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YAZIM KURALLARI

1 Makale İngilizce veya Türkçe olarak yazılabilir.
2 Tüm makalede, her sayfada bireysel satır numarası, normal kenar boşluğu ve çift aralık kullanılmalıdır. Bu kurallar Özet, Dipnot ve Kaynaklar bölümlerinde de uygulanmalıdır. Makalenin Kaynaklar ve Tablolar dahil her sayfası numaralandırılmalıdır.
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6 SI birimleri ve standart kısaltmalar kullanılmalıdır.

Başlık Sayfası

Başlık; Yazar(lar)ın ad(lar)ı; İşyeri açık posta adres (ler)i; Sorumlu yazarın telefon numarası, faks numarası ve e-posta adresi.

Ana Metin

Özet:Özet 300 kelimeyi aşmamalıdır ve kaynak kullanımından kaçınılmalıdır. Türkçe sunulan makalelerde ayrıca İngilizce Özet (Abstract) bölümü hazırlanmalıdır.

Giriş:Bu bölümde sonuçların anlaşılabilmesi ve yorumlanabilmesi için o konu ile ilgili (daha önceden) yapılmış olan çalışmalar hakkında bilgilere yer verilmelidir. Giriş'te çalışmanın hipotezi belirtilmelidir. Çalışmanın amacı bu bölümün en sonunda açık olarak yazılmalıdır. Sadece gerekli olan kaynaklara atıf yapılmalıdır.

Materyal ve Metot: Bu bölümde deneysel çalışmalar diğer araştırmacılar tarafından tekrarlanabilecek yeterlilikteki detayı ile verilmelidir. İstatistik çalışmalar ayrı bir alt başlık şeklinde sunulabilir. Uluslararası indekslerde yer alan dergilerde yayınlanmış bir makalede açıklanan bir teknik yeniden kullanıldığında, metodun çok kısa açıklanması ve ilgili orijinal makaleye atıf yapılması yeterlidir. Hayvan deneylerini içeren çalışmalarda yerel etik kurul kararlarına uyulmalıdır.

Sonuçlar:Araştırma bulguları açık ve anlaşılabilir şekilde, Materyal ve Metot bölümünde verilen sıra ile sunulmalı, tartışma veya yorumlara yer verilmemelidir. Bulgular tablo ve şekillerle desteklenerek kısa olarak sunulmalıdır.

Tartışmave Sonuç: Bulgular gereksiz ayrıntıya girmeden tartışılmalı, bulguların önemi ve literatür içindeki yeri vurgulanmalıdır.

Teşekkür:Parasal destek, çalışmalara veya makaleye kişisel katkılar burada belirtilmelidir.

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1 Makalede atıf yapılan tüm kaynaklar makale sonunda bir Kaynaklar Listesi halinde verilmelidir. Kaynak listesinde yer alan tüm kaynaklara da makale içinde atıf yapılmış olmasına dikkat edilmelidir.
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Kitap:Adkinson N, Yunginger J, Busse W, Bochner B, Holgate S, Middleton E, eds. Middleton's Allergy: Principles and Practice. 6thed. St Louis, MO: Mosby; 2003.

Kitap Bölümü:Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. Allergens and Allergen Immunotherapy. 3rded. New York, NY: Marcel Dekker; 2004:585-606.

Yayına kabul edilmiş ama basılmamış çalışmalar referanslarda "(basımda)" olarak belirtilmelidir. Carrau RL, Khidr A, Crawley JA, Hillson EM, Davis JK, Pashos CL. The impact of laryngopharyngeal reflux on patient-reported quality of life. Laryngoscope. In press.

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Abstract:Manuscripts of original research papers should include an Abstract of 300 or fewer words. References should not be cited in this section.

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Materials and Methods:This section should describe the experimental procedures in sufficient detail to allow other scientists to repeat the experiments. Statistical methods can be given in a separate subtitle. Where techniques that have already been described in an indexed journal are used, this section should be concise and provide relevant references, only.

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For book chapters:Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. Allergens and Allergen Immunotherapy. 3rded. New York, NY: Marcel Dekker; 2004:585-606.
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Articles available online but without volume and page numbers may be referred to by means of their Digital Object identifier (DOI) code.Smeeth L, Iliffe S. Community screening for visual impairment in the elderly. Cochrane Database Syst Rev. 2002;(2):CD001054. doi:10.1002/14651858.CD1001054.
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REVIEW ARTICLE

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RESEARCH ARTICLES

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Effect of Central and Peripheral Injected Nesfatin-1 on Electrocardiography in Rats

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Abstract

Nesfatin-1 is an anorexic nucleobindin-2 -derived peptide and it has directly and centrally effect on the heart. The current study was designed to determine the effect of centrally and peripherally administered nesfatin-1 on electrocardiography (ECG) of healthy both fasted rats for 12 h and satiated rats fed ad libitum. In order to record ECG, the electrodes were placed limbs of at lead II under ketamine (50 mg/kg; im) and xylazine (20 mg/kg; im) anesthesia mix.

Centrally administered different doses of nesfatin-1 (100 and 200 pmol; icv) resulted in dose- and time-dependently a statistically significant increase ($p < 0.05$) in T wave, Q-T interval, and R-R interval duration without changing in ECG waves' amplitude in both satiated and fasted rats. In a similar way, peripheral administration of nesfatin-1 (80 µg/kg; iv) in satiated rats prolonged statistically significant ($p < 0.05$) T wave, Q-T interval, and R-R interval without producing a change in ECG waves' amplitude. Moreover, icv administered nesfatin-1 in fasted and satiated rats, and iv injected nesfatin-1 in satiated rats induced a statistically significant decrease in heart rate ($p < 0.05$).

In conclusion, our findings suggest that centrally and peripherally administrated nesfatin-1 caused a delay in T wave, Q-T interval and two R-waves interval duration in ECG so that leading to a bradycardic effect in heart rate.

Keywords: Nesfatin-1, Electrocardiography, Intracerebroventricular, Intravenous, Heart rate.

Introduction

Nesfatin-1 is an 82 amino acid anorexigenic endogenous peptide produced by the proteolytic processing of nucleobindin-2 (NUCB-2).[1] Nesfatin-1 and its precursor molecule NUCB-2 show widespread distribution throughout the central nervous system[2,3] and the peripheral tissues such as gastric mucosa[4] and heart ventricles.[5] The central and peripheral nesfatin-1 expression is modulated by starvation and refeeding.[1,4] Experimental evidence supports the involvement of nesfatin-1 in the modulation of feeding, neuroendocrine functions, stress, metabolic responses and cardiovascular control.[1,6-9]

Indeed it was reported that central administration of nesfatin-1 increases blood pressure and renal sympathetic nerve activity following intracerebroventricular (icv) administration in conscious and urethane-anesthetized rats.[10,11] Moreover, nesfatin-1 modulates the excitability of nucleus tractus solitarius (NTS) neurons and produces hypertensive and tachycardic responses upon microinjection into the NTS.[12] When nesfatin-1 is injected intravenous (iv), the peptide also increases peripheral arterial resistance through a direct action arterioles.[13] Nesfatin-1 expression in the heart has been correlated with negative inotropism and protection against ischemia-reperfusion injury.[5] Recently it was reported that central administration of nesfatin-1 exerts pressor and bradycardic effects in normotensive animals[8,9] and produces pressor and tachy-

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cardic effects under hypotensive conditions produced by severe hemorrhage.[8] It was also shown that icv administered nesfatin-1 increased the plasma catecholamines, vasopressin, and renin concentrations and those hormones contributed to the pressor effects of the peptide in both normotensive and hypotensive conditions.[8] Previously it was reported that nesfatin-1 expression in the heart has been correlated with negative inotropism and protection against ischemia-reperfusion injury.[4] Evidence on the effects of cardiovascular parameters following central administration of nesfatin-1 raises the possibility that it may modulate arterial pressure and heart function. However, there is no any report showing the effect of nesfatin-1 on electrical activity of the heart when it was administrated via icv or iv. Moreover, it was not known how central or peripheral injected nesfatin-1 was able to affect electrocardiography (ECG) waves in fasting or satiety condition.

Considering the above data, the primary aim of the current study was to show the effect of centrally or peripherally administrated nesfatin-1 on ECG in fasted for 12h and in satiated fed ad libitum anesthetized rats.

Materials and Methods

Animals

The experiments were performed on 56 adults, male Sprague–Dawley rats (3 months old, 280–340 g). The rats were provided from Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey. The animals were accommodated as 4-5 rats in a cage and maintained on a 12 h light/dark cycle at 20–22 °C with 60–70% humidity. All experimental protocol was approved by The Animal Care and Use Committee of Uludag University (2018 – 01/07). Each group had 7 rats and each rat was used in a single experimental protocol.

Surgical procedures

The rats anesthetized with ketamine (50 mg/kg) and xylazine (20 mg/kg) mixture. Under anesthesia, rats were placed in a stereotaxic frame to insert the icv guide cannula. For this reason, a burr hole was drilled through the skull 1.5 mm lateral to midline and 1.0 mm posterior to bregma, which those coordinates were used from the atlas of Paxinos and Watson.[14] Then, the guide cannula, which was made 22-gauge stainless steel hypodermic tubing, was directed through the hole toward the lateral ventricle and lowered 4.5 mm below the surface of the skull and fixed to the skull with acrylic cement.

Experimental protocol

In the present study, first, the dose and time relation of ECG responses to icv injected nesfatin-1 were studied in fasted for 12 h or/and satiated fed ad libitum rats. Following baseline ECG measurements, nesfatin-1 (100 and 200 pmol) or saline (5 μL) was delivered i.c.v. and changes of ECG were recorded for the next 60 min. Hence to determine the effect of centrally injected nesfatin-1 on ECG, total 6 groups were constituted as so 3 groups from fasted for 12 h and as so 3 groups from satiated fed ad libitum rats: 1, control (saline 5 μL; icv); 2, nesfatin-1 (100 pmol; icv); and 3, nesfatin-1 (200 pmol; icv).

Secondly, to determine the effect of peripheral injected nesfatin-1 on ECG of satiated fed ad libitum rats, saline (1 mL/kg) or 80 μg/kg dose of nesfatin-1 were iv injected via tail vein. Before and after injections, changes of ECG were recorded for the next 60 min. So total 2 groups were formed from satiated fed ad libitum rats as 1, control (saline 1 mL/kg; iv); and 2, nesfatin-1 (80 μg/kg; iv) in terms of the effect of peripherally injected nesfatin-1 on ECG.

ECG recording

In order to record ECG, the electrodes (SS2L, BIOPAC Systems Inc. California, USA) were inserted limbs of at lead II. Findings were recorded and analyzed by using the MP36 system and AcqKnowledge software (BIOPAC Systems Inc.). The ECG was used to determine the P waves, P-R intervals, QRS complexes Q-T intervals, T waves, R-R intervals durations. Heart rate was calculated from R-R intervals according to the following formula: heart rate=60/(R-R intervals in seconds) and was expressed beats per minute (bpm).

Drug and icv injections

Nesfatin-1 (Sigma-Aldrich Co., Deisenhofen, Germany) solutions were prepared in saline on the day of the experiment. The dose of nesfatin-1 was chosen from the previous studies.[8,9]

Icv injections were made by using hand-made injection cannula (28 gauge stainless steel tubing). Injection cannula was connected to polyethylene tubing, which was filled with saline or saline solution of the studied agent in a 10 μL microsyringe. For the icv injection, 5 μL volume of the solution was infused within 60 s. During the injection, an air bubble moving in the polyethylene tubing was closely watched to ensure the drug was delivered in its entirety.

Data and statistical analysis

All values are given as mean ± standard error of the mean (SEM) with $p<0.05$ considered as the level of significance. Statistical evaluation was performed by analysis of variance (RM-ANOVA; two-way) and the post-ANOVA test of Bonferroni by using Sigma Stat 3.5 software (CA, USA).

Results

Effects of central or peripheral injected nesfatin-1 on ECG

There was no difference in basal value of the P waves (Table 1), P-R intervals (Table 2), QRS complexes (Table 3), Q-T intervals (Table 4), T waves (Table 5), R-R intervals (Table 6) durations and heart rate of both fasted (Fig 1A) and satiated (Fig 1B) rats. Icv injected nesfatin-1 led to dose- and time-dependently statistically significant increase ($p < 0.05$) in Q-T interval (Table 4), T wave (Table 5) and R-R interval (Table 6) duration without changing in P wave (Table 1), P-R interval (Table 2) and QRS complex (Table 3) duration in both fasted and satiated rats. Also centrally injected nesfatin-1 induced dose- and time-dependently statistically significant ($p < 0.05$) decrease in heart rate of both fasted (Fig 1A) and satiated (Fig 1B) rats.

Table 1. The effect of central and peripheral injected nesfatin-1 on P wave duration.

P wave duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.022±0.001	0.022±0.002	0.022±0.001	0.023±0.001	0.023±0.003	0.023±0.002	0.022±0.002	0.023±0.001
1	0.022±0.003	0.022±0.003	0.023±0.002	0.023±0.002	0.022±0.003	0.023±0.002	0.022±0.002	0.023±0.002
3	0.022±0.001	0.022±0.001	0.023±0.003	0.023±0.002	0.021±0.002	0.022±0.001	0.022±0.001	0.023±0.002
5	0.023±0.002	0.022±0.002	0.022±0.003	0.024±0.002	0.023±0.003	0.020±0.002	0.022±0.003	0.024±0.001
10	0.023±0.001	0.022±0.004	0.021±0.002	0.023±0.001	0.022±0.003	0.021±0.001	0.023±0.003	0.024±0.003
20	0.024±0,004	0.021±0.002	0.023±0.002	0.023±0.003	0.022±0.004	0.022±0.002	0.023±0.002	0.024±0.002
30	0.023±0.003	0.022±0.001	0.023±0.002	0.023±0.004	0.023±0.001	0.022±0.001	0.023±0.002	0.025±0.003
60	0.024±0.001	0.022±0.002	0.024±0.002	0.023±0.002	0.024±0.002	0.023±0.003	0.023±0.002	0.023±0.001

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven P wave duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test.

Table 2. The effect of central and peripheral injected nesfatin-1 on P-R interval duration.

P-R interval duration (second)								
Ti me	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.051±0.002	0.052±0.002	0.052±0.001	0.052±0.001	0.051±0.003	0.051±0.007	0.053±0.003	0.051±0.005
1	0.051±0.003	0.053±0.004	0.052±0.003	0.051±0.003	0.052±0.003	0.052±0.007	0.055±0.002	0.051±0.006
3	0.051±0.006	0.053±0.004	0.054±0.003	0.053±0.003	0.052±0.004	0.053±0.004	0.052±0.004	0.050±0.005
5	0.053±0.003	0.054±0.003	0.052±0.003	0.053±0.001	0.053±0.002	0.054±0.005	0.050±0.006	0.052±0.003
10	0.054±0.002	0.054±0.007	0.055±0.002	0.052±0.003	0.051±0.003	0.053±0.006	0.051±0.008	0.052±0.005
20	0.053±0.004	0.055±0.005	0.054±0.003	0.052±0.002	0.053±0.004	0.053±0.007	0.052±0.006	0.053±0.004
30	0.053±0.006	0.054±0.005	0.055±0.002	0.053±0.001	0.051±0.006	0.052±0.006	0.052±0.005	0.053±0.005
60	0.054±0.002	0.056±0.006	0.056±0.002	0.050±0.002	0.052±0.004	0.051±0.008	0.053±0.003	0.050±0.001

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven P-R interval duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test.

Table 3. The effect of central and peripheral injected nesfatin-1 on QRS complex duration.

QRS complex duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.026±0.002	0.026±0.001	0.026±0.001	0.025±0.001	0.025±0.001	0.025±0.002	0.023±0.001	0.023±0.002
1	0.026±0.002	0.026±0.001	0.026±0.002	0.024±0.001	0.025±0.001	0.025±0.001	0.023±0.002	0.023±0.001
3	0.025±0.001	0.025±0.002	0.027±0.003	0.024±0.001	0.026±0.001	0.025±0.001	0.024±0.001	0.025±0.002
5	0.025±0.001	0.026±0.002	0.027±0.003	0.022±0.001	0.026±0.002	0.026±0.003	0.025±0.001	0.025±0.003
10	0.025±0.001	0.026±0.001	0.026±0.003	0.024±0.002	0.026±0.001	0.027±0.002	0.023±0.001	0.025±0.002
20	0.025±0.001	0.026±0.002	0.026±0.003	0.023±0.001	0.025±0.001	0.025±0.001	0.023±0.001	0.025±0.002
30	0.025±0.003	0.026±0.002	0.027±0.003	0.023±0.001	0.026±0.002	0.025±0.002	0.023±0.002	0.024±0.001
60	0.026±0.002	0.027±0.001	0.026±0.001	0.025±0.002	0.025±0.003	0.0245±0.002	0.023±0.002	0.024±0.001

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven QRS complex duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test.

Table 4. The effect of central and peripheral injected nesfatin-1 on Q-T interval duration.

Q-T interval duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.066±0.002	0.066±0.002	0.065±0.003	0.067±0.004	0.067±0.002	0.067±0.001	0.065±0.002	0.065±0.001
1	0.065±0.002	0.064±0.003	0.065±0.002	0.066±0.002	0.066±0.004	0.067±0.001	0.064±0.005	0.064±0.004
3	0.065±0.004	0.065±0.002	0066±0.004	0.067±0.002	0.067±0.005	0.067±0.002	0.063±0.005	0.065±0.005
5	0.066±0.001	0.066±0.001	0.069±0.001*	0.066±0.001	0.069±0.001*	0.069±0.001*	0.064±0.001	0.067±0.001*
10	0.064±0.001	0.067±0.001*	0.071±0.001*	0.065±0.001	0.069±0.001*	0.070±0.001*	0.065±0.001	0.069±0.001*
20	0.065±0.001	0.070±0.001*	0.072±0.001*	0.066±0.001	0.070±0.001*	0.072±0.001*	0.066±0.001	0.070±0.001*
30	0.064±0.001	0.069±0.001*	0.070±0.001*	0.065±0.001	0.065±0.001	0.070±0.001*	0.064±0.001	0.068±0.001*
60	0.062±0.001	0.070±0.001*	0.069±0.001*	0.065±0.003	0.065±0.004	0.063±0.003	0.064±0.001	0.065±0.003

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven Q-T interval duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *$p<0.05$, significantly different from the value of the saline-treated group.

Iv injected nesfatin-1 had a similar effect on the ECG parameters with icv injected nesfatin-1. Iv injected nesfatin-1 also prolonged Q-T interval (Table 4), T wave (Table 5) and R-R interval (Table 6) duration but no effect on P wave (Table 1), P-R interval (Table 2) and QRS complex (Table 3) duration in satiated rats. Moreover, iv injected nesfatin-1 caused the bradycardia in satiated rats (Fig 1C).

Both central and peripheral injection of nesfatin-1 did not cause any change in amplitude and shape of the ECG waves and intervals (Fig 2).

Table 5. The effect of central and peripheral injected nesfatin-1 on T wave duration.

T wave duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.048±0.002	0.048±0.002	0.048±0.003	0.049±0.003	0.048±0.002	0.049±0.002	0.048±0.002	0.048 ±0.003
1	0.048±0.002	0.047±0.002	0.048±0.003	0.048±0.001	0.048±0.003	0.048±0.002	0.047±0.003	0.048±0.003
3	0.049±0.003	0.047±0.002	0.048±0.004	0.046±0.002	0.047±0.002	0.047±0.001	0.047±0.003	0.048±0.004
5	0.047±0.002	0.049±0.002	0.047±0.003	0.047±0.001	0.048±0.003	0.049±0.001	0.047±0.005	0.049±0.005
10	0.046±0.001	0.049±0.001*	0.049±0.001*	0.046±0.001	0.049±0.001*	0.051±0.001*	0.046±0.001	0.049±0.001*
20	0.047±0.001	0.051±0.001*	0.049±0.001*	0.046±0.001	0.050±0.001*	0.051±0.001*	0.046±0.001	0.050±0.001*
30	0.047±0.001	0.051±0.001*	0.049±0.001*	0.046±0.001	0.049±0.001*	0.050±0.001*	0.047±0.003	0.049±0.003
60	0.048±0.004	0.048±0.004	0.049±0.003	0.047±0.004	0.048±0.003	0.049±0.001	0.048±0.001	0.049±0.004

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven T wave duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *$p<0.05$, significantly different from the value of the saline-treated group.

Table 6. The effect of central and peripheral injected nesfatin-1 on R-R interval duration.

R-R interval duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.264±0.02	0.262±0.03	0.269±0.02	0.259±0.01	0.253±0.05	0.258 ±0.05	0.251±0.04	0.250±0.01
1	0.269±0.01	0.274±0.02*	0.271±0.02	0.255±0.01	0.261±0.02*	0.263±0.02*	0.260±0.03	0.259±0.02
3	0.254±0.01	0.274±0.01*	0.277±0.02*	0.252±0.02	0.265±0.04*	0.271±0.05*	0.263±0.02	0.265±0.01
5	0.242±0.01	0.271±0.02*	0.278±0.03*	0.240±0.05	0.267±0.04*	0.273±0.06*	0.260±0.01	0.265±0.01*
10	0.241±0.03	0.271±0.06*	0.278±0.04*	0.231±0.05	0.269±0.04*	0.276±0.05*	0.260±0.01	0.269±0.03*
20	0.243±0.02	0.269±0.06*	0.276±0.03*	0.242±0.05	0.269±0.09*	0.279±0.04*	0.261±0.01	0.269±0.03*
30	0.245±0.01	0.253±0.02*	0.271±0.04*	0.235±0.04	0.256±0.03*	0.281±0.06*	0.268±0.01	0.271±0.03
60	0.247±0.01	0.248±0.03	0.263±0.05*	0.239±0.04	0.247±0.04*	0.286±0.05*	0.268±0.01	0.265±0.03

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven R-R interval duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *$p<0.05$, significantly different from the value of the saline-treated group.

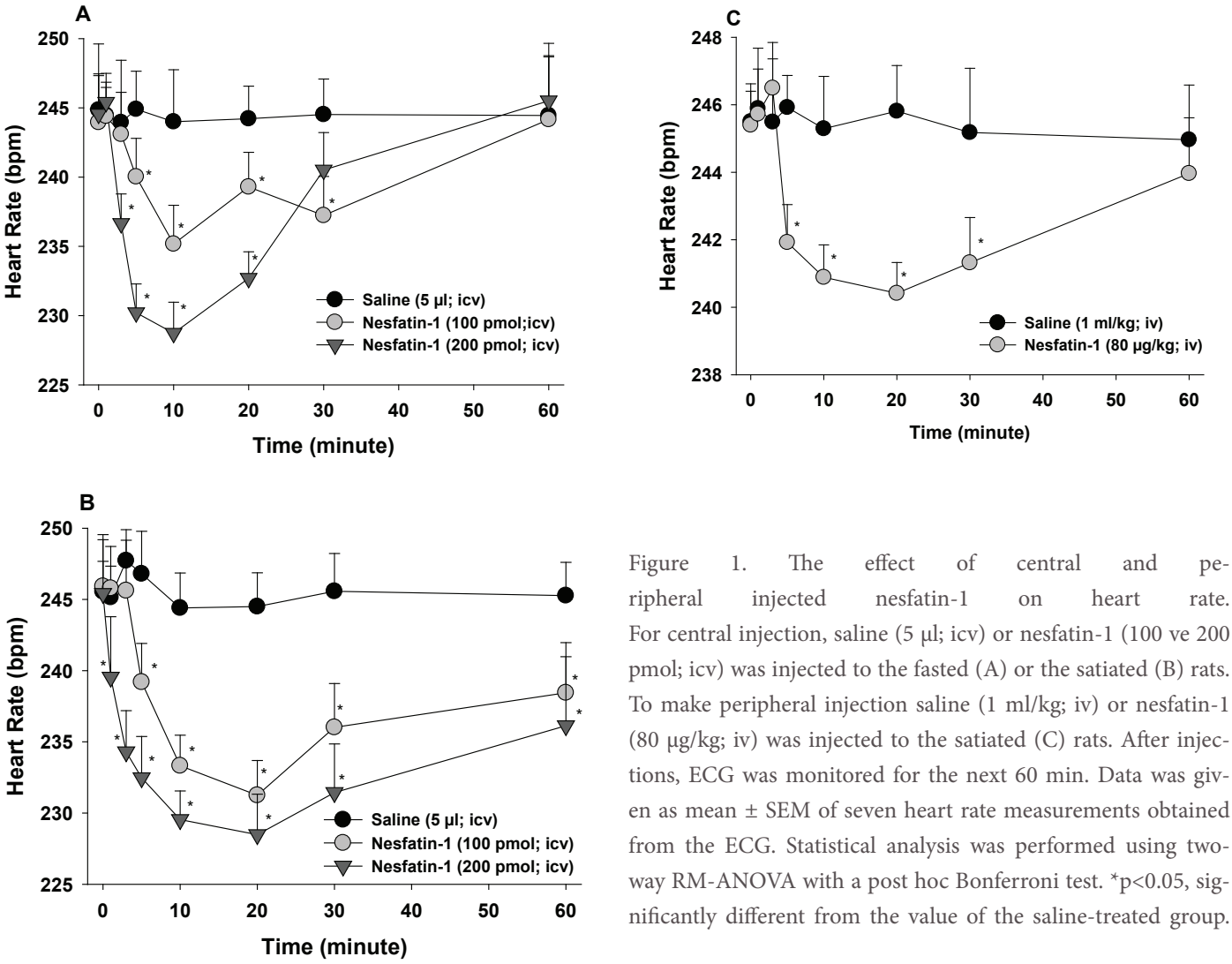

Figure 1. The effect of central and peripheral injected nesfatin-1 on heart rate. For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted (A) or the satiated (B) rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated (C) rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven heart rate measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *p<0.05, significantly different from the value of the saline-treated group.

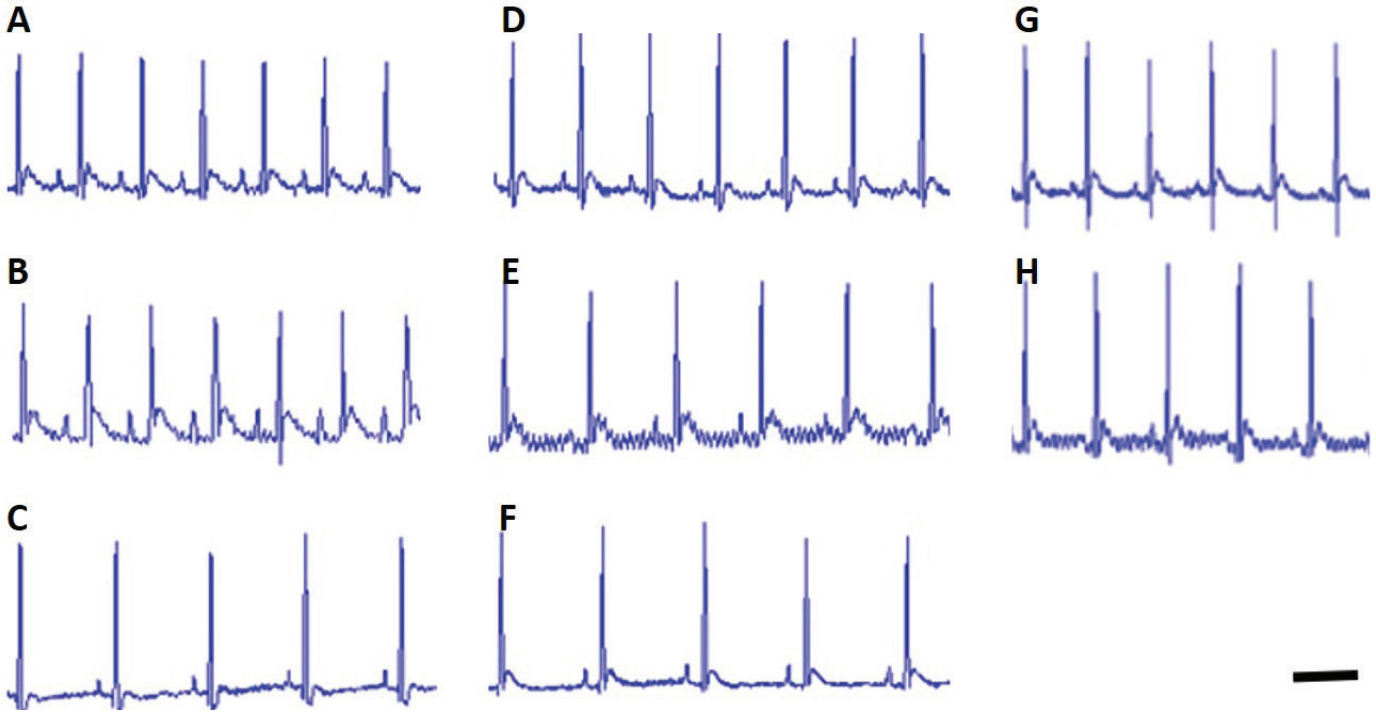

Figure 2. The effects of central and peripheral injected nesfatin-1 on ECG waves and intervals. The fasted rats were treated with saline (5 µl; icv) (A), nesfatin-1 (100 pmol; icv) (B) or nesfatin-1 (200 pmol; icv) (C); the satiated rats were treated with saline (5 µl; icv) (D), nesfatin-1 (100 pmol; icv) (E) or nesfatin-1 (200 pmol; icv) (F); the satiated rats were treated with saline (1 ml/kg; iv) (G) or nesfatin-1 (80 µg/kg; iv) (H). The bar shows 0.3 second.

