Journal of Advances in VetBio Science and Techniques

E-ISSN: 2548 - 1150 - Period: Tri-annual

J Adv VetBio Sci Tech - Volume 9(1) - April 2024

JOURNAL OF ADVANCES IN VETBIO SCIENCE AND TECHNIQUES

e-ISSN 2548-1150 | **Period** Tri-annual | **Founded:** 2016 | *J Adv **VetBio** Sci Tech*

Volume 9 | **Issue 1** | **Year 2024**

EDITORIAL ARCHIVE

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JOURNAL OF ADVANCES IN VETBIO SCIENCE AND TECHNIQUES

e-ISSN 2548-1150 | **Period** Tri-annual | **Founded:** 2016 | *J Adv **VetBio** Sci Tech*

J Adv VetBio Sci Tech, 9(1), 2024

CONTENTS

Effects of prophylactic propylene glycol administration at calving on subclinical ketosis in Holstein dairy cows

Research Article

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Article info
Submission: 11-10-2023
Accepted: 15-12-2023
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

ABSTRACT

Forty-four Holstein dairy cows were randomly enrolled in the treatment group (group 1, n=19) or control group (group 2, n=25) at calving. Group 1 received prophylactic propylene glycol treatment (PPGT) (300 ml/cow, beginning at calving, total 3 days). The group 2 remained untreated. All animals were tested on blood and milk beta-hydroxybutyric acid (BHBA) at postpartum week 2 and 4 (PW2 and 4), body condition scores (BCS), average daily milk production (ADMP) and postpartum health disorders were monitored in 90 days in milk (90 DIM). The incidence of subclinical ketosis (SCK) was 8% and 4% in blood test (BHBA≥1.2 mmol/L) and by 12% and 24% in milk test (BHBA≥200 µmol/L) in group 2 at PW2 and PW4 respectively. SCK was not detected in group 1. The study cows lost BCS on postpartum days 30 and 60. ADMP was remarkably different between group 2 with SCK (28.36 kg), without SCK (34.36 kg) and group 1 without SCK (33.92 kg). Mastitis, metritis and laminitis incidence were observed both in group 1 and 2, but clinical ketosis and culling rate were observed in group 2 only. Mastitis incidence was 32% and 10.5% in group 2 and group 1 respectively. Culling rate was 12% in group 2, no culling was observed in group 1. Conclusively, although there wasn't a significant effect of PPGT on the averages of blood and milk BHBA at PW2 and 4, the observed incidence of SCK in group 2 in association with postpartum health disorders and ADMP loss may require selective PPGT in cows at risk of SCK, rather than treatment the entire whole population.

Keywords: Beta-hydroxybutyric acid, Holstein, milk yield, propylene glycol, subclinical ketosis

INTRODUCTION

Propylene glycol (PG) was frequently used in the pharmaceutical industry for different formulations (Jimenez et al., 2020; Mikuła et al., 2020), and it was also recommended for the prevention and treatment of hyperketonemia in dairy cattle (El-Kasrawy et al., 2020; Gordon et al., 2017; McArt et al., 2011; Zhang et al., 2020). Studies showed that oral PG application significantly changed the rumen fermentation pattern and decreased the molar ratio of acetate/propionate by increasing the predominant end-product propionate of PG fermentation in the rumen (Christensen et al., 1997; Nielsen, 2004). Hyperketonemia is a metabolic disease diagnosed by elevated ketone bodies in blood, milk and urine in dairy cattle (Deniz et al., 2020; Duffield et al., 2009). Subclinical ketosis (SCK) is defined by the increased ketone bodies in blood and milk without clinical signs, but it can cause production losses in dairy cow (Aksoy et al., 2022; Uyarlar et al., 2018). It is manifested by a high betahydroxybutyric acid (BHBA) concentration ≥1.2 mmol/L in the blood (Brunner et al., 2018; Gordon et al., 2017; Şentürk et al., 2016) and 100 µml/L (light) or ≥200 µml/L (severe) in the milk (Aksoy et al., 2022; Berge et al., 2014; McArt et al.,

How to cite this article
Aksoy, K., Deniz, A. (2024). Effects of prophylactic propylene glycol administration at calving on subclinical ketosis in Holstein dairy cows. *Journal of Advances in VetBio Science and Techniques, 9*(1), 1-9. https://doi.org/10.31797/vetbio.1374657

2012). SCK can cause milk production losses in average up to 300 kg per lactation (Deniz et al., 2020; Duffield et al., 2009), as well as increases the risk for metabolic and reproductive diseases and the culling rate (Aksoy et al., 2022; McArt et al., 2012; Uyarlar et al., 2018).

Worldwide prevalence of SCK was reported to be 22-24 % in blood (Brunner et al., 2018; Suthar et al., 2013; Şentürk et al., 2016) and 39% in milk tests (Berge et al., 2014). Oral propylene glycol drenching was frequently used in controlling SCK (Gordon et al., 2017; McArt et al., 2014). However, there were contradictory reports about the significant efficacy of prophylactic PG treatment (PPGT) in dairy cattle in the literature (Fonseca et al., 2004; Jeong et al., 2018; Lomander et al., 2012; Østergaard et al., 2020). Many well-cared-for and managed integrated dairy cattle farms use PPGT in the entire population at calving to reduce hyperketonemia and associated metabolic diseases in Turkey despite contradictory and doubtful reports are available about its significant effects.

The present study was conducted to investigate the effects of PPGT at calving on blood and milk BHBA concentrations, as well as on SCK incidence and associated milk production, body condition scores (BCS), culling rate, and metabolic diseases in a dairy Holstein farm.

MATERIALS AND METHODS

Study animals and groups

The present randomized and controlled study was conducted on an integrated dairy Holstein farm with an average of 315 lactating cows per period. Forty-four pregnant primiparous and multiparous dairy Holstein cows, which were close to calving were randomly enrolled in the study. Cows in the treatment group (group 1) (n=19) received 300 ml of oral PG drench (99.9%, Yongjam-ro, Nam-gu, Ulsan, Korea) on the day of calving, one day and two days after calving (total 3 consecutive days). Control group cows (group 2) (n=25) remained untreated. Subgroups for SCK positive or negative (with or without SCK) were created to evaluate the effects of hyperketonemia on milk production and postpartum metabolic problems (Aksoy et al., 2022; Brunner et al., 2018; Suthar et al., 2013).

Animal feeding

Ration content and calculated energy intake were presented in Table 1. Water was served ad libitum. The content of the ration was prepared by farm a veterinarian and consultant animal feeding expert. As a standard protocol of the farm for the controlling of milk fever, anionic feeding was initiated at the last 21 days of gestation and oral calcium boluses were administered at calving and 12 h later to all animals in the farm.

Blood collection, analysis and health checks

Blood was collected from the coccygeal vein to analyse BHBA by a cow-side BHBA-analyser (Khol et al., 2019) (Medtrust Wellionvet Belua, Med Trust Handelsges.m.b.H., Austria) at postpartum week 2 (PW2) and 4 (PW4). On the same days, milk BHBA was tested in 50 ml of freshly taken milk with milk-test-strips (Ketotest, Elanco Animal Health, Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan). According to the manufacturer's instruction, this test strip showed different colours indicating 0, 50, 100, 200, 500, and 1000 µmol/L BHBA in the milk, which refer to the BHBA scores of 0, 0.5, 1.0, 2.0, 5.0, 10.0 respectively. SCK was defined by a cut-off point of BHBA≥1.2 mmol/L in the blood (Brunner et al., 2018; Gordon et al., 2017) and ≥200 µmol/L (BHBA score ≥2.0) in the milk (Aksoy et al., 2022; Benedet et al., 2019; Berge et al., 2014) as also recommended by the manufacturer of the test kits. BCS was evaluated according to Edmonson et al. (1989) on a scale from 1 to 5 at calving, 1 month and 2 months after calving.

Table 1. Content of the ration of the study cows as dry matter in close-up and early lactation.

Contents	Close-up	Early lactation
Maize silage (kg/day)	3.63	4.95
Hay (kg/day)	2.78	0.70
Alfalfa (kg/day) (17% protein)	2.08	4.06
*Concentrated milk feed (kg/day)	3.10	3.54
Maize flake (kg/day)	1.63	3.00
Cotton seed (kg/day)	0.00	1.35
Limestone (kg/day)	0.15	0.20
Soy sauce 46% protein (kg/day)	0.00	2.45
ByPass fat (kg/day)	0.00	0.50
**Vitamin, mineral and amino acid premix (kg/day)	0.12	0.12
Calcium chloride 77-80% (kg/day)	0.15	0.00
Ammonium sulphate (kg/day)	0.15	0.00
ME (Mcal/day)	32.1	54.3
NEI (Mcal/day)	20.1	34.9
DMI (kg/day)	13.7	20.8
Ca (%DM)	1.30	1.00
P (%DM)	0.30	0.40
DCAD (mEQ/kg)	-169	158

DM: dry matter, DMI: dry matter intake, ME: metabolizable energy, NEI: net energy intake, DCAD: dietary cation-anion difference. *Concentrated milk feed composed of 21 % crude protein, 4.2 % crude fat, 7.5 % crude cellulose, 23.4 % starch, 8.2 % crude ash. **Premix content: each 7,5 kg premix contains 1 mio IU of vitamin A, 350.000 IU of vitamin D3, 4.800 mg of vitamin E (50%), 100 mg of biotin (2%), 20 mg of vitamin B12 (1%), 4.000 mg of ferrous oxide (55%), 750 mg of organic ferrous (17%), 1.800 mg of copper oxide (21%), 6.000 mg manganese oxide 60%, 10.000 mg zinc oxide 75%, 1.000 mg of organic zinc (21%), 70 mg of sodium selenite (4.5%), 20 mg of organic selenium 0.3%, 100 mg of calcium iodate (62%), 40 mg of ethylene diamine dehydrate iodate (79.5%), 40 mg of cobalt sulphate 20%, 85.000 mg of choline chloride (25%), 30.000 mg of organic lysine (40%), 80.000 mg of organic methionine (55%), 2.000 g sodium bicarbonate (27% Na), 600.000 mg of magnesium oxide (82%), 87.500 mg of calcium (D.C.P 18% P and 15% Ca), 63.000 mg of phosphorous (D.C.P 18% P and 15% Ca), 200.000 mg sodium chloride (38% Na).

