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Research article

The Effects of Stocking Density on Some Blood Stress Parameters of Meat Turkeys[a]

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Abstract. Stocking density is one of the important issues in turkey meat production which is enjoyed by consumers as a source of animal protein. This issue is examined in terms of both animal welfare and efficiency in this research. The commercial hybrid white broiler turkey line Hybrid Converter poults were individually numbered following hatch. Study took place on 200 poults. 25 males and 25 females were set in 4 different floor sized cages according to sex. Slaughter age weights in those cages were set to be 30, 40, 50 and 60 kg m^{-2} respectively. Blood was collected in 2nd and 6th weeks and slaughter ages of 16 weeks for females and 20 weeks for males. Blood laboratory analyses were made after collection where each numbered individual in the pens was taken as a replicate. Findings obtained from the study were interpreted for animal welfare and economic issues based on general practice and stress level in the world and our country by subjecting statistical analysis. Stress on the poults have been found to be increasing depending on increasing stocking density and it was concluded that it would be appropriate in terms of animal welfare and ethical conviction for not exceeding certain limits of stocking density within terms of efficiency.

Etlik Hindilerde Yerleşim Sıklığının Bazı Kan Stres Parametrelerine Etkileri

Anahtar kelimeler:
Hindi, stres, refah, kan parametreleri

Özet. Yerleşim sıklığı, dünyada ve ülkemizde sevilerek tüketilen bir hayvansal protein kaynağı olan hindi eti üretiminde üzerinde durulan önemli konulardan birisidir. Bu konu, hem hayvan refahı bakımından incelenmekte, hem de genel performans ve verimlilik bakımından değerlendirilmektedir. Bu araştırmada, konu iki açıdan da değerlendirilmeye çalışılmıştır. Çalışmada ticari melez hatlardan Hybrid Converter beyaz etlik hindi palazları, yumurtadan çıkışı takiben numaralandırılarak kullanılmıştır. Çalışma 200 adet hindi palazı üzerinde yapılmış olup kesim yaşında metrekareye 30, 40, 50 ve 60 kg canlı ağırlık gelecek şekilde farklı ebatlarda yapılmış bölmelere erkek ve dişi ayrı olmak üzere, 25'er adet palaz yerleştirilmiştir. 2. Haftalık yaşta hayvanlar bu bölmelere alınarak yerleşim sıklığı etkisi ortaya çıkarılmıştır. Kan alım işlemi 2. ve 6. hafta ile kesim yaşında yapılmış olup, analizler bakımından her bir birey yetiştirildiği şartlar altında tekerrür olarak değerlendirilmiştir. Çalışmadan elde edilen bulgular istatistik analize tabi tutularak, dünyada ve ülkemizdeki genel uygulama ve stres düzeyine bağlı hayvan refahı bakımından değerlendirilmiştir. Hindilerde stresin yerleşim sıklığının artışına bağlı olarak yükseldiği tespit edilmiş, hayvan refahı ve verimlilik bakımından belirli sınırların aşılmamasının etik olarak uygun olacağı sonucuna varılmıştır.

[a]This article was compiled from Ph.D. Thesis of the researcher.

INTRODUCTION

Turkey meat production is an important branch in the poultry meat industry in Turkey as it is in the world. As the world's population grows, demand for animal protein increases more than the production does. As the world's population was 6.127 Million in 2000, the turkey meat production was 5.061 Mton worldwide, where the population went up to 7.052 Million as the production just increased to 5.609 Mton. This shows the increasing output gap for protein coming from turkey meat in the world and in Turkey as it can clearly be seen on figure 1 representing the population and production change from 2000 to 2012 (FAO 2015; TUIK 2016).