Discussion and Conclusion

These data demonstrate that icv administered nesfatin-1 dose- and time-dependently increases T wave, Q-T and R-R intervals durations but not any changes in P wave, P-R intervals, and QRS complex durations in both fasted and satiated rats. Furthermore, central injection of nesfatin-1 causes dose- and time-dependently bradycardic responses in fasted and satiated rats. When nesfatin-1 injected iv, it produces a similar effect with its central administration and causes the delay in T wave, Q-T and R-R intervals along with bradycardia. Central and peripheral injection of nesfatin-1 does not alter the ECG waves' amplitude of the rats.

ECG in rats is a widely applied experimental method in basic cardiovascular research. It has become one of the most widespread diagnostic tools in clinical medicine. ECG recording reflects the electrical activity of the heart and may provide important insights into the functional and structural characteristics of the myocardium. The physiological and pathological criteria of ECG recordings have been thoroughly described in multiple handbooks and research papers.[15,16] An action potential in the heart is generated in the sinoatrial node and subsequently conducted through the atrioventricular node, His bundle, His bundle branches, and Purkinje fibers, finally reaching ventricular cardiomyocytes. A typical ECG tracing mirrors the repeating cycle of three major electrical events, including atrial depolarization (P wave), ventricular depolarization (QRS complex) and ventricular repolarization (T wave). In the current study, central or peripheral injection of the nesfatin-1 prolonged the T wave and Q-T intervals without changing other waves and intervals durations. It could be considered that nesfatin-1 has a direct effect on heart tissue. It was reported that heart, particularly ventricles, had the presence of both nesfatin-1 protein and NUCB-2 mRNA in rat cardiac extracts according to western blotting and QT-PCR analyses.[5] On isolated and Langendorff-perfused rat heart preparations, it was found that exogenous nesfatin-1 depressed contractility and relaxation without affecting coronary motility by inducing dose-dependent negative inotropism.[5] Our results are consistent with this report. Because the iv injected nesfatin-1 had to have affected the ventricles of the heart so that there was a delay in T wave and Q-T interval. Also, it could be considered that nesfatin-1 has indirectly effect heart tissue via sympathetic or parasympathetic neurons. Principally, parasympathetic stimulation causes a marked decrease in heart rate (negative chronotropic effect) and a slight decrease in heart muscle contractility (negative inotropic effect). Activation of sympathetic[10,11] and parasympathetic cardiac tones[17] with the central injection of nesfatin-1 has been observed. It appears that injected nesfatin-1 has a potency of a parasympathetic effect on the heart. Recently it was reported that the central cholinergic system modulated the centrally injected nesfatin-1 induced cardiovascular responses.[9] Because icv injected nesfatin-1 increased the level of the hypothalamic acetylcholine and choline, and also central muscarinic and nicotinic receptors mediated pressor and bradycardic responses to centrally injected nesfatin-1.[9] Activation of the central cholinergic parasympathetic pathway with the icv injection of nesfatin-1 might lead to a decrease in T wave and Q-T interval in ECG.

Heart rate represents the number of heart contractions in 1 min. There is a correlation between R-R interval duration and heart rate. In the present study, both central and peripheral injection of the nesaftin-1 caused the bradycardia. Central administration of nesfatin-1-evoked bradycardic effect in normotensive animals was demonstrated.[8,9] It was also reported that nesfatin-1 decreases heart rate when injected intracerebroventricularly.[10,11,18,19] Moreover, microinjection of nesfatin-1 into the NAmb generated a decrease in heart rate in conscious rats.[17] Those reports and our current data clearly explain that nesfatin-1 might have a bradycardic effect.

The first report determined the anorexigenic effects of nesfatin-1 and its precursor NUCB-2.[1] In this first report, it was shown that NUCB-2 mRNA expression in the hypothalamus was significantly down-regulated after 24-h fasting in rats.[1] On the other hand refeeding after a 48-h fast resulted in an increase of activated nesfatin-1 immunoreactive neurons in the hypothalamus.[20] It was also reported that gastric mucosa abundantly expressed NUCB-2 mRNA.[4] Again NUCB-2 mRNA expression in gastric mucosal tissue was significantly down-regulated after 24-h fasting in rats. Those reports evidently indicate that the fasting suppresses the production of nesfatin-1 in central and peripheral. Considering the change in the amount of nesfatin-1 in both periphery and central according to the condition of fasting and satiety, we performed nesfatin-1 application to both fasted and satiated animals, but we did not obtain any significant differences between the two conditions.

In summary, the present results suggest that icv or iv administration of nesfatin-1 causes an increase in T wave, Q-T and R-R intervals durations in ECG without affecting fasting or satiety conditions. Moreover, central or peripheral injected nesfatin-1 produces the bradycardic response by directly affecting the heart or indirectly activating the central nervous system.

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Genotip ve Zemin Tipinin Etlik Piliç Büyüme Performansı ve Ekonomik Verimlilik Üzerine Etkisi*

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Abstract

The aim of this study was to evaluate the effects of genotype and floor type on broiler growth performance and production economics. In the study, slow-growing (Hubbard JA57) and fast-growing (Ross 308) broilers with slatted floor and deep litter were used, so there were four main groups (2x2), and each main group consisted of 5 replicates. Each replicate consisted of 10 male chicks, so 200 birds were used in total. The experiment lasted for 8 weeks. Live weight gain, feed consumption, and dead birds were recorded throughout the experiment. Variable costs of each group and its percentages within total costs were calculated, gross profit and profitability were calculated to analyze economic performance. As expected, the growth performance of fast-growing broiler was found to be better than slower-growings, and the effect of floor type on growth performance was found to be insignificant. In general, slow-growing genotypes were found to be better in terms of economic benefit. As a result of this study, even though fast-growing broilers had a better production performance, slower-growing broilers had a better eco-nomic performance at the end of the experiment. However, considering that the slaughter ages of slow- and fast-growing birds are different in commercial conditions, it would be more beneficial to make an economic comparison with the total production in the same unit in one year. Key words: Broiler, genotype, floor, growth performance

Özet

Bu çalışma, piliç eti üretiminde genotip ve zemin tipinin büyüme performansı ve ekonomik verimlilik üzerine etkilerini incelemek amacıyla yapılmıştır. Çalışmada; yavaş gelişen Hubbard JA57 genotipi ve hızlı gelişen Ross 308 etlik piliç genotipleri ile ızgaralı ve geleneksel derin altlık zemin sisteminin etkisi incelenmiş, çalışmada dört ana grup yer almış (2 genotip x 2 zemin tipi) ve her ana grup 5 tekrarlı gruptan oluşmuştur. Her tekrarlı grupta 10 adet erkek civciv olmak üzere çalışmada toplamda 200 adet günlük civciv kullanılmıştır. Büyütme dönemi süresince (8 hafta) gruplarda haftalık tartımlar ile canlı ağırlık izlenmiş, yem tüketimi ve ölenler kaydedilmiştir. Ekonomik verimliliği belirlemek için, gruplarda değişken giderler ve toplam içindeki payları belirlenmiş, brüt kar ve karlılık oranı hesaplanmıştır. Çalışmada beklenildiği gibi hızlı gelişen genotiplerin büyüme performansı yavaş gelişenlere göre daha yüksek bulunmuş, zemin tipinin büyüme performansı üzerine etkisi ise önemsiz bulunmuştur. Hızlı gelişenlere göre yavaş gelişenlerin ekonomik verimliliği daha yüksek bulunmuştur. Sonuç olarak; hızlı gelişen genotiplerin büyüme performansı daha yüksek bulunsa da deneme sonu itibari ile ekonomik verimlilikleri yavaş gelişenlere göre daha düşük bulunmuştur. Ancak yavaş ve hızlı gelişenlerin ticari koşullardaki kesim yaşlarının farklı olduğu dikkate alınarak aynı birim alanda bir üretim yılındaki toplam üretime göre ekonomik karşılaştırma yapılması daha faydalı olacaktır.

Anahtar Kelimeler: Etlik Piliç, Genotip, Zemin, Performans

*"Izgaralı Zemin Sistemi ve Yavaş Gelişen Etlik Piliç Gen tiplerinin Büyüme Performansı,Hayvan Refahı ve Davranışları, Ayak Sağlığı ve Üretim Ekonomisi Üzerine Etkileri" konulu doktora tezinden özetlenmiştir.

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Giriş

Ekonomik ve biyolojik verimliliğin ön planda olduğu modern ve entansif piliç eti üretimi geleneksel olarak kapalı barınaklarda zemine serilen altlık materyali (derin altlık) üzerinde hızlı büyüyen genotipler ile yapılmaktadır.[1] Derin altlık zeminde bir altlık materyali üzerinde hayvanlar yetiştirilmektedir. Piliç eti üretiminde altlık materyali olarak genellikle çeltik kavuzu, talaş, ağaç kabuğu, yerfıstığı kabuğu, ezilmiş mısır koçanı, saman ve kâğıt gibi malzemeler kullanılmaktadır.[2] İyi bir altlık malzemesi, tozlanmaya yol açmayacak, rutubeti iyi çekebilecek ve çabuk kuruyabilen yapıda, zeminden civcivlerin etkilenmemesi için ısı iletkenliği düşük, ucuz ve kolay bulunabilir olmalıdır.[3] Kümeslerde altlığın iyi yönetilememesine bağlı altlık ve hava kalitesi bozulmakta, hayvan sağlığı ve refahı olumsuz etkilenmektedir. İyi yönetilemeyen altlık hayvanların ayak tabanı, diz eklemi ve göğüs eti üzerinde leke, lezyon ve yaralanmalara yola açarak hayvan sağlığı ve refahını olumsuz etkilemesi yanında, et kalitesini de düşürmektedir.[4-6] Bundan dolayı piliç eti üretiminde derin altlık yanında kafes sistemi, ızgara altlık gibi değişik barındırma sistemlerinin kullanılabileceği düşünülmektedir. Kafes sistemi zemin üzerinde yapılan teknolojik iyileştirmelerin de etkisi ile piliç eti üretiminde yeniden kullanılmaya başlanmış, Türkiye ve Rusya gibi ülkeler yanında bazı Asya ülkelerinde de ticari üretimde kullanımı giderek yaygınlaşmaktadır.[7] Ancak kafes sistemi hayvanların hareket alanını sınırladığından hayvan refahı yönünden eleştirilmekte, ızgaralı zeminin daha uygun olabileceği düşünülmektedir. Almeida ve ark.[8] odun talaşı kullanılan derin altlık zemin ile plastik ızgaralı zeminin etlik piliçlerin büyüme performansına etkilerini incelediği çalışmada 42 günlük büyütme dönemi sonunda derin altlık sistemde yetiştirilen hayvanlardan erkeklerin canlı ağırlıklarının 3111 gr, dişilerin ise 2708 gr, plastik zemin üzerinde yetiştirilen erkek hayvanların 3167, dişi olanların 2760 gr canlı ağırlığa ulaştıklarını bildirmişler ve gruplar arası farklılıkları önemli bulmuşlardır. Yine aynı çalışmada, derin altlık zeminde yetiştirilen hayvanların yem tüketimleri erkeklerde 5082 gr, dişilerde 4665 gr, yemden yararlanma oranının erkeklerde 1,63; dişilerde 1,72; ölüm olanlarını erkeklerde %5,96; dişilerde %1,08 olarak bulunmuştur. Bu çalışmada plastik zeminde yetiştirilen gurubun yem tüketiminin erkeklerde 5180 gr, dişilerde 4720 gr; yemden yararlanma oranının erkeklerde 1,64; dişilerde 1,71; yaşama gücünü erkeklerde %100,00; dişilerde %94,05 olarak bulmuşlardır. Li ve ark.[9] yaptıkları bir çalışmada derin altlık ve ızgaralı altlık üzerinde yetiştirilen hızlı gelişen etlik piliç genotiplerinin 6 haftalık üretim dönemi sonunda performanslarını karşılaştırmış, derin altlık ve ızgaralı altlık sitemlerde yetiştirilen piliçlerin, canlı ağırlıklarını 2510 gr ve 2500 gr, yem tüketimlerini 4360 gr ve 4290 gr, yemden yararlanma oranını 1,79 ve 1,78; ölüm oranlarını %7,02 ve 7,53 olarak tespit etmişlerdir. Bu çalışmada gruplar arasında incelenen özellikler yönünden istatistiki düzeyde bir farklılık bulunamamıştır.

Ticari piliç eti üretiminde gelenekselin dışındaki üretim yöntemlerinde genelde yavaş gelişen hibrit genotipler kullanılmaktadır. Bookers ve De Boer[10] yaptıkları çalışmada geleneksel ve organik üretimi ekonomik açıdan karşılaştırmış ve geleneksel üretimde hızlı gelişen genotipleri, organik üretimde ise yavaş gelişen genotipleri kullanmışlardır. Bu çalışmada büyüme periyodu geleneksel üretimde 43 gün, organik üretimde 70 gün devam etmiş ve incelenen özellikler yönünden gruplar arası farklılıklar önemli bulunmuştur. Bu çalışmada kesim ağırlığı hızlı gelişen genotiplerde 2100 gr iken, yavaş gelişenlerde 2600 gr, yemden yararlanma oranı hızlı gelişenlerde 1,73; yavaş gelişenlerde 2,45 bulunmuştur. Hızlı gelişen genotipler 43 günlük sürede 3634 gr yem tüketmişken, yavaş gelişenler 6370 gr yem tüketmişlerdir. Berri ve ark.[11] yaptıkları çalışmada, 6 haftalık yaşta kesime gönderilen hızlı gelişen broyler genotiplerinde kesim ağırlığını 2496 gr, 8 hafta sonunda kesilen yavaş gelişen broyler genotiplerinde 2650 gr bulmuşlardır. Goscik ve ark.[12] hızlı ve yavaş gelişen broyler hibritlerin kullanıldığı geleneksel ve organik üretimde, hızlı gelişenleri 40 günde, yavaş gelişenleri 70 günde kesime göndermişlerdir. Bu çalışmada hızlı gelişen ve yavaş gelişen etlik piliçlerin kesim ağırlıkları sırası ile 2200 gr ve 2600 gr, yemden yararlanma oranı 1,69 ve 2,6; ölüm oranları % 3,7 ve 2,8 olarak bulunmuş ve gruplar arası farklılıklar önemli bulunmuştur. Fanatico ve ark.[13] yavaş gelişen etlik piliç genotiplerinin kullanıldığı bir çalışmada 91 günlük büyütme döneminde; canlı ağırlık kazancını 2888 gr, yem tüketimini 7959 gr, ölüm oranını %3, yemden yararlanma oranını 2,76 bulmuşlardır. Aynı çalışmada 63 gün beslenen hızlı gelişen etlik piliç genotiplerinin kullanıldığı grupta canlı ağırlık kazancı 2808 gr, yem tüketimi 5546 gr, ölüm oranı %19, yemden yararlanma oranı 1,97 bulunmuştur. Yine aynı çalışmada genotipler arasında canlı ağırlık kazancı, yem tüketimi, yemden yararlanma ve ölüm oranı arasındaki farklılıklar istatistiksel olarak önemli bulunmuştur. Petek[14], Bursa il merkezine yakın çevre 16 adet broyler işletmesinin karlılık ve verimliliğini araştırdığı bir çalışmada ticari üretim işletmelerinde brüt kar oranını %25,95; sözleşmeli işletmelerde %68,43 bulmuştur. Yine aynı çalışmada ticari ve sözleşmeli işletmelerde sırası ile net kar oranı %17,91 ve %26,39; değişken giderlerin toplam içindeki payı %91,64 ve %56,75; sabit giderlerin payı ise %8,36 ve %43,25 bulunmuştur. Bu çalışmada ticari üretim işletmelerinde civciv giderinin payı %26,26, yem giderlerinin toplam içindeki payı ise %58,92 bulunmuştur.

Bu çalışma, piliç eti üretiminde genotip ve zemin tipinin büyüme performansı ve ekonomik verimlilik üzerine etkisini araştırmak amacı ile yapılmıştır.

Materyal ve Metot

Bu çalışma için Uludağ Üniversitesi, Hayvan Deneyleri Yerel Etik Kurulundan, 01.09.2015 tarih ve 2015-10/12 karar numaralı izin belgesi alınmıştır. Ayrıca, çalışma için Uludağ Üniversitesi Veteriner Fakültesi'nden 81516830-050/7855 sayılı kararları ile izin alınmıştır. Projenin deneysel kısmı Uludağ Üniversitesi Veteriner Fakültesi, Hayvan Sağlığı ve Hayvansal Üretim Araştırma ve Uygulama Merkezi Tavuk Yetiştirme Ünitesinde yer alan deneme kümesinde gerçekleştirilmiştir.

Yönetim

Çalışmada iki zemin sistemi (ızgara ve geleneksel derin altlık) ile iki etlik piliç genotipinin (yavaş gelişen Hubbard JA57 ve hızlı gelişen Ross 308) broyler büyüme performansı ve üretim ekonomisi üzerine etkisi incelenmiştir. Bu şekilde çalışmada dört ana grup yer almış (2x2) ve her ana grup 5 tekrarlı gruptan oluşmuştur. Her tekrarlı grup 1x1 metre ölçülerinde tasarlanmış ve her tekrarlı grupta 10 adet erkek civciv yer almıştır. Her ana grupta, günlük yaşta, 50 adet civciv olacak şekilde çalışmada 100 adet yavaş gelişen (Hubbard JA57) 100 adet hızlı gelişen (Ross 308) etlik piliç genotipi civciv kullanılmıştır. Yavaş ve hızlı gelişen genotipler ticari kuluçkacı bir firmadan temin edilmiştir.

Izgaralı zemin olarak tavuklar için geliştirilmiş plastik ızgara kullanılmıştır ve yerden 50 cm yükseklikte olacak şekilde tasarlanmıştır. Izgaralı zeminin üzeri, civcivler bir haftalık yaşa ulaşana kadar gazete kâğıdı ile kapatılmıştır. Derin altlık zeminde altlık olarak 7 kg/m2 olacak şekilde pirinç kavuzu kullanılmıştır. Gruplarda yer alan hayvanlar; etlik piliçler için standart bakım/besleme koşullarında 56 gün süre ile büyütülmüştür. Kümes sıcaklığı, civcivlerin geldiği ilk gün 33 C° olacak şekilde ayarlanmış ve devam eden günlerde her hafta 3-3,5 C° düşürülerek, deneme sonuna kadar 21 C°'de sabit tutulmaya çalışılmıştır. Denemede başlangıçta civciv yemlik ve sulukları, bir haftalık yaştan deneme sonuna kadar yuvarlak askılı tipte suluk ve yuvarlak askılı kovalı yemlikler kullanılmıştır. Isıtma kaynağı olarak da doğalgazlı radyan ısıtıcılar kullanılmıştır. Denemede ticari bir yem firmasından temin edilen etlik piliç yemleri kullanılmıştır.

Veri toplama

Büyüme Performansı

Deneme başlangıcında civcivler bireysel olarak tartılarak gruplara rastgele dağıtılmış ve deneme süresince haftalık bireysel tartımlar ile canlı ağırlık kazancı izlenmiştir. Her tekrarlı guruba ilave edilen yemler kaydedilmiş, haftalık olarak gruplarda kalan yemler tartılarak yem tüketimi belirlenmiştir. Tekrarlı gruplarda tavuk başına ortalama kümülatif yem tüketimi ortalama canlı ağırlığa bölünerek yemden yararlanma oranı hesaplanmıştır. Ölen hayvan sayılarına göre haftalık olarak ölüm oranı hesaplanmıştır. Gruplarda haftalık ve dönem sonu verim indeksi;

(Yaşama Gücü (%) x Canlı Ağırlık (kg)) / (Yaş (gün) x Yemden Yararlanma Oranı) x 100 formülü ile hesaplanmıştır15.

Ekonomik Verimlilik

Gruplarda ekonomik verimliliği karşılaştırmak için brüt kar ve rantabilite/karlılık oranı üzerinden değerlendirme yapılmıştır. Bunun için, deneme gruplarında önce piliç başına değişken giderler hesaplanmış, perakende kg karkas satış gelirinden, kg değişken giderler toplamı çıkartılarak kg karkas için brüt kar hesaplanmıştır. Perakende kg piliç satış fiyatı yavaş ve hızlı gelişen piliçler için ayrı ulusal bir marketler zincirinden alınmıştır. Değişken giderler olarak bu çalışmada gerçekleşen ya da ölçülebilir olan yem gideri, civciv maliyeti ve altlık gideri dikkate alınmıştır[16].

Brüt Kar = kg/karkas perakende satış fiyatı — kg/ karkas için değişken giderler toplamı (yem, civciv ve altlık gideri toplamı)

Rantabilite (%) = Kar/Maliyet x 100

Giderler toplamı yapılan gerçek harcamalardan hesaplanmıştır.

İstatistiki Karşılaştırmalar

Tesadüf parselleri deneme desenine göre yürütülen çalışmadan alınan veriler varyans analiz metodu ile genel doğrusal model (General Linear Model) kullanılarak analiz edilmiş, gruplar arası farklılıkların önemli bulunması halinde çoklu karşılaştırmalar için Duncan testi uygulanmıştır.[17] Gruplar arası karşılaştırmalar SPSS istatistik programı ile bilgisayar ortamında yapılmıştır.[18]

Bulgular

Büyüme Performansı

Izgaralı ve derin altlık zeminde yetiştirilen yavaş ve hızlı gelişen etlik piliç genotiplerinin 6. Hafta büyüme performansları tablo 1' de sunulmuştur. Çalışmada yer alan yavaş ve hızlı gelişen hibritler 6. haftada sırası ile 1855,92 gr ve 3234,29 gr canlı ağırlığa ulaşmışlar, zemin tipinin canlı ağırlık üzerine etkisi önemsiz bulunmuştur. Gruplarda 6. hafta yemden yararlanma yavaş ve hızlı gelişenlerde 1,80 ve 1,55; derin altlık ve ızgaralı zeminde 1,66 ve 1,70 hesaplanmıştır. Yemden yararlanma üzerine genotip ve zemin tipinin etkisi önemli bulunmuştur. Gruplarda 6. hafta ölüm oranı üzerine genotip ve zemin tipinin etkisi önemsiz bu-

Tablo 1: Gruplarda büyüme performansı (6.hafta)

	Canlı Ağırlık g	Yem Tüketimi, g	Yemden yararlanma	Ölüm Oranı (%)	Verim İndeksi
Genotip					
Yavaş Gelişen	1855,92±25,43	3357,15±57,10	1,80±0,008	1,00	242
Hızlı Gelişen	3234,29±25,83	5022,25±40,38	1,55±0,006	4,00	476
Zemin Tipi					
Derin Altlık	2561,26±25,71	4173,43±49,45	1,66±0,007	3,00	356
Izgara Altlık	2528,94±25,56	4205,97±49,45	1,70±0,007	2,00	345
Genotip X Zemin Tipi					
Yavaş X Derin	1868,62±35,793	3334,50±80,76	1,78±0,012	0,00	240
Yavaş X Izgara	1843,22±36,15	3379,81±80,76	1,83±0,012	2,00	229
Hızlı X Derin	3253,91±36,91	5012,37±57,10	1,54±0,008	6,00	465
Hızlı X Izgara	3214,67±36,15	5032,14±57,10	1,56±0,008	2,00	478
ANOVA					
Genotip	0,001	0,001	0,001	0,369	
Zemin Tipi	0,374	0,646	0,001	1,000	
Genotip X Zemin Tipi	0,849	0,857	0,201	0.403	

lunmuştur. Derin altlık ve ızgaralı zeminde yetiştirilen hızlı gelişenlerin verim indeksleri yavaş gelişenlere göre daha yüksek bulunmuştur.

Deneme gruplarında 7. Hafta büyüme performansına ait değerler tablo 2' de gösterilmiştir. Ölüm oranı hariç büyüme performansı ile ilgili incelenen canlı ağırlık, yem tüketimi ve yemden yararlanma üzerine genotipin etkisi önemli (P<0.001), zemin sisteminin etkisi ise önemsiz bulunmuştur. Yemden yararlanma için genotip x zemin tipi interaksiyonu önemli bulunmuştur (P<0.002). Izgaralı zeminde yetiştirilen hızlı gelişenlerin verim indeksi en yüksek hesaplanmıştır.

Deneme gruplarında yer alan hayvanların deneme sonu (8.hafta) büyüme performansları tablo 3' te sunulmuştur. Deneme sonu itibari ile ölüm oranı hariç canlı ağırlık, yem tüketimi ve yemden yararlanma üzerine genotipin etkisi önemli (P<0.001), zemin tipinin etkisi önemsiz bulunmuş, canlı ağırlık ve yemden yararlanma için genotip x zemin tipi interaksiyonlarının önemli olduğu tespit edilmiştir (P<0.006 ve P<0.001).

Ekonomik Verimlilik

Deneme gruplarında kg karkas maliyeti, brüt kar ve karlılık oranları tablo 4' te sunulmuştur. Gruplarda kg karkas maliyetini ile brüt kar ve karlılık düzeyini hesaplayabilmek için; hızlı gelişen piliç karkası kg perakende satış fiyatı 7 TL, yavaş gelişen piliç karkası kg satış fiyatı 9 TL, yem kg alış fiyatı 1,4 TL, yavaş ve hızlı gelişen civciv adet fiyatı 1,2 TL, altlık kg alış fiyatı 0,32 TL olarak dikkate alınmış olup, bu fiyatlar ticari koşullardaki gerçek gider ve gelirlerdir. Denemenin altıncı haftası sonunda; kg karkas maliyet, brüt kar ve karlılık oranları yavaş gelişen x derin altlık grupta sırası ile 4,33; 4,66 ve 107,5; yavaş gelişen x ızgara altlık grubunda sırası ile 4,29; 4,71 ve 109,7; hızlı gelişen x derin altlık grupta sırası ile 3,45; 3,54 ve 102,6; hızlı gelişen x ızgara altlık grubunda sırası ile 3,42; 3,58 ve 104,6 hesaplanmıştır. Deneme sonu (sekizinci hafta) kg karkas maliyet, brüt kar ve karlılık oranları yavaş gelişen x derin altlık grubunda sırası ile 4,45; 4,55 ve 102,2; yavaş gelişen x ızgara altlık grubunda sırası ile 4,63; 4,36 ve 94,3; hızlı gelişen x derin altlık grubunda sırası ile 4,06; 2,93 ve 72; hızlı gelişen x ızgara altlık grubunda sırası ile 3,85; 3,14 ve 81,6 hesaplanmıştır.