Average daily total milk production (ADMP) per cows was recorded with the automatic milking system (DeLeval 2 x 20 parallel speedy system) in 90 days in milk (90 DIM). All study cows in the groups were monitored and evaluated from the clinical health point of view, and any diseases or culling were registered immediately up to 90 DIM.

Statistical analysis

Statistical analyses were performed using the SPSS (SPSS 22, IBM SPSS Statistics®, Chicago, IL, USA) software and the results were evaluated for α=0.05. Mean (m) and standard error (se) were presented as descriptive statistics. Normality of the data were evaluated by Kolmogorov-Smirnov and Shapiro-Wilks tests. The nonparametric tests (Mann-Whitney, Wilcoxon, Friedman) were used for statistical analysis because of non-normality of the data and small sample size. Blood and milk BHBA were analysed by Mann-Whitney-U test to compare treatment and control groups including subgroups (with SCK or without SCK). Change of BCS from calving to postpartum day 30 and 60 was tested by Friedman test. Wilcoxon test was used for pairwise comparisons. Differences in BCS between group 1 and group 2 was evaluated by Mann-Whitney-U test. Incidence of the diseases in the groups was presented as percentage. Odds ratio (OR) was determined for each of diseases (for those with sufficient data for computation) in the groups. Mann-Whitney-U test is used to compare the average daily and weekly milk production between the groups and subgroups.

RESULTS

The average lactation numbers were 2.42±0.90 and 2.96±1.84 in the group 1 and 2 respectively (P>0.05). Blood and milk BHBA levels were not significantly different between group 1 and 2 at PW2 and PW4 (Table 2). The incidence of SCK in group 2 was 8% and 4% at PW2 and 4 in the blood test, it was 12% and 24% at PW2 and 4 in the milk test respectively (Table 3). However, SCK was not observed in group 1 in the blood and milk. The OR for SCK at PW4 in milk test was 5.68 times greater in group 2.

Table 2: BHBA concentrations (mean±standard error, mmol/L) and milk BHBA scores at postpartum week 2 and 4 and body condition scores (BCS, mean±standard error) at postpartum day 30 and 60 of treatment group (group 1, n=19) and control group (group 2, n=25).

Parameters		Group 1	Group 2	P
Blood BHBA	PW2	0.453±0.046	0.679±0.167	0.755
Blood BHBA	PW4	0.405±0.030	0.388±0.020	0.990
Milk BHBA*	PW2	0.463±0.043	1.333±0.580	0.488
Milk BHBA*	PW4	0.316±0.290	1.229±1.014	0.948
BCS	Calving	3.276±0.039[a]	3.248±0.039[a]	0.279
BCS	PPD 30	3.066±0.038[b]	3.010±0.049[b]	0.351
BCS	PPD 60	2.921±0.027[c]	2.850±0.086[c]	0.856
	P**	<0.0001	<0.0001	-

PW2: postpartum week 2. PW4: postpartum week 4. PPD30: postpartum day 30. PPD60: postpartum day 60. BHBA: blood beta-hydroxybutyric acid. BCS: body condition score. *Milk test strips indicate colours for 0, 50, 100, 200, 500, 1000 µmol/L of BHBA in the milk, which meet to BHBA scores 0, 0.5, 1.0, 2.0, 5.0 and 10.0 respectively. [a, b, c:] different letters refer to the significant difference within the group 1 and 2. **: refers BCS between calving, PPD30, PPD60

There was no significant difference between group 1 and group 2 concerning BCS at calving, including postcalving day 30 and 60 (Table 2), as well as in the subgroups (with and without SCK) (data not shown in tables). Almost all animals in the groups lost significantly BCS (P<0.01) on postcalving days 30 and 60 (Table 2).

Table 3: Incidence of subclinical ketosis, postpartum diseases and culling rate in treatment group (group 1) and control group (group 2) up to postpartum day 90.

Diseases	Postpartum	Group 1	Group 2
SCK (blood BHBA≥1.2 mmol/L)	Week 2	0.0%	8.0%
SCK (milk BHBA≥200 µmol/L)*	Week 2	0.0%	12.0%
SCK (blood BHBA≥1.2 mmol/L)	Week 4	0.0%	4.0%
SCK (milk BHBA≥200 µmol/L)*	Week 4	0.0%	24.0%[1]
Mastitis	90 days	10.5%	32.0%[2]
Metritis	90 days	5.3%	8.0%[3]
Laminitis	90 days	5.3%	4.0%[4]
Clinic ketosis	90 days	0.0%	4.0%
Culling rate	90 days	0.0%	12.0%
SCK in culled cows (blood)	Week 2	0.0%	0.0%
SCK in culled cows (milk)	Week 2	0.0%	33.3%
SCK in culled cows (blood)	Week 4	0.0%	33.3%
SCK in culled cows (milk)	Week 4	0.0%	66.7%

BHBA: betahydroxybutyric acid concentration. SCK: subclinical ketosis. *Milk test strips indicate colours for 0, 50, 100, 200, 500, 1000 µmol/L of BHBA in the milk, which meet to BHBA scores 0, 0.5, 1.0, 2.0, 5.0 and 10 respectively. SCK: subclinical ketosis. Odds ratio: [1]5.68, [2]4.00, [3]1.56, [4]0.75

Three cows in group 2 (12 %) and none of cows in group 1 were culled in 90 DIM. Cows were culled by the farm veterinarian at 73, 48, and 35 days postcalving due to udder problems, very low milk production and downer cow syndrome. Incidence of SCK based on the blood and milk tests on PW4 was 33.3 and 66.7% in the culled cows of group 2 respectively. No OR for culling

was generated in the statistical analysis with respect the SCK. The most common disease was mastitis in group 1 (10.5%) and group 2 (32%). OR respecting mastitis was 4.0 in group 2 with much higher incidence in 90 DIM. Metritis, laminitis and CK were observed at an incidence of 8%, 4%, and 4% in group 2 respectively, but without a significant OR (Table 3).

Table 4: Average daily milk yield (mean ± standard error, kg) in treatment group (group 1) and control group (group 2) and subgroups with and without SCK in the control group up to postpartum day 90.

Groups	n	Milk yield (kg)	P***
Group 1 (total)	19	33.92±1.65	
Group 2 (total)	25	30.69±2.49	>0.05
Group 2 with SCK*	6	19.06±6.44	<0.05
Group 2 without SCK	19	34.36±2.03	>0.05
Group 2 with SCK**	6	28.36±4.11	=0.07

SCK: subclinical ketosis (milk BHBA ≥200 µmol/L at postpartum week 4). *: Milk yield was taken zero as of culling day until 90 DIM. **: Milk yield was not included in the calculation after culling day. ***: P value refers in comparison with group 1 (treatment group)

Average daily and weekly milk production was presented in Table 4 and Figure 1. There was no significant difference between group 1 and group 2 concerning ADMP in 90 DIM. The number of SCK positive cows in group 2 was not enough at PPW2 to conduct a statistical comparison for milk yield. SCK positive cows in the milk testing at PW4 as a subgroup in group 2 had significantly reduced ADMY (19.06±6.44 kg) in 90 DIM (Table 4). The difference between the average daily and weekly milk production of group 1, 2 without SCK and group 2 with positive SCK was meaningfully different (P=0.026) if milk yield of culled cows with SCK tested in the milk or blood (n=2) was accounted for as zero from culling date to postcalving day 90th. The difference between average daily milk production of cows with positive SCK (28.36±4.11 kg) and without SCK (34.36±2.03) in group 2 was moderately significant (p=0.07) if the milk yield of culled cows in this group was not included in the calculation from culling date to postcalving day 90th.