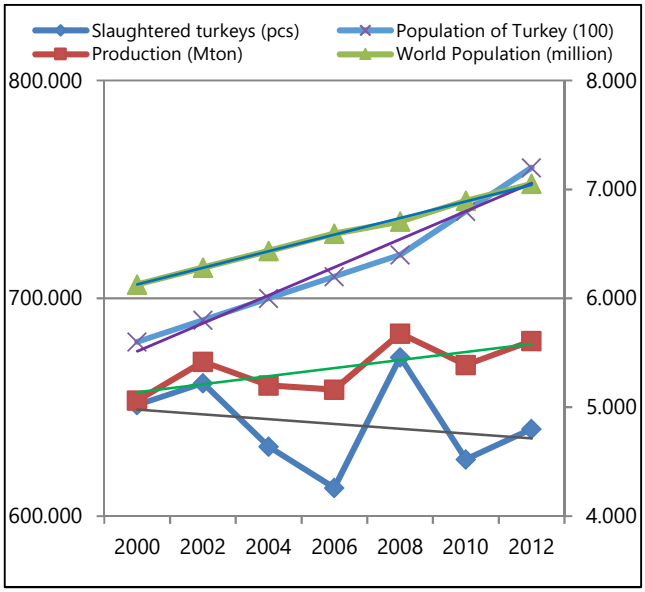

Figure 1. Turkey, World Population and turkey meat production from 2000 to 2012 (FAO 2015; TUIK 2016).
Şekil 1. 2000 – 2012 yılları arasında Türkiye'de ve dünyada hindi eti üretim miktarları.

Turkey meat is a nutritive and precious source of animal protein as seen on Table 1. It has a delicious unique aroma and taste as well. The protein level is similar to cows but less in fat. This makes turkeys a better option for people on a diet (Eratalar and Bulut 2007) as seen on Table 1.

Table 1. Nutritional value of 100 g meat from different farm animals (Ertugrul 1997; Ergün *et al.*, 2001).
Çizelge 1. Farklı çiftlik hayvanlarına ait 100 g etin besin değeri.

	Broiler	Turkey	Cattle	Lamb
Energy (kcal kg^{-1})	215	160	194	228
Protein (g)	18.6	20.4	20.0	14.0
Fat (g)	15.1	8.0	12.0	18.0

In Turkey, public request is mostly leg and wing meat of poultry where European countries and United States mostly demand on breast meat. This may give Turkey an option of selling wings and legs in the country and exporting breast meat to Europe soon for a better profit. Meanwhile, it should be taken into consideration to make more advertisements to achieve a better community understanding of how healthy turkey meat is.

Turkeys can only show their genetic potential of growth and meat yield under the best environmental conditions. In the production farms, stocking density is one of the most important primary factors affecting the birds' performance as well as lighting, ventilation and optimum heating.

Surely, animal ethics and welfare should be taken into consideration when organizing the optimum stocking density for production with economical aspects.

In a research, Noll *et al.* (1991) set 2.2 and 4.8 male turkeys per square meters. Researchers arrived at the data that the birds at higher stocking density had less live weight than the others. As well, a supporting research result comes from Dogrul *et al.* (2005). Researchers have designed a work with 3 and 4 birds m^{-2} and concluded that birds reared at higher stocking density (SD) resulted in worse growth performance.

Also Mirabito *et al.* (2002) shares the data that decreasing the levels of stocking density brings no difference in performance and health issues but worsens the economic aspects in production. Hafez and Hagen (2003) also showed that there is no significant difference between SD groups for turkey poults in terms of health and immune response. Cetin and Ogan (1997) reported that increasing stocking density conditions made the antibody titers fall after vaccination in broiler chickens. Houshmand *et al.* (2012) found that there has been no difference between stocking density groups of 10 and 16 broiler chickens m^{-2} on blood glucose levels and H/L ratios. In contrast Qaid *et al.* (2016) found that except blood glucose levels stress indicators as decreasing H/L levels and increasing heterophyl levels showed that there is an impact of stocking density on stress of the broiler chickens. Azzam and Gogary (2015) also reported that there has been no change on blood glucose levels of chickens reared at stocking densities of 11.90 birds m^{-2} and 16.66 birds m^{-2}.

As stress indicators, blood glucose, lymphocyte count, leukocyte count, heterophyl count and Heterophyl/Lymphocyte (H/L) ratio were by Maxwell

et al. (1990), Martrenchar *et al.* (1997), Patterson and Siegel (1998) and Houshmand *et al.* (2012).

As seen there have been several researches about SD for chickens and turkeys. But, there is no similarity for these researches for bird races, SD levels, environmental conditions etc. This research is important for introducing the present condition for determining the stocking density effect on stress levels of turkeys in contrast to turkey production in Turkey.