Tablo 2: Gruplarda büyüme performansı (7.hafta)

	Canlı Ağırlık g	Yem Tüketimi g	Yemden yararlanma	Ölüm Oranı (%)	Verim İndeksi
Genotip					
Yavaş Gelişen	2247,32±38,91	4422,91±94,00	1,96±0,013	1,00	230
Hızlı Gelişen	3693,87±39,94	6478,26±66,46	1,75±0,009	4,00	413
Zemin Tipi					
Derin Altlık	2917,76±39,44	5382,05±81,40	1,86±0,011	3,00	309
Izgara Altlık	3023,42±39,42	5519,12±81,40	1,86±0,011	2,00	324
Genotip X Zemin Tipi					
Yavaş X Derin	2243,07±54,68	4357,40±132,93	1,94±0,018^{c}	0,00	231
Yavaş X Izgara	2251,56±55,38	4488,43±132,93	1,99±0,018^{d}	2,00	221
Hızlı X Derin	3592,45±56,86	6406,70±94,00	1,78±0,013^{b}	6,00	377
Hızlı X Izgara	3795,28±56,11	6549,81±94,00	1,73±0,013^{a}	2,00	427
ANOVA					
Genotip	0,001	0,001	0,001	0,369	
Zemin Tipi	0,060	0,245	0,806	1,000	
Genotip X Zemin Tipi	0,083	0,959	0,002	0.403	

a-c: Aynı sütünda değişik harf taşıyan gruplar arası farklılıklar önemlidir (P<0,05).

Deneme gruplarında başlıca ölçülebilir değişken giderlerin toplam maliyet içindeki payları ise tablo 5' de sunulmuştur. Altıncı hafta sonunda yavaş gelişenlerde yem giderleri toplam maliyetin %76-79'u olarak hesaplanmış, hızlı gelişenlerde yem maliyetinin % 83-85 arasında değiştiği belirlenmiştir. Sekizinci haftada, yavaş gelişen genotiplerde yem giderleri, toplam ölçülebilir giderlerin %85-87'sine ulaşmıştır. Hızlı gelişenlerde sekizinci hafta sonunda yem giderleri toplam ölçülebilir giderlerin %89-91 arasında bulunmuştur.

Tablo 3: Gruplarda büyüme performansı (8.hafta)

	Canlı Ağırlık	Yem Tüketimi	Yemden yararlanma	Ölüm Oranı (%)	Verim İndeksi
Genotip					
Yavaş Gelişen	2755,20±58,13	5765,59±133,39	2,09±0,016	1,00	232
Hızlı Gelişen	4370,43±60,21	8333,20±94,32	1,91±0,011	4,00	392
Zemin Tipi					
Derin Altlık	3505,55±59,21	6920,08±115,52	1,99±0,014	3,00	304
Izgara Altlık	3620,08±59,14	7178,71±115,52	2,02±0,014	2,00	313
Genotip X Zemin Tipi					
Yavaş X Derin	2814,96±81,512[a]	5700,23±188,65	2,02±0,023[c]	0,00	247
Yavaş X Izgara	2695,44±82,90[a]	5830,95±188,65	2,16±0,023[d]	2,00	210
Hızlı X Derin	4196,14±85,92[b]	8139,94±133,39	1,95±0,016[b]	6,00	352
Hızlı X Izgara	4544,71±84,37[c]	8526,46±133,39	1,87±0,016[a]	2,00	421
ANOVA					
Genotip	0,001	0,001	0,001	0,369	
Zemin Tipi	0,174	0,126	0,119	1,000	
Genotip X Zemin Tipi	0,006	0,441	0,001	0.403	

a-c: Aynı sütünda değişik harf taşıyan gruplar arası farklılıklar önemlidir (P<0,05).

Tablo 4. Denemede yer alan gruplarda kg karkas geliri ve maliyeti ile brüt kar ve karlılık oranları.

Gruplar	Kg karkas geliri (TL)	Kg karkas maliyeti (TL)	Brüt Kar (TL)	Karlılık (%)
6. Hafta				
Yavaş Gelişen X Derin Altlık	9,00	4,337	4,663	107,5
Yavaş Gelişen X Izgara Altlık	9,00	4,290	4,710	109,7
Hızlı Gelişen X Derin Altlık	7,00	3,454	3,546	102,6
Hızlı Gelişen X Izgara Altlık	7,00	3,420	3,580	104,6
7. Hafta				
Yavaş Gelişen X Derin Altlık	9,00	4,464	4,536	101,6
Yavaş Gelişen X Izgara Altlık	9,00	4,433	4,567	103
Hızlı Gelişen X Derin Atlık	7,00	3,852	3,148	81,7
Hızlı Gelişen X Izgara Altlık	7,00	3,643	3,357	92,1
8. Hafta				
Yavaş Gelişen X Derin Altlık	9,00	4,450	4,550	102,2
Yavaş Gelişen X Izgara Altlık	9,00	4,632	4,368	94,3
Hızlı Gelişen X Derin Atlık	7,00	4,068	2,932	72
Hızlı Gelişen X Izgara Altlık	7,00	3,854	3,146	81,6

Tablo 5. Denemede yer alan gruplarda yem, civciv ve altlık giderinin toplam içindeki payları (%).

Gruplar	Yem	Civciv	Altlık
6. Hafta			
Yavaş Gelişen X Derin altlık	76,79	19,74	3,47
Yavaş Gelişen X Izgara altlık	79,76	20,24	0
Hızlı Gelişen X Derin atlık	83,26	14,24	2,5
Hızlı Gelişen X Izgara altlık	85,44	14,56	0
7. Hafta			
Yavaş Gelişen X Derin altlık	81,22	15,98	2,8
Yavaş Gelişen X Izgara altlık	83,96	16,04	0
Hızlı Gelişen X Derin atlık	86,41	11,56	2,03
Hızlı Gelişen X Izgara altlık	88,42	11,58	0
8. Hafta			
Yavaş Gelişen X Derin altlık	84,98	12,77	2,25
Yavaş Gelişen X Izgara altlık	87,18	12,82	0
Hızlı Gelişen X Derin atlık	88,98	9,3	1,72
Hızlı Gelişen X Izgara altlık	90,86	9,14	0

Tartışma ve Sonuç

Bu çalışmada beklenildiği gibi hızlı gelişen etçi genotiplerin kümülatif canlı ağırlıkları deneme süresince yavaş gelişenlerden önemli düzeyde daha yüksek bulunmuştur.[19-20] Michalczuk ve ark.[21] yavaş ve hızlı gelişen Hubbard JA 957 ve Cobb 500 genotiplerini 8 hafta süre ile beslemişler ve deneme sonu canlı ağırlıklarını sırası ile 2279 ve 3100 gr bulmuşlardır. Anderle ve ark.[22] yavaş gelişen genotiplerde 49 günlük yaş için canlı ağırlığı 2340 gr bulmuşlardır. Hoan ve ark.[23] yavaş ve hızlı gelişen genotiplerin 90. gün canlı ağırlıklarını 1919 ve 5318 gr bildirmişlerdir. Berri ve ark.[11] hızlı ve yavaş gelişen genotipleri 6 ve 8 hafta süre ile büyütmüşler, bu sürede ulaşılan canlı ağırlıkları; 2694 ve 2650 gr bildirmişlerdir. Fanatico ve ark.[13] hızlı ve yavaş gelişenler için kümülatif canlı ağırlıkları 63 ve 91 günlük büyütme dönemi için 2808 ve 2888 gr bulmuşlardır. Bu çalışmada elde edilen canlı ağırlık değerleri, özellikle de yavaş gelişenlerin canlı ağırlıkları, genel olarak yavaş ve hızlı gelişenler için bildirilen bulgulardan daha yüksek bulunmuştur. Bu çalışmada sadece erkek cinsiyette hayvanların yer alması ve hızlı gelişenler genotipler için hazırlanmış standart yemin kullanılması bu farklılığın başlıca nedeni olabilir. Yavaş gelişenler ile karşılaştırıldığında, hızlı gelişenler hem derin altlık, hem de ızgaralı zeminde daha yüksek canlı ağırlığa ulaşmışlardır. Yavaş gelişen hibritlerin ızgara ve derin altlık zeminde canlı ağırlık kazançları arasında deneme süresince önemli bir farklılık yok iken, hızlı gelişen genotiplerde 8. haftada kümülatif canlı ağırlık bakımından farklılıklar önemli bulunmuştur. Deneme sonu itibari ile (8.hafta) canlı ağırlık yönünden genotip x zemin interaksiyonu önemli bulunmuş, yavaş gelişenler derin altlıkta, hızlı gelişenler ızgara altlıkta daha yüksek canlı ağırlığa ulaşmışlardır. Yaş ve artan canlı ağırlığın etkisi altlık kalitesi hızlı gelişenlerin yetiştirildiği derin altlık

zeminde daha fazla kötüleşmiş ve hızlı gelişenlerin canlı ağırlık kazancı olumsuz etkilenmiş olabilir. Izgaralı ve derin altlıkta yetiştirilen yavaş gelişen hibritlerin canlı ağırlık ortalamaları ile yine aynı zeminlerde yetiştirilen hızlı gelişen hibritlerin canlı ağırlık ortalamaları arası farklılıklar önemsiz bulunmuştur. Bu çalışmada elde edilen bulgular ile benzer olarak Almedia ve ark.[8] derin altlık ve plastik ızgaralı zeminde yetiştirilen hızlı gelişen hibritlerde 6. hafta canlı ağırlık değerleri arası genelde fark bulunmadığını bildirmişlerdir. Cengiz ve ark.[24] derin altlık, ızgaralı ve tahta ızgaralı zeminde büyütülen hızlı gelişen genotiplerde 49 günlük yaş için, derin altlık zeminde rakamsal olarak daha düşük olsa da, aradaki farklılığı önemsiz bulmuşlardır.

Genelde; yavaş ve hızlı gelişen genotiplerde deneme sonu yem tüketimi; 5765 gr ve 8333 gr hesaplanmıştır. Deneme sonunda, genotipin yem tüketimi üzerine etkisi önemli bulunurken, zeminin etkisi önemsiz bulunmuştur. Yem tüketiminde olduğu gibi bu çalışmada yemden yararlanma bakımından deneme süresince genotipin etkisi önemli, zeminin etkisi önemsiz bulunmuştur. Deneme sonu itibari ile hızlı gelişen genotiplerin yemden yararlanma değeri yavaş gelişenlere göre istatistiki olarak daha iyi bulunmuştur ($p<0,001$) (Tablo 3). Bu bulgu genel olarak literatür bulgular ile benzerdir. Bokkers ve De Boer10 organik ve geleneksel üretimde, yavaş ve hızlı gelişen genotiplerin yemden yararlanma oranlarını 2,45 ve 1,73 bulmuşlardır. Goscik ve ark.[12] yaptıkları bir çalışmada, yavaş ve hızlı gelişen genotiplerin yemden yararlanma oranını 2,60 ve 1,69; Fanatico ve ark.[13] ise 2,76 ve 1,97 bulmuşlardır. Mikulski ve ark.[19] yaptıkları bir çalışmada 42 günlük dönem sonunda yavaş ve hızlı gelişen genotiplerin yemden yararlanma oranını 1,71 ve 1,68; 65 günlük dönem sonunda ise 2,54 ve 2,48 bildirmişlerdir. Almeida ve ark.[8] derin altlık ve plastik ızgara üzerinde yetiştirilen hızlı gelişen genotiplerin kullanıldığı bir çalışmada, derin altlık zeminde yetiştirilen erkek ve dişi hayvanlarda yemden yararlanma oranını sırası ile 1,63 ve 1,72; plastik ızgaralı zeminde erkeklerde 1,64; dişilerde 1,71 bulmuşlar ve yemden yararlanma için zemin sistemleri arasındaki farkın önemsiz olduğunu bildirmişlerdir. Mikulski ve ark.[19] organik ve kapalı derin altlık sistemde yetiştirilen hayvanların yemden yararlanma oranını, 42 günlük büyütme dönemi sonunda 1,71 ve 1,69; 65 günlük dönem sonunda 2,52 ve 2,51 bulmuş, barınak sistemleri açısından yemden yararlanma oranları arasındaki farklılıkların önemsiz olduğunu bildirmişlerdir. Denemenin 7 ve 8. haftalarında yemden yararlanma bakımından genotip x zemin interaksiyonu önemli bulunmuş, yavaş gelişenler derin atlıkta, hızlı gelişenler ızgaralı zeminde kg canlı ağırlık kazancı için daha az yem tüketmişlerdir. İlerleyen yaş ve canlı ağırlıkla birlikte derin altlık grupta altlık kalitesinin düşmesine bağlı olarak hızlı gelişenlerin canlı ağırlık kazancının düşmesi bu hayvanlarda yemden yararlanmayı olumsuz etkilemiş olabilir.

Guruplarda kg karkas ya da canlı ağırlık maliyetini hesaplamak için, bütün guruplar eşdeğer koşullarda yetiştirildiğinden, gruplar arasında ölçülebilir ve değişken olan yem, civciv ve altlık giderine göre toplam maliyet hesaplanmıştır. Deneme sonu (8.hafta) itibari ile derin altlık ve ızgaralı zeminde kg karkas maliyeti; yavaş gelişen genotipler için 4,45 TL ve 4,63 TL, hızlı gelişen genotipler için 4,06 TL ve 3,85 TL bulunmuştur. Toplam maliyet içinde yem giderinin payı beklenildiği gibi bütün guruplarda en yüksek bulunmuştur.[14,25,26] Hem derin, hem de ızgaralı zeminde yetiştirilen hızlı gelişen genotiplerde yem giderinin toplamdaki payı daha yüksek bulunmuştur. Haftalar ilerledikçe yem giderlerinin toplam gider içindeki payı artarken, civciv giderlerinin payı azalmıştır. Bu çalışmada perakende kg piliç eti satış fiyatı yavaş gelişenler için 9 TL, hızlı gelişenler için 7 TL alınmıştır. Üretim maliyetleri yüksek olsa da hem ızgaralı hem de derin altlık zeminde yetiştirtilen yavaş gelişen hibritlerde brüt kar ve rantabilite hızlı gelişenlere göre daha yüksek bulunmuş, haftalar ilerledikçe karlılık oranının azaldığı gözlenmiştir. Toplam maliyet ve satış fiyatlarına göre değişmekle birlikte, azalan verimler kanunu (marjinal masraf/marjinal fayda) dikkate alınırsa, özellikle hızlı gelişen genotiplerin 8. haftaya kadar yetiştirilmesi ekonomik olmayabilir. Sakarya[27], piliç eti üreten işletmelerin ekonomik analizi üzerine yaptığı bir çalışmada, karlılık oranının %102 ile %109 arasında değiştiğini bildirmiş, toplam giderler içinde yem giderlerinin payını %64, civciv giderlerini %19 hesaplamıştır. Ike ve ark.[26] etlik piliç işletmelerinde yaptıkları çalışmada, toplam giderler içinde yem giderlerini %78, civciv giderlerini %17, ilaç giderlerini %2,24 hesaplamışlar; bu işletmenin karlılık oranını da %41 bildirmişlerdir. Baéza ve ark.[28] etlik piliçlerin ilerleyen yaşlardaki ekonomik analizini karşılaştırdıkları bir çalışmada, 42 günlük yaşta bir metrekareden elde edilen toplam geliri 36,69 Amerikan Doları, toplam maliyeti 27,67 Amerikan Doları, brüt kar oranını %22, net kar oranını %9 bulmuşlardır. Sarıca ve ark.[20] yavaş ve hızlı gelişen etlik piliç genotiplerinin ekonomik analizi üzerine yaptıkları bir çalışmada, 49 günlük büyütme döneminde, değişken giderler içinde yem giderlerinin oranını yavaş gelişenlerde %74,09 ve %74,03; hızlı gelişenlerde %73,35 ve %77,18; civciv giderlerinin değişken giderler içindeki payını yavaş gelişenlerde %11,83 ve %11,85; hızlı gelişenlerde %13,80 ve %11,20 bildirmişlerdir.

Sonuç olarak; bu çalışmada hızlı gelişen genotiplerin yavaş gelişenlere göre büyüme performansının daha iyi olduğu,

haftalar ilerledikçe özellikle altıncı haftadan sonra her iki genotipte de yemden yararlanma kabiliyetinin düştüğü görülmüştür. Zemin tipi ise büyüme performansı üzerine önemli bir etki göstermemiştir. Ekonomik verimlilik açısından, hızlı gelişen genotipler daha iyi büyüme performansı göstermesine rağmen, brüt kar oranı bakımından yavaş gelişenlerin daha karlı olduğu sonucuna ulaşılmıştır. Bu durum, yavaş gelişen genotiplerin markette kg karkas satış fiyatının daha yüksek olmasından kaynaklanmaktadır. Ayrıca ticari koşullarda hızlı gelişen genotiplerin ideal kesim yaşının beş-altı hafta, yavaş gelişen genotiplerin sekiz-on iki hafta olduğu, hızlı gelişen genotiplerin kullanılması durumunda yılda 6-7 dönem, yavaş gelişen genotiplerin kullanılması durumunda en fazla 5 dönem üretim yapılabileceği dikkate alınarak, aynı üretim alanında bir yıl boyunca gerçekleştirilecek toplam üretim üzerinden karlılığın hesaplanması daha gerçekçi sonuçlara ulaştıracaktır.

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The Influence of Calving Year on Milk Yield and Milk Components in Dairy Cattle

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Abstract

The aim of this study was to identify the effect of calving year on milk yield and milk composition traits in Holstein and Jersey cattle raised in the Marmara and Black Sea Regions of Turkey, respectively. The data set consisted of 582 dairy cows, which were 306 Holstein raised and 276 Jersey cows calving from 2011 to 2013. Except for fat yield, test day milk yield (TDMY), 305-day milk yield (305 DMY), fat percentage, protein percentage and protein yield of Jersey cows were affected by calving season. The effect of calving year on TDMY, 305 DMY, fat percentage, fat yield, protein percentage and protein yield of Holstein cows were found to be statically significant. In conclusion, in 2011 to 2013, milk yield and its composition in both Holstein and Jersey cows were significantly related to calving year.

Key words: Calving year, DGAT1, milk yield, fat yield, protein yield

Introduction

Dairy cow breeding is the forefront of milk production under the conditions of our country. Milk is one of the important animal-based nutriment sources in terms of content and most milk and dairy products are produced from cattle. According to data from Turkish Statistical Institute for 2018, the total number of cattle was 17.042.506 head in Turkey. 90.65% of these animals are culture and crossbred. In addition, a total of 22.120.716 tons of milk produced annually in Turkey and 90.58% of these productions are obtained from cattle.[1] The highest milk production rate in our country is obtained from Holstein cows grown in the west part of country and Jersey cows raised especially in the Black Sea Region.

The increasing milk yield in terms of quantity and quality has always been the desired target for producers. To increase dairy productivity of animals, it is important to work with all the parameters affecting milk yield and all its components. Those factors are genetic and non-genetic factors which affect directly or indirectly to milk production of cows. All the genetic factors are in quantitative nature, thus many genes also influence reproductive performance of animals directly or by the manner of interacting with each other. Although some of the genes has a major effect, most of them has a very small effect on milk yield. Those types of genes are cumulatively affecting the level of production.

Even if genetic capacity of animal determined the utmost limit of quantitative traits like milk yield, the observation of genetic capability depends entirely on environmental factors. Thus, many non-genetic factors including calving year, calving interval, season, parity, age of cows, feeding, housing, climate change, disease, days from parturition to

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first service, days open, number of services per conception, and management conditions are crucial for this type of traits. There are many studies conducted to search not only the effect of genetic factors but also all possible environmental parameters which somehow influence on the milk yield and its component. One of the studies was conducted to search milk yield and reproductive performance of crossbred cows and it was reported that the significant effect of calving year on lactation duration.[2] Some studies were also showed that calving year significantly influenced on all milk yield traits such as 305 DMY, dry period, and lactation period.[3-5] Inci et al[6] also found that calving year were important on 305 DMY and lactation period but not significantly affecting on dry period. In another study, it was reported that various environmental factors including calving year significantly affected on 100, 200, 305 DMY in Holstein cows raised in different regions of Turkey.[7] As optimizing all the environmental effects will help to increase milk yield, the levels of milk fat, milk protein, and all other composition traits.[8] Overall this process will be possible by selecting animals through the breeding programs, improving of genetic structures of animals, and besides adapting of all environmental factors that affect the yield of animals. Therefore, the aim of this study was to determine the effect of non-genetic factor which is calving year on milk yield and milk composition traits of animals genotyped for DGAT1 (K232A) gene polymorphism in Jersey and Holstein cows raised in Marmara and Black Sea regions, respectively.

Materials and Methods

Animals Sources and Phenotypic Data

The study involved a total of 582 dairy cows, which were 306 Holstein raised in Marmara region and 276 Jersey raised in Black Sea region of Turkey. The number of Holstein and Jersey cows according to five parity (from 1 to ≥5) in this study were 128, 127, 29, 15 and 7, and 29, 54, 47, 51 and 95, respectively. Holstein cow is raised especially the west part of country. On the other hand, Jersey breed is raised mostly in Black Sea region due to well adaptation to environmental condition. Within the scope of this study, animals were identified and selected based on farm records. Milk yield records were obtained monthly basis during the lactation periods from 2011 to 2013. Collected milk samples at monthly milk test days were used to detect milk fat and protein percentage by ultrasonic milk analyzer (Foss MilkoScan FT1, Hillerod, Denmark). Moreover, milk fat and protein yields were calculated based on the milk yield levels for each cow. Milk fat yield (TDMY*Fat%) and milk protein yield (TDMY*Protein%) were calculated by the use of the values obtained as a result of the analyses.

The following model was used to examine the influence of calving year on TDMY, 305 DMY, fat percentage, fat yield, protein percentage and protein yield;

$Yij = \mu + Ai + \varepsilon ij$

Yij is the observation value

μ is the overall mean

Ai is effect of calving year (i= 2011, 2012, 2013)

eij is random error.

The statistical analyses were performed by the general linear model (GLM) procedure of SPSS9 package program (SPSS, 2004). The means were compared by Duncan's multiple range test.

Results

In this study, the change in milk yield and its composition according to calving year in Jersey cows are presented in Table 1. TDMY, 305 DMY, fat percentage, protein percentage and protein yield were affected by calving year. However, fat yield not effected by calving year. The effect of calving year on TDMY, 305 DMY, milk fat percentage, fat yield, protein percentage and protein yield were significantly important in Holstein cows (Table 2).

As a seen Table 1, the TDMY in Jersey cows was the lowest in 2011 (14.56 kg) and the highest in 2012 (16.34 kg) and 2013 (16.83 kg). The highest 305 DMY was determined in 2011 (5116.2 kg) and 2012 (5484.2 kg), but the lowest in 2013 (4604.8 kg). The fat percentage was the highest in 2011 (5.36%) and decreased gradually from 2012 (4.90%) to 2013 (4.74%). The protein percentage was the highest in 2011 (3.44%), but the lowest in 2012 (3.33%). The highest protein yield was determined in 2012 (0.54 kg) and 2013 (0.57 kg), but the lowest in 2011 (0.50 kg).

The highest TDMY for Holstein cows was found to be in 2012 (27.48 kg) and 2013 (27.94 kg) and the lowest in 2011 (20.71 kg). The 305 DMY was also determined the highest in 2012 (9143.8 kg) and 2013 (9119.0 kg), but the lowest in 2011 (8000.1 kg). The fat percentage was the highest in 2011 (3.80%), but the lowest in 2012 (3.52%). The fat yield was the lowest in 2011 (0.78 kg) and increased gradually in 2012 (0.96 kg) and 2013 (1.02 kg). The highest protein percentage was determined in 2011 (3.28%) and 2012 (3.21%), while the protein yield was determined the highest in 2012 (0.88 kg) and 2013 (0.86 kg).

Table 1. Milk yield and its components by calving year in Jersey cows.

Calving Year		TDMY (kg)	305 DMY (kg)	Fat %	Fat Yield* (kg/d)	Protein %	Protein Yield* (kg/d)
2011	n	93	93	93	93	93	93
	$\bar{X}$	14.56^{b}	5116.2^{a}	5.36^{a}	0.77	3.44^{a}	0.50^{b}
	S	2.97	1395.7	0.69	0.15	0.21	0.10
	Min.	6.60	2268.0	3.94	0.28	2.91	0.21
	Max.	22.37	10226.0	7.48	1.17	4.03	0.72
2012	n	108	108	108	108	108	108
	$\bar{X}$	16.34^{a}	5484.2^{a}	4.90^{b}	0.80	3.33^{c}	0.54^{a}
	S	3.16	1491.9	0.58	0.16	0.16	0.10
	Min.	3.74	1480.0	3.41	0.18	2.94	0.13
	Max.	23.26	11874.0	6.52	1.25	3.79	0.74
2013	n	75	75	75	75	75	75
	$\bar{X}$	16.83^{a}	4604.8^{b}	4.74^{c}	0.80	3.40^{b}	0.57^{a}
	S	3.29	954.6	0.41	0.16	0.09	0.11
	Min.	8.08	2126.0	3.94	0.34	3.14	0.27
	Max.	23.47	7255.0	5.96	1.12	3.58	0.78
	P	**0.004**	**<0.001**	**<0.001**	**0.808**	**<0.001**	**0.018**
	b**	**-0.006 (0.013)**	-	**0.001 (0.060)**	**<0.001 (0.208)**	**<0.001 (0.034)**	**<0.001 (0.046)**

a.b.c The differences between the group means with different letters in the same column are significant (P<0.05).

TDMY: Test day milk yield, 305 DMY: 305-day milk yield, Min: Minimum, Max: Maximum.

**b: The regression coefficient calculated for the lactation period and the values given in brackets indicate the significance level.

Calving Year		TDMY (kg)	305 DMY (kg)	Fat %	Fat Yield* (kg/d)	Protein %	Protein Yield* (kg/d)
2011	n	59	59	59	59	59	59
	$\bar{X}$	20.71[b]	8000.1[b]	3.80[a]	0.78[c]	3.28[a]	0.68[b]
	S	5.75	2249.4	0.60	0.22	0.28	0.19
	Min.	11.19	3711.0	2.04	0.36	2.46	0.37
	Max.	41.13	13585.0	5.23	1.30	4.08	1.16
2012	n	71	71	71	71	71	71
	$\bar{X}$	27.48[a]	9143.8[a]	3.52[b]	0.96[b]	3.21[a]	0.88[a]
	S	3.79	1772.3	0.57	0.15	0.28	0.11
	Min.	17.68	3659.0	2.45	0.61	2.46	0.56
	Max.	35.76	13775.0	5.01	1.52	4.00	1.13
2013	n	176	176	176	176	176	176
	$\bar{X}$	27.94[a]	9119.0[a]	3.69[ab]	1.02[a]	3.09[b]	0.86[a]
	S	5.54	2090.1	0.59	0.21	0.29	0.16
	Min.	11.87	3179.0	2.42	0.42	2.40	0.38
	Max.	43.00	15809.0	5.89	1.65	3.97	1.35
	P	**<0.001**	**0.003**	**0.017**	**<0.001**	**0.002**	**<0.001**
	b**	**0.004 (0.286)**	-	**<0.001 (0.724)**	**<0.001 (0.376)**	**<0.001 (0.512)**	**<0.001 (0.127)**

[a.b.c] The differences between the group means with different letters in the same column are significant (P<0.05).

TDMY: Test day milk yield, 305 DMY: 305-day milk yield, Min: Minimum, Max: Maximum.

**b: The regression coefficient calculated for the lactation period and the values given in brackets indicate the significance level.

Discussion and Conclusion

The effect of calving year on TDMY (P=0.004), 305 DMY (P<0.001), fat percentage (P<0.001), protein percentage (P<0.001) and protein yield (P<0.018) genotyped according to DGAT1 gene for Jersey cows were found to be statically significant. However, fat yield was not affected by calving year (Table 1). Similar conclusions were reached by Gurses et al[10] and Teke and Akdag[11], who documented that the effect of calving year was significant on 305 DMY in Jersey cow. Missanjo et al[12] found similar results and concluded that calving year had a significant effect on milk, fat and protein yields of Jersey cattle. The results found in this study are also consistent with literature.[13] The differences between the years may be related to care-feeding, selection and management.

In the present study, the TDMY (P<0.001), 305 DMY (P=0.003), milk fat percentage (P=0.017), fat yield (P<0.001) protein percentage (P=0.002), and protein yield (P<0.001) genotyped according to DGAT1 gene in Holstein cows were affected by calving year (Table 2). Results clearly indicate that an elevated milk production according to years reflected as a decrease in milk fat and protein percentage in the investigated farms. This result for 305 DMY was similar to results with reported by many literatures.[4,14-18] Contrary to the present finding, Arslan and Cak[19] reported that effects of calving year on 305 DMY was not significantly important in Holstein cows. Koc and Kizilkaya[20] observed that calving year on TDMY was sig-

nificantly important. Atasever and Stadnik[21] determined that calving year on daily milk yield, fat and protein was significantly important in Holstein cows. Adediran et al[22] reported that highly significant effects of year of calving on protein and fat yields in Holstein breed. On the contrary, Sekerden[23] reported that effect of calving year on fat yield and protein yield was not important in Holstein cows. This difference between studies may be related to different environment, nutritional, management and barn conditions.