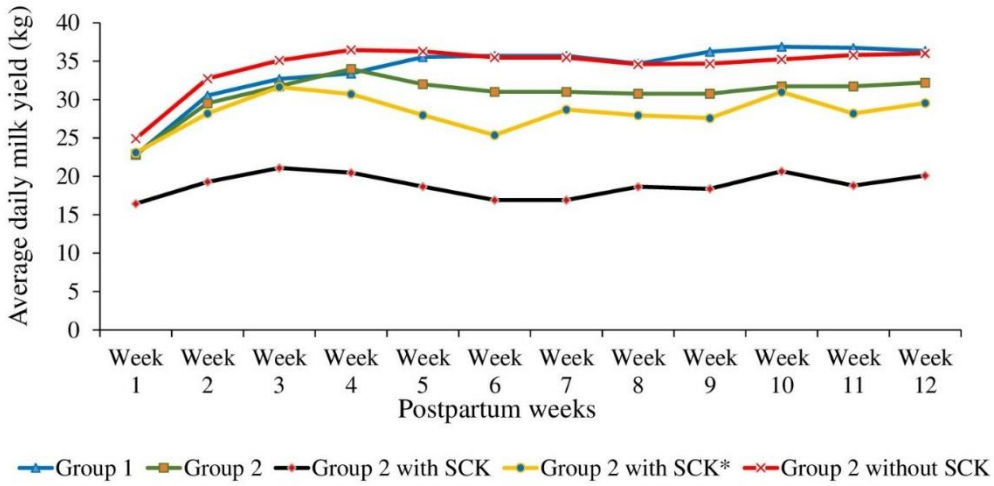

Figure 1: SCK: subclinical ketosis. Average daily milk production (kg) per week in the group 1 (treatment group), group 2 (control group with and without SCK) between postcalving week 1 to 12. *Group 2 with SCK: The milk production of culled cows with positive SCK in the control group was not included in the calculation after culling until to 90 DIM. Group 2 with SCK: The milk production of culled cows with positive SCK (n=2) in the control group was taken '0' in the rest of days after culling until to 90 DIM. SCK: cows with subclinical ketosis (milk BHBA ≥200 µmol/L at postpartum week 4). P<0.05 for the weeks 2 to 10 between group 1 (treatment group) and group 2 (control group) with SCK.

DISCUSSION

In the present study, blood and milk BHBA testing time points were the most prevalent period after calving and in line with previous reports in dairy cows (Brunner et al., 2018; McArt et al., 2012; Suthar et al., 2013). The cut-points for milk and blood BHBA for SCK were also in compromise with the previous reports (Benedet et al., 2019; Berge et al., 2014; Brunner et al., 2018; Gordon et al., 2017).

The present study did not find a significant effect of PPGT on average blood and milk BHBA at neither PW2 nor 4 that results were in line with the previous studies (Jeong et al., 2018). But, SCK was not observed in group 1 compared to group 2 in the present study, this was not reported by others because of the lack of classification of cows for SCK by the cut-points of BHBA. PG has been used for the treatment and control of SCK in dairy cows using different protocols (Gordon et al., 2017; Lomander et al., 2012; McArt et al., 2014; Zhang et al., 2020). However, there were contradictory and unsatisfactory results reported worldwide about the efficacy of PG in dairy cattle (Jeong et al., 2018; Lomander et al., 2012; Østergaard et al., 2020), although many dairy farms use preventive PG at calving in Türkiye. However, Gordon et al. (2017) stated that the PG treatment was beneficial in decreasing blood BHB concentrations in more severely affected animals with high blood BHBA and low glucose concentrations. That was a treatment regime with 2 more days of applications rather than a prevention. Oral drench of PG (400 ml) in lactating dairy cows (126 DIM) with positive energy balance reduced blood BHBA concentration within 2.5 h after the drenching, however blood BHBA concentrations increased in the 11 h post-treatment (Mikuła et al., 2020). PG drenching had short efficacy on the blood BHBA and treated cows were in 126 DIM and had positive energy balance, which is not comparable to the fresh cows treated preventively in the present study.

In the present study, the incidence of SCK observed in the blood and in the milk tests of group 2 looked a little lower than the previously reported prevalence in Turkey (Aksoy et al., 2022; Suthar et al., 2013; Şentürk et al., 2016; Uyarlar et al., 2018) and in the world (Brunner et al., 2018; Suthar et al., 2013). That might be a reason for the lack of efficacy of PPGT on the average milk and blood BHBA, or due to the dosage of PG. The prevalence of SCK was reported to be much higher when tested in the milk (Benedet et al., 2019; Berge et al., 2014) compared to blood test as reported by the present study as well. PPGT did not effect on BCS that was consistent with studies of Fonseca et al. (2004) and Jeong et al. (2018). However, it was inconsistent with the results of El-Kasrawy et al. (2020), who observed positive significant effect on BCS in 30 DIM at a much higher dosage of PG at pre- and postcalving.

Deniz et al. (2020) reported that cows with SCK have a significantly higher risk for displaced abomasum (DA), retained placenta (RP), milk fever (MF) and cystic ovarian (CO). McArt et al. (2011; 2014) reported that oral PG supported to cure hyperketonemia and reduced risks for DA. But, DA, RP, MF and CO were not observed in the present study. The study cows received oral calcium boluses at calving and had anionic feeding at close-up as standard protocol of the farm which can prevent from milk fever (Goff, 2008). Metritis and laminitis were observed both in group 1 and group 2 respectively, but without a significant odds ratio between groups. Suthar et al. (2013) reported 1.7 times higher risk for metritis and Brunner et al. (2018) reported 5.3 % incidence of metritis in SCK worldwide, and Uyarlar et al. (2018) reported 25 % incidence of metritis in SCK. The inconsistence with the literature might be due to different hyperketonemia incidence between the studies.

The most frequently observed postpartum disease was mastitis in group 1 and 2 thus group 2 cows had 4 times higher risk for mastitis compared to the cows in group 1. Similar results were reported by Uyarlar et al. (2018) in Türkiye. Suthar et al. (2013) did not find a significant correlation between mastitis and SCK. The high culling rate in group 2 and higher SCK incidence in culled cows were consisten with the previous studies (McArt et al., 2011; Uyarlar et al., 2018). Uyarlar et al. (2018) reported a 26.4 % culling incidence in cows with SCK. The results of the present study were similar with the previous reports. McArt et al. (2011) reported a reduction of culling rate in 30 DIM in cows treated with PG against SCK between 3-16 DIM. However, it was not a prevention study, rather a cure protocol of SCK. Although the result of the present study did not create a significant OR for culling rate and risk, the descriptive data showing a higher incidence of culling in group 2 is in line with the previously reported data.

The average daily and weekly milk production between group 1 and group 2 was not significantly different at 90 DIM. This was consistent with the data of Jenkins et al. (2015) and Jeong et al. (2018). Another study by Fonseca et al. (2004) indicated limited increased milk yield in cows treated with much higher oral PG dosages in the first 4 weeks of lactation, which was inconsistent with the results of our present study. A much higher dosage of PG (300 g, twice a day, from calving to 21 DIM) has slightly tended to increase the milk yield in 90 DIM (Lomander et al., 2012). Although a much higher dosage of PG used in those studies than in the present study, a limited increase in milk production was observed. Once the subgroups with and without SCK were created in group 2, a significant reduction in average daily and weekly milk production was observed in the SCK positive group. These results of the present study were in line with the previous studies (El-Kasrawy et al., 2020; McArt et al., 2014; Zhang et al., 2020), but many previous studies focused on the treatment of SCK rather than its prevention. SCK caused a significant loss of up to average 300 kg milk in 305 DIM (Deniz et al., 2020).

In terms of the overall effects of PPGT, the results of the present study are in line with others (Fonseca et al., 2004; Jenkins et al., 2015; Jeong et al., 2018; Østergaard et al., 2020). Other studies indicated also limited effects of PG, even no significant effect, but certain tendencies in the effects on the average milk production, BCS and postpartum health disorders were observed. Preventive treatment of all cows at 5 DIM with PG was the most cost-effective strategy when herd hyperketonemia incidence was >50% (McArt et al., 2014). No satisfactory effects were observed by PG application to balance the metabolic status and NEB in dairy cattle by others (Østergaard et al., 2020). Looking at the average worldwide prevalence (average 22–24%) as well as in the present study, the statement of McArt et al. (2014) that PPGT requires >50% incidence of hyperketonemia in the farm seems currently to be unrealistic.

CONCLUSION

In conclusion, PPGT at calving did not significantly affect the averages of blood and milk BHBA at PW2 and 4, BCS and ADMP. These results were in agreement with some previous studies that reported unfavourable results about PG effect. However, once the study cows were classified for SCK by blood and milk BHBA cut-off points, a clear effect of PPGT was observed on SCK incidence in association with postpartum health disorders and ADMP. Therefore, a risk assessment for the incidence of SCK should be performed in the respective farms based on cost-benefit calculations for PPGT in

advance. Consequently, a selective PPGT can be used in cows at risk or predisposed of SCK at calving or early postpartum rather than treating the entire population. PPGT in dairy cows can be an option and a next study hypothesis for small family business-dairy farming, in which professional tailor-made dairy management and continuous monitoring by veterinarians might be inadequate.

ACKNOWLEDGMENT

We would like to thank farm veterinarian and owner for their support in this study.

Financial support: Authors did not receive funding for this study.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical statement: This study was approved by the Muğla Sıtkı Koçman University Animal Experiments Local Ethics Committee (23.09.2021/34-21). In addition, the authors declared that Research and Publication Ethical rules were fully followed.