MATERIALS AND METHODS

The animal material of the research was obtained from Bolca Hindi which is a turkey meat production company integration founded in Bolu, Turkey. The hatching eggs were obtained from 42 week old Hybrid Converter breeders which were reared in the same breeder house. 200 hatching eggs of these breeders were hatched in the company's hatchery and were transferred to the production farm of the company immediately after automatic vaccination, sexing and beak trimming at the hatchery. The birds were randomly aluminum wing banded, numbered from 1 - 200. So, all the birds would be personally tested as a replicate for the connected blood parameters.

Research took place in commercial company's turkey farm and cages were built in before the research was set. Female cages were 8.245 m^2, 6.179 m^2, 4.943 m^2 and 4.125 m^2 for 30, 40, 50 and 60 kg m^{-2} estimated live weight at the slaughter age of the birds. Male cages were 15.443 m^2, 11.575 m^2, 9.363 m^2 and 7.727 m^2 for 30, 40, 50 and 60 kg m^{-2} estimated slaughter weight of the poults.

Birds were placed in 8 pens 4 male pens and 4 female pens with 25 birds in each pen. In the first 2 weeks birds were reared in rings for better start and the rings were removed at the 2nd week. So, the SD (stocking density) effect was put on in the 2nd week. The SD levels were arranged for the birds' estimated slaughter weights of 30, 40, 50 and 60 kg m^{-2}.

The slaughter weights (SW) of the birds came up higher but not more than 2.5% than the estimated SW set at the beginning of the study.

Vaccination program for the birds used by the company is shown at Table 2.

Male birds were fed with 8 different types of feed where females were fed with 7 different feed in the rearing period. Males were slaughtered at the 20th week where females were at 16th week which were the suitable and the present application at the time of the study.

Feed and water were given ad-libitum. All the feed were obtained from Bolca Hindi's feed mill. The chemical and physical composition of the feed is shown in Table 3.

Lighting program was the same for all the birds with a beginning of 100 lux florescent light at bird level and after 2nd day dark period began with 30 minutes and increased 30 minutes every day up to 6 hours of dark and 18 hours of light which was continued till the end of the rearing period. 100 lux was decreased to 75 lux at day 7 and it was decreased to 50 lux at the 2nd week and went on till slaughter age.

The poults arrived at the farm were reared at 37.0±0.5 °C and the temperature was decreased 0.5 °C daily till the rearing environment is finally 20.0 °C and this temperature (20.0 °C ± 0.5 °C) was kept till the slaughter age.

Proper ventilation was obtained by an automatic environment control system controlling the side curtains during the whole period of the study.

Health control was done by the company's veterinarian where no drugs and feed additives were used during the study.

The bedding material was disinfected, dry pinewood shavings which was spread about 5 kg m^{-2} to the ground homogeneously.

Blood sampling was done in 2nd, 6th weeks and at the slaughter age of 16th week for females and 20th week for males. Blood was taken by veterinarian of the company and 2.5 cc sterile single use injectors were used with green tip needles. 2 cc bloods were taken from each bird and were transferred into 3 ml EDTA tubes containing EDTA for the blood not to coagulate until reaching the lab. Samples were taken from a total of 40 and 10 birds of each stocking density group. The bloods were immediately taken to the lab in an environment controlled carrying box. Blood sugar, leucocyte, erythrocyte, hemoglobin, thrombocyte, lymphocyte counts were taken, hematocrit value and erythrocyte volume was determined by the private laboratory experts with suitable experimental blood kits for poultry.

Table 2. The vaccination program used in the experiment.
Çizelge 2. Denemede kullanılan aşılama programı.

Time	Vaccine	Type	Method	The disease
After hatch	HB1	Active	Sprav	Newcastle D.
7. Day	TRT	Active	Spray	TRT
21. Day	Clone 30	Active	Spray	Newcastle D.
35. Day	TRT	Active	Spray	TRT
56. Day	Lasota	Active	Spray	Newcastle D.

Table 3. Feeds' chemical and physical contents for the rearing period.
Çizelge 3. Yetiştirme dönemi boyunca kullanılan yemin fiziksel ve kimyasal özellikleri.