In conclusion, in 2011 to 2013, milk yield and its composition in both Holstein and Jersey cows were significantly related to calving years. It may be said that the feeding, maintenance-management factors between years have an important effect on the productivity of the dairy cows.

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RESEARCH ARTICLES

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Comparison of Serum IgG Concentration, Total Protein, Glutaraldehyde Coagulation Test and Gamma Glutamyl Transferase in Neonatal Foals

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Abstract

The purpose of the present study was to detect the passive transfer status in healthy neonatal foals by comparing serum immunoglobulin G (sIgG) concentration, serum total protein (STP), glutaraldehyde coagulation test (GCT) duration and gamma glutamyl transferase (GGT) activity . Fifteen neonatal foals (0-15 days old)' blood samples were collected before suckling (day 0), 12th hour (hr), 24th hr (1st day), 7th and 15th days. Serum IgG and STP levels significantly increased after the 12th hr. Conversely, serum GCT duration significantly decreased ($p< 0.05$) in neonatal foals after the 12th hr. The result of the present study was shown that measurements of sIgG, STP concentration and GCT duration are useful parameters to detect Failure of Passive Transfer (FPT) in neonatal foals. While GCT and STP provide a simple and inexpensive field test, serum GGT measurement is not a beneficial test to determine colostrum intake in newborn foals.

Key Words: Foals, Newborn, Failure of Passive Transfer, serum Immunoglobulin G

Introduction

Foals are born without appreciable serum concentrations of immunoglobulin (Ig). Colostral Ig is required to supply humoral immunity during the neonatal period. Immunoglobulin requirements are satisfied by ingesting and absorbing colostral Igs. The absorption of Igs from colostrum is a vital importance for foals' health. Failure of passive transfer was increased the rate of illness and death during 6-7 weeks of age.[1,2] The majority of Ig in equine colostrum is serum immunoglobulin G (IgG), which provides protection against infection for the first one to two months of life.[3] One of the most important causes of infectious diseases (septicemia, enteritis, pneumonia, arthritis etc.) are complete (sIgG:0-400 mg/dl) or partial (sIgG: 401-800 mg/dl) FPT in the neonatal period of foals.[1] The major cause of neonatal death is septicemia in the first week of their life.[4,5] Many studies have shown that FPT occurs in 10-20% of foals and rises to 30% in those animals that require hospitalization.[3]

There are several methods for determining of sIgG levels in foals. Diagnostic tests include single radial immunodiffusion6, GCT,[1,7] zinc sulfate turbidity, 8 latex agglutination,1 Enzyme Linked Immunosoebent Assay,[9,10] turbidimetric immunoassay,[3,6] determination of STP,[6,10] and serum globulin concentration.[10] Glutaraldehyde coagulation test (GCT), which is introduced for detection of hypergammaglobulinaemia using whole blood, is a semiquantitative, practical, inexpensive, fast and especially amenable to field conditions. Serun total protein (STP) measurement is an alternative method for detecting the concentration of IgG for diagnosing FPT in foals and it can be measured in the laboratory but may also be approximated using a refractometer.[6,11] In decade, GGT is the most popular diagnostic method to diagnose of the FPT in cattle. However, there

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are limited scientific knowledge about importance in diagnosis of FPT in foals.

The aims of this study were to determine the suitability of the GCT, STP, and GGT activity based on total IgG in foal blood.

Materials and Methods

Study Design

In this study, 15 foals (0-15 days) born on a breeding farm located at Karacabey, Bursa, Turkey. All foals and their mothers were clinically examined daily until 1 month of age after birth, and they were healthy. All applications were performed under the control and approval of the University of Ethical Committee, in accordance with the Animal Welfare Guidelines (2014-10/03). The blood samples were collected from the jugular vein using plain vacuum tubes at before suckling (day baseline), after 12th hr, 1st (24th hr), 7th and 15th day of the study. Blood samples were centrifugated and then serum samples were stored at -20° C until analysis.

Total IgG, TP, GCT, and GGT measurement

The concentrations of sIgG were measured by an immunoturbidimetric method (MBC QTII®)[3,6]. The range of measurement is 200 to 1700 mg/dl. This method is a linear representation of Equine IgG levels until 1700 mg/dl. Samples higher than 1700 mg/dl were re-assayed after dilution with 0.9% saline. Serum GGT activity was analyzed by an enzymatic colorimetric test (Roche Cobas Integra 400 Plus). Serum GCT duration was measured by using a 10% glutaraldehyde solution (Merck). 0,5 ml of each serum sample were mixed with 50 µl solution into the test tubes and examined for coagulation during 1 hour. A positive coagulation reaction was said to occur when a solid gel. Serum TP was measured by a hand refractometer. After calibration, a few sample drops were placed on the surface of the prism. The daylight plate was closed gently and the protein scale was read.

Statistical analyzes

The data were analyzed using the Friedman test, a non-parametric test, because the data was not passed the normality test (SPSS, windows version 13.0). Pearson's correlation analysis was calculated between sIgG concentrations, STP activity, GCT duration and GGT activity. The level of significance was set at $P<0.05$.

Results

The results about IgG concentrations, STP levels, GCT duration, and GGT activities are summarized in Table 1. Serum IgG levels were too low before suckling in this study. It is important to note that the commercial kit (MBC QTII®) did not provide IgG results measuring less than 200 mg/dl, so the mean for this measurement is not entirely accurate. The correlations were indicated between sIgG concentrations, GGT activities, GCT duration and STP activities in

Table 1. Serum IgG, STP, GCT, and GGT findings (mean±SE) in newborn foals (n:15)

	Presuckling (n:15)	12hour (n:15)	1st day (n:15)	7th day (n:15)	15th day (n:15)
IgG (mg/kg)	<200[b,c,d,e]	1215.06±151,3[a,c,d,e]	1643.9±64[a,b,e]	1561.9±75[a,b]	1491.2±84.96[a,b,c]
GGT (IU)	13.62±4.7[c,d,e]	14.527±1.36[c,d,e]	9.697±0.94[a,b,d,e]	19.952±6.9[a,b,c,e]	24.6±6.7[a,b,c,d]
GCT (minutes)	50±00[b,c,d,e]	18.933±6.4[a,c,d,e]	6.467±1.8[a,b,e]	6.7±1.6[a,b,e]	4.66±0.37[a,b,c,d]
(STP) (g/dl)	3.89±0.12[b,c,d,e]	7.2±0.2[a]	7.4±0.1[a]	7.547±0.13[a]	7.56±0.1[a]

a, b, c, d, e are refered to value belong to each column and indicated statistically significance between rows, $P<0.05$.

Table 2: Correlations between serum IgG concentrations, STP levels, GCT durations, and GGT activities.

	12 hour	1st day	7th day	15th day
IgG and GGT	0.141	-0.206	0.146	0.156
IgG and GCT	-0.729*	-0.901*	-0.955*	-0.288
IgG and STP	0,830*	0.732*	0.575*	0.610*
GGT and GCT	-0.291	-0.0456	-0.229	-0.373
GGT and STP	0.229	-0.148	0.496	0.358
GCT and STP	-0.689*	-0.778*	-0.727*	-0.332

*p< 0.05 represents correlation between the different two variables on same period that are found to be statistically significant.

Discussion and Conclusion

Measurement of serum IgG levels are very important for detecting an early diagnosis and treatment of FPT. Maximum ingestion of colostral IgG consists by 12 hours after birth. So, it has been recommended that a neonatal foal's serum should be measured 8 to 12 hours after birth to assess passive transfer status.12 In this study, serum IgG concentrations were detected as 1215.06±151.3 mg/dl at 12 hours after birth, and maximum concentrations were reached 1643.93±64.48 mg/dl in 24 hours. The absorption of Igs decreases 24 to 36 hours after birth because the specialized enterocytes are replaced by epithelial cells with pinocytosis deficiency.[2,13] The first 24-hours sIgG results of this study are similar findings to other studies (Table 1) .[2,13] In line with these results, it may be possible to assess serum IgG levels as a gold standard in relation to previous studies. Early diagnosis of FTP is very important in decreasing the rate of disease in foals. Several studies have suggested that a STP concentration less than or equal to 4.5 g/dL was suggestive of FPT, whereas values greater than or equal to 6.0 g/dL indicated adequate IgG concentrations and that serum TP concentration can be used as an alternative to potentially assess the IgG concentration if no other test can be performed.[6,14]

In this study, STP concentrations were measured as 3.89±0.122 g/dl before suckling and the concentrations increased at 12 h (7.227±0,21 g/dl) after suckling. This demonstrates that the concentrations of STP increased after 12 hours, as did sIgG concentrations. The major advantages of this test are that it does not contain very special procedures for STP measurement and can be applied in a farm or a field condition.[11] Also, in this study, a positive correlation was detected between sIgG concentrations and STP levels (p< 0.05), and a negative correlation was found between serum TP and GCT duration (p< 0.05) at the following time points: 12 hours after birth, and the 1st, 7th and 15th days of the experiment as shown in Table 2. Serum GCT duration is a useful test to evaluate passive transfer status of calves, goat kids and foals.[15] The effect of GCT duration in detecting failure to acquire colostral immunoglobulin in neonatal foals was investigated by comparing and correlating results from the GCT with those obtained by equine IgG. The GCT duration was found to be a practical, inexpensive, semi-quantitative test with a high specificity and sensitivity at critical IgG levels.[7,16]

In this study, the foals' serum did not clot until reaching 50 minutes in GCT duration before colostrum feeding indicated hypogammaglobulinemia. There was found a negative relationship between the GCT duration and sIgG concentration in the present study. The GCT duration decreased, while serum IgG concentrations and STP activities increased at 12 hours after birth (Table 1). A negative correlation was detected between the GCT duration and sIgG concentrations (p< 0.05) from 12 hours after birth, and the 1st, 7th and 15th days of the experiment as shown in Table 2. Hurcombe et al. (2012) 5 proposed that STP levels can be used to estimate the sIgG concentrations in neonatal foals. Moreover, Beetson et al5 determined that it is possible to identify foals which have successful colostral transfer by using the GCT. Although we could not find any study STP and GCT are evaluated together, we detected that our results are consistent with the results of both studies.[5,7]

Serum GGT concentration was very high in colostrum in-

gested through suckling and it was reported to be a useful parameter to assess the passive transfer status of calves and lambs. [15,17,18] We aimed to determine whether GGT activity assessment is a useful method for determining FPT and whether any correlation with other parameters was observed in neonatal foals. The result of our study was found to be parallel with a study by Braun et al[19] who reported that GGT was not a useful method for determining the FPT in foals and we detected that there was no correlation between GGT and other parameters.

In conclusion, the results of this study clearly demonstrate that measurements of sIgG, STP concentration and the GCT duration before and after suckling are useful parameters for detecting FPT in foals. In addition, GCT and STP provide a simple and inexpensive field test. Simultaneously, the results of the present study showed that determination of GGT activity in foals is not a useful method to diagnose FPT for neonatal foals.

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Farklı Dozlardaki Gonadotropinlerin Ovaryum Follikül Sayıları Üzerine Etkisi

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Özet

Çalışmamızın amacı farelere, farklı dozlarda Pregnant Mare Serum Gonadotropin (PMSG) ve takiben Human Chorionic Gonadotropin (hCG) uygulamalarının, ovaryumda gelişmekte olan folikül ve korpus luteum sayılarına olan etkilerinin istatistiksel yöntemler ile belirlenmesidir. Çalışma materyali olarak 7 haftalık 40 adet BALC/c soyu dişi fare kullanıldı. Fareler, rastgele dört gruba ayrılarak, kontrol grubuna, 0,2 ml serum fizyolojik enjekte edildi. Diğer üç gruba subkutan yolla sırasıyla 2,5; 5 ve 7,5 I. U. PMSG hormonu verildi. PMSG enjeksiyonunun izleyen 48'inci saatte, deney gruplarına, PMSG'nin artan dozuna paralel olarak, gruplara sırasıyla 2,5; 5 ve 7,5 I. U. hCG, kontrol grubuna ise 0,2 ml serum fizyolojik subkutan yolla enjekte edildi. Anestezi altında alınan ve tespit edilen ovaryumlara rutin histolojik metodlar uygulanarak; ovaryum foliküllerinin ve korpus luteumların sayılması için Crossmon'ın üçlü boyama tekniği uygulandı. Çalışmamızda 3 farklı dozda yapmış olduğumuz uygulamalarda tüm deney gruplarında foliküler gelişimin kontrole oranla daha fazla olduğu görüldü. Gelişen folikül sayısının I. deney grubunda en çok olduğu sırasıyla III. ve II. deney grubu ile en az kontrol grubunda olduğu saptandı. Ovulasyon açısından korpus luteumlar değerlendirildiğinde en çok III. deney grubu sırasıyla II. ve I. deney grupları bulunurken en az kontrol grubunda olduğu görüldü. Uygulanan protokollerin hepsinde kullanılan gonadotropin dozuna bağlı olarak foliküler gelişimin ve ovulasyonun arttığı saptandı. Sonuç olarak; çalışmada en çok korpus luteum oluşumunun saptandığı III. deney grubunun (7,5 I.U. PMSG ve ardından 7,5 I.U. hCG'nin s.c.enjeksiyonunun) histolojik değerlendirmeler sonucunda, BALC/c soyu dişi fareler için en uygun süperovulasyon protokolü olduğuna karar verildi.

Anahtar Kelimeler: Süperovulasyon, PMSG, hCG, ovaryum, fare.

Abstract

The aim of our study is to determine the effect of PMSG and following hCG administration to seven-week-old mice follicle development and corpus luteum formation by statistically methods. Seven-week-old, fourty BALB/c breed female mice were used in this study. Mice were divided in to four groups randomly, and 0,2 ml buffer saline was injected to control group. PMSG hormone was administrated at doses of 2,5; 5 and 7,5 I.U. respectively to other three groups. hCG was injected 2,5; 5 and 7,5 I.U. subcutaneous doses respectively to the experiment groups after 48 hours later than PMSG administration and 0,2 ml buffer saline was injected subcutaneously to control group. Ovaries were collected and fixed for histological examination and Crossmon's triple staining method was applied for examination of follicle and corpus luteum developments in all experiment groups were more than control group. First experiment group had the most developing follicle number, followed by third and second experiment group respectively and at least control group. When corpus luteums were evaluated in terms of ovulation, it was seen that the third, second and first experiment groups were the most respectively and the control was the least. In all procedures, follicular development and ovulation were increased depending on the applied dose of gonadotropin. As a result of this study; it was decided, third experimental group had the most number of corpus luteum formation (7,5 I.U. PMSG and following 7,5 I.U. hCG subcutanous administration) was the most appropriate superovulation procedure for BALB/c breed female mice.

Key Words: Superovulation, PMSG, hCG, ovarium, mouse.

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Giriş

Deneysel çalışmalarda, özellikle üreme tıbbı, genetik ve toksikoloji alanlarında fare, iyi bilinen bir deney hayvanı olup süperovulasyon protokolleri için başarılı bir modeldir.[1] Süperovulasyon, follikül gelişimi ve ovosit salınımı için hormon tedavilerini içerir.[2,3] Follikül gelişimini teşvik etmek için kullanılan hormonlar; FSH, gebe kısrak serum gonadotropin (PMSG) veya insan menopozal gonadotropindir (HMG).[4,5] PMSG/hCG kombinasyonu ile süperovulasyon, farenin siklus döneminin her aşamasında ovulasyonu uyardığı bilinmektedir.[6,7] Yaş ve fare soyları süperovulasyon protokol sonuçlarını etkileyen önemli faktörler arasındadır.[8,10] Genellikle 3-6 haftalık dişilerden maksimum sayıda yumurta elde edilebilmektedir. Fare soyları, süperovulasyon uygulamalarına yüksek seviyede yanıt verenler (30-50 yumurta/fare), düşük seviyede yanıt verenler (<15 yumurta/fare) olmak üzere iki kategoriye ayrılır. C57BL/6J, BALB/cByJ ve SJL/J soyları yüksek seviyede ovulatuvar iken, A/J, C57/L ve 129/J düşük seviyede ovulatuvar soylardır.[11] Çalışmamızda 7 haftalık yaşta çok sayıda yumurta elde edilebilen BALB/c soyu dişi fareler kullanıldı.[12,13]

Multifolikülasyon ve süperovulasyonu sağlamak amacıyla hipofiz kökenli gonadotropinler; Folikül Uyarıcı Hormon (Follicule Stimulating Hormone-FSH), Lüteinleştirici Hormon (Luteinizing Hormone-LH) ve İnsan Menopozal Gonadotropini (Human Menopausal Gonadotrophin-HMG), plasental kökenli gonadotropinler; Gebe Kısrak Serum Gonadotropini (Pregnant Mare Serum Gonadotrophin -PMSG) ve İnsan Koriyonik Gonadotropini (Human Chorionic Gonadotrophin-hCG), Gonadotropin Salgılatıcı Hormon (Gonadotrophin Releasing Hormone-GnRH) agonisti ile klomifen sitrat, difenileilen türevleri gibi bazı sentetik preparatlar da kullanılmaktadır.[14-17] PMSG, kompleks bir glukoprotein olup PMSG içeren ticari preparatlar yüksek oranda FSH ve takiben LH etkisi gösterirler. FSH aktivitesi ovaryum intersitisyel hücrelerinin gelişmesini, foliküllerin büyümesini ve olgunlaşmasını stimüle eder. LH aktivitesi ise ovulasyonu indükleyerek süperovulasyon oluşturur.[18,19] LH, diğer bir hipofiz hormonu olan FSH ile birlikte, üreme organlarının faaliyetlerini kontrol altında tutar. hCG içeren ticari preparatlar kadınların ve erkeklerin hipofiz bezinde üretilen, LH ile aynı etkileri gösterir.[20] Vücudun yeterli miktarda FSH ve LH üretememesi büyük ölçüde üreme kapasitesinin azalmasına yol açar. Süperovulasyon protokollerinde FSH içeren preparatın (Folligon®-Intervet, Kanada) enjekte edilmesi, oositin olgunlaşmasını, takiben LH içeren preparatın (Pregnyl-Organon, İngiltere) uygulanması ile olgunlaşan oositin ovule olmasını sağlaması nedeniyle oldukça sık kullanılmaktadır.[20]

Bu çalışma farklı hormon dozlarının superovülasyon protokollerine olası etkisini göstermek ve kullanılan dozlardan uygun olanını saptamak için hazırlanmıştır. 7 haftalık farelere, farklı dozlarda PMSG takiben hCG uygulamalarının, ovaryumda foliküler gelişime ve korpus luteum oluşumuna olan etkilerinin istatistiki yöntemler aracılığı ile belirlenmesi amaçlanmaktadır.

Materyal ve Metot

Deney Hayvanları ve Bakım Koşulları

Çalışma Uludağ Üniversitesi Deney Hayvanları Yetiştirme Uygulama ve Araştırma Merkezi ve Uludağ Üniversitesi Veteriner Fakültesi Histoloji ve Embriyoloji Anabilim Dalı laboratuvarında yürütülerek 7 haftalık 40 adet BALB/c soyu dişi fareler kullanıldı. Fareler standart fare yemi ile beslenerek, önlerinde su sürekli bulunduruldu. Çalışmadaki tüm deneysel uygulamalar Uludağ Üniversitesi Hayvan Bakımı ve Kullanımı Komitesi tarafından onaylandı (Karar No: 2012- 02/06).

Deney Planı

Fareler, rastgele dört gruba ayrılarak, tüm deney boyunca 14 saat aydınlık/10 saat karanlık döngüsüne maruz bırakıldı. Östrus döngülerini saptamak amacıyla smear ile proöstrus ve östrus dönemindeki hayvanlar belirlenerek kontrol grubuna, 0.2 ml serum fizyolojik enjekte edildi. Diğer üç gruba subkutan (s.c.) yolla sırasıyla 2.5; 5 ve 7.5 Internasyonal Ünite (I. U.) PMSG (Folligon®-Intervet, Kanada) hormonu (1000 I.U.) verildi. PMSG enjeksiyonunu izleyen 48'inci saatte, deney gruplarına, PMSG'nin artan dozuna paralel olarak, gruplara sırasıyla 2.5 I. U. (I. deney grubu); 5 I. U. (II. deney grubu) ve 7.5 I.U. (III. deney grubu) hCG (Pregnyl-Organon, İngiltere) (l500 I. U./ ml) ; kontrol grubuna ise 0.2 ml serum fizyolojik s.c. yolla enjekte edildi. Deney ve kontrol gruplarından 10'ar farenin hormon enjeksiyonundan önce canlı ağırlıkları ölçüldü (canlı ağırlık I). hCG enjeksiyonunu takiben 17 ile 20. saatlerde servikal dislokasyondan hemen önce canlı ağırlıkları tekrar ölçülerek (canlı ağırlık II) di-etil eter anestezisi altında servikal dislokasyon yapıldı. Takiben süratle ovaryumlar çevresindeki yağ dokusu temizlenip, tartımları yapılarak Bouin tespit solüsyonuna alınarak tesbit edilen her bir farenin sağ ve sol ovaryumları stereo (Nikon SMZ1000, Japonya) mikroskopta incelendikten sonra, rutin histolojik metodlar uygulanarak doku takibi yapıldı. Her bir grupta ovaryumların tam ortasından geçecek şekilde parafin bloklardan 5-6 mikrometre kalınlığında (Leica RM 2155, U.S.A.) kesitler alındı. Her grupta bu ovaryum doku kesitlerine genel yapının gözlenmesi ve follikül sayımlarının yapılması için Crossmon'ın üçlü boyama tekniği[21] uygulandı. Araştırma mikroskobunda (Nikon Eclipse 80i,

Japonya), her gruba ait farelerin ovaryumlarından hazırlanan preparatlarda özellikle oosit çekirdeğinin görüldüğü primer, sekonder ve graaf foliküller ile korpus luteum sayıldı.[22,23]

İstatistiksel Analizler

Deney ve kontrol gruplarına ait farelerin enjeksiyon öncesi (canlı ağırlık I), servikal dislokasyon öncesi canlı ağırlıkları (canlı ağırlık II), ovaryum ağırlıkları ile ovaryumlarda gelişmekte olan foliküller (primer ve sekonder,) graaf folikül ve korpus luteum sayıları istatistiksel olarak değerlendirildi. Tüm veriler SPSS 20.0 programı kullanılarak analiz edilerek enjeksiyon öncesi ve servikal dislokasyon öncesi canlı ağırlıklarının karşılaştırılmasında Wilcoxon Signed Ranks Testi kullanıldı. Gruplar arası fark olup olmadığının belirlenmesinde Kruskal-Wallis Testi yapılarak fark çıkan gruplarda Mann-Whitney U Testi uygulandı[24].

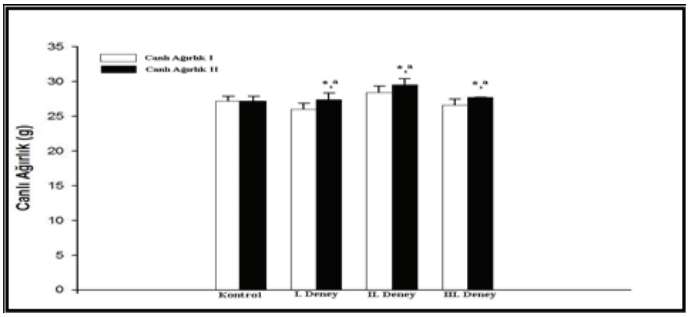
Bulgular

Morfolojik ve İstatistiksel Bulgular

Canlı Ağırlık

Kontrol ve deney gruplarına ait canlı ağırlık I ve II değerleri istatistiki açıdan değerlendirildiğinde, deney grupları kendi aralarında p<0,05 düzeyinde önem gösterirken kontrol grubunda önem görülmemiştir (Şekil 1, Tablo1). Ancak canlı ağırlık II değerlerinde kontrol grubu ile I. , II. ve III. deney grupları arasında p < 0,05 düzeyinde önem saptanmıştır (Şekil 1, Tablo1).

Şekil-1: Kontrol ve deney gruplarına ait farelerin canlı ağırlıkları (g).



* Deney grupları ile kontrol grubu arasında önem, p<0,05
a Deney gruplarında "canlı ağırlık I" değerleri ile "canlı ağırlık II' değerleri arasında önem, p<0,05

Tablo1: Kontrol ve deney gruplarına ait farelerin canlı ağırlıkları (g)

	n	Kontrol Grubu ±SE	I. Deney Grubu ±SE	II. Deney Grubu ±SE	III. Deney Grubu ±SE
Canlı Ağırlık I	10	27.2±0.64	26.0±0.85*	28.4±0.92*	26.6±0.89*
Canlı Ağırlık II	10	27.2±0.67	27.4±0.94*,a	29.5±0.90*,a	27.7±0.01*,a

* Deney grupları ile kontrol grubu arasında önem, p<0,05
a Deney gruplarında "canlı ağırlık I" değerleri ile "canlı ağırlık II' değerleri arasında önem, p<0,05

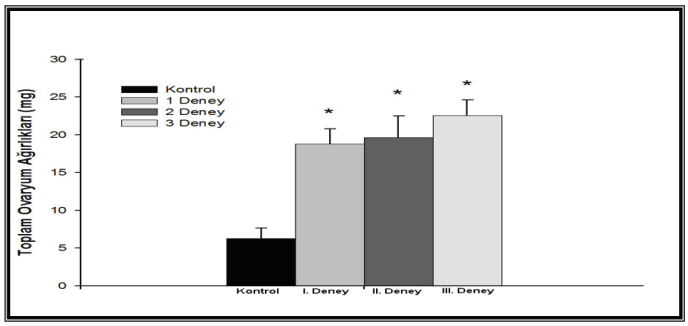
Ovaryum Ağırlığı

Kontrol ve deney gruplarına ait farelerin ovaryum ağırlıkları şekil 2 ve tablo 2'de gösterilmiştir. Deney gruplarında ovaryum ağırlık ortalamaları kontrol grubu değerlerine oranla oldukça fazla bulunmuştur ve gruplar arasında p < 0,05 düzeyinde istatistiki önem saptanmıştır.

Gelişmekte Olan Folikül ve Korpus Luteum Sayıları

Deney gruplarında uygulanan PMSG ve hCG hormonlarına bağlı patolojik bir durum gözlenmemiş olup deneysel uygulamaya bağlı olarak ölüm de görülmemiştir. Kontrol ve deney gruplarına ait ovaryumların makroskopik görünümleri stereo mikroskop (Nikon SMZ1000) şekil 3'de gösterilmiştir. Crossmann'ın üçlü boyama yöntemi(1937) uygulanan, kontrol ve deney gruplarına ait ovaryum kesitlerinde ise primer, sekonder, graaf folikül ve korpus luteum sayılarak tablo 3'de gösterilmiştir.

Şekil-2: Kontrol ve deney gruplarına ait farelerin toplam ovaryum ağırlıkları (mg)



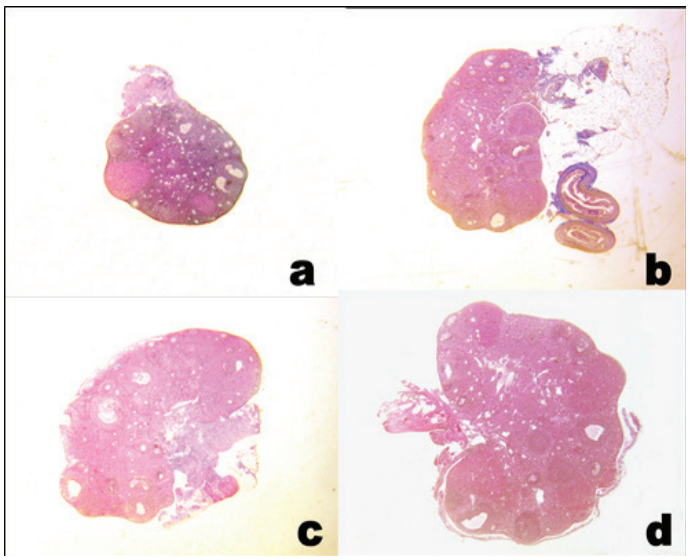
*Deney grupları ile kontrol grubu arasında önem, p < 0,05

Tablo-2 : Kontrol ve deney gruplarına ait farelerin toplam ovaryum ağırlıkları (mg)

	n	Kontrol Grubu ±SE	I. Deney Grubu ±SE	II. Deney Grubu ±SE	III. Deney Grubu ±SE
Ovaryum Ağırlığı	10	6.25±1.37	18.75±2,04*	19.66±2.85*	22.56±2.02*

*Deney grupları ile kontrol grubu arasında önem, p < 0,05

Şekil-3: a)Kontrol b) I.deney c II.deney d) III.deney grubuna ait ovaryumların genel görünümü, üçlü boyama, stereo mikroskop, X 1.5 obj.