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Anöstrüs dönemindeki Kıvırcık ırkı koyunlarda kısa ve uzun süreli progesteron uygulamalarının üreme değerleri üzerine etkileri

The effects of short and long-term progesterone applications on reproductive values in Kıvırcık sheep in the anoestrus period

Research Article

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ÖZET

Yapılan araştırmada, koyunlarda Anöstrüs (üreme mevsimi öncesi) uygulamalar ile ovaryumun hormonal değerlerinin artırılabilme imkanlarının araştırılması ve uygulanan farklı yöntemlerin fertilite üzerine etkisinin belirlenmesi amaçlandı. Materyal olarak, 2-4 yaşlı laktasyonda olmayan 210 adet Kıvırcık ırkı koyun ve fertilitesi kanıtlanmış 3-5 yaşlı 25 adet koç kullanıldı. Koyunlar EBC6 (Eazi-breed 6 gün süreli), EBC12 (Eazi-breed 12 gün süreli) ve kontrol grubu olmak üzere 3 gruba bölündü. Eazi-Breed Controlled Internal Drug Release (CIDR)'lar (0,33 g progesteron) koyunların anterior vaginasına grup EBC12'de 12 gün, grup EBC6'da 6 gün süre ile yerleştirildi. CIDR'ların uzaklaştırılmasını takiben 400 IU PMSG intramuskuler olarak enjekte edildi. Arama koçu yardımıyla 6 gün süre ile östrüsler tespit edildi, östrüste olduğu tespit edilen koyunlara elde aşım uygulandı. Kontrol grubunda ise 5 gün aralıklarla 4 kez kan örnekleri alınarak spontan ovaryum aktivitesine sahip koyunlar tespit edildi. Koyunların gebelikleri, aşımları takiben 40. günde reel-time B mod ultrason yöntemiyle kontrol edildi. Deneme grupları arasında, östrüs gösterim oranları istatistiki olarak önemsiz iken ($p>0,05$), östrüs gösterim oranları açısından kontrol grubu ile diğer gruplar arasındaki farklılıklar önemli bulundu ($p<0.05$). İkiz doğumlar açısından gruplar arasında istatistiki bir fark bulunmamış iken ($p>0,05$), tekil doğum oranları açısından EBC12 ile EBC6; ikiz, çoklu doğum ve kuzu verimi oranları açısından ise EBC6 ile EBC12 grupları arasındaki farklılıklar önemli bulundu ($p<0.05$). Sonuç olarak, Kıvırcık ırkı koyunlarda anöstrus döneminde ovaryum aktivitesinin uyarılmasında kısa süreli progesteron uygulamalarının oldukça etkili olduğu tespit edildi.

Anahtar Kelimeler: CIDR, kısa süreli 5-8 gün, Kıvırcık koyun, progesteron, uzun süreli 12-14.

ABSTRACT

In the research, it was aimed to investigate the possibilities of increasing the hormonal values of the ovary with anestrus (before the breeding season) applications in ewes and to determine the effects of different methods applied on fertility. As a material, 210 Kıvırcık ewes aged 2-4 years not in lactation and 25 rams aged 3-5 years old with proven fertility were used. Ewes were divided into 3 groups: EBC6 (Eazi-breed for 6 days), EBC12 (Eazi-breed for 12 days), and the control group. Eazi-Breed Controlled Internal Drug Release (CIDR) (0.33 g progesterone) were placed in the anterior vagina of ewes for 12 days in group EBC12 and 6 days in group EBC6. Following the removal of the CIDRs, 400 IU PMSG was injected intramuscularly. Oestrus was detected for 6 days following the removal of CIDRs using search ram. The ewes, which were found to be in estrus with the help of a search coach, were hand-measured. In the control group, blood samples were taken 4 times at 5-day intervals and ewes with spontaneous ovarian activity were detected. The pregnancies of the ewes were checked by real-time B-mode ultrasound on the 40th day following their breeding. While the rates of estrus representation were statistically insignificant between the trial groups ($p>0.05$), the differences between the control group and the other groups in terms of oestrus display rates were significant ($p<0.05$). While there was no statistical difference between the groups in terms of twin births ($p>0.05$), EBC12 vs. EBC6 for single birth rates; The differences between EBC6 and EBC12 groups were significant in terms of twin, multiple birth, and lamb yield rates ($p<0.05$). As a result, it was determined that short-term progesterone applications were quite effective in stimulating ovarian activity during anoestrus period in Kıvırcık ewes.

Keywords: CIDR, Kıvırcık ewes, long term 12-14, progesterone, short term 5-8 days

Article info
Submission: 18-06-2023
Accepted: 21-12-2023
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

How to cite this article
Kırkpınar, S., Ataman, MB., (2024). The effects of short and long-term progesterone applications on reproductive values in Kıvırcık sheep in the anoestrus period. *Journal of Advances in VetBio Science and Techniques*, *9*(1), 10-17. https://doi.org/10.31797/vetbio.1329176

GİRİŞ

Tüm dünyada hayvanlardan elde edilen gıdaların artırılmasına yönelik birçok çalışma bulunmaktadır. Bu amaçla flushing, östrus senkronizasyonu, suni tohumlama, embriyo transferi gibi reprodüktif uygulamalar yapılmaktadır. Reprodüktif amaçlı farklı üreme yöntemlerinin yaygın bir şekilde uygulanmasıyla ülkemizdeki mevcut koyun popülasyonunun artırılması mümkün olabilir. Koyunlarda ovaryum aktivitesinin üreme sezonu dışında uyarılmasının, kuzu üretimini yılın tüm aylarına yaymak ve yıllık damızlık hayvan sayısı üretimini artırmak gibi başlıca amaçları vardır (Castonguay, 2000). Bununla birlikte planlı ve düzenli suni tohumlama uygulamalarının yapılması ile, mevcut et ve süt üretimini artırarak üreme sezonu dışında da bu ürünlerin pazarlanmasına olanak sağlamaktadır (Baril, 2003).

Özellikle ülkemizde kısa dönem CIDR uygulama yöntemleri ile anöstrüs döneminde yapılan çalışmalar oldukça azdır. Progesteron veya Analogları uzun süreli olarak kullanıldığında, bu preparattan salgılanan progesteron miktarı zaman geçtikçe azalırken, kısa süreli uygulamalarda kan progesteron seviyesi yüksek konsantrasyonda kalmaktadır (Christenson, 1976). Uygulanan progesteronun düşük dozda kalması östrus senkronizasyon aralığını azaltmakta, dişi genital kanalda spermatozoon taşınmasını engellemekte ve spermatazoonun yaşam sürelerini azaltarak fertilite oranlarını düşürdüğü ifade edilmektedir (Allison ve Robinson, 1970). Altı gün süre ile progestagen uygulamalarının intravaginal progesteron salan süngerin uzaklaştırılması anında yüksek progesteron seviyesi sağladığı, 12-14 gün süreli uygulamalar kadar etki gösterdiği, saha şartlarında daha kolay ve pratik olarak kullanılabileceği ifade edilmektedir (Ungerfeld ve Rubianes, 1999). Kısa süreli uygulamalar 5-7 gün sürmekte, uygulamanın başında veya sonunda PGF2α enjeksiyonları, uygulama sonlarında ise Equine Chorionic Gonadotropin (eCG) enjeksiyonları yapılmaktadır (Dixon vd., 2006). Üreme sezonu dışında yapılan östrus senkronizasyonu, koç etkisi ve ovaryum senkronizasyonu uygulamalar ile östrus ve gebelik oranları imkanlarının araştırılması ve bu uygulanan yöntemlerin fertiliteye olan etkisinin belirlenmesi sunulan çalışmada amaçlandı.

MATERYAL VE METHOD

Yapılan çalışma Trakya bölgesi Çanakkale ili Korubaşı (39°30’55’’ enlem ve 26°15’37’’ boylamı) köyünde gerçekleştirildi. Materyal olarak, 2-4 yaşlı laktasyon döneminde olmayan 210 Kıvırcık ırkı koyun ve fertilitesi ispatlanmış 3-5 yaşlı 25 koç kullanıldı. Anöstrus dönemindeki koyunlar Eazi-breed 6 gün süreli (EBC6), Eazi-breed 12 gün süreli (EBC12) ve kontrol grubu olmak üzere 3 gruba bölündü. CIDR uygulanacak koyunlar özel bir bölüme alındı. CIDR (Eazi-breed^{TM}, CIDR^{R}, Zoetis USA)’lar, uygulama aplikatörleri, antiseptik solüsyon, kayganlaştırıcı jel, lateks eldivenler bir masa üzerinde hazırlandı. Uygulama yapılacak koyunların zapturaptı bir yardımcı tarafından sağlandı. Koyunların vulva bölgesi antiseptikli suyla temizlendi, CIDR’lar ilgili aparatına yerleştirildi, aparatlar jel ile kayganlaştırıldı. Aplikatörler vulva dudakları ikiye ayrılarak zemin ile 30-45 derece açı olacak şekilde vulvadan içeriye doğru cranio-dorsal yönde 4-5 cm yavaşça ilerletildi, daha sonra aplikatörler yere paralel duruma getirilerek serviks uteriye kadar ilerletildi ve çekme ipleri vulva dudaklarından dışarıya sarkıtılarak görünecek şekilde CIDR’lar yerleştirildi. Her bir uygulamada kullanılan aplikatörler temizlenip antiseptik ile dezenfekte edildi.