Feed No	301	302	303	304	305	306	307	308
Weeks	0-2	2-4	4-6	7-9	10-12	13-14	15-16	17+
Crude protein (%)	28.5	27.5	26	23.5	21.5	19.5	18	17
ME (kcal kg^{-1})	2750	2850	2950	3050	3125	3225	3350	3400
Methionine	0.74	0.69	0.63	0.56	0.5	0.44	0.4	0.37
Meth. + Syst.	1.21	1.17	1.07	1.0	0.9	0.8	0.72	0.68
Lysine	1.85	1.8	1.66	1.55	1.4	1.2	1.02	0.9
Calcium	1.45	1.4	1.4	1.3	1.2	1.1	1.0	1.0
Digestible Phosphorus	0.78	0.75	0.75	0.65	0.6	0.55	0.5	0.5
Sodium	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.18
Threonine	1.11	1.1	1.04	1.0	0.93	0.76	0.64	0.58
Tryptophan	0.34	0.3	0.27	0.25	0.23	0.2	0.18	0.17
Arginine	1.98	1.94	1.79	1.63	1.44	1.24	1.05	0.93
Structure of the feed	Crumble	Pellet	Pellet	Pellet	Pellet	Pellet	Pellet	Pellet

The data achieved from blood sampling and analyses were computed with Minitab 14 statistical analysis software program using variance analysis and Duncan Test (Düzgüneş *et al.*, 1987; Sheskin 2000; Minitab 2014).

The non-homogeneous data of blood parameters were transformed using square root transformation Y = √P equality. All blood samples were analyzed separately by a linear model as shown below.

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Y_{ij} : ith stocking density group, jth week observed value
μ : population mean for the blood parameter
α_i : ith stocking density group effect
e_{ij} : random error

RESULTS AND DISCUSSION

The findings of the research suggests that blood glucose levels of female turkeys are not affected by the SD at 6 weeks of age where male turkeys are at the same age as seen on Tables 4 and 5 respectively. As another outcome, blood glucose levels of female turkeys have changed statistically at the age of slaughter where males were not affected at the slaughter age. This consequence may have come forth because males were slaughtered at the age of 20 weeks where females were at 16 weeks. So, by the passing 4 weeks males may have overcome stress and may not have showed any indicator at the slaughter age after 16 weeks.

The findings of the research are in harmony of the results of the research made by Khadjeh *et al.* (2004) on local turkeys. They found BC (Blood Glucose) levels of 342.3 ± 152.6 g 100 ml^{-1}. Assuming the standard deviation these findings are numerically similar as seen.

Table 4. Blood glucose levels of female turkeys reared under different SDs.
Çizelge 4. Farklı yerleşim sıklıklarında yetiştirilen dişi hindilerde kan şeker düzeyleri.

SD, kg m^{-2}	Blood glucose Levels, g 100 ml^{-1} ($\overline{X}$±S$\overline{X}$)		
Week	2 (start)	6	16
60	274.79±4.44	290.18±6.04	294.18±2.48^{a}
50	276.18±4.36	293.87±3.38	174.31±5.32^{b}
40	271.72±1.66	296.06±2.63	282.34±12.73^{a}
30	266.40±4.67	297.70±3.41	195.54±8.03^{b}
S$\overline{X}$	2.00	2.01	9.24
F	1.19	0.63	56.05
P	>0.05	>0.05	<0.01

The statistically different data is shown as small uppercase characters and P values are as given. (SD: Stocking Density).

As another outcome of the experiment, Lymphocyte counts (LC) were found to be changing during the lifespan of the birds. At slaughter age, LC was found to be higher in both male and female birds reared at a SD of 30 and 40 kg m^{-2}. Also at 6 weeks where LC of females were not significantly different, LC of males were found to be significantly different as birds reared at lower SD levels had higher LC levels. Decreasing LC shows the stress on the birds reared on higher SD. However there has been no other parameter backing up the statistically important differences in LC, itself can be told to be an indicator of stress as the increasing H/L ratio which may occur

by decreasing LC by itself Gross and Siegel (1983). LC values can be seen on Table 6.

Table 5. Blood glucose levels of male turkeys reared under different SDs.
Çizelge 5. Farklı yerleşim sıklıklarında yetiştirilen erkek hindilerde kan şeker düzeyleri.