Tablo-3: Kontrol ve deney gruplarına ait ortalama folikül, korpus luteum ve gelişen folikül sayısı.

	N	Primer Folikül Sayısı±SE	Sekonder Folikül Sayısı±SE	Graff Folikül Sayısı±SE	Korpus Luteum Sayısı±SE	Gelişen Folikül Sayısı ±SE
Kontrol Grubu	10	20,10±2,63	17,00±2,23	1.00±0,00	11,30±1,56	37,50±3,45
I. Deney Grubu	10	28,70±2,92	21,70±2,78	2,00±0,36	11,50±1,84	52,60±4,83*
II. Deney Grubu	10	26.10±2,40	15,40±2,43	1,25±0,25	12,90±1,60	42,00±2,93
III.Deney Grubu	10	29,90±2,36	12,60±1,24	1,85±0,55	13,20±1,33	43,80±2,51

* I. deney grubu ile kontrol arasında önem, p<0,05

Kontrol Grubu Bulguları

Sadece 0,2 ml serum fizyolojik uygulanan kontrol grubunda, toplam primer folikül sayısının ortalama değeri 20,10±2,63 olduğu, maksimum primer folikül sayısının 36'ya kadar çıktığı, minimum primer folikül sayısının 9 olduğu gözlemlendi. Toplam sekonder folikül sayısının ortalama değerinin 17,00±2,23 olduğu, maksimum sekonder folikül sayısının 29'a kadar yükseldiği, minimum sekonder folikül sayısının 8 olduğu görüldü. Toplam korpus luteum sayısının ortalama median değeri 11,30±1,56 iken maksimum korpus luteum sayısı 20, minimum korpus luteum sayısı 4 olarak tespit edildi. Gelişmekte olan foliküllerin ortalama değeri 37,50±3,45 olarak bulunurken, en düşük ve en yüksek folikül sayısı 23-57 arasında değişkenlik gösterdiği saptandı.

I. Deney Grubu Bulguları

Foliküler gelişmeyi uyarmak ve ovulasyon oluşturmak amacıyla 2.5 I. U. PMSG ve 48. saatte 2.5 I. U. hCG uygulanan I. deney grubuna ait 10 adet farenin ovaryumlarının mikroskobik incelenmesinde, toplam primer folikül sayısının ortalama değeri 28,70±2,92 olduğu, bu grupta maksimum folikül sayısının 44'e kadar çıktığı minimum primer folikül sayısının 10 olduğu gözlemlendi. Toplam sekonder folikül sayısının ortalama değeri 21,70±2,78 olduğu, maksimum sekonder folikül sayısının 37'ye kadar yükseldiği, minimum sekonder folikül sayısının 5 olduğu görüldü. Toplam korpus luteum sayısının ortalama değeri 11,50±1,84 iken maksimum korpus luteum sayısı 24, minimum korpus luteum sayısı 5 olarak tespit edildi. Gelişen folikül sayısının değeri 52,60±4,83 iken, en yüksek gelişen folikül sayısının 75, en düşük 17 olduğu saptandı.

II. Deney Grubu Bulguları

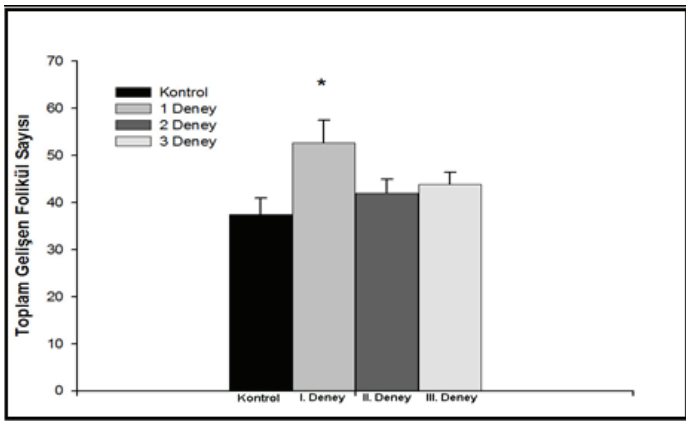
Deri altı yolla 5 I. U. PMSG ve 5 I. U. hCG uygulanan II. deney grubunun toplam primer folikül sayısının ortalama değeri 26.10±2,40 olduğu, bu grupta maksimum primer folikül sayısının 43'e kadar çıktığı, minimum primer folikül sayısının 19 olduğu gözlemlendi. Toplam sekonder folikül sayısının ortalama değerinin 15,40±2,43 olduğu, maksimum sekonder folikül sayısının 28'e kadar yükseldiği, minimum sekonder folikül sayısının 7 olduğu görüldü. Toplam korpus luteum sayısının ortalama değeri 12,90±1,60 iken maksimum korpus luteum sayısının 22, minimum korpus luteum sayısı 5 olarak tespit edildi. Gelişen folikül sayısının değeri 42,00±2,93 iken, en yüksek gelişen folikül sayısının 52, en düşük 26 olduğu saptandı.

III. Deney Grubu Bulguları

En yüksek dozda PMSG ve hCG hormonlarının 7.5 I. U. uygulandığı III. deney grubunda toplam primer folikül sayısının ortalama değeri 29,90±2,36 olduğu, bu grupta maksimum primer folikül sayısının 43'e kadar çıktığı, minimum primer folikül sayısının19 olduğu gözlemlendi. Toplam sekonder folikül sayısının ortalama değerinin 12,60±1,24 olduğu, maksimum sekonder folikül sayısının 28'e kadar yükseldiği, minimum sekonder folikül sayısının 7 olduğu görüldü. Toplam korpus luteum sayısının ortalama değeri 13,20±1,33 iken maksimum korpus luteum sayısının 22, minimum korpus luteum sayısı 10 olarak tespit edildi. Gelişen folikül sayısı ortalama değeri 43,80±2,51 olarak belirlendi. Bu grupta, en yüksek gelişen folikül sayısının 52, en düşük 31 olduğu saptandı.

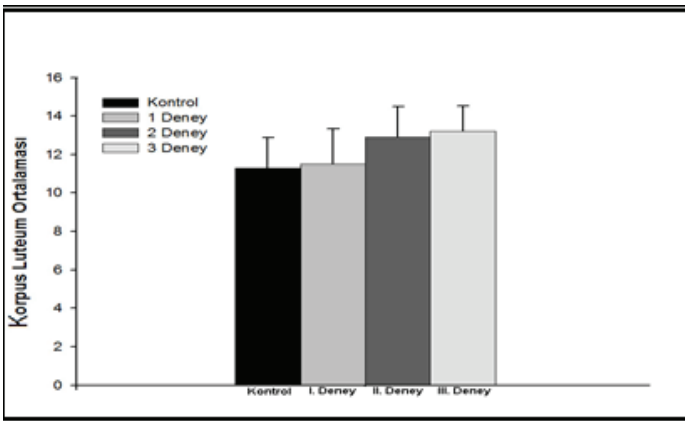
İncelenen preparatlarda gelişmekte olan folikül (primer, sekonder, graaf folikül) ve korpus luteum sayımları istatistiksel olarak değerlendirildiğinde I. deney grubu ile kontrol arasında istatistiki önem bulurken II. ve III. deney grupları ile kontrol grubu arasında istatistiki önem görülmemiştir(-Şekil 4-5). Ortalama değerlere bakıldığında I. deney grubunda folikül gelişiminin en fazla olduğu belirlenmiştir.

Şekil-4: Kontrol ve deney gruplarına ait toplam gelişen folikül sayısı.



I. Deney grubu ile kontrol grubu arasında önem, p<0,05

Şekil-5 Kontrol ve deney gruplarına ait korpus luteum ortalaması.



Tartışma ve Sonuç

Ovaryumdaki foliküllerin gelişmesi, memelilerde üreme fonksiyonunun gerçekleştirilmesinde vazgeçilmez öneme sahiptir. Foliküllerin gelişmesi sırasında bir yandan döllenme yeteneği olan bir ovum üretilirken, diğer yandan da üreme sistemine ait diğer organlarda eş zamanlı değişim ve gelişmeler sağlanır.[25] IVF uygulamalarının hız kazanmasıyla birlikte ovaryumun hormonal yönden uyarılması Üremeye Yardımcı Teknikler (ÜYTE)'in vazgeçilmez bir parçası olmuştur. Ovaryumun uyarılması ile foliküller gelişimin arttırılması hedeflenmektedir. Ayrıca hem in vivo hem de in vitro olarak elde edilen fazla sayıdaki oositlerin döllenme şansı da yüksek olacaktır.[26,27] Yardımcı üreme tekniklerinde süperovulasyon, nesli tükenmekte olan türlerin korunması, transgenesis, nükleer transfer, üreme amaçlı klonlama rejeneratif tıpta, embriyonik kök hücre üretiminde ve klonlamanın korunmasında oldukça önemlidir.[27] Deneysel çalışmalarda, özellikle üreme tıbbı, genetik ve toksikoloji alanlarında fare, iyi bilinen bir deney hayvanı olup süperovulasyon protokolleri için başarılı bir modeldir.[1] Süperovulasyon, folikül gelişimi ve oosit salınımı için hormon tedavilerini içerir.[2,3] Folikül gelişimini teşvik etmek için kullanılan hormonlar; FSH, PMSG veya HMG.4,5 PMSG/hCG kombinasyonu ile süperovulasyon, farenin siklus döneminin her aşamasında ovulasyonu uyardığı bilinmektedir.[6,7] Yaş ve fare soyları süperovulasyon protokol sonuçlarını etkileyen önemli faktörler arasındadır.[8-10] Genellikle 3-6 haftalık dişilerden maksimum sayıda yumurta elde edilebilmektedir. Fare soyları, süperovulasyon uygulamalarına yüksek seviyede yanıt verenler (30-50 yumurta/fare), düşük seviyede yanıt verenler (<15 yumurta/fare) olmak üzere iki kategoriye ayrılır. C57BL/6J, BALB/cByJ ve SJL/J soyları yüksek seviyede ovulatuvar iken, A/J, C57/L ve 129/J düşük seviyede ovulatuvar soylardır.[11] Çalışmamızda 7 haftalık yaşta çok sayıda yumurta elde edilebilen BALB/c soyu dişi fareler kullanıldı.[12,13]

Süperovulasyon uygulaması ile birden fazla ovulasyonun gerçekleşmesi hedeflenir. Bu uygulama sayesinde hastaların büyük çoğunluğuna birden çok embriyo aktarılabilir, ayrıca artan embriyolar süperovulasyon uygulaması yapılmadan tekrar hamilelik elde etmek için dondurularak saklama imkânı da sağlamaktadır.[28,29] Bununla birlikte bu yöntem, oosit olgunlaşmasında, IVF'de, embriyo kültüründe, IVF tedavisi uygulanmış kadına embriyoların aktarılmasında ve implantasyon süreçlerinde yardımcıdır.[30] Bu avantajlarından dolayı IVF'in ilk dönemlerinden beri süperovulasyon uygulaması yöntemi çok önemli bir yer tutmaktadır.[31,32] Çalışmamızda 3 farklı dozda yapmış olduğumuz uygulamalarda tüm deney gruplarında foliküler gelişimin kontrole oranla daha fazla olduğu görüldü.

Lehtonen ve Kankondi[33] gonadotropinlerin intraperitonal uygulamalarının, subkutan uygulamaya göre anormal yumurta oranını artırdığı bildirmişlerdir. Bu bulgular ışığında çalışmamızda s.c. uygulama tercih edilmiş olup anormal yumurta görünümü de saptanmamıştır.

Barnes ve arkadaşları[34] hipofizektomi yapılmış kadınlara dışardan verilen FSH ile folikül gelişimini sağlayarak ovulasyon öncesi aşamaya kadar uyarılabildiğini göstermişlerdir. Stern ve Schuetz[35], süperovulasyon amacı ile gonadotropinle uyarılan farelerde oositlerin ovulasyonunu, iki aşamalı olarak gerçekleştirdikleri protokoller ile değerlendirmişlerdir. Birinci aşamada 5 I.U. PMSG enjeksiyonunu izleyen 20 saat içinde, ikincisi ise 48 saat sonra uygulanan hCG'ye yanıt araştırılmıştır. Farelere enjekte edilen 5 I.U. PMSG enjeksiyonuyla (20 saat içinde) %.77,9 oranında ovulasyon meydana gelmiş ve bu hayvanlardan yumurta kanalında ortalama 8,56 oosit elde edilmiştir. Serum fizyolojik enjekte edilen kontrol grubunda bu oran % 5,5 olup ortalama 7,64 oosit elde edilmiştir. PMSG ve 48 saat sonra uygulanan hCG ile çiftleşme oranlarına bakıldığında serum fizyolojik verilen kontrol grubu ile karşılaştırıldığında, göze çarpan bir artış olmuştur. Sadece PMSG enjekte edilen hayvanlardaki çiftleşme davranışı salin verilen kontrol grubundan farklı bulunmamıştır. Bizim çalışmamızda özellikle korpus luteumu, ovule olan oosit olarak düşünüp değerlendirildiğinde, en yüksek ortalama korpus luteum sayısının 13,20±1,33 değeri ile III. deney grubunda, sonra 12,90±1.60 değeriyle II. deney grubunda, 11,50±1,84 değeri ile I. deney grubunda ve son olarakta 11,30±1,56 değeriyle kontrol grubunda saptadık. Bu da bize süperovulasyon amaçlı olarak belli dozlarda arttırdığımız PMSG hormonunun tedaviye olumlu yönde cevap verdiğini ve artan dozların oosit oluşumuna pozitif etki sağlayabileceğini düşündürmektedir.

Puberteye yakın fareler ile yapılan çalışmalarda, PMSG ve hCG uygulamalarına hayvanların % 95'inin süperovulasyon ile yanıt verdiğini bildirilmiştir.[36,37] Çalışmamızda kullanılan fareler puberte döneminde olup PMSG ve hCG

uygulanan deney grubundaki farelerin tümü süperovulasyona pozitif cevap vermiştir.

Araştırmamızda kullanılan hayvanlar rastgele olarak kontrol ve deney gruplarına ayrılmıştır. Aynı bakım-besleme koşullarında canlı ağırlık artışları 2 günlük kısa bir süre içerisinde özellikle deney gruplarında kontrol grubundan daha fazla bulunmuştur. Aynı şekilde ovaryum ağırlıkları da bu artışa paralellik göstermektedir. Çalışmamızda da 7 haftalık puberte dönemindeki farelerde, kontrol ve deney grupları, ovaryum ağırlıklarına göre kıyaslandığında, deney gruplarında istatistiki olarak da önem gösteren belirgin bir artış saptanmıştır. Çalışmamızdaki bulgulara paralel olarak Neal ve Challoner[37] uygulanan gonadotropinlere bağlı olarak antral foliküllerin ve eş zamanlı olarak ovaryum ağırlığının kademeli olarak arttığını bildirmişlerdir.

Sonuç olarak bu çalışmada en çok korpus luteum oluşumunun saptandığı 3.deney grubunun (7,5 I.U. PMSG ve ardından 7,5 I.U. hCG'nin s.c. enjeksiyonunun) histolojik değerlendirmeler sonucunda, en uygun süperovulasyon protokolü olduğuna karar verilmiştir.

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Survey of Ochratoxin a in Coffee, Dried Grapes and Grape Pekmez Samples in Burdur, Turkey

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Abstract

The aim of this study was to investigate the occurrence and levels of ochratoxin A (OTA) in coffee (roasted and instant), dried grapes and grape pekmez samples consumed in Burdur city markets. During 2015, a total of 86 samples including 43 coffee (30 instant coffee and 13 roasted coffee), 17 dried grapes and 26 grape pekmez were randomly collected from different markets of Burdur. The occurrence and contamination levels of OTA in the samples were investigated by the competitive enzyme-linked immunoabsorbent assay (ELISA) method. OTA was detected in 24 (55 %) coffee samples (13 roasted coffee and 11 instant coffee samples) and in 1 (3 %) grape pekmez samples. The range OTA levels were 8.34 and 22.54 µg/kg in coffee samples and 20.48 µg/kg in one grape pekmez sample, respectively. The highest recorded OTA concentration was 22.54 µg/kg in instant coffee. Furthermore, 13 roasted coffee, 11 instant coffee and 1 grape pekmez samples were contaminated at levels above the Turkish legal limits of 5 µg/kg,10 µg/kg and 2 µg/kg, respectively. In contrast, OTA was not detected in all dried grape samples. It is concluded that the occurrence of OTA, coffee samples, in particular may be considered as a possible hazard for public health.

Keywords: Ochratoxin A, Coffee, Dried Grapes, Grape Pekmez

Introduction

Mycotoxins are naturally occurring toxins produced by flamentous fungi. So far, more than 300 mycotoxins have been isolated and identified. One of the mycotoxins that cause harmful effects in humans and animals is ochratoxins.[1] Ochratoxins are a group of mycotoxins produced as secondary metabolic products mainly by some species of *Aspergillus* and *Penicillium*.[2] Ochratoxin A (OTA) is the most commonly found in foods and feeds among ochratoxins, and it is considered to be the most toxic compound of them.[3] Chemically, OTA is a chorophenolic compound in which a dihydroisocumarin component is joined to L-phenylalanyl in an amide-linkage.[4] This mycotoxin was reported in 1965 by van der Merwe et al.[5] from maize based products contaminated with *Aspergillus ochraceus* and in 1974 was found coffee.[6] In 1987, OTA was also reported in commercial roasted coffee.[7]

OTA is a secondary metabolite produced mainly by *Penicillium verrucosum* and *Penicillium nordicum*, and several species of the genus *Aspergillus*, such as *A. ochraceus, A. niger, A. carbonarius, A. sulphureus and A. sclerotiorum*.[8] OTA is frequently found a wide variety of food commodities including cereals (wheat, barley, rice, sorghum), cereal-derived products, bread, dried fruits, coffee, coffee beans, chocolate, beer, cacao, wine, grape juice, spices,

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beer and products animal origin.[9-11] OTA has been detected in green coffee beans,[12-15] roasted coffee,[16,17] instant coffee,[3,17-24] grape[10,25-29] and grape pekmez.[10,30,31]

The presence of OTA in human blood has been suggested as a contamination risk indicator. The results of analyses of human serum samples have demonstrated wide and continued OTA exposure through the ingestion of contaminated foods.[3]

OTA has received increasing interest from the scientific community on food.[32] The International Agency for Research on Cancer has classified OTA in the group 2B of substances as a possible human carcinogen.[33] OTA has been shown to be nephrotoxic, hepatotoxic, genotoxic, teratogenic, fetotoxic and immunosuppressive in several animal species.[3,11] The most important toxic effect of this mycotoxin is its nephrotoxicity.[9] It may be associated with Balkan Endemic Nephropathy, a chronic kidney disease, and the development of urinary tract tumors in humans.[3] For this reason, many countries and international organisations have regulations to control OTA in commodities and food. The European Commission[34] have established regulations for OTA in roasted coffee beans (5 µg/kg), instant coffee (10 µg/kg), dried grapes (10 µg/kg), grape juice and concentrated grape juice (2 µg/kg). The Turkish of Ministry of Food, Agriculture and Livestock adopted the EU levels of OTA in food and food stuffs.[35]

The occurrence of OTA in coffee beans can be due to both environmental conditions and processing conditions. OTA present before storage, indicates the possibility that harvesting and post-harvest handling of coffee cherries could be the critical steps leading to contamination.[36] OTA is found at stages of coffee production and processing in cherry, green coffee, roasted coffee[37.]

The aim of this study was to assess the occurrence and levels of OTA in different food samples consumed in Burdur, a western city of Turkey.

Materials and Methods

Materials

In 2015, a total of 86 samples including 43 coffee (30 instant coffee and 13 roasted coffee), 17 dried grapes and 26 grape pekmez were randomly collected from different markets of Burdur. All these samples were stored at 4 °C in a dark and dry place until analysis.

Method

The quantitative analysis of OTA in the samples was performed by competitive enzyme-linked immunosorbent assay (ELISA) method according to the procedure described by Helica Biosystems Inc. USA (Helica Biosystems Inc, Ochratoxin A Cat No.: 961OC01COF).

Preparation of samples was conducted according to the instructions of the HELICA kit (Helica Biosystems Inc. USA). The samples (1 g each) were diluted in 50 ml of deionised water and were stirred for 5 min. Afterwards, the extracts were diluted 10:1 with 70 % methanol. An aliquot of this solution was used in the test.

Two hundred µL of the assay diluent into each mixing well was added. Then, that 100 µL standard solutions and prepared samples in separate wells were added to each well mixed by priming pipettor at least 3 times. One hundred µL of contents from each mixing well were transferred antibody coated well and incubated at room temperature for 30 minutes. At the end of incubation, the liquid in the wells was poured out, the microwell holder was tapped upside down, and an absorbent paper was used to remove the reminder of the liquid. The wells were washed three times with PBS-Tween washing buffer. After washing steps, 100 µL of the conjugate was added to the wells and incubated for 30 min at room temperature in the dark. At the end of incubation, the wells were washed three times with washing buffer. Then, 100 µL of substrate reagent was added to each well and mixed thoroughly and incubated for 10 min at room temperature in the dark. Following this step, 100 µL of the stop solution was added to each well and mixed. The absorbance was measured at 450 nm by an ELISA (ELX-800, Bio-Tek Instruments Inc., Winooski, VT, USA) against air blank within 15 min.

The samples were evaluated according to the computer program, prepared by Helica Biosystems Inc. The levels of aflatoxin standards used were 0, 0.02, 0.05, 0.1, 0.2 and 0.4 µg/L. The detection limit of this ELISA method was 1 ng/L.

Results

The occurrence and distribution of OTA in coffee (roasted and instant), dried grapes and grape pekmez samples were presented in Tables 1 and 2, respectively. Although OTA was detected in 24 (55.8 %) coffee samples (13 roasted coffee and 11 instant coffee samples) in concentrations ranging from 8.34 to 22.54 µg/kg (mean level: 17.76 ng/L) and only 1 (3 %) grape pekmez sample in concentration at 20.48 µg/kg. The highest recorded OTA concentration was 22.54 µg/kg in instant coffee. In addition, 3 roasted coffee, 11 instant coffee and 1 grape pekmez samples were contaminated at levels above the Turkish legal limits of 5 µg/kg,10 µg/kg and 2 µg/kg. In contrast, OTA was not founded in all dried grape samples.

Table 1. Occurrence of OTA in coffee, grape pekmez and dried grapes samples.

Samples	Tested *n*	Positive *n* (%)	Contamination (µg/kg)		Exceed regulation[a]
			Range	Mean±SD[b]	*n* (%)
Instant coffee	30	11 (36.6)	14.01-22.54	18.42±2.97	11 (36.6)
Roasted coffee	13	13 (100)	8.34-18.54	12.66±3.19	13 (100)
Grape pekmez	26	1 (3.8)	20.48	20.48	1 (3.8)
Dried grapes	17	-	-	-	-

[a] The Turkish limits for OTA are 5, 10 and 2 µg/kg for roasted coffee, instant coffee and grape pekmez.
[b] SD: Standart deviation

Table 2. Distribution of OTA in coffee and grape pekmez samples.

Samples	Distribution of samples (µg/kg) *n* (%)				
	<1[a]	1-5	6-10	11-20	>20
Instant coffee	19 (63,3)	-	-	7 (23,3)	4 (13,3)
Roasted coffee	-	-	3 (23,1)	10 (76,9)	-
Grape pekmez	25 (96,2)	-	-	-	1 (3,8)

[a] Distribution of negative samples.

Discussion and Conclusion

OTA is a nephrotoxic and nephrocarcinogenic mycotoxin produced by *Penicilium* in temperate climates and by the species of *Aspergillus* in warmer climates.[3]

In this study, OTA was detected in 24 of 43 (55.8 %) coffee samples at levels ranging from 8.34 to 22.54 µg/kg. The concentrations of OTA were 14.01-22.54 µg/kg (mean level:18.42 µg/kg) in instant coffee samples and 8.34-18.54 µg/kg (mean level: 12.66 µg/kg) in roasted coffee samples. Also, 13 roasted coffee and 11 instant coffee samples were contaminated at levels above the Turkish legal limit 5 µg/kg and 10 µg/kg, respectively. In this study, OTA was found in all of the roasted coffee samples and it was determined that the total of the samples exceeded the Turkish legal limit. However the highest recorded OTA concentration was 22.54 µg/kg in instant coffee. Many studies have been conducted about the existence of OTA in various coffee samples in countries in the literature.[1,3,11,17-24,38,39] In England, Pittet et al.[18] analysed 101 instant coffee samples and detected OTA 0.2-6.5 µg/kg in 75 (74.3 %) samples and Patel et al.[19] examined instant coffee samples and found in 64 of 80 samples contaminated with OTA in concentrations of 0.1-8.0 µg/kg. In Brazil, Leoni et al.[20] observed that in all of 16 instant coffee samples, OTA was detected in a concentration 0.5-5.1 µg/kg; Prado et al.[21] analysed 37 samples of instant coffee and the OTA in 31 (83.8 %) of the samples ranged from 0.31 to 1.78 µg/kg and de Almeida et al.[3] observed that in 81 of 82 (98.8 %) coffee samples in Brazil, OTA was detected in a concentration range of 0.17-6.29 µg/kg. In Canadian study, Lombaert et al.[38] analysed 30 samples of instant coffee and the OTA in 20 of the samples ranged from <0.1 to 3.1 µg/kg. In Japan, Kawamura[22] evaluated the occurrence of OTA in 12 samples at levels of 0.11-4.41 µg/kg; Tabata et al.[23] found the toxin in 5 out of 7 coffee samples and contained in the range of 0.16-1.1 µg/kg and Aoyama et al.[24] detected OTA in 90 % of 63 samples ranged from 0.1-4.23 µg/kg. In Italy, Vecchio et al.[1] observed OTA occurrence at levels between 0.32 to 6.40 µg/kg in 48 out 50 analyzed instant coffee samples. In Argentina, OTA was detected in 35 of 51 coffee samples at levels 0.11-20.30 µg/kg.[39] In Chile, Galarce-Bustos et al.[17] evaluated the occurrence of OTA from 63 samples of coffee (24 roasted and 39 instant coffee). All of the roasted and instant coffee samples were contaminated with OTA at range of 0.30-0.84 µg/kg and 0.28-5.58 µg/kg, respectively. In Ivory Coast, OTA was found in all of the coffee samples and contained in the range of <5-12 µg/kg.[11] In this study, the concentration levels of OTA in coffee samples were higher than results reported in England, Canada, Italy, Brazil, Argentina, Chile, Japan and Ivory Coast.[1,3,11,17-24,38,39] Higher OTA content of coffee in Burdur could be due to

a number of reasons: OTA-producing, microorganisms could be more or hygenic conditions of coffee productions and storage could be poor or our detection method could be sensitive or false.