CIDR’lar (0,33 g progesteron) koyunların anterior vaginasına grup EBC12’de 12 gün, grup

EBC6'da ise 6 gün süre ile yerleştirildi. CIDR'ların uzaklaştırılması ile birlikte 400 IU Pregnant Mare Serum Gonadotropine (PMSG, Merck Sharp Dohme (MSD), USA) intramuskuler olarak enjekte edildi. Arama koçu yardımı ile CIDR'ların uzaklaştırılmasını takiben sekiz saat aralıklarla günde üç kez 6 gün süreyle 8/9 koyuna 1 koç olacak şekilde östrüsler takip edildi. Östrüste olduğu tespit edilen koyunlara elde aşım uygulandı. Aşımları takiben geri dönen koyunları (östrüs gösteren) belirlemek amacıyla sonraki iki siklus boyunca östrüsler izlendi. Kontrol grubunda spontan ovaryum aktivitesine sahip koyunları belirlemek amacıyla 5 gün aralıklarla 4 kez (-15,-10,-5,0.günler) kan örnekleri toplandı. Koyunların vena jugularisden 10 ar mililitre kan örnekleri heparinli cam tüplere aktarıldı. Alınan bu örnekler 3000 rpm de 15 dakika süreyle santrifüj edilerek plazmaları ayrıldı, plazmalar 2,5 mililitrelik assay tüplerine aktarılarak analiz gününe kadar -20°C de saklandı. Progesteron hormon ölçümleri, Lalahan Hayvan Sağlığı ve Nükleer Araştırma Enstitüsünde Enzimimmunoassay (EIA) yöntemiyle yaptırıldı. Kan progesteron seviyesi 0,5 ng/ml seviyesi üzerinde olan koyunların ovaryum aktivitesine sahip oldukları kabul edildi. Gebe koyunların gebelik muayeneleri aşımları takiben 40. günde 5 megaherztlik (MHz) sektör transüdörü (prob) bulunan bir reel-time B mod ultrason cihazı kullanılarak belirlendi. Çoklu doğum yapan koyun sayısı ise doğumları takiben tespit edildi. Östrüs, gebelik, doğum oranları ve kuzu verimleri arasındaki farklılıklar SPSS 21.0 programında Ki-kare testi yardımıyla belirlendi. Östrüs, gebelik, doğum oranları ve kuzu verimleri aşağıda belirtilen yöntemle hesaplandı.

Östrüs oranı = Östrus olduğu belirlenen koyun sayısı / uygulama yapılan koyun sayısı
Gebelik oranı = Gebe kalan koyun sayısı / uygulama yapılan koyun sayısı
Doğum oranı = Doğuran koyun sayısı / uygulama yapılan koyun sayısı
Kuzu verimi = Doğan kuzu sayısı / doğuran koyun sayısı.

BULGULAR

Grup EBC6, grup EBC12 ve kontrol grubu arasındaki östrus oranları ile, Grup EBC6 ve grup EBC12 arasındaki gebelik, doğum, teklik, ikizlik, çoklu doğum ve kuzu verimi oranları Tablo 1 de belirtilmiştir.

Tablo 1. Çalışmada elde edilen bazı fertilite parametreleri

Grup/Oran	EBC6 Grubu (n=80)	EBC12 Grubu (n=80)	Kontrol Grubu (n=50)
Östrus, %	78/80[a] (97,50)	80/80[a] (100)	8/50[b] (16)
Gebelik, %	75/80[a] (93,75)	70/80[b] (87,50)	-
Doğum, %	72/80[a] (90,00)	65/80[b] (81,25)	-
Tek, %	24[a] (33,33)	37[b] (56,92)	-
İkiz, %	34 (47,22)	24 (36,92)	-
Çoğul, %	14[a] (19,44)	4[b] (6,15)	-
Kuzu verimi, %	1,91 (1,91)	1,49 (1,49)	-

[a-b]: Aynı satırda değişik harf taşıyan gruplar arası farklılık önemlidir ($p<0.05$).

Sunulan çalışmada spontan ovaryum aktivitesi oranı %16 olarak belirlenerek uygulamaların yapıldığı zaman aralığının anöstrus sezonuna uygun bir dönem olduğu tespit edildi. EBC6 ve EBC12 gruplarında toplam östrüs oranları sırasıyla %97,50 ile %100 iken kontrol grubunda ise bu oran %16 olarak tespit edilmiştir. Östrüs oranları açısından deneme grupları arasında istatistiki açıdan herhangi bir farklılık gözlenemez iken ($p>0.05$), gebelik ve doğum oranları açısından gruplar arasındaki farklılık önemli ($p<0.05$) bulundu.

Tekil doğum oranları açısından EBC6 ile EBC12 grupları arasındaki farklılıklar istatistiki açıdan önemli bulundu ($p<0.05$). İkiz doğumlar açısından gruplar arasında istatistiki açıdan

önemli bulunmadı (p>0.05). Çoklu doğum oranları açısından EBC6 ile EBC12 grupları arasındaki farklılıklar istatistiki açıdan önemli bulundu (p<0.05). Kuzu verimleri açısından gruplar arasında istatistiki açıdan herhangi bir farklılık belirlenmemiş ancak grup EBC6'da grup EBC12'ye göre bir oransal olarak artma mevcuttur (p>0.05).

TARTIŞMA

Koyunlarda üremenin denetlenmesi amacıyla anöstrüs döneminde progestagen preparatları kullanılarak kan-progesteron seviyelerinin yükseltilmesiyle; üreme sezonunda ise progesteron uygulanması veya koyunlarda mevcut olan luteal dokuların prostaglandinler ile lize edilmesi (Baril vd., 1993) ya da melatonin hormonunun (Kaya, 1996) gonadotropik etkilerinden yararlanmak suretiyle gerçekleştirilmektedir. Küçük ruminantlarda CIDR uygulamaları kısa süreli (5-7 gün) ya da uzun süreli (12-19 gün) olarak uygulanmaktadır (Abecia vd., 2011; Carlson vd., 1989; Jackson vd., 2014; Vilariño vd. 2011). Kısa süreli progesteron uygulamalarının başlıca yararlarından birisi de kısa bir zaman dilimi içerisinde koyunların topluca senkronize edilmesidir. Bu durum üreticilere planlı suni tohumlama ve embriyo nakli programları uygulanabilmesine olanak sağlar. CIDR uygulamaları PG600 ve $PGF_{2\alpha}$ hormonları ile kombine olarak kullanılabilmektedir. Kısa süreli uygulamalar ile farklı hormonların kombine olarak kullanılmaları luteal dönemin ve folliküler dinamiğin kontrolüne imkan sağlamaktadır (Vilariño vd., 2011). Yapılan çalışmada gruplar arasındaki östrus, gebelik, doğum oranları, tek, ikiz ve çoklu doğum oranları ile kuzu verimi oranları farklılıkların araştırılması amaçlandı.

Çalışmada spontan ovaryum aktivitesi gösteren koyunların oranı %16 (8/50) olup, bu oran koyunların hakiki anöstrüste olduğunu gösteren bir gerçekliktir ve bu oran deneme gruplarında elde edilen oranların tamamından düşüktür. Çalışmada östrüs oranları sırasıyla EBC6, EBC12, gruplarında sırasıyla %97,50, %100 olarak belirlenmiştir. Elde edilen değerler (Akbaş ve Köse, 2017) (%88, %72), (Doğan ve Nur, 2006) (%77,8, %85,7), (Fleisch vd., 2013) (%91,7, %93,8), (Júnior vd., 2019) (%95.23, %92.85), (Tajaddodchelik vd., 2017) (%82,69, %86,53), (Wei vd.,2016) (%71,43, %85,72) elde ettikleri değerlerden yüksek, (Çevik vd., 2017) (%97, %100) elde ettikleri değerleri ile benzer ve (Wei vd., 2016) (% 100) elde ettikleri değerlerden düşüktür. Araştırmacıların elde ettikleri tüm değerler yapılan çalışmada EBC12 grubundan elde edilen %100 östrüs oranı ile benzerlik gösterirken EBC6 grubundan yüksektir. Değerler arası farklılıkların oluşmasında; çalışmalarda farklı ırktan koyunları kullanılmaları, çalışmaların üreme sezonunun farklı dönemlerinde ve farklı coğrafik bölgelerde yapılması, östrüs senkronizasyon amacıyla sünger ya da CIDR kullanımının sebep olduğu düşünülmektedir.

Yapılan çalışmada EBC6, EBC12 gebelik oranları sırasıyla %93,75, %87,50 olarak elde edilmiştir. Bu değerler (Akbaş ve Köse, 2017) (%60, %64), (Pinna vd., 2012), (%42,9, %61,5), (Tajaddodchelik vd., 2017) (%47,11, %56,73) elde ettikleri oranlardan yüksek, (Jackson vd., 2014), (%87, %88) elde ettikleri değerlere yakın, (Omontese vd., 2014), (%100)'nın değerlerinden düşük olarak tespit edilmiştir.