SD, kg m^{-2}	Blood glucose levels, g 100 ml^{-1} ($\overline{X}$±S$\overline{X}$)		
Week	2 (start)	6	20
60	272.69±1.59	291.43±5.79^{ab}	268.17±7.89
50	272.03±2.81	304.71±6.69^{a}	281.49±14.64
40	278.64±4.12	286.19±5.31^{b}	251.43±13.94
30	278.94±3.90	289.85±4.28^{ab}	257.17±3.78
S$\overline{X}$	1.63	2.91	5.6
F	1.34	2.09	1.45
P	>0.05	<0.05	>0.05

The statistically different data is shown as small uppercase characters and P values are as given. (SD: Stocking Density).

As it is specified by some researchers (Maxwell *et al.,* 1990, Martrenchar *et al.*, 1997) H/L ratio is a stress indicator for poultry. The increase in H/L ratio is known to be the indication of stress and the inclination of this parameter numerically is told to be because of the drop in lymphocyte counts where stress is imminent. As it can be seen on the Tables 6 and 7 the stress on turkeys are increasing especially on females and there is a permanent stress on birds during the whole rearing period where the SD effect is actual.

CONCLUSION

Stress is one of the main points of animal welfare. In Turkey birds are reared under stocking densities of

Table 6. Lymphocyte count of female turkeys reared under different SDs.
Çizelge 6. Farklı yerleşim sıklıklarında yetiştirilen dişi hindilerde lenfosit sayıları

SD, kg m^{-2}	Lymphocyte levels, g 100ml^{-1} ($\overline{X}$±S$\overline{X}$)		
Week	2 (start)	6	16
60	81.44±3.96^{a}	88.18±2.36	72.48±0.74^{b}
50	78.53±4.22 ^{a}	100.00±0.01	73.17±2.57 ^{b}
40	73.26±3.76^{ab}	92.9±2.68	88.37±2.10 ^{a}
30	64.24±3.40^{ab}	90.65±8.45	96.38±5.09 ^{a}
S$\overline{X}$	2.12	2.31	2.19
F	4	1.35	15.12
P	<0.05	>0.05	<0.01

The statistically different data is shown as small uppercase characters and P values are as given. (SD: Stocking Density).

Table 7. Lymphocyte count of male turkeys reared under different SDs.
Çizelge 7. Farklı yerleşim sıklıklarında yetiştirilen erkek hindilerde lenfosit sayıları.

SD, kg m^{-2}	Lymphocyte levels, g 100 ml^{-1} ($\overline{X}$±S$\overline{X}$)		
Week	2 (start)	6	20
60	79.01±4.31	57.04±5.27^{d}	62.12±0.37^{ab}
50	74.98±7.12	80.47±0.90^{c}	57.73±3.89^{b}
40	70.04±5.37	96.93±1.78^{b}	66.11±0.84^{a}
30	65.95±3.72	108.80±1.59^{a}	65.15±2.85^{a}
S$\overline{X}$	2.66	3.41	1.29
F	1.2	46.61	2.59
P	>0.05	<0.01	<0.05

The statistically different data is shown as small uppercase characters and P values are as given. (SD: Stocking Density).

40–50 kg m^{-2}. The experiment was designed to evaluate the current situation in the country on the term of stress and to find out if there is a change in stress on animals by SD if the SD is increased or decreased.

As it is told by many researchers, the main stress parameters are BC, LC and H/L ratios where mainly BC and LC specify the stress (Maxwell *et al.,* 1990; Martrenchar *et al.*, 1997).

In the experiment LC counts were found to be decreasing in parallel with the increasing SD levels. The stress on animals can be seen during the whole rearing period where LCs were found to be higher in lower SDs and less at higher SDs of 50 and 60 kg m^{-2} as it can be seen on Tables 6 and 7.

Also, BC was found to be higher in various periods of rearing period on higher SDs. This is also a sign of stress on birds just as LC counts demonstrate.

As a summary it can be said that the stress on turkey broilers tends to increase with the increase in SDs. The break point of incipience of stress seems to be between 40 – 50 kg m^{-2} SDs and there should be further and more detailed researches should be undertaken to determine the point of commencing stress as a SD level. So this level should be used as a barrier for animal welfare as a limiting parameter.

From the outcome of the research it can surely be told that in terms of animal welfare and stress the amount of stress on turkey broilers increase at higher SDs. However, there has been no significant change on performance parameters during the research of turkeys reared under different SDs, stress have been ongoing for the whole rearing period which should be taken into consideration as an animal welfare aspect.

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