Some studies have been carried out in Turkey, [10,26,27] Argentina, [25,28] Iran[40,41] and Greece[29] with regard to occurrence of OTA in grapes. In this study OTA was not founded in none of dried grape samples. The results of the present study were not in agreement with the grape-OTA results obtained by other researchers.[10,25-28,40] In an earlier survey of grapes in Turkey Aksoy et al.[26] reported that 1712 processed sultana grapes samples out of 1885 (90.82 %) were contaminated with OTA levels between 0.02 and 10 µg/kg and however only 0.6 % of them exceed the EU level. In addition, Bircan[27] found OTA in 28 (53 %) of 53 samples ranged from 0.51-58.04 µg/kg, 2 samples to be contaminated above 10 µg/kg. Another study carried out in Turkey, Akdeniz et al.[10] detected OTA in 8 % of 50 dried grapes samples ranged from 0.19-2.59 µg/kg (mean level 1.15 µg/kg). In Argentina, Magnoli et al.[25] found OTA in 37 of 50 (74 %) dried vine fruit samples with levels of 1.4-14 µg/kg and Ponsone et al.[28] detected OTA in 9 out of 15 (60 %) dried wine fruits samples ranging between 0.26-20.28 µg/kg. In Iran, Rahimi and Shakerian[40] revealed 17 (44.7 %) of 38 dried grapes samples contain of OTA with a range of 2.9-18.2 µg/kg and, Heshmati and Nejad[41] detected 39 out of 66 (59 %) to be contaminated with OTA in concentrations of >0.16-8.4 µg/kg (mean level 2.98 µg/kg). Also Hesmati and Nejad[41] reported that the levels of OTA in 5 dired grapes samples were above the maximum tolerance accepted by the national standard levels of Iran (5 µg/kg). In Greece, OTA was found in 100 % of dried grapes samples (n: 26), and contained in the range of 2.8 to 138.3 µg/kg and 18 samples to be contaminated above the EU regulation limit for OTA[29]. None existence OTA in Burdur dried grape samples could be due to better hygenic conditions in Burdur.

There are few studies on the occurrence for OTA in grape pekmez. In this study, OTA was detected in 1 of 26 (3.8 %) grape pekmez samples at level 20.48 µg/kg. This one OTA contaminated grape pekmez sample has a level above the Turkish legal limit 2 µg/kg. In an earlier survey of grape pekmez in Turkey, Arici et al.[30] found in the grape juices produced from mouldy grapes contaminated between 2.1-9.8 µg/l with OTA, was also used in pekmez production. They reported that the levels of OTA in pekmez samples were detected to be 5-6 times higher than OTA amounts of grape juice. Similarly, Akdeniz et al.[10] found OTA in 23 of 25 grape pekmez samples ranged from 0.44-5.32 µg/kg and 12 samples exceed the EU level (2 µg/kg). Futhermore, Tosun et al.[31] detected OTA in 37 out of 82 grape pekmez samples and contained in the range of 2-31.2 µg/kg. In this study, the incidence of OTA in grape pekmez samples was lower than the studies above. However, the level of OTA in our one OTA contaminated pekmez sample was higher than the results reported by Arici et al.[30] and Akdeniz et al.[10], but were lower than the results reported by Tosun et al.[31]

The results of this study revealed that the incidence and levels of OTA in coffee samples were a serious public health hazard. In contrast, the occurrence of OTA in dried grapes samples was not detected and only one samples of grape pekmez was exceeded the legal limit. Although the number samples analyzed is limited, the fact that OTA was detected in 24 (55.8 %) of 43 coffee samples at a level of (8.34-22.54 µg/kg) indicated that this may be serious issue. These OTA levels in coffee samples of Burdur were above the maximum limit of the EU. Therefore, the occurrence of OTA in more coffee samples should be carried out by authorities and industries to safeguard human health.

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Screening of Veterinary Growth-Promoting Agent and Antibacterial Residues in Beef Cattle and Broiler Meats Consumed in Bursa, Turkey

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Abstract

This study was aimed to determine residues of growth-promoting agents and some antibacterials in beef cattle and broiler meats consumed in Bursa, as well as to evaluate their hazards on public health. A total of 45 meat samples consisting of 36 beef cattle meat and 9 broiler meat samples were collected from supermarkets and butchers between November and December in 2016. The analysis was carried out by biochip array-based immunoassay technique. This system is also currently used for simultaneous detection and quantitation of different anabolics consisting of β-agonists, boldenone, corticosteroids, nandrolone, ractopamine, stanozolol, stilbenes, trenbolone and zeranol, and six group of antimicrobials consisting of quinolones, cephalosporins, amphenicols, aminoglycosides, macrolides and tetracyclines. Although residues of growth-promoting agents could not be detected in any of the samples, antimicrobial residues from all groups were detected in 10 beef cattle meat samples and tetracycline residues were detected in two broiler meat samples at various levels. In conclusion, there is no risk to consumers for growth-promoter residues according to the results. The detected antibacterial levels were generally lower than hazardous concentrations of residue. However, some detected levels for quinolone, amphenicol, macrolide and tetracycline groups in beef meat samples, and detected concentrations for tetracycline group in two broiler meat samples exceeded the maximum residue limits, and could pose a risk for public health.

Keywords: Growth-promoting agents, antibacterials, biochip array-based immunoassay, beef cattle and broiler meat, residue analysis.

Introduction

Veterinary drugs are being extensively used in livestock for the treatment and prevention of diseases and to improve feed efficiency and promote growth. As a main group of veterinary drugs, antibacterials are mainly used for treatment of infectious diseases in animals. Antibacterials and hormonal substances can also be used legally or illegally for the growth promotion in food producing animals. When the veterinary drugs are being misused or abused in food-producing animals, the drug residues in edible products could lead to health hazards such as allergic reactions and the development of resistant bacterial strains. Hormonal substances may also cause cancer in humans. Hormones or their metabolites could be discharged by excretion into water with effects on environmental pollution, which can lead to hermaphroditism in wildlife.[1-3] Furthermore, some cases by consumption of lamb and bovine meat containing residues of clenbuterol resulted intoxications in humans in different countries with symptoms such as gross tremors of the extremities, tachycardia, nausea, headaches and dizziness.[4-9] In addition, the consumption of trace levels of antibacterial residues in foods of animal origin may have consequences on the indigenous human

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intestinal microflora which constitutes an essential component of human physiology. In view of all these circumstances, foods of animal origin must be monitored for the presence of drug residues.[10]

Simultaneous analysis of different groups of antimicrobials and growth promoters is a difficult task but highly desirable in diagnostic laboratories. A biochip array-based immunoassay test (Antimicrobial Array II, Growth Promoter Agents, Randox Laboratories Ltd., Crumlin, UK) can quantitatively analyse different groups of antimicrobials and growth promoters simultaneously in selected matrices. This apparatus was recently developed and has been used for analysis of foods of animal origin. The test can be used to simultaneously quantify multiple analytes from a single sample.[11,12] Biochip array-based immunoassay technique is a screening test method and was approved by Bornova Veterinary Control Institute (İzmir, Turkey) for antimicrobial array-I, II, III, IV and beta lactam kits in 2015. Many countries have regulated the use of antibacterials as well as growth-promoting agents to prevent hazardous effects on human health. The Codex Alimentarius Commission of the Food and Agriculture Organization, the World Health Organization (WHO)[13] and the European Community (EC)[14] have set maximum residue limits (MRLs) for veterinary drugs used in edible products of food-producing animals. Growth promoters use in food-producing animals has been prohibited by the European Union (EU) by Council Directive 96/22/EC and has also been prohibited in China in 2002 and in Turkey in 2003.[15-17] On the other hand The Codex Alimentarius International Food Standards[13] allows the use of naturally occurring growth promoters and synthetically derived hormones for animal production.

Some news related with beef cattle and broiler meats containing residues of growth-promoting agents and antimicrobials have appeared in visual and written media in Turkey recently. There is not enough data about beef cattle and broiler meats containing residues of growth promoters and antibacterials. Therefore, objectives of this study were to determine residues of antibacterial and growth-promoting agents in beef cattle and broiler meats consumed in Bursa and to evaluate their risks on public health.

Material and Methods

Sample Collection

A total of 45 samples consisting of 36 beef cattle meat samples (18 from different supermarkets and 18 from butchers) and 9 broiler meat samples (9 from different supermarkets) were randomly collected as customer in November and December of 2016 in Bursa. Each sample was 100 to 200 grams and was brought to the laboratory for analysis in cold chain. Samples were kept frozen at -20 °C until nalysis.

Growth Promoter Multiple Matrix Screen (GPMMS)

Biochip array analysis steps were carried out according to the GPMMS EV 3726 manufacturer's instructions.[18] GPMMS kit quantitatively analyse different groups of growth-promoting agents including β-agonists (clenbuterol, carbuterol, brombuterol, salbutamol, methly-clenbuterol, cimbuterol, terbutaline etc.), boldenone (17β-boldenone, 1,4-androstadiene-3, 17-dione and 17α-boldenone), corticosteroids (dexamethasone, flumethasone, betamethasone, dexamethasone 21 acetate and betamethasone 21 acetate), nandrolone (19-nortestosterone (17β), trenbolone, trenbolone acetate, 19-nor-4-androstene,3,17-dione, 19-nortestosterone (17α) and 19-nortestosterone (17β) sulphate), ractopamine (ractopamine and ractopamine hydrocloride), stanozolol (stanozolol and 16β-hydroxystanozolol), stilbenes (hexestrol, diethylstilbestrol and dienestrol), trenbolone (trenbolone (17β) and trenbolone (17α)) and zeranol (zeranol and α-zearalenone) individually or in groups in selected matrices. Immunoaffinity column, column wash buffer, assay diluent, working strength conjugate, working strength reagent were supplied by the manufacturer of the kits. Assay ranges were between 0-5 µg/kg for β-agonists, boldenone, corticosteroids, ractopamine, stanozolol, stilbenes, zeranol and 0-4.5 µg/kg for trenbolone, 0-7 µg/kg for nandrolone and 0-11.5 µg/kg according to GPMMS EV 3726.

Antimicrobial Array-II (AM-II)

Biochip array analysis steps were carried out according to the AM-II EV 3524 manufacturer's instructions.[19] AM-II kit quantitatively analyse different groups of antibacterials including quinolones (norfloxacin, enrofloxacin, ciprofloxacin, oflaxacin, enoxacin and danofloxacin, etc.), ceftiofur, amphenicols (florfenicol and tiamphenicol), streptomycin (streptomycin and dihydrostreptomycin), macrolides (tylosin and tilmicosin) and tetracyclines (tetracycline, 4-epitetracycline, rolitetracycline, 4-epioxytetracycline, oxytetracycline, chlortetracycline, demeclocycline, doxycycline and 4-epichlortetracycline) individually or in groups, simultaneously. Assay diluent, working strength conjugate, working strength reagent were supplied by the manufacturer of the kits. Assay ranges were between 0-7 µg/kg for ceftiofur, 0-5 µg/kg for florfenicol and tylosin, 0-75 µg/kg for streptomycin, and 0-2.5 µg/kg for tetracycline according to AM-II EV 3524.

Three steps immunoaffinity column procedure are available for growth-promoting agents' extraction, consisting of pre-column, column and post-column. In the pre-column step the sample is run through a series of extraction steps to prepare for the column procedure. Column is the

step where the sample is free of impurities and the eluent is obtained. Post-column is the last step where the sample is prepared for the biochip array-based immuonoassay test procedure.[18]

AM-II Extraction Procedure

Nine ml of working strength wash buffer was added to one g of homogenised tissue sample by ultrathorax (Janke and Kunkel Ika-werke, Germany) and mixed by vortex (Boeco Vortex, V1 plus, Germany) for 30 seconds and centrifuged (Sigma, 2-16K, Germany) for 10 minutes at 400 rpm at room temperature. Two hundred µl of supernatant was collected and diluted with 200 µl of working strength wash buffer. Then, the extract was ready for biochip array test procedure.[19]

Biochip Array-Based Immunoassay Test Procedure

Biochip array analysis steps were the same for GPMMS and AM-II. Biochips were equilibrated to room temperature for approximately 30 minutes. After extraction, 100 µl of "assay diluent" was pipetted into the biochip wells. One hundred µl of calibrator or sample was pipetted into the wells. Biochips were incubated at 25°C and 370 rpm for 30 minutes in a thermoshaker (Randox Laboratories Ltd., Crumlin, UK). One hundred µl of working strength conjugate was pipetted into the wells. Biochip wells were incubated at 25°C and 370 rpm for 60 minutes in the thermoshaker. Reagents were discarded to the waste container. Two quick wash cycles were immediately carried out with "diluted wash buffer". Four additional wash cycles were used, then biochips were left to soak in wash buffer for 2 minutes. After the final wash, 250 µl "working signal reagent-EV 805" was added to each well and covered to protect from light in the thermoshaker. After two minutes, the carrier was placed into the Evidence Investigator (Randox Laboratories Ltd., Crumlin, UK). Captures of images were automatically initiated as defined by the dedicated software. Dilution factor was 2.5 for GPMMS and 20 for AM-II.

Results

Biochip array-based immunoassay technique is a screening test and the detected growth promoter and antibacterial levels are different for each substance due to sensitivity differences (%) of the substances according to the method. The analysis system detect the results for a substance for each group that has 100% sensitivity as shown as bold in Table 1. Therefore, equals of a detected other active substance levels for sensitivity of % and positive samples results are presented in Table 1. As a result, we can calculate substance concentrations in a group for positive samples depends on the percent (%) sensitivity and used active substance in veterinary medicine in Turkey. Therefore, some positive results were determined for main molecule by the instrument, and suspicious results should confirm by Liquid Cromatography systems as much as possible.

Growth-promoting agents' residues were not detected in any of the samples. However, antibacterial residues were detected in 10 of 36 beef cattle meat samples (27.7%) and in two of nine broiler meat samples (22.2%). Six antibacterial group residues were detected in 10 beef cattle meat samples and tetracycline residues were only detected in two broiler meat samples. Nine samples were from supermarkets and three samples were from butchers. In beef cattle meat samples, seven positive samples of 18 samples (38.8%) were from markets and three positive samples of 18 samples (16.6%) were from butchers (Table 1).

Discussion

Food safety is important for public health due to outbreaks of meat, liver and offal poisonings with growth promoter residues.[4-9,20-22] and presence of anabolic and antibacterial agents' residues.[23-27]

The use of veterinary medicinal products within the EC is governed by directives and regulations that describe the requirements for application, safety, quality, and efficacy of these products. In Turkey, regulations for veterinary medicinal products are harmonious with European Community directives. The residue of veterinary drugs in meat and related products should be monitored by government authorities and related sections of universities in Turkey.

Any residues of growth-promoting agents were not detected either in beef cattle or broiler meat samples analysed in this study. These results are totally safe for public health for the anabolic agents' residues in beef cattle and broiler meats sold in Bursa. In Turkey, growth promoter substances are forbidden in food producing animals for the growth promotion of livestock, and the detected results can be due to consciousness of veterinarians and management of farms for drug residues and related regulations, and sensitivity of the consumer in Turkey. However, although clenbuterol use as a growth-promoting agent is forbidden, there have been various intoxications caused by the ingestion of liver, meat and offal containing clenbuterol residues in Spain[4,7], France[5], Italy[6,8,21], China[22] and Portuga[l9]. In previous studies in Turkey, although Akkaya et al.24 found higher levels of diethylstilbestrol (DES) (e.g. 1500 ng/kg), zeranol (e.g. 2500 ng/kg), ostrediol (e.g. 1500 ng/kg) and clenbuterol (e.g. 2500 ng/kg) residues in broiler meats, Oruç et al.[25], Mor et al.[28], and Sever et al.[29] detected lower concentrations of zeranol, DES and trenbolen residues in beef cattle meats. Quinolon group was detected a beef cattle meat sample

Table 1. Analysis results of positive samples (µg/kg).

AM-II Groups	Number of Positive Samples		% Sensitivity of Antimicrobial Substances		Positive Sample Results				MRL for Cattle and Chicken Muscle	
					Beef		Broiler			
	(M)	(B)			(M)	(B)	(M)	(B)	Turkey and EU	Codex
QNL	1	-	Norfloxacin	**%100**	**M1: 78.5**	-	-	-	-	-
			Enrofloxacin	%76	M1: 103,3	-	-	-	100	-
			Danofloxacin	%20	M1: 392,8	-	-	-	200	200
CEF	2	2	Ceftiofur	**%100**	**M1: 64.8 M2: 66.0**	**B1: 65.7 B2: 65.0**	-	-	1000	1000
TAF	1	-	Florfenicol	**%100**	**M1: >92**	-	-	-	200	-
			Tiamphenicol	%53	M1: >173,5	-		-	50	-
STR	1	-	Streptomycin	**%100**	**M1: 251.7**	-	-	-	500	600
			Dihydrostreptomycin	%182	M1: 138.3	-	-	-	500	600
TYL	1	-	Tylosin	**%100**	**M1: 72.5**	-	-	-	100	100
			Tilmicosin	%37	M1: 196.1	-	-	-	75	100
TCN	3	1	Tetracycline	**%100**	**M1: >57.6**	**B1: 52.2**	**M2: 49.8 M3: 50.9**	-	100	200
			Oxytetracycline	%52	M1: >110,7	B1: 100.4	M2: 95.8 M3: 97.9	-		
			4-Epioxytetracycline	%52	M1: >110,7	B1: 100.4	M2: 95.8 M3: 97.9	-		
			Doxycycline	%23	M1: >250,4	B1: 227.0	M2: 216.6 M3: 221.3	-		

QNL: Quinolones, CEFT: Ceftiofur, TAF: Thiamphenicol, STR: Streptomycin, TYL: Tylosin, TCN: Tetracycline, (-): Negative, M: Market, B: Butcher.

obtained from market. Among the detected quinolone antibacterial residues, enrofloxacin and danofloxacin are used for cattle in Turkey and both of their levels (103.3 µg/kg and 392.8 µg/kg, respectively) exceed MRL of Turkish, EU (100 µg/kg and 200 µg/kg, respectively) and Codex Alimentarius MRL (Table 1). Especially, detected level of danofloxacin may be a problem for public health. Ceftiofur residues were detected in four samples, but none of them

exceeded the MRL (Table 1). For amphenicols, there was a positive sample and the detected level (>92 µg/kg) was above the assay range of the system. This result may exceed the MRL of 200 µg/kg for florfenicol and could have risk for public health (Table 1). Tiamphenicol evaluation was not done as this substance is not used systemically in beef cattle in Turkey. Detected streptomycin (251.7 µg/kg) and dihydrostreptomycin (138.3 µg/kg) concentrations did not exceed the MRL of 500 µg/kg. Tylosin and tilmicosin from macrolides, were detected in a sample. Tylosin level (72.5 µg/kg) did not exceed the MRL of 100 µg/kg, although tilmicosin level (196.1 µg/kg) exceeded the MRL of 75 µg/kg. Tilmicosin residue level could include risk for public health (Table 1). The detected tetracycline group level (52.2 µg/kg) in a positive sample collected from a butcher exceeded the MRL of 100 µg/kg for oxytetracycline (100.4 µg/kg) and 4-epioxytetracycline (100.4 µg/kg) and doxycycline (227.0 µg/kg). However, concentration of another positive sample (>57.6 µg/kg) supplied from market was above the assay range of the system. This result may exceed the MRL of 100 µg/kg for oxytetracycline (>110.7 µg/kg) and 4-epioxytetracycline (>110.7 µg/kg), and would exceed for doxycycline (250.4 µg/kg). These results could have a risk for public health.

Two broiler meat samples were positive from nine samples and tetracycline residues were the only detected residues. The detected tetracycline group concentrations were 49.8 and 50.9 µg/kg in positive samples collected from markets and did not exceed the MRL of 100 µg/kg for oxytetracycline (95.8 µg/kg and 97,9 µg/kg) and 4-epioxytetracycline (95.8 µg/kg and 97,9 µg/kg). However, doxycycline levels (216.6 and 221.1 µg/kg) exceed the MRL of 100 µg/kg (Table 1). Doxycycline is extensively used in broilers for antibacterial therapy in Turkey. If this broilers treated with doxycycline, the detected levels could have risk for public health. Two doxycycline results in this study could exceed the MRL of Codex Alimentarius and similarly MRL of EU (Table 1). In Turkey, detected positive antibacterials are commonly used in beef cattle and broilers and determination of antibacterial residues is ordinary. However, when the food producing animals are treated with antibacterials and the withdrawal time of the used drug is not considered, drug residues can be found in meats above the MRL. Higher concentrations above the MRL observed in this study may be due not considering the withdrawal time or applying higher doses of antibacterials in beef cattle and broiler. In previous studies[27,30-31], antibacterial residue levels were generally lower than MRL in Turkey. However, some residue results in cattle and sheep meats could exceed MRL[26], such as our results. In previous studies in Oman[32] and Bangladesh[33] in broiler and other meats, antimictobial residues were detected under the MRL. When the results from the market and butchers were considered, although the number of beef cattle meat samples from markets and butchers were same (n=18) positive samples (38.6%) from markets were higher than positive samples from butchers (16.6%).

In conclusion, growth-promoting agents' residues were not detected in any of the samples. Therefore, these beef cattle and broiler meats are safe for public health. However, the detected antibacterial levels including danofloxacin or enrofloxacin residue in a sample, florfenicol residue in a sample, tilmicosin residue in a sample, oxytetracycline or doxycycline residue in two samples in beef cattle meats, and doxycycline residue in two samples in broiler meats exceeded the MRL of Turkey and EU, and Codex Alimentarius and these levels could have risk for public health. For this reason, foods of animal origin should be closely monitored by the Turkish Government authorities for the presence of drug residues especially antibacterials in Turkey.

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The Study of Histopathologic Changes of Experimental Infection with *Listonella (Vibrio) anguillarum* in Rainbow Trout

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Abstract

The rainbow trout production increased more than 100% in the last decade and total rainbow trout production was shown as 107.013 tons according to 2016 statics. According to Federation of European Aquaculture Producer, Turkey was determined as the biggest rainbow trout producer into the European Countries in 2016. Together with high production capacity, a number of outbreaks were reported causing *L. anguillarum* (Vibriosis). The pathogenesis of *L. anguillarum* which were experimentally infected in rainbow trout were examined during 15 days in our study. Spreading of agent that was injected with intraperitoneally into tissue and organs were studied by using histopathological methods. The mortality rate of agent was determined above 70% and deaths were seen in 2-3th days of experiment. In addition of these, liver, spleen, kidney and gills were determined as the most affected organs and tissues. In the present study was obtain for original pathological findings of *L. anguillarum.*

Key words: *L. anguillarum*, Vibriosis, Pathology, Rainbow trout

Introduction

Vibriosis is an infectious bacterial disease causing Vibrio species which seen aquaculture industry with important economic loss in over the World. The most important agent is *Listonella (Vibrio) anguillarum* in Vibrionaceae family.[1,2] *L. anguillarum* causing by hemorrhagic septicemia both warm water and cold water fishes like Atlantic salmon, rainbow trout, turbot, sea bass, sea bream, striped bass, cod, Japan and Europe eel fishes and ayu fishes.[2-5] There are much of information for disease outbreak in fresh water, brackish-water and marine water fish species.[7,8]

L. anguillarum infections can successfully be identified with conventional methods, rapid identification kits and Polymerase chain reaction.[9,10] Histopathologic changes must work for screening disease process and understanding tissue lesions in rainbow trout in case of vibriosis. While there are some research for histopathologic changes in vibriosis[11-14] which subjects were agent colonization and penetration to skin,[15-17] yet there is limited information for disease progression in gill, muscle, liver, spleen, kidney tissues.[18]

We researched pathogenesis of Vibriosis (*L. anguillarum*) in rainbow trout tissues (muscle, kidney, liver, spleen, and gill) by experimental infection for 1, 2, 3, 4, 5, 7, 9, 11, 13 and 15 days. We think that this study provides detailed information study for progression of *L. anguillarum* diseases.

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Material and Methods

Create experimental groups, inoculum preparation of *L. anguillarum* and experimental infection

The study was carried out 170 rainbow trout on average weight of 208.7±8.11 g for determining histopathologic changes of *L. anguillarum*. The bacterial strain isolated in rainbow trout and identified before by Ekici et al.,[14] The fishes were taken from Faculty of Egirdir Aquaculture and adapted completely for 10 days in 1 m^3 fiber plastic tanks contain 900-liter water are located in Aquatic animal disease unit of Egirdir Aquaculture Research Institute. Experimental fishes were examined for parasitic and bacterial diseases (conventional bacteriologic and parasitic methods) in random selected 20 fishes. 0.2 ml volume of 101 and 102 cfu/ml *L. anguillarum* strain was injected for suitable infective dose (LD50) via intraperitoneal separated in two fish group (25 fish). After determination of infective dose as 2,3x102 cfu/ml, experimental injections were done two groups into two separated tanks (total 50 fishes).[19] For control group, 50 fishes separated two groups in two tanks (each of 25 fishes) injected 0.2 ml PBS buffer (pH 7.2) solution.[20-24] Pathologic examinations were made in 1, 2, 3, 4, 5, 7, 9, 11, 13 and 15 days by euthanized in disease and control groups after experimental injection. Dissolved oxygen, pH and water temperature measured 5.72±0.46 mg/lt, 7.51 and 13.04±0.22°C respectively in experiment tanks.

Histopathology

Fishes euthanatized by Quinidine were necropsied and liver, kidney, spleen, gill and muscle tissue samples were collected and fixed in 10% buffered formalin solution and further processed following standard techniques. The tissue samples were embedded in paraffin wax, cut into 5 µm thick tissue sections, mounted on slides and stained with hematoxylin-eosin (H&E) and Brown & Brenn stains for bacteria. Tissue sections were then examined under light microscope.

Results

Macroscopic Findings

In diseased group; 36 fishes were died injected with 10^2 live bacteria. Clinical signs, inappetence and motionless were observed in experimental groups. In addition, hemorrhagic lesions were located on anal, ventral and lateral region of body, eyes and fin root were seen in the progressive days of infection (Fig 1, 2). Petechial hemorrhage was especially remarkable on liver in experimental group as necropsy findings (Fig 3). There was no clinical finding in control group injected with sterile PBS before necropsy.

Figure 1. A: Erosion in lateral line
B: Hemorrhage on eye,
C: Hemorrhage on fin

Figure 2. Hemorrhage on eye

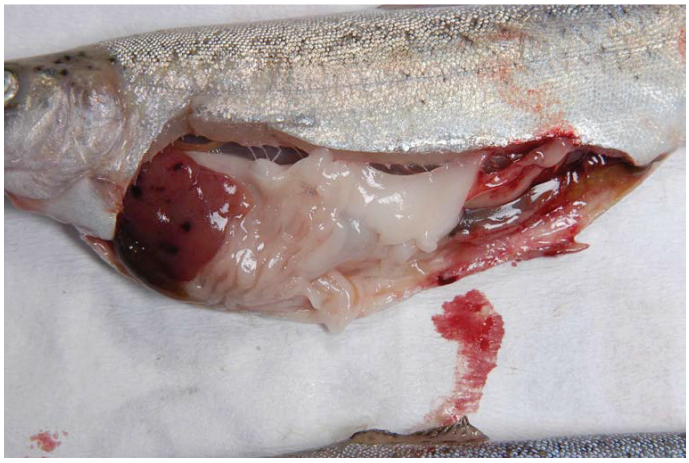

Figure 3. Petechial hemorrhage on liver

Table 1. Lesions of fish in experimental group

Days of Infection	Gills								Skeletal muscle						Spleen				Liver							Kidney					
	Desquamation of seconder	Mononuclear cell	Atrophy Seconder lamella	Hyperemia	Hemorrhage	Necrosis	Telangiectasia of Seconder	Exudation of Seconder	Exudation	Mononuclear Cell	Hemorrhage	Zenger's Degeneration	Necrosis	Calcification	Hemorrhage	Hyperemia	Siderosis	Necrosis	Hemorrhage	Necrosis	Dissociation	Vacuolar Degeneration	Bile stasis	Mononuclear Cell	Siderosis	Siderosis	Hyperemia	Necrosis	Hemorrhage	Mononuclear Cell	Vacuolar Degeneration
1a	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1b	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
1c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2a	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2b	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2d	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3a	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3b	+	+	-	+	+	+	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3c	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3d	+	+	-	-	-	-	-	-	-	-	-	+	-	-	++	-	++	-	-	-	-	+	-	-	++	-	-	-	-	-
4a	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-
4b	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-		++	-	-	-	-	-	-
4c	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	++	-	++	-	-	-	-
4d	-	-	+	-	-	-	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	-	-	++	-	-	-	-
4e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-
5a	-	++	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-
5b	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
5c	-	++		-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	++	-	-	-	-	-	+
5d	-	+	+	-	-	-	-	-	-	-	-	+	-	-	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
5e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	++	-	-	-	-	-	
7a	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
7b	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	++	-	-	-	-
7c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	-	++	+	++	-	-	-	-	-	-
7d	-	-	++	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
7e	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
9a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
9b	+	+	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	+	-	+	-	+		-	-	-	-	-	-
9c	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	++	+	-
9d	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
9e	-	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	-	-	-	+	+	-	+	-	+	-	-	+	-	-	-	-	-	+	++	-	-	++	-	-	-	-	-	-	-	-
11	-	+	-	-	-	-	+	+	-	+	-	-	-	-	+	-	++	-	+	-	+	+	++	-	-	-	-	-	-	-	-
11	+	+	-	-	-	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	+	++	-	-	-	-	+	-	-	+	-
11	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-
11	+	+	-	-	+	-	-	-	-	-	-	++	+	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-
13	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-
13	++	+	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
15	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	++	-	+	-	-	-	-	-	-	++
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15	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+

-: no lesion, +: mild lesion, ++: severe lesion; a, b, c, d, e: refer to each of sampled fish

Microscopic findings

Control group; Desquamation of gill seconder lamella, hyperemia of primer lamella, and cell infiltration of the gill lamella were examined in control groups during 2, 4, 5, 7, 11, 13, 15th days after experimental PBS injection. These fairly mild lesions were seen on histopathological examination in several fishes.