Üreme sezonu dışındaki farklı ırk koyunlarda kısa (7 gün) ve uzun (14 gün) süreli CIDR uygulamalarını takiben sırasıyla %85,19, %96,15 östrüs ve %81,48, %88,46 gebelik oranı elde ettiğini ifade etmektedir (Harl, 2014). Gerek östrüs gerekse gebelik oranları çalışmamızda elde edilen gebelik ve doğum oranlarına yakın ve benzer olarak bulunmuştur. Oranların benzerlikleri her iki çalışmada da CIDR kullanılmasına bağlı olabileceği

düşünülmektedir. Üreme sezonu dışında 5 günlük CIDR uygulamalarının fertilite oranlarında azalmaya sebep olmadığını vurgulamaktadır (Vilariño vd., 2011). Sunulan çalışmada da kısa süreli uygulamaların fertilite oranlarını düşürmemesi Vilarino vd (2011) çalışmasını destekler niteliktedir. Gebelik oranları arasındaki farklılıkların; östrüslerin belirlenmesini takiben yapılan elde aşım ya da suni tohumlama uygulamalarına, senkronize östrüslerde ise östrüs belirlenmesinde kullanılan koç/koyun oranlarına, spontan embriyonik ölüm/abort oranlarına, kullanılan gonadotropin ve progesteron kaynaklarının farklı olmasına ve östrüslerin uyarılması amacıyla farklı ve modifiye yöntemlerin kullanılmasına bağlı olarak değiştiği düşünülmektedir.

Yapılan çalışmada EBC6, EBC12 gruplarından elde edilen doğum oranları sırasıyla %90, %81,25 olarak belirlenmiştir. 5 gün süreli CIDR ve CIDR-$PGF_{2\alpha}$ uyguladıkları anöstrüs dönemindeki Columbia ve Hampshire ırkı koyunlarda sırasıyla %87 ve %90 doğum oranı elde etmişlerdir (Jackson vd., 2014). Üreme sezonu dışında Lacaune ırkı koyunlarda yaptıkları çalışmada CIDR'ları 12 ve 6 gün süreyle uygulamışlar ve doğum oranlarını sırasıyla %83,30, %72,90 elde etmişlerdir (Fleisch vd., 2013). Değerler arası farklılıklara kullanılan hayvan materyalinin farklı ırktan olması, hayvanların prolifik ve nonprolifik özelliklerine bağlanabilir. Suffolk ırkı koyunları 9 gün süreli 500 mg progesteron içeren sünger ve CIDR kullanarak östrusları senkronize etmişler, östrüsteki koyunlara elde aşım uygulamışlardır ve sırasıyla % 54,2 ve % 61,5 doğum oranı elde etmişler (Fukui vd., 1994). Araştırmacıların elde ettikleri oranlar sunulan çalışmadaki gruplarda (EBC6-%90, EBC12-%81,25) elde edilen doğum oranlarından oldukça düşüktür. Oranlar arasındaki farklılık, Fukui vd (1994) çalışmalarını hakiki anöstrüs sezonu içinde yapmaları, sunulan çalışmanın ise anöstrüs sezonunun sonuna doğru yapılmasına bağlanabilir. Bununla birlikte doğum oranları arasındaki farklılıklara; erken ya da geç embriyonik ölüm oranlarının, fertilizasyon hatalarının, elde aşımlarda koçların aşırı kullanımlarının, çalışmaların yapıldığı bölgelerdeki iklim değişiklikleri ve ısı streslerinin sebep olabileceği düşünülmektedir.

Yapılan çalışmada EBC6, EBC12 gruplarında elde edilen tekil doğum oranları sırasıyla %33,33 %56,92 olarak belirlenmiştir. Sezon dışında Dorset, Suffolk, Cheviot, Polypay, Romney ve Hampshire melezi koyunlarda 9 gün süreyle tuttukları norgestomet implant ve CIDR'ların uzaklaştırılması sonrası PG600'ün (Intervet/Merck Animal Health, Madison, NJ) 1,5 ml'den 5 ml'ye kadar değişen dozlarını denedikleri araştırmada en yüksek tekil doğum oranını 2,5-3 ml uyguladıkları grupta %26 olarak elde etmişlerdir (Cross vd., 2019). Araştırmacılar tekil doğum oranlarının diğer gruplarda %20–23 arasında değiştiğini bildirmişlerdir. Sunulan çalışmada her iki gruptan elde edilen tekil doğum oranları Cross vd (2019) yılında yapmış oldukları araştırmaya göre yüksek bulunmuştur. Oranlar arası farklılıklara Cross vd (2019) yılındaki çalışmalarında yüksek dozda PG600 kullanmalarının etkili olabileceği söylenebilir.

Sunulan çalışmada EBC12 (%36,92) grubundan elde edilen ikiz doğum oranı anöstrus döneminde İran Shal ırkı koyunlarda yapmış oldukları araştırmada elde ettikleri değerler (MAP-% 36; MAP+eCG-% 41) (Garoussi vd., 2019) ile benzerlik gösterirken, sunulan çalışmada EBC6 (%47,22) ve EBC12 (%36,92) gruplarından elde edilen değerler, Sezon dışında Tuj ırkı koyunlarda yaptıkları araştırmadan (%6,66) yüksek olarak elde edilmiştir (Kaya vd., 2013). Sunulan çalışmada elde edilen ikiz doğum oranları (EBC6-%47,22 EBC12-%36,92), Santos vd (2010) çalışmalarında sezon dışında Texel ırkı koyunlarda yaptıkları araştırmalardan (%33,33) yüksek, Shahneh vd (2008) çalışmalarında sezon içinde Nadooshan ırkı koyunlarda yaptıkları araştırma (% 42,9) ile benzerlik göstermektedir. Oranlar arası

değişikliklere sezon farklılıkları, araştırmaların farklı coğrafik bölgelerde yapılmış olması ve farklı ırk hayvanların kullanılmasının sebep olduğu düşünülmektedir. Ayrıca follikül sayısını ve ovulasyon oranlarını artırmak için farklı gonadotropin türevlerinin kullanılmasının da etkili olabileceği söylenebilir.

Yapılan çalışmada EBC6, EBC12 gruplarında elde edilen çoğul doğum oranları sırasıyla %19,44, %6,15 olarak belirlenmiştir. Sunulan çalışmanın EBC6 grubunda elde edilen oran Öztürkler vd (2003) araştırmalarında FGA+$PGF_{2\alpha}$ (%22,22) ve FGA (%20) gruplarından elde ettikleri oranlar ile benzer, sunulan çalışmanın EBC6 ve EBC12 gruplarından elde edilen oranlar $PGF_{2\alpha}$ (%0) gruplarından elde ettikleri değerden yüksek olarak belirlenmiştir. Çoğul doğum oranları arasındaki farklılıklara çalışmaların yapıldığı sezonların farklı olması, kullanılan gonadotropinlerin farklı olması ve ırk farklılıklarının etkileyebileceği düşünülmektedir. Geçiş dönemi başında Akkaraman melezi ırkı koyunları 5 gruba ayırmışlar; 1. grupta (n=15) koyunlara 30 mg FGA içeren süngerleri 12 gün süreyle, 2. grupta (n=15) 40 mg FGA içeren süngerleri 12 gün süreyle, 3. grupta (n=15) 3 mg norgestomet içeren implantları 9 gün süreyle, 4. grupta (n=15) 9 gün arayla çift doz prostaglandin uygulamış ve 5. grupta (n=15) ise sadece vena jugularis'ten kan alınarak spontan ovaryum aktivitesi gösteren koyun oranı belirlemiştir (Ataman vd., 2009). Araştırmacılar 1, 2, 3, 4 ve 5. gruplarda çoklu doğum oranlarını sırasıyla %27,7; %18,18; %41,66; %0,0; ve %0,0 olarak tespit etmişlerdir. Sunulan çalışmada tüm gruplarda elde edilen çoğul doğum oranları (EBC6-%19,44, EBC12-%6,15,) araştırmacıların FGA30 (%27,7) ve N-implant (%41,66) gruplarından elde ettikleri değerlerden düşük olarak belirlenmiştir. Araştırmacıların FGA (40 mg) grubunda elde ettikleri çoklu doğum oranı (%18,18), sunulan çalışmadaki EBC6 (%19,44) ile benzerlik göstermekte, araştırmacıların diğer gruplarda elde ettikleri çoğul doğum oranları sunulan çalışmada elde edilen tüm oranlardan düşük olarak belirlenmiştir. Oranlar arasındaki farklılıkların Ataman vd (2009) çalışmalarında sunulan çalışmadan daha yüksek oranda PMSG kullanmalarından kaynaklanabileceğini düşündürmektedir. Bunlara ilaveten ırk farklılıkları ve progesteron kaynaklarının farklı olmalarının da etkili olması muhtemeldir.

Yapılan çalışmada EBC6, EBC12 gruplarında elde edilen kuzu verimleri 1,92; 1,49 olarak belirlenmiştir. EBC6 grubunda elde edilen değer Öztürkler vd (2003) çalışmalarında $PGF_{2\alpha}$ (1,3) ve FGA (1,3) gruplarından yüksek olarak belirlenmiştir. FGA (1.8) ve PGF2α (1.8) grubundan elde ettiği değer sunulan çalışmada EBC12 grubundan yüksektir (Öztürkler vd., 2003). Kısa süreli sünger ve CIDR uygulamalarından sırasıyla 1,60 ve 1,55 kuzu verimi elde ettiklerini ifade etmektedirler (Fukui vd., 1994). Sunulan çalışmada ise kısa süreli CIDR (EBC6) uygulamaları ile elde edilen 1,92 değeri Fukui vd (1994) çalışmalarında elde ettikleri (1,60 ve 1,55) değerlerden yüksek bulunmuştur. Bulgulardaki farklılığın nedenlerden bir tanesi aşım sonrası ilk östrusta gebe kalanların oranları ve izleyen östrusta gebe kalanların oranları detayına girilmediği için ortaya çıkmıştır. Anöstrüsteki İvesi ırkı koyunlarda 12 gün süreli CIDR ve sünger uygulamalarını takiben sırasıyla 1,21, 1,18 kuzu verimi elde etmişlerdir (Özyurtlu vd., 2010). Sunulan çalışmada ise 6 ve 12 gün süreli CIDR (EBC6 ve EBC12) uygulamaları ile elde edilen 1,92 ve 1,49 kuzu verimleri Özyurtlu vd (2010) yılında elde ettikleri değerlerden yüksek bulunmuştur. Sunulan çalışmada materyal olarak kullanılan Kıvırcık ırkı koyunların İvesi ırkı koyunlara göre daha çok prolifik olmalarının kuzu verimlerini artırması doğaldır.

SONUÇ

Sonuç olarak, anöstrüs döneminde Kıvırcık ırkı koyunlarda ovaryum aktivitesinin uyarılmasında kısa süreli progesteron uygulamalarının oldukça etkili olduğu tespit edildi.

AÇIKLAMALAR

Bu makale "Anöstrus döneminde kıvırcık ırkı koyunlarda farklı uygulamalar ile ovaryum aktivitesinin uyarılması" isimli doktora tez verilerinin bir bölümü kullanılarak hazırlanmıştır.

Çanakkale İli Ayvacık İlçesi Korubaşı ve Koruoba köylerinde ikamet eden; İsmail Adalı, Recep Adalı, Fatih Bıçak, Alper Uysal ve Adnan Avakoşan isimli sürü sahiplerine bu çalışmada gösterdikleri özverili çalışmalarından ve katkılarından dolayı teşekkür ederim.

Finansal destek: Bu çalışmada herhangi bir finansal destek alınmamıştır.

Etik beyan: Bu çalışma, Selçuk Üniversitesi Veteriner Fakültesi Deney Hayvanları Üretim ve Araştırma Merkezinin 2018/49 etik kurul karar numarası ile yapılmıştır.

Çıkar çatışması: Yazarlar arasında çıkar çatışması yoktur.

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Feke ilçesinde alabalık işletmelerinin organik yetiştiricilik için su özelliklerinin belirlenmesi

Determination of water characteristics of trout farms for organic farming in Feke

Research Article

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ÖZET

Organik su ürünleri yetiştiriciliği, en iyi çevresel uygulamaları birleştiren, biyolojik çeşitliliği koruyan, doğal kaynakları koruyan ve tüketicilerin tercihi ile yüksek balık refahı gerektiren, çiftlik yönetimi ve gıda üretimine yönelik bütüncül bir yaklaşımdır. Bu çalışmada, Adana ili Feke ilçesinde bulunan iki farklı alabalık üretim işletmesinin su kalitesi ve organik balık yetiştiriciliğine uygunluğu araştırılmıştır. Su kalite parametrelerinden sıcaklık, çözünmüş oksijen, pH, Ca, Mg, Na, K, alkalinite, nitrat azotu, nitrit azotu, amonyum azotu, ortofosfat fosforu ve alkalinite değerlendirilmiştir. Yapılan örnekleme sonucunda seçilen kaynak sularında elde edilen su parametreleri sırasıyla sıcaklık 11.7°C ve 11.3°C, pH 7.12 ve 7.11, çözünmüş oksijen 11.10 mg/L ve 10.63 mg/L olarak saptanmıştır. Kaynak sularının tüm fiziksel ve kimyasal özellikleri açısından yüksek su kalite standardına sahip olduğu görülmektedir. Böylece her iki istasyona giren suların organik balık yetiştiriciliğine uygun olduğu saptanmıştır.
Anahtar kelimeler: Alabalık üretimi, organik yetiştiricilik, su kalitesi.

ABSTRACT

Organic aquaculture represents a comprehensive approach to farm management and food production. It integrates the best environmental practices, promotes biodiversity conservation, preserves natural resources, and places a strong emphasis on ensuring the well-being of the fish, all in alignment with the preferences of discerning consumers. In this study, water quality and suitability of two different trout production enterprises in Feke/Adana city in Turkey for organic fish culture was investigated. The water quality parameters evaluated include temperature, dissolved oxygen, pH, Ca, Mg, Na, K, alkalinity, nitrate nitrogen, nitrite nitrogen, ammonium nitrogen, orthophosphate phosphorus, and alkalinity. The results of the sampling indicated that the water parameters for the selected spring sources were as follows: temperature 11.7°C and 11.3°C, pH 7.12 and 7.11, dissolved oxygen 11.10 mg/L and 10.63 mg/L, respectively. It is seen that the head waters have a high-water quality standard in terms of all their physical and chemical properties. Therefore, it was concluded that the water entering both stations is suitable for organic fish farming.
Keywords: Organic aquaculture, trout production, water quality

Article info
Submission: 09-11-2023
Accepted: 19-01-2024
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

How to cite this article
Erçen, Z., Özlüer Hunt, A., Tabakoğlu, ŞS., Güzel, E., Çevik, F., (2024). Determination of water characteristics of trout farms for organic farming in Feke. *Journal of Advances in VetBio Science and Techniques*, *9*(1), 18-25. https://doi.org/10.31797/vetbio.1388454

GİRİŞ

Su ürünleri yetiştiriciliği, insan beslenmesinde hayvansal protein kaynağı olarak en hızlı büyüyen sektörlerden biri olmaya devam etmektedir. Su ürünleri yetiştiriciliğinden elde edilen balık üretiminin istikrarlı büyümesi ve yakalanan balık üretimine ulaşması nedeniyle, dünyadaki toplam balık arzı son on yılda sürekli bir artış eğilimi göstermiştir. Küresel olarak balıketi tüketimi, hayvansal protein tüketiminin %20'si ile yaklaşık 3.3 milyar insanın beslenmesini sağlamaktadır. Ayrıca kişi başına düşen balık tüketimi 1961'de 9 kg iken 2018'de 20,5 kg'a, 2020'de ise 20.2 kg'a yükseldiği gözlenmektedir (FAO, 2022). Su ürünleri yetiştiriciliği, 2001–2018 döneminde yıllık ortalama %5.3 büyüme oranıyla dünya genelinde en hızlı büyüyen gıda üretim sektörüdür. Dünya genelinde gıda için yapılan su ürünleri üretimi 1990'da 13 milyon metrik ton iken 2018'de 82.1 milyon tona, 2020'de ise 87,5 milyon tona çıkarak altı kattan fazla artmıştır. Dünya çapında su ürünleri üreten ülkeler arasında Çin ilk sırada (toplam üretimin %58'i) yer almaktadır, ardından Hindistan, Endonezya, Vietnam, Bangladeş, Mısır, Norveç, Şili, Myanmar ve Tayland gelmektedir (FAO, 2020; FAO, 2022).

Su ürünleri yetiştiriciliği artan balık üretimine paralel olarak, ekosistem değişikliği, habitat tahribatı, ötrofikasyon, su kirliliği ve hastalık salgınları gibi bir dizi çevresel zorlukla karşılaşmaktadır (Ahmed vd., 2019; Hall vd., 2011; Naylor vd., 2000).

Ticari bir kültür balıkçılığı faaliyetinin başarısı, en düşük kaynak maliyetiyle hızlandırılmış büyüme için en uygun çevre koşullarına bağlıdır. Su kalitesi, kültür balığının sağlık ve büyüme koşullarını belirleyerek balığın genel durumunu etkilemektedir. Bu nedenle su kalitesi, yüksek bir su ürünleri üretimi planlanırken göz önünde bulundurulması gereken temel bir faktördür (Timmons vd., 2002).

Balık yetiştiriciliği ortamı, bazı su kalitesi değişkenlerinden oluşan karmaşık bir sistem olmasına rağmen, bunlardan sadece birkaçı önemli bir rol oynamaktadır. Kritik parametreler sıcaklık, çözünmüş oksijen, nitrit, amonyak, alkalinite ve karbondioksittir. Bununla birlikte, çözünmüş oksijen balık aerobik metabolizması için gerekli olduğundan en önemli parametredir ve su ürünleri üretim sistemlerinde sürekli izlenmesi gerekmektedir (Timmons vd., 2002).

Tablo 1: Alabalık yetiştiriciliğinde su kalitesi parametreleri (Anonim, 2023c).

Su Parametreleri	1. İstasyon	2. İstasyon
pH	7.12	7.11
Çözünmüş Oksijen (mg/L)	11.10	10.63
Sıcaklık (°C)	11.7	11.3
Alkalinite	90.39	92.21
Nitrat Azotu (mg/L)	1.79	1.65
Nitrit Azotu (mg/L)	ALA	ALA
ortofosfat fosforu (mg/L)	0.035	0.033
Amonyum Azotu (mg/L)	ALA	ALA
Kalsiyum (Ca) (mg/L)	25.86	26.08
Magnezyum (Mg) (mg/L)	15.63	16.12
Sodyum (Na) (mg/L)	3.05	2.94
Potasyum (K) (mg/L)	1.77	0.85

*ALA: Analiz Limiti Altında

Organik su ürünleri yetiştiriciliği, organik tarım hareketinden kaynaklanmaktadır. Organik balık yetiştiriciliği, yoğun su ürünleri yetiştiriciliğinin karşılaştığı çevresel kısıtlamaları ele almak için potansiyel bir alternatif olarak geliştirilen ekosistem tabanlı bir yönetim sistemidir. Ancak konvansiyonel kültür balıkçılığından organik kültür balıkçılığına geçiş çok boyutlu, karmaşık ve pahalı bir süreçtir. Organik su ürünleri yetiştiriciliğinin daha da geliştirilmesi, tek tip organik su ürünleri standartları oluşturarak geliştirilebilir. Organik su ürünleri yetiştiriciliğine geçiş, çok çeşitli çevresel avantajlar sağlar. Artan insan nüfusunun balık ve deniz ürünlerine yönelik küresel talebini karşılamak için, avlanan balıkçılıktan elde edilen üretim durgun kaldığından, su ürünleri yetiştiriciliğinden elde edilen gıda üretimi artırılmalıdır (Arslan ve Akhan, 2018).

Organik su ürünleri yetiştiriciliği, en iyi çevresel uygulamaları birleştiren, biyolojik çeşitliliği ve doğal kaynakları koruyan, çiftlik yönetimi ve gıda üretimine yönelik bütüncül bir yaklaşımdır (Lembo ve Mente, 2019; Mente vd., 2019). Organik su ürünleri yetiştiriciliği dört ilkeye dayanır: (1) sağlık, (2) ekoloji, (3) uygunluk ve (4) bakım. Yaşayan ekolojik sistemlere dayanan ve ekosistemlerin, hayvanların ve insanların sağlığını gözeten, gerçekten bütüncül bir sistem olmalıdır (Gould vd., 2019).

Ayrıca, Avrupa Birliği Konseyi tarafından EEC 2092/91 numaralı bir yönetmelikte belirtilmiştir ki, Avrupa Birliği üyesi ülkelere organik tarım ürünleri ihraç eden üçüncü ülkelerin, kendi hükümetleri tarafından oluşturulan bir organizasyona sahip olmaları gerekmektedir. Avrupa Birliği, organik üretimin altyapısını bu yönergeye uymayan ülkelerden organik ürünler ithal etmemektedir (Anonim, 1991). Bu bağlamda, Türkiye'de de Tarım ve Orman Bakanlığı tarafından yürütülen çalışmalar sonucunda, 11 Temmuz 2002 tarihli ve 24812 sayılı Resmi Gazete'de "Organik Tarımın Esasları ve Uygulanmasına İlişkin Yönetmelik" yayınlanmıştır. Bu yönetmelik, en son olarak 28 Nisan 2020 tarihli ve 31112 sayılı Resmi Gazete'de "Organik Tarımın Esasları ve Uygulanmasına İlişkin Yönetmelikte Değişiklik Yapılmasına Dair Yönetmelik" başlığı altında güncellenmiştir (T.C. Resmi Gazete, 11 Temmuz 2002, sayı: 24812; 28 Nisan 2020 sayı: 31112). Böylece organik üretimde Avrupa Birliğine uyum sağlanmış ve Bakanlığımız bünyesinde Ekolojik Tarım Komitesi (ETK) kurulmuştur. Bugünkü konumu ile ülkemiz, Avrupa Birliği ülkelerine 1980'li yılların ortalarından beri organik ürün ihraç eden ülkeler arasında üçüncü sıradadır. İhraç edilen organik ürünlerin tamamına yakını bitkisel kökenlidir. Ülkemizde organik hayvansal üretim noktasındaki çalışmalar başlangıç aşamasındadır (Özlüer Hunt, 2022).

Organik su ürünleri yetiştiriciliğinden elde edilen gıda üretimi son yıllarda hızla artmaktadır. Gerçekten de dünya düzeyinde hala düşük hacimleri temsil etmesine rağmen, 2014'ten bu yana neredeyse iki katına çıkmıştır. Dünya çapında organik su ürünleri yetiştiriciliğinden elde edilen toplam gıda üretimi 2016 yılında 415 bin metrik tonun üzerindedir (Lernoud ve Willer, 2017; Lernoud ve Willer, 2018). Bununla birlikte, 2020 yılında dünya genelinde organik su ürünleri yetiştiriciliğinde 306.000 tonun üzerinde bir üretim gerçekleştiği bildirilmiştir. En fazla organik üretimi yapılan su ürünleri arasında somon, midye ve mersin balığı öne çıkmaktadır. Bu alanda en büyük üretime sahip ülke, 169.400 ton ile Çin'dir. Çin'i sırasıyla 43.000 ton ile Ekvador ve 30.000 tonun üzerinde üretimle İrlanda takip etmektedir. Ancak, organik su ürünleri yetiştiriciliğiyle ilgili veriler Brezilya ve Endonezya gibi büyük su ürünleri üreticisi olan ülkeler için sağlanamamıştır. Bu nedenle, organik su ürünleri üretim hacminin, açıklanan verilerden daha yüksek olabileceği tahmin edilmektedir (Willer vd., 2022).

Organik balık üretiminin tarihçesine baktığımızda, 1990'lı yılların ortalarında ilk kez sazan balığının "organik" olarak sertifikalanması Bio Ernte tarafından Avusturya'da yapılmıştır. Organik alabalık üretimi ise 1998 yılında İngiltere'de gerçekleşmiştir (Tacon ve Brister, 2002). Su ürünlerinin organik yetiştiriciliğindeki gelişmelere baktığımızda organik tarıma oldukça benzer olduğu görülse de organik su ürünleri yetiştiriciliği, sertifikalandırılmış ürünlerin çeşitliliği ve kalitesi açısından tarım sektörünün gerisinde olduğu bir durum sergilemektedir (Bergleiter, 2001; Brister ve Kapuscinski, 2001).

Kaliteli protein içeren balığın akuakültür sistemlerinde daha kaliteli yetiştirilebilmesi için, özellikle su kaynaklarının kalitesinin belirlenmesi ve değerlendirilmesi gerekir. Su kalitesi; türlerin bileşimini, verimliliğini, bolluk durumlarını ve sucul türlerin fizyolojik durumlarını etkilemektedir (Yılmaz, 2004). Endüstriyel, tarımsal ve evsel atıklar nedeniyle oluşan kirleticiler akarsular, göller ve denizlere ulaşmaktadır. Su ürünleri yetiştiriciliğinde kullanılan ve doğal kaynaklardan temin edilen suların özellikleri çok iyi bilinmelidir. Suyun fiziksel, kimyasal ve biyolojik parametrelerinin periyodik olarak ölçülmesiyle gerekli önlemler alınması sağlanabilir. Su ürünleri yetiştiriciliği yapılan su ortamlarında suyun kalitesi, kirliliğin tespiti ve organik balık yetiştiriciliğine ne şekilde etki ettiği ortaya konulmalıdır (Yılmaz, 2004).

Ülkemizde yapılan organik tarım üretimi, ihracatı ve ithalatı ele alındığında, Dünya ve Avrupa Birliği ülkeleri arasında oldukça iyi bir yerdedir. Fakat bu durum organik akuakültür üretimi ve pazarlanması açısında henüz gelişme gösterememiştir. Ülkemizde su ürünleri yetiştiriciliğinden elde edilen üretimin yaklaşık %50'si yurtdışına pazarlanmaktadır. Organik ürünlerin sertifikalanması ile sağlıklı ve güvenilir bir gıda olduğu belirlenmekte ve böylece Avrupa ülkelerinde kolaylıkla pazar bulması söz konusu olmaktadır (Şahinöz vd., 2017). Ülkemiz su ürünleri üreticilerinin geleneksel üretimden organik balık yetiştiriciliği metotlarını uygulamaları sektörün gelişiminde önemli bir rol oynayacaktır. Bu çalışmada Adana ili Feke ilçesinde - bulunan iki alabalık işletmesinin yetiştiricilik amacıyla kullanmış oldukları su kaynaklarının su kalitesi açısından organik balık yetiştiriciliğine uygunluğu incelenmiştir.

MATERYAL VE METHOD

Adana ili Feke ilçesinde bulunan iki farklı alabalık işletmelerine alınan suyun bazı fiziksel ve kimyasal parametrelerini belirlemek için bu alabalık işletmelerinin su girişindeki kaynak suyundan örnekleme yapılmıştır. Su örnekleri iki litrelik temiz plastik kavanozlara alınmış ve standart metotlara göre en kısa zamanda Çukurova Üniversitesi Su Ürünleri Fakültesi Temel Bilimler laboratuvarında analizleri gerçekleştirