1.33±0.3
		P Values					
Treatment		0.025	0.016	0.232	0.599	0.684	0.312
Observation Day		0.099	0.901	<.0001	0.0001	0.0004	0.024
Treat.* Observ.		0.001	0.005	0.007	0.599	0.940	0.635

[a, b] : means with different superscripts in the columns significantly differ (P≤0.05)

In conclusion, administration of 3 mg melatonin per kg body weight improved stress status of cockerels as shown by decreased heterophil percentage and H:L ratio and increased lymphocyte percentage. However we did not observed a decrease in body temperatures measured 4 to 5 h after melatonin administration in which the hottest hours of the day. Although significant increase in normal live sperm percentage and decreases in abnormal and dead sperm percentages had been observed in the melatonin treated males, improvement in sperm quality parameters did not result in a better fertility rate in melatonin treated males. Higher hatch weight and relative spleen weight of progeny from melatonin treated roosters needs further investigation.

References

ALTAN, Ö., ALTAN, A., ÖZKAN, S., AÇIKGÖZ, Z. and ÖZKAN, K., (2000a) Effect of dietary sodium bicarbonate on egg production and egg quality of laying hens during high summer temperature. Arch. Geflügelk. 64 (6), 269-272.

ALTAN, Ö., ÖZKAN, S., ALTAN, A., AKBAŞ, Y., AYHAN, V., ÖZKAN, K., (2000b) Effects of short-term fasting and midnight lighting on egg production traits of laying hens during summer season. Arch. Geflügelk. 64 (2), 85-89.

APELDOORN, E.J., SCHRAMA, J.W., MASHALY, M.M. and PARMENTIER, H.K., (1999) Effect of melatonin and lighting schedule on energy metabolism in broiler chickens. Poult. Sci. 78: 223-229.

AL-MURRANI, W.K., KASSAB, A., AL-SAM, H.Z. and AL-ATHARI, A.M.K., (1997) Heterophil/lymphocyte ratio as a selection criterion for heat resistance in domestic fowls. Br. Poult. Sci. 38:159-163.
BAKST, M.R. and CECIL, H.C., editors (1997) Techniques for semen evaluation, semen storage, and fertility determination. Poultry Science Association, Inc. Savoy, Illinois.
BARRENETXE, J., DELAGRANGE, P. and MARTINEZ, J.A., (2004) Physiological and metabolic functions of melatonin. J. Physiologic. Biochem. 60 (1), 61-72.
BARRIGA, C., MARCHENA, J.M., LEA, R.W., HARVEY, S. and RODRIGUEZ, A.B., (2002) Effect of stress and dexamethasone treatment on circadian rhythms of melatonin and corticosterone in ring dove (Streptopelia risoria). Molecular and Cellular Biochemistry 232: 27–31.
BRENNAN, C.P., HENDRICKS III, G.L., EL-SHEIKH, T.M., and MASHALY, M.M., (2002) Melatonin and enhancement of immune responses in immature male chickens. Poult. Sci. 81: 371-375.
BREQUE, C., SURAI, P. & BRILLARD, J.P., (2003) Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. Molecular Reproduction and Development, 66: 314-323.
GROSS, W.B. and SIEGEL, P.B., (1983) Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases, 27: 972.
GROSS, W.B. and SIEGEL, P.B., (1993) General principle of stress and welfare. In Livestock, Handling and Transport. Ed. Grandin, T. pp: 21-34. CAB International, Wallingford, UK.
KARACA, A.G., PARKER, H.M., YEATMAN, J.B. and MCDANIEL, C.D., (2002a) The effects of heat stress and sperm quality classification on broiler breeder male fertility and semen ion concentrations. Br. Poult. Sci. 44:621-628.
KARACA, A.G., PARKER, H.M., YEATMAN, J.B., and MCDANIEL, C.D., (2002b) Role of Seminal Plasma in Heat Stress Infertility of Broiler Breeder Males. Poult. Sci. 81:1904-1909.
KOVACIKOVA, Z., KUNCOVA, T., LAMOSOVA, D., ZEMAN, M., (2003) Embryonic and Post-Embryonic development of Japanese quail after in ovo melatonin treatment. Acta Vet. Brno, 72: 157-161.
MCDANIEL, C.D., BRAMWELL, R.K., WILSON, J.L. and HOWARTH JR. B., (1995) Fertility of male and female broiler breeders following exposure to an elevated environmental temperature. Poult. Sci. 74:1029-1038.
MCDANIEL, C.D., PARKER, H.M., (2004) The effects of dietary acetylsalicylic acid on heat stress infertility of broiler breeder males. International Journal of Poultry Science 3 (9): 570-577.
MCDANIEL, C.D., HOOD, J.E., PARKER, H.M., (2004) An attempt at alleviating heat stress infertility in male broiler breeder chickens with dietary ascorbic acid. Int. J. Poult. Sci. 3 (9): 593-602.
ÖZKAN, S., AKBAŞ, Y., ALTAN, Ö., ALTAN, A., AYHAN, V. and ÖZKAN, K., (2003) The effect of short-term fasting on performance traits and rectal temperature of broilers during the summer season. British Poultry Science 44: 88-95.
SAHIN, N., ONDERCI, M., SAHIN, K., GURSU, M.F. and SMITH, M.O., (2004) Ascorbic acid and melatonin reduce heat-induced performance inhibition and oxidative stress in Japanese Quails. Br. Poult. Sci., 45:116-122.
SAS INSTITUTE, (2000) JMP®, Statistics and Graphics Guide Version 4. SAS Inst. Inc. Cary, NC:
SIEGEL, H. S., (1995) Stress, strains and resistance. Br. Poult. Sci. 36:3–22.
SURAI, P.F., (2002). Natural Antioxidants in Avian Nutrition and Reproduction. pp:305-358. Nottingham University Press, UK:
YALÇIN, S., ÖZKAN, S., ÇABUK, M., BUYSE, J., DECUYPERE, E. and SIEGEL, P.B., (2005) Pre- and Postnatal conditioning ınduced thermotolerance on body weight, physiological responses and relative asymmetry of broilers originating from young and old breeder flocks. Poultry Science 84: 967-976.
ZEMAN, M., BUYSE, J., HERICHOVA, I., DECUYPERE, E., (2001) Melatonin decreases heat production in female broiler chickens. Acta Vet. Brno, 70: 15-18.