Hyaline degeneration, necrosis, mononuclear cell infiltration and hemorrhagic exudation were observed in muscle tissue samples in 2, 3, 4, 11, 15th days. Slightly hemorrhage and erythrocyte loaded macrophages only were recorded only on 4th day in spleen tissues. Necrosis, mononuclear cell infiltration, hyperemia and bile stasis were seen in 2, 4, 7, 9, 11th days in liver tissues. Hyperemia, necrosis, mononuclear cell infiltration and melanin pigmentation were observed on 9, 11, 15th days in kidney tissues of control and infected fish groups.

Experimental group; mononuclear cell infiltration and intensively desquamation of gill tissues were seen (Table 1, Fig 5, 6) in the infected group. In some cases, atrophy and desquamation were noticed (Fig. 5). Hyperemia and necrosis were seen in afferent and efferent arteriols of primer and seconder lamella (Fig. 6). Hemorrhages were observed sporadically in some fishes gill tissue. There was enlargement of primer lamella because the mononuclear cell infiltration was observed (Table 1, Fig. 9). Zenker's degeneration, mononuclear cell infiltration hemorrhage and calcification between muscle bundles were observed (Fig 12, 15), (Table 1).

Hemorrhage, siderosis and necrosis were major findings from 3th day of experimental infection in spleen tissue (Table 1, Fig. 4).

Vacuolar degenerations were observed from 3th day of infection depicting beginning of the degeneration of hepatocytes (Fig. 14). From 4th day of infection, vacuolar degeneration, necrosis and bile stasis, depending on inflammation on liver tissue, were determined (Fig. 8, 11, 14). There were increasing multifocal necrosis area, invading mononuclear cell infiltrations and progressive bile stasis showing severely progressive infection (Fig. 8, 10).

Exudation and mononuclear cell infiltration of some livers capsule demonstrating peritonitis were noticed (Table 1).

In kidney, siderosis were increased from 1 day to 4, 5, 7, 9, 11 and 15th days. Besides siderosis, hydropic degenerations of tubules, hyperemia, mononuclear cell infiltration hemorrhage and necrosis in kidney were observed (Fig. 7, 13), (Table 1).

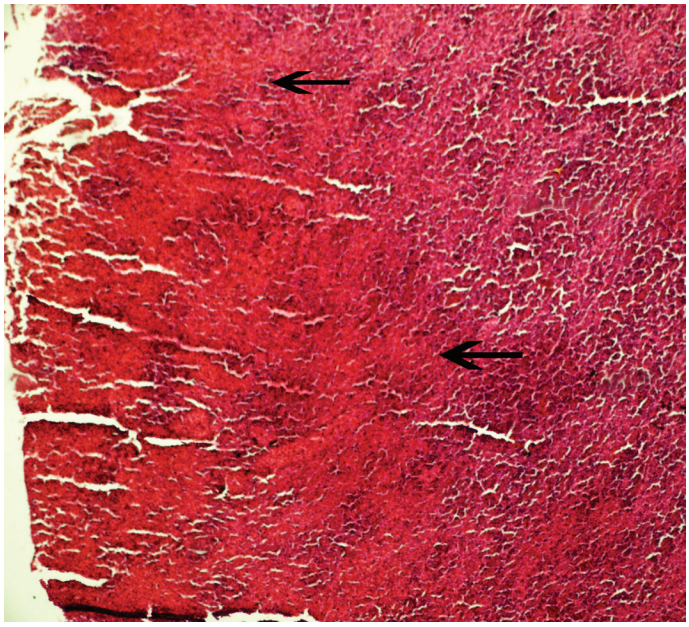

Fig. 4. Hemorrhage on spleen (arrows) H&E x100

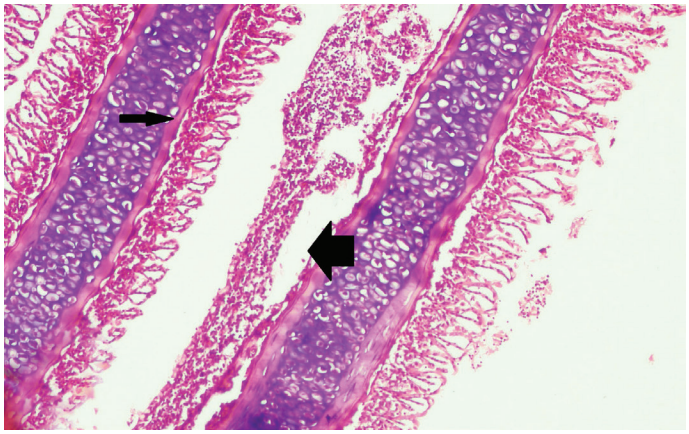

Fig. 5. Desquamations on seconder lamella (thick arrows), Normal seconder lamella (thin arrows) H&E x100

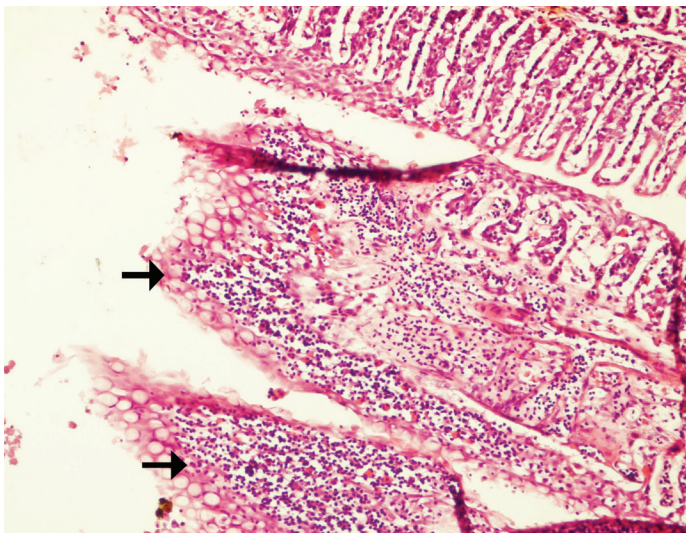

Fig. 6. Severe mononuclear cell infiltration on primer lamella, (arrows) H&E x200

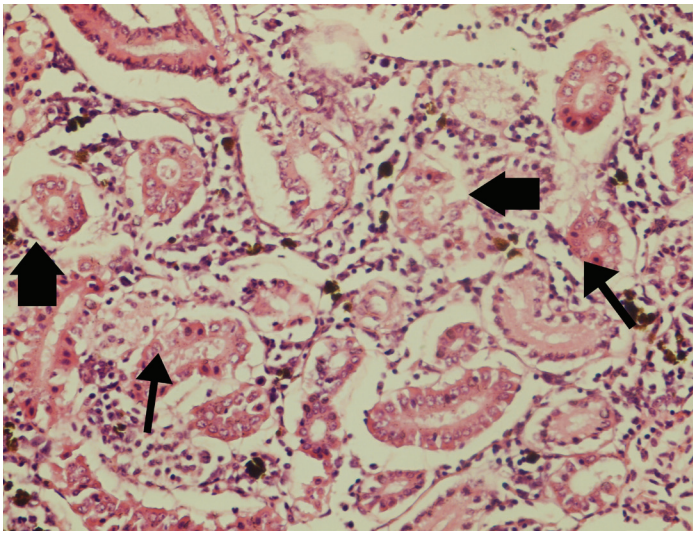

Fig. 7. Vacuolar degeneration on kidney tubules (thin arrows), necrosis (thick arrows) H&E x400

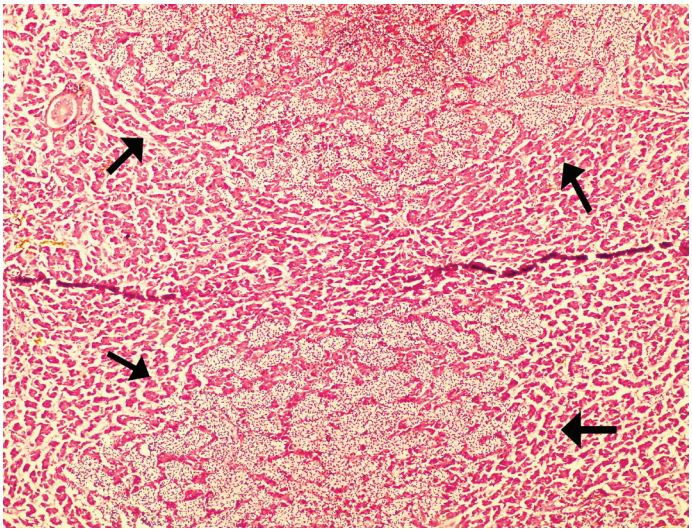

Fig. 10. Multifocal necrosis area in liver that contain inflamma tion cells on center (arrows) H&E x100

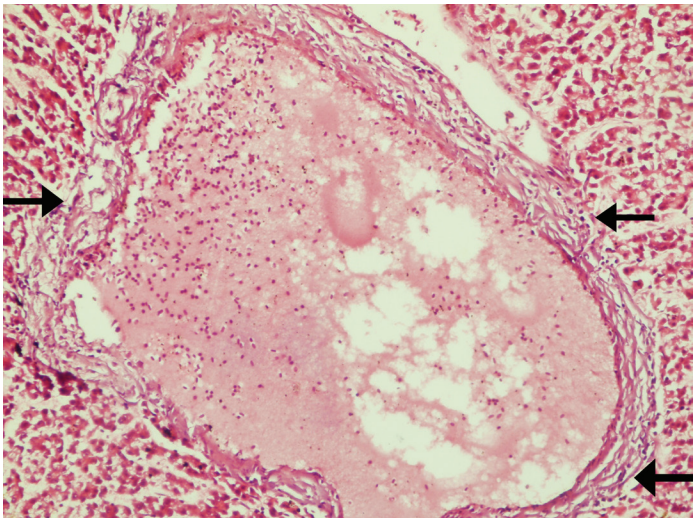

Fig. 8. Mononuclear cell infiltration on portal region of liver (arrows) H&E x200

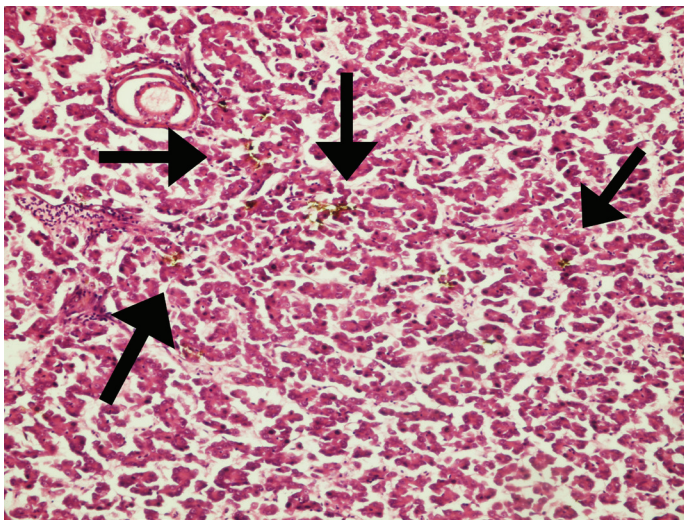

Fig. 11. Gall stasis on liver (arrows) H&E x200

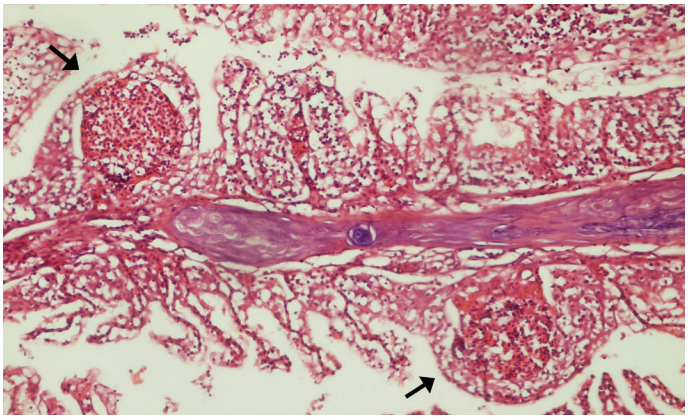

Fig. 9. Telangiectasia on seconder lamella (arrows) H&E x200

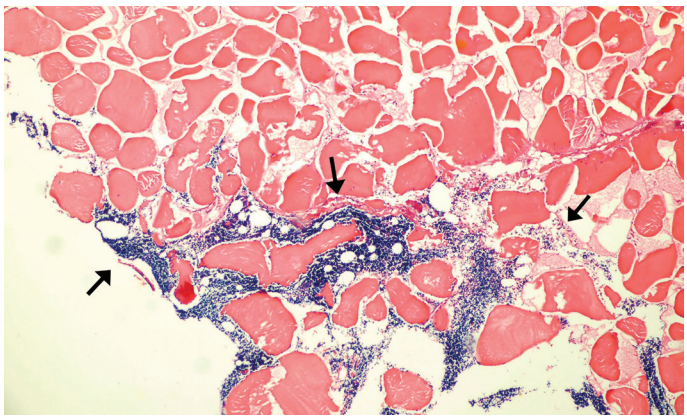

Fig. 12. Severe mononuclear cell infiltrations on muscle (arrows) H&E x100

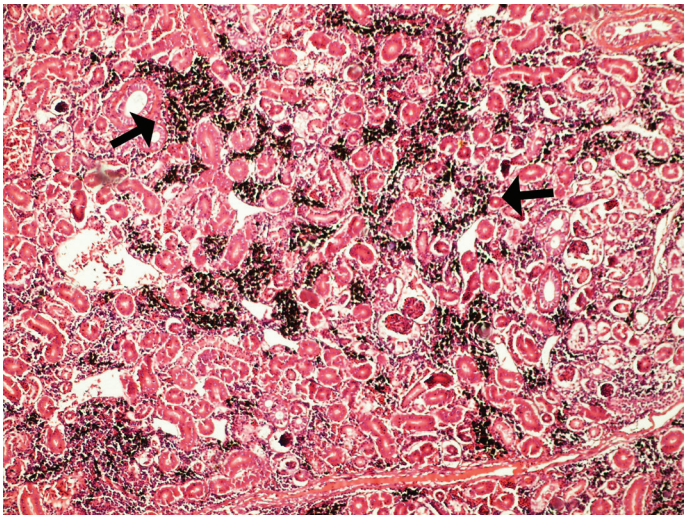

Fig. 13. Severe melanin pigmentation on kidney tubules H&Ex100

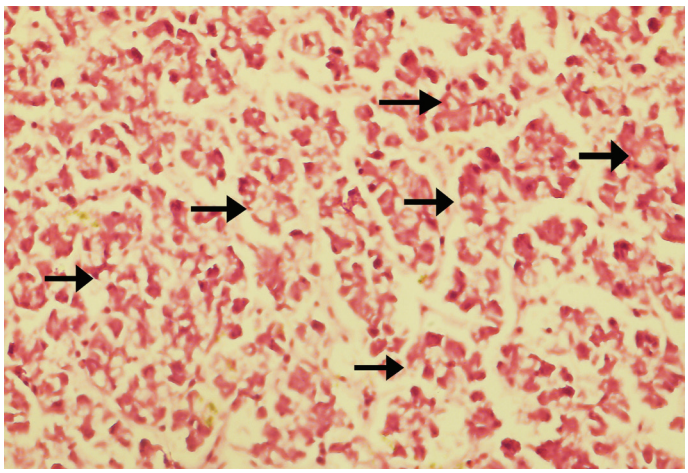

Fig. 14. Vacuolar degenerations on liver epithelials (arrows) and dissociation H&E x400

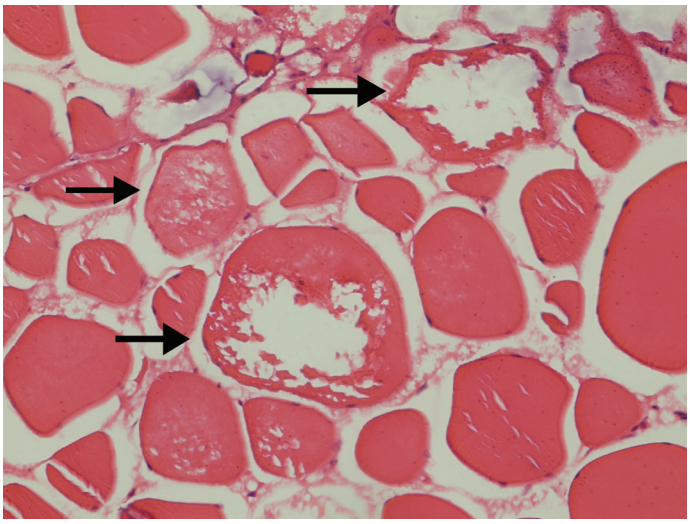

Fig. 15. Piecemeal necrosis on muscle fibrils H&E x400

Discussion

The detailed pathogenesis of *L. anguillarum* which is one of the most deadly agent of Vibriosis for rainbow trout was examined that was injected via intraperitoneally. Austin and Austin[33] were reported that there is limited information about pathogenesis of *L. anguillarum*, that so; we would like to give macroscopic clinical signs and microscopic findings in different tissue and organs during experimental infection from one to 15 days.

Generally, *L. anguillarum* causes about 40-70% mortality in natural infection for fish[6,25,26], mortality could be reach 100% in experimental infections.15,27-30 Avci et al.,[11] were reported that L. anguillarum caused 40% mortality in experimental infection by immersion and 55% mortality by intraperitoneal injection. As similar these findings, our results showed that mortality reached above 70% by intraperitoneally.

Experimental infections with *L. anguillarum* showed that mortality generally observed between one and 15th day,[31,32] similarly, we observed mortality was shown after injection up till 15th day of experiment. The results showed that *L. anguillarum* caused acute mortality when agent enter the fish body and has short time for incubation and spread all of the body. *L. anguillarum* is also named as red pest because of agent causing hemorrhage in different region of body.[33] We observed motionless, loss of appetite, swimming abnormality, hemorrhages on anal, ventral, lateral region, eyes and fin rots as seen previously disease outbreaks.[6,7,11,34-36] Prolapsus of anus and adhesion in abdominal cavity also reported clinical symptoms[35-38] while we could not detected them, inflammation in liver's capsula was detected which is caused peritonitis. We argued that if infection would progressive, peritonitis could cause adhesion on abdominal cavity and may exudate should be seen. Primarily there is some report showed that agent enter the body via gill tissue, while some of them reported that anus and skin mainly route of entry.[15,16,27,37] As similar pathological signs of desquamation of seconder lamella, atrophy in lamella epithelia, exudation, hyperemia on seconder lamella and submucosa vessel showed that *L. anguillarum* has high affinity of gill tissue regardless of route of entry in the fish body.

One of the most effected tissue is skeletal muscle due to *L. anguillarum* infection was reported[5,11,13,30,40], zenker's degeneration and mononuclear cell infiltration were the one of the most observed findings on skeletal muscle with the experimental infection of our study. But in addition of these findings on skeletal muscle, there are severe lesions were determined on gill, liver and spleen tissue distinct from previous report. There is limited information about pathogenesis of *L. anguillarum* infection on liver tissue,[6,37,40] so we presented dissociation, bile stasis and severe accumulation of siderocyte in liver tissue in the first time. Generally hyaline degeneration and mononuclear cell infiltration were mostly occurred pathological findings in kidney,[6,30] we determined accumulation of siderocyte, hydrobic and vacuolar degeneration and after 11th day mononuclear cell infiltration in our experiment.

In the control group of experiment, the showing microscopic findings demonstrated that fish could easily affected any stressor factor like as PBS injection. There is no macroscopic findings and clinical signs in fish of control group. As a consequence of our study, the pathogenesis of *L. anguillarum* by experimental infection were presented from day to day in the first time. After entry of agent into the fish body, agent reached all body regions via blood stream from 2. and 3th days and especially skeletal muscle, liver and spleen are the most effected tissue were observed. These findings showed that *L. anguillarum* is an acute infection that can affect all tissue severely in short time.

Acknowledgement

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RESEARCH ARTICLES

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Evaluation of Some Synthetic Acaricides Against *Varroa Destructor* (Acari: Varroidae) in Turkey: an Indication of Resistance or Misuse of Fumigant Amitraz?

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Abstract

The parasitic mite, Varroa destructor is one of the most important agents for substantial losses in honeybee colonies throughout the world. Several acaricides consisting of synthetic and organic compounds are being used to combat mite. This study was conducted to determine the efficacies of three synthetic acaricides in naturally infested honeybee colonies at consecutive two autumn seasons. Acaricides were commercial preparations of coumaphos (liquid and plastic strips), amitraz (fumigation and plastic strip) and flumethrin (plastic and wooden strips) as a treatment group consisting of eight hives per drug. A control group was kept in both seasons. All drugs were applied as prescribed to the homogenised Varroa-infested honeybee colonies at consecutive two years. The evaluation of efficacies was based on the collected mite percentage obtained with powdered sugar method and it was calculated through Henderson-Tilton's formula. Dropped mites onto the pollen drawers were also evaluated statistically and drugs were compared to each other. Results showed us the effective drugs (up to 90%) were amitraz plastic strip, flumethrin wooden strip and coumaphos plastic strip with 98.5%, 96.5%, and 93.2% averages, respectively according to formula if evaluated within two autumn seasons. Fumigation of amitraz is not sufficient if compared to others and the control group. This data is discussed for suspicion of the possible resistance of mites or misuse of the product with this kind of application.

Keywords: Acaricide, chemical, honeybee, Turkey, Varroa destructor

Introduction

Honeybee *Apis mellifera* L. is the most critical insect that has benefited humanity for medicinal and nutritional purposes for thousands of years. It has significant economic value in agriculture not only for honey production but also they play a vital role in crop pollination.[1] Many insect pests and microbes may attack honeybees and cause considerable yield losses. An ectoparasite, *Varroa destructor* is posing a significant threat to the beekeeping industry throughout the world, also in Turkey, for the last four decades.[2] *V. destructor* is causing severe complications in beekeeping all over the world and can easily be observed in adult bees, broods and also in hive debris. Pupa cannot develop into an adult form or if developed and emerge, bees with deformed wings/legs/abdomens in the case of heavy infestation. Untreated honey bee colonies which are infested with *V. destructor* may perish within two years.[3] *V. destructor* has been proved an important vector for different viral and fungal pathogens spread among honeybees like acute bee paralysis virus and deformed wing virus.[4] The infected bees also have a reduced foraging ability to collect nectar and pollinate crops.[5] The usage of synthetic acaricides to mites is legal and common in Turkey if appropriately used. The misuse of synthetic acaricides may cause severe problems in bees such as bee toxicity; may increase the probability of disease-resistance and may leave residues on bee products.[6] Natural plant products/organic

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acids can be the desired alternative or supplementary to synthetic acaricides with low mammalian toxicity and negligible environmental effects.[7]

Some synthetic chemicals licensed on honeybees like coumaphos, flumethrin, amitraz, tau-fluvalinate are widely used in the world. Coumaphos is a neurotoxic organophosphate that inhibits acetylcholinesterase, thus interfering with nerve signalling and function, while amitraz is a formamidine, octopaminergic agonist. A synthetic pyrethroid, flumethrin, acts by inhibiting gated sodium channels in the nervous system of the mite and being widely used as an acaricide also to Varroosis in the field.[8]

Despite the often efficient Varroa control promoted by synthetic acaricides, numerous side effects may be observed due to misuses. Another negative consequence of the indiscriminate use of acaricides to control Varroa infestation is the repeated selection of mites that are resistant to each of these compounds.[9-11] It has also been demonstrated that combined exposure to pesticides may synergise, resulting in the compounds being even more toxic to honey bees than when administered individually.[12,13]

The present study was designed to find out the efficacies of commercial synthetic Varroacidal drugs in Turkey market and compare them to themselves. Possible long-term effects of the drugs were also evaluated.

Material and Methods

This experiment was conducted during consecutive two autumns, 2012 and 2013 at Bursa - Turkey under field conditions. The colonies of *Apis mellifera* which are settled in the wooden Langstroth hives were used. The bottom boards had drawers enabling to monitor the numbers of dead *Varroa destructor* specimens. The strength of the colonies (with 6-7 frames with bees and less brood or broodless) was assessed before the study in order to establish a homogenous experimental and control group. Natural mite falls were checked by counting them in pollen-drawers at one-week duration. Thus, groups were created with similar mite burdens and bee/brood population.

Six commercial products consist of three synthetic acaricides; coumaphos (liquid-32 mg/ml and plastic strip-13.6 gr/strip), amitraz (fumigation-265 mg/cardboard and plastic strip-500 mg/strip) and flumethrin (plastic strip-3.6 mg/strip and wooden strip-3.6 mg/strip) were applied at recommended applications/doses as treatment group consisting eight hives per drug. A control group consisting of the same number of hives was kept in both seasons. Totally 56 Langstroth-type hives with 6-7 combs, without super and large-size pollen drawers were used, which were highly infested with *V. destructor.*

Approximately 200 adult worker bees from outer frames of each hive were collected into special jars containing icing (powdered) sugar to determine the rate of Varroa infectivity before and after treatment. Aliquoted adult bees and mites were counted as described in Dietemann et al.[14] Additionally, the bottoms of the drawers were cleaned before the trial and were covered with white paper to count dead mites that dropped into the drawer after each drug application.

In each season, the dead mites that had dropped into the pollen drawers were counted on days 1, 3, 5, 7, 14, 21, 28, and 35. Mites on bees were counted on day 35 after treatment. Strips were removed on recommended time when the treatment period finished.

The efficacies of drugs were measured with the Henderson-Tilton formula[15] and significance between the drugs was determined via Tukey's multiple comparison tests defined at the level of 0.05. The formula was:

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}}\right) \times 100$$

Where: n = mite population, T = treated, Co = control

The mite mortality data was recorded on 1, 7, 14, 21, 28 and 35th days of post-treatment by counting the fallen mites from the bottom drawers of the hives.

Results

The present work was conducted in the South-east Marmara Region conditions; the province of Bursa to test the efficacy of commonly used anti-Varroosis chemical products. These efficacy results are summarized in Table 1. If evaluated to average percentages, the most efficient product was amitraz plastic strip (98.5%), following with flumethrin wooden strip (96.5%), coumaphos plastic strip (93.2%), flumethrin plastic strip (88.3%), coumaphos liquid (76.8%) and amitraz fumigation (30.0%). The efficacies of coumaphos liquid and amitraz fumigation in the second season were markedly lesser than those of the other drugs. Except then those two lesser efficient drugs, other drugs have demonstrated almost a high effect in both autumn seasons. Amitraz plastic strip reached 99.0% efficacy during the second autumn season.

During the experiment, dropping mites on drawers were counted for 35 days. The mean numbers of mites dropped in each treatment in each season were shown in Table 2. Means in all treated colonies were decreased in the second season if compared to the first autumn season, interestingly. Amitraz plastic strip treatment caused the highest mean number of mites to drop throughout the seasons, followed by flumethrin plastic strip, coumaphos liquid, flumethrin wooden strip, coumaphos plastic strip and amitraz fumi-

Table 1. % Efficacy of chemical acaricides by the Henderson-Tilton formula for five weeks period

Season	Coumaphos (liquid)	Coumaphos (plastic strip)	Amitraz (fumigation)	Amitraz (plastic strip)	Flumethrin (plastic strip)	Flumethrin (wooden strip)
1st autumn	91.2[a]	93.3[a]	37.7[b]	98.1[a]	89.7[a]	96.1[a]
2nd autumn	62.5[a]	93.1[b]	22.3[c]	99.0[b]	86.9[b]	97.0[b]
Average	**76.8[a]**	**93.2[a]**	**30.0[b]**	**98.5[a]**	**88.3[a]**	**96.5[a]**

a, b, c values with different letters in each category are significantly different.

Table 2. Mean number of dead mites on pollen drawers on the 1st, 3rd, 7th, 14th, 21st, 28th and 35th days (mean ± Standard Error Mean)

Drugs	1st autumn	2nd autumn	Both seasons
Coumaphos (liquid)	58.95 ± 15.95[a]	40.12 ± 5.75[a]	**49.53 ± 10.85[a]**
Coumaphos (plastic strip)	69.78 ± 13.65[a]	14.50 ± 1.60[b]	**42.14 ± 7.62[a]**
Amitraz (fumigation)	17.87 ± 5.81[b]	14.01 ± 2.27[b]	**15.94 ± 4.04[b]**
Amitraz (plastic strip)	107.83 ± 37.48[a]	28.46 ± 3.95[a]	**68.14 ± 20.71[a]**
Flumethrin (plastic strip)	91.75 ± 21.93[a]	30.62 ± 3.74[a]	**61.18 ± 12.83[a]**
Flumethrin (wooden strip)	66.6 ± 24.47[a]	21.37 ± 5.45[a]	**43.98 ± 14.96[a]**
Control	**49.95 ± 11.87[b]**	**18.57 ± 2.61[b]**	**34.26 ± 14.48[b]**

a, b values with different letters in each category are significantly different.

gation. Unexpectedly, a mean number of dead mites in control group is higher than the amitraz fumigation group. The differences between the control group and the drugs were significant in the first season except for amitraz fumigation. Besides, in the second autumn season, the differences between the control group and the drugs were significant for coumaphos liquid, amitraz plastic strip and flumethrin plastic strip, but not significant for coumaphos plastic strip, amitraz fumigation and flumethrin wooden strip ($p>0.05$). Finally, there were no observable side-effects or abnormal bee deaths during any of the trials in the treated or control colonies.

Discussion

There is always a potential hazard of the usage of synthetic acaricides like building-up residue in bee products and/or mite resistance. However, Varroa mites are widely being controlled by using synthetic acaricides which were applied in formulated different forms like plastic/wooden strips, liquid or soaked cardboard. It has been noted that the efficacy of some drugs (coumaphos liquid, amitraz fumigation and flumethrin plastic strip) can be variable in different seasons.

Our results obtained for plastic strips of amitraz, coumaphos and flumethrin either in first and second autumn agreed with other published works as 90.6%, 82.8% and 99.9%, respectively.[16-18] We detected a drop in the efficacy of coumaphos liquid in the second autumn, probably due to the lower external temperatures or internal (in-hive) conditions like colony population. A similar observation has also been reported by Semkiw et al.[17] and Leza et al.[19] with the studies of amitraz plastic strip.

Although the results of the assessments of amitraz strips in our experiment are high/not variable, some researchers have gained variable results as 83.8% on average (78.8 – 87.3%) in Italy and maximum 60.1% efficacy in Portugal.[20,21]

Amitraz fumigation is still being used in Turkey since the 1980's. According to its short-time effect, its efficacy can be different in the whole year's Varroa combat management. There is limited data on the efficacy of amitraz fumigation applications on honeybees in Turkey such as by Kumova[22] conducted in an autumn period and reached 91.1% efficacy. In contrast to our study, although, low efficacy of amitraz fumigation seems like a possible mite resistance, we think that using this fumigation is not related to resistance. Amitraz fumigation is also not suitable for a long time period (during autumn) Varroacide. It can be used as a short time and fast mite determiner. To ensure this theory, further investigations into the specific resistance of the mites should be needed.

Chemical acaricides with plastic strips possess some advantages, such as the simplicity of application and the low economic cost. However, the main disadvantages of these products are their limited efficacy after continuous use due to the development of resistance,[9,10,21] as well as the residues in bee products. Due to the prescribed use, there were no side-effects on adults/broods or abnormal bee deaths during treatment.

All drugs except amitraz fumigation were proved proper methods to control Varroa mites. Our results showed that the effective drugs (up to 90%) were amitraz plastic strip, flumethrin wooden strip and coumaphos plastic strip with 98.5%, 96.5%, and 93.2% averages, respectively according to formula if evaluated within two autumn seasons. According to formulated data, efficacies of high affected drugs are almost stable in both autumn seasons, except

for coumaphos liquid one. That decrease can be explained by the result of some environmental variables like climate, brood population and colony strength. Although usage of chemical acaricides is tending to be reduced due to its possible side-effects, if they are appropriately used and rotated, they are adequately effective, especially in long-term autumn treatment.

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The Effect of Saccharomyces Cerevisiae and Spirulina Platensis on Glutathione and Leucocytes Count in Rabbits

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Abstract

Glutathione is the important antioxidant agent that is used for body detoxification system. Because of the fact that ıt is crucial for protecting health. A feeding trial was conducted to evaluate the effect of natural additives such as live yeast culture Saccharomyces cerevisiae (SC) and microalgae Spirulina platensis (SP) on the glutathione and leukocytes counts of rabbits. Forty male New Zealand white rabbits, aged 5-6 weeks, were studied in 4 groups. Treatments were control group, SC (added 3 g/kg diet), SP (added 5% of the diet) and, SC and SP (added 3 g/kg diet and added 5% of the diet) respectively. The experiment lasted for 90 days and the blood samples were obtained by ear venipuncture on the 90th day.

In conclusion, according to the results of this study, although not statistically significant, supplementing rabbit with S. cerevisiae or S. platensis had increased on glutathione values. Glutathione tend to be positively correlated with the addition of SC or SP. No significant difference in white blood cell counts was evidenced, even if lymphocyte counts tended to increase and neutrophil counts to decrease in rabbits fed SC or SC+SP. The determination of biological consequences (antioxidant potential, resistance to diseases, and improvement of nutritional status) requires further investigations.

Keywords: Saccharomyces cerevisiae, Spirulina platensis, glutathione, leukocyte.

Introduction

Antioxidants such as glutathione provide a defence against the damage of cells by reactive oxygen species (ROS) in living systems.[1-3] Glutathione (GSH) has several biological roles: detoxifies electrophiles, maintains the essential thiol by preventing oxidation, modulates the cellular prosess (DNA synthesis), and modulates immunity.[4,5] Blood glutathione protects cellular proteins against oxidation through glutathione redox cycle.[6]

It is suggested that ROS has a role in the development of various diseases such as cardiovascular, arteriosclerosis, cancers, and many others, and the process of aging. Therefore, in recent years, researchers' increasing attempts to prevent diseases caused by ROS have been drawing attention.[7,8] Therefore, there have been many attempts to prevent against chronic diseases, by reducing cellular oxidative stress.[9,10] There are positive correlation between dietary intake of natural antioxidants and health. Especially, presence of antioxidants in the plant foods have a beneficial role in resistance of the some cardiovascular diseases, cancer or degenerative diseases of aging.[11]

Spirulina platensis (SP, S. platensis) is rich in fatty acids, amino acids, vitamin and selenium.[12] Recently, attention has been placed on the antioxidant potential of spirulina. Indeed, many of the chemical components of spirulina, such as phenolic compounds, tocopherols, A-carotenes, and phycocyanins exhibit antioxidants properties.[13] S.

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platensis recommended for health due to their effects on growth,[14] immunity[15,16] and antioxidant mechanism.[14,17-20] In our previous studies serum CD4+/CD8+ increased in the animals fed SP, and it was concluded that S. platensis may be used as an immune enhancer.[16]

The yeast culture, Saccharomyces cerevisiae (SC, S. cerevisiae) is a well-known probiotic having positive effects in the treatment and prevention of diseases.[21] S. cerevisiae, inparticular, has proven to benefit health in several ways including stimulation of the growth of intestinal microflore in mammals; pH modulation in ruminants as well as reduction in the number of pathogenic microorganisms in monogastric animals.[22-24] In addition, in our previous experimental studies on S. cerevisiae, addition of SC at a level of 3 g/kg of feed in rabbits increased the total mucosa, villus height, and gland depth of duodenal mucosa,[25] improvement of ruminal cellulolytic activity[26] and haematopoiesis.[27]

The combined effect of S. platensis and S.cerevisiae on antioxidant mechanism and defense cells in the blood have not been addressed yet. Therefore, the aim of this study is to investigate the effect of natural additives such as live yeast culture S. cerevisiae and microalgae S. platensis on the glutathione and leukocytes counts of rabbits.

Materials and Methods

Animals, Groups and Feeding

Forty male New Zealand white rabbits aged 5-6 weeks were randomly allocated on a weight basis to four groups: I. Control, II. Saccharomyces cerevisiae (SC, added 3 g/kg diet), III. Spirulina platensis (SP, added 5% of the diet), IV. Combination of SC and SP (added 3 g/kg diet and 5% SP of the diet), respectively. The rabbits were housed individually in metal cages and, feed and water were offered ad libitum to the rabbits throughout the 90 day trial. Basal diet (pelleted) was formulated to contain 2.500 kcal ME/kg metabolizable energy, 16% crude protein and was designed to meet maintenance requirements according to the NRC.28 Chemical composition and ingredients of the diet are provided in Table 1 and Table 2. Chemical analyses of diets were carried out according to AOAC.29 Basal diet was supplemented with Saccharomyces cerevisiae live yeast culture (Yea Sacc1026 Altech. Nicholasville: 1x109 CFU g-1) and/or Spirulina platensis.

The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was carried out with the permission of Uludag University Animal Experimentation Local Ethics Committee (Approval No: 2010-09/01).

Measurements

The blood samples were collected by ear venipuncture on the 90th day. Counts of leukocytes were estimated according to the methods reported by Jain[30]. Reduced glutathione in erythrocytes was determined spectrophotometrically (Shimadzu UV 1201 V, Japan). The absorbances were read in a spectrophotometer at 412 nm.[31] The values were determined from standard curve. The results were expressed as micromole per gram of protein (μmol/g protein).

Statistical Analysis

Statistical analyses were performed with SPSS (Version 17.0; Chicago.IL).[32] Data were tested for normality distribution and variance homogeneity assumptions. All the values were grouped and the means and standard errors were calculated. One-way ANOVA was applied to the parameters to examine the difference between groups.[33]

Results

Glutathione concentration in erythrocytes and leukocytes counts of blood of the control and experimental groups (SC, SP and SC+SP) are presented in Table 3. There was no significant difference in the glutathione and leukocyte counts in all groups ($P<0.05$) but glutathione (control, SC and SP; 5.75, 5.96 and 5.91 (μmol/g protein) , respectively) and leukocytes counts (control, SC and SP; 6860, 7011 and 7010, respectively) tended to increase with the addition of SC or SP . In addition, lymphocyte counts tended to increase, and neutrophil counts to decrease in rabbits fed SC or SC+SP ($P>0.05$; Table 3). As a result, S. cerevisiaea and S. platensis had changed glutathione and leukocytes counts.

Table 1. Chemical composition of basal diet (%DM)

	Diet
Dry matter%	88.89
Crude protein%*	16.00
Ether extracts%*	3.52
Crude fiber%*	10.95
Ash	7.68

* Based on %Dry Matter

Table 2. Ratio of feed ingredients (%)

Ingredients	Usage rate, %
Barley	30.00
Alfalfa meal	25.00
Corn14	17.61
Soybean meal 46	10.83
Rice bran	10.00
Corn bran	3.60
Limestone	1.40
Salt	0.80
Dicalcium phosphate 18	0.28
Vitamin premix*	0.25
Methionine	0.09
Anticoccidial	0.03
Antioccidial	0.03
Total	100.00

* Premix: Vit A 4.800.000 IU, Vit D 800.000 IU, Vit E 14.000 mg, Biotin 18 mg, CH-CL 50.000 mg, Folic acid 400 mg, Niacin 8.000 mg, Pant.Acid 4.000 mg, Riboflavin 2.800 mg, Thiamin 1.200 mg, Pyridoxine 2.000 mg, Vit K 1.600 mg, Zinc 24.000 mg, Iron 2.000 mg, Iodine 400 mg, Manganese 32.000 mg, Selenium 60 mg, Copper 24.000 mg.

Table 3. Glutathione values (µmol/g protein) and leukocytes counts (mm3 of blood) in Rabbits fed yeast and spirulina supplemented diets (mean±standard error).

Treatment groups				
Parameters	**C[1]**	**SC[2]**	**SP[3]**	**SC + SP[4]**
GSH[5]	5.75±0.23	5.96±0.30	5.91±0.33	5.75±0.24
Leukocytes	6860 ± 192	7011 ± 228	7010 ± 212	6780 ± 223
Lymphocytes	4328 ± 385	5126 ± 234	4430 ± 332	4712 ± 229
Neutrophils	2127 ± 299	1605 ± 279	2243 ± 350	1844 ± 226
Eosinophils	213 ± 87	91 ± 62	147 ± 75	95± 50
Basophiles	55 ± 33	39 ± 24	49 ± 26	47 ± 21
Monocytes	137 ± 00	148 ± 39	140 ± 36	81 ± 33

1Control group, 2Saccharomyces cerevisiae , 3Spirulina platensis , 4Saccharomyces cerevisiae and Spirulina platensis, 5Reduced glutathione values in erythrocytes.

Discussion

Neutrophils, monocytes and macrophages derived monocytes work under inflammatory sites of high oxidative stress because of the production of ROS. Yang et al.[34] found that glutathione protects monocytes and macrophages against to hypochlorite formed by neutrophils and macrophages within inflammatory environments. In the same study, it was postulated that glutathione protects these cells from oxidative stress. Also, Kim et al.[35] indicate that the GSH status of cells play a crucial role in the differentiation and phagocytosis of macrophages. Leukocytes release chemical oxidants that have potent antibacterial, antiviral, and antifungal, but over production of these oxidants may cause the cellular damage that can lead to disease such as arthritis, cardiovascular disease, cancers and many others[36,37]. Antioxidants have a beneficial role in resistance of many diseases and aging process.[11]

S. platensis is a rich source of protein and has been found to be a rich source of vitamins, minerals, essential fatty acids, and antioxidant pigments such as carotenoids.[12,13,38] In particular, this alga is a rich source of phycocyanin, an antioxidant biliprotein pigment.[17,39,40] Recently, it was reported that the protein phycocyanin played a crucial role in the antioxidative action of Spirulina.[41] In studies, it was focused on its therapeutic properties, especially antioxidant effects.[42,43] Riss et al.[41] observed that SP (7.14 mL/kg/ day) significantly increased plasma antioxidant capacity compared with controls. Also, Kim et al.[18] found that 5% Spirulina significantly increased glutathione in erythrocytes compared with controls in rabbits fed a high cholesterol diet, and they suggested that dietary supplementation with Spirulina may be useful to protect the cells from lipid peroxidation and oxidative DNA damage. In addition, Kalafati et al.[44] reported significant increases of glutathione level in red blood cell in human supplemented with spirulina (6 g/day, for 4 wk) at rest and 24 h after exercise, and they suggested that these increases would be related to enhance glutathione synthesis by SP.

Al-Masri et al.[24] indicated that blood glutathione level significantly increased in groups administered S.cerevisiae in rats, and these researchers proposed that yeast beta glucan enhances the glutathione synthesis. On the other hand, in a study related to nicotine reported that nicotine caused significant reductions in the glutathione levels in rat when compared to control group, while beta glucan treatment significantly reversed the glutathione back to control level, and they suggested that beta glucan, S. cerevisiae cell wall component, protects against chronic nicotine- ınduced oxidative damage in tissue.[45]

The present study is an attempt to identify natural effects of SP, SC and SP+SC combinations for rabbits. According to the results of this study, although not statistically significant, supplementing rabbit with S. cerevisiaea and S. platensis had increased on glutathione values (Table 3).

A study related to aflatoxin, glukomannan, derived from cell wall of Saccharomyces cerevisiae, at a dose of 2g/kg did not statistically significant effect on reduced glutathione value in the blood compared with controls in quails , but it reverse the aflatoxin decreased glutathione.[46] Relating the present experimental results with our previous

studies in which rations of high forage or high concentrate and sheep were used, we observed that the addition with a daily dose of 4g SC for every animal had no significant effect on reduced glutathione in erythrocytes.[27]

According to some researchers feeding Spirulina enhanced nonspecific cellular immune responses such as chemotaxis and phagocytosis.[47,48] In a study involving the effect of Spirulina in fish, Duncan and Klesius[15] found that fish fed Spirulina had a higher percentage of lymphocytes than fish fed a control diet. Şahan et al.[49] found that the addition of 5.0 g/kg, 7.5 g/kg and 10 g/kg spirulina elevated leukocytes levels. In the same study, percentages of lymphocytes were significantly higher in the groups fed with 5.0 and 10 g/kg of spirulina whereas percentages of monocytes level were significantly lower in the same groups.

Onifade et al.[50] studied in rabbit with a diet containing 0 g/kg, 1.5 g/kg, and 3.0 g/kg S.cerevisiae yeast, and they found that leucocyte counts were similar on the treatments.

Also in the present study, addition of S.cerevisiae and S.platensis alone or in combination had no significant effect on leucocyte count (Table 3). Although not statistically significant, we found the higher counts of leucocytes in rabbits fed SC and SP, the higher counts of lymphocytes and the lower counts of neutrophils in rabbits fed SC or SC+SP.

In conclusion, according to the results of this study, although not statistically significant, supplementing rabbit with S. cerevisiaea and S. platensis had increased on glutathione values. Glutathione values are likely to rise as a result of feeding rabbit with SC and SP. No significant difference in white blood cell counts was evidenced, even if lymphocyte counts tended to increase and neutrophile counts to decrease in rabbits fed SC or SC+SP. The determination of biological consequences (antioxidant potential, resistance to diseases, and improvement of nutritional status) requires further investigations.

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The Utilization Areas of Cabergoline in Veterinary Gynecology

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Abstract

Cabergoline is a potent dopamine agonist and a synthetic ergot derivative, and it acts by inhibiting prolactin secretion from the pituitary gland. It reduces the amount of plasma prolactin and inhibits progesterone secretion at the same time. Because of these effects, it has found a widespread area of use in the treatment of hyperprolactinemic disorders both in the human medicine and veterinary medicine.

Keywords: cabergoline, veterinary, gynaecology

Introduction

Prolactin is a hormone in the polypeptide structure that is secreted from specific lactotroph cells in the adenohypophysis. The main function of the prolactin is the onset and continuation of lactation; however, numerous of its tasks have been reported both inside and outside the reproductive system. The secretion of prolactin is under control of tonic inhibition of prolactin inhibitory factors. The most important prolactin inhibitory factor is dopamine. Dopamine inhibits adenylate cyclase by binding to the dopamine D2 receptors on lactotroph membranes and reduces the prolactin release. In this review, indications of cabergoline in veterinary gynaecology were evaluated by a clinician. It is aimed to draw attention to the importance of cabergoline and to shed light on the works that can be planned in the future.

Cabergoline

Cabergoline (CAB) (1-[(6-allelylergolin-8 b-yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethyl-urea) is a potent dopamine-2 (D2) receptor stimulant and synthetic ergot derivative. It creates an effect by inhibiting the prolactin secretion from the pituitary gland.[1,2]

Cabergoline is the newest dopamine agonist used in the treatment of hyperprolactinemic disorders both in human and veterinary medicine. The inhibition of prolactin secretion occurs via dopamine. Cabergoline has high selectivity and affinity against the dopamine D2 receptors that are in pituitary prolactin secreting cells and acts through these receptors.[3,4] Cabergoline inhibits prolactin secretion in vivo and in vitro pathways and constitutes a stronger prolactin inhibition than other dopamine agonists. It has been reported in the studies carried out on rats that the treatment of single-dose 0.6 µg/kg CAB reduce the concentrations of prolactin for 6 days, and the CAB application inhibits the progesterone secretion in pregnant cats and dogs.[3-7]

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Utilization Areas Of Cabergoline In Veterinary Gynecology

Estrus Induction in Bitches

Bitches are monoestrus type animals that are not dependent on the season. In general, they reach puberty at 6-14 months of age. The time between two standing heats is 7 months on average and varies between 5-12 months.[8,9] The bitches enter the anestrus period after the diestrus period of the cycle. The anoestrus period is long and approximately 120 (40-270) days. Duration of this time may vary depending on the races and the individuals.[10] The prolongation of the anestrus period is an infertility cause and is divided into two categories as the primary and secondary anestrus. The primary anestrus is the state that the female dog does not have any estrus until 24 months of age, and the secondary anestrus is the state that it does not have oestrus even though 10-18 months have passed after the previous estrus period.[9]

One of the drugs used in bitches for the termination of an anestrus to form estrus is CAB which is a dopamine agonist. Cabergoline is an ergot alkaloid and has an anti-prolactinergic effect.[11,12] Suppression of prolactin secretion via dopamine agonists shortens the duration of anestrus and induces the estrus.[13] It has been reported that the use of CAB in the treatment of both primary and secondary anestrus cases in dogs induces estrus and provides a high pregnancy rate. Cabergoline is recommended at 5 μg/kg doses to stimulate the estrus in dogs and reported to be effective also at low doses (0.6 μg/kg).[14-17] Unlike dogs, CAB has not been shown to be effective in inducing estrus in cats.[5]

Termination of Pregnancy Dogs and Cats

In dogs, prolactin is the luteotropic hormone in the second half of the luteal phase. Prolactin provides the continuity of progesterone by supporting the corpus luteum. Cabergoline, which has an anti-prolactin effect, suppresses luteal functions, causing the decrease of the progesterone concentration and the termination of the pregnancy.[6-18,19] Cabergoline is effective in the second half of pregnancy for the termination of the pregnancy in dogs, and it can be used to terminate advanced and middle term pregnancies.[20]

Onclin et al.[21] reported that the treatment of pregnant dogs between 25 and 40 days by subcutaneous injection of CAB for 5 days at 1.65 μg/kg dose displayed a 1/4 abortion at 25th day, 4/6 at 30th day and 5/5 at 40th day. Moreover, it was reported that the abortion was achieved by the oral use of 160 μg of CAB for 7 days after the 40th day of pregnancy and no side effects were observed.[22] It has been reported that CAB can be used in combination with PGF2α analogues in abortion prophylaxis in dogs. This combination is effective from 25th day of the pregnancy in which is applied at 5 μg/kg orally for 9-10 days whereas 1 μg/kg subcutaneous cloprostenol is used at 28th and 32nd days of the pregnancy.[23]

Because of the harmfull side effects, the uses of estradiol and PGF2α has been reported to be unsafe in preventing pregnancy in cats, hence cabergoline and aglepristone are better tolerated.[24] Verstegen et al.[6] indicates that there is no adverse effect or behavioural disturbance in the cats on the 30th day of gestation, at dose of 1.65 μg/kg subcutaneous injection of Cabergoline for five consecutive days and 80% abortion is available. Cabergoline is effective in termination of pregnancy in female cats between 34th and 42th days of pregnancy, when it is used alone, however the combination with PGF2α is more effective between 25th and 40nd days of pregnancy.[25]

Cystic Endometrial HyperplasiaPyometra Complex Treatment in Queen and Bitch

Cystic endometrial hyperplasia-pyometra complex (CEH-P) is a disease characterized by purulent fluid accumulation in uterine lumen, cystic dilatation and endometrial hyperplasia in endometrial gland. This case is often seen during the diestrus period, since the cycle is under effect of the influence of progesterone. The frequency of this disease in cats is lesser than those in dogs.[26]

The most important luteotropic hormone in dogs is prolactin. Cabergoline is revealed to be inhibiting the action of prolactin and reducing the plasma progesterone concentration. [21] In the medical treatment of CEH-P, CAB is used to reduce the serum progesterone concentration (luteolytic effect) and its combination with prostaglandins is recommended to relieve the uterine contents. England et al.[27] found that the combination of CAB-prostaglandin (5μg/kg of CAB once a day for 10 days, and 5μg/kg cloprostenol for 3 times SC. in 3 days intervals) decreased plasma progesterone concentration, increased vaginal secretion and reduced uterine diameter. Jena et al.[28] reported that the most effective treatment protocol for their studies comparing various treatment protocols in pyometra cases of dogs was using cloprostenol (1 μg/kg SC. 7 days) in combination with CAB (5 μg/kg oral 7 days).

It has been reported that the dopamin agonist CAB (5 μg/kg oral) can be used alone, combined with prostaglandins or combined with anti-progestins, a rapid luteolysis is provided by the combination of PGF2α and CAB and the cervix is opened in 24-48 hours, in pyometra cases of cats.[26]

Pseudo-Pregnancy Treatment in Dogs

Pseudo-pregnancy (PSP) is a common syndrome characterized by nesting, gaining weight, growth in udders,

increasing milk secretion and maternal behaviour in non-pregnant dogs at the end of the metestrus stage. It may show subclinical or clinical courses. Although the exact physiopathology of the PSP is not fully understood, it has been determined that prolactin hormone levels increase, whereas progesterone hormone levels decrease and it occurs in 6-12 weeks after estrus.[29,30]

Diuretics, progestagens, estrogen, and androgen combinations have been used in the medical treatment of pseudo-pregnancy, however, their usage is contraindicated due to the incidence and severity of side effects. Today, prolactin antagonists are effective in the treatment of symptoms within the next 10 days from the start of treatment.[31] These drugs have been proven to decrease the level of prolactin, lower the serum levels and thus regress udder gland development, reducing pseudo-pregnancy behaviours.[32] It was reported that, in the treatment of pseudo-pregnant dogs, application of daily 5 mg/kg, prolactin antagonist,CAB for 5 days was highly effective and no side effects were observed.[33-35]

Treatment of Mammary Hyperplasia in the Cats

In cats, fibroepithelial udder hyperplasia is a benign disease characterized by progesterone related sudden and rapid fibroglanduler proliferation of one or more mammary glands.[36] It is reported that this is more common in young female cats but may be seen in male cats.[3] This disease is often found in animals treated with progesterone or analogues. Progesterone antagonists (aglepristone) and prolactin inhibitors (cabergoline) can be used to treat the disease. Or ovariohysterectomy may be recommended. Medical treatment of this disease is frequently recommended in cats especially that are breed for production.[36-38, 39]

In mammary hyperplasia cases with milk secretion, it was reported that successful results were gained with the combined use of Aglepristone (two days a week for three weeks, at the dose of 15 µg/kg, subcutaneously) and CAB (at the dose of 5 µg/kg once a day per os, at the first week).[40]

Utilization of Cabergoline in Drying Off the Dairy Cows

Dairy cows need a dry period between the two lactations to provide high milk yield.[41] This period, in which the udders of the cows are rested between the two lactation periods and not milked for the last 45-60 days of the pregnancy, is called the dry period, and the process at the beginning of this period is called drying off. In order to dry the cows off from the milking, there are methods such as intermittent milking, not evacuating the milk in the udder completely or sudden cease of milking.[42] In recent years, it has been reported that, as an alternative to conventional drying off methods, successful results have been obtained with a single CAB injection in transition to the drying off phase.[41-43,44] Cabergoline is a high-affinity ergot derivative for D2 dopamine receptors, whose dopaminergic effects cause the inhibition of prolactin secretion. Therefore, as a result of studies carried out considering CAB as a potential drying off molecule, it was concluded that the intramuscular injection of a single dose of 5.6 mg of CAB on the cows, which gives over 16 kg of milk in the transition to dry period, Cabergoline treatment positively influences dry period mammary health and reduces risk factors for animal welfare.[44]

In similar studies, it was reported that the same dose of CAB facilitated the drying of the udder gland and udder gland involution, reduced prolactin release and leakage from the udder, and prolonged the resting time after the drying off. [41-43] Cabergoline treatment will be beneficial because it accelerates mammary involution and decreases milk secretion in the transition to dry period.

Conclusion

As a result, CAB has found a utilization area in veterinary gynaecology, especially in the medical treatment of various gynaecological disorders of carnivorous, and it is advantageous for it to have fewer side effects than other hormone drugs. However, it is not used in dairy cattle in our country as it is used in European countries. Since the CAB is thought to be useful as a medicine to increase the health and comfort of the cows to be dried off, to increase the number of the studies in this area and to use it in cattle in our country is deemed to be beneficial.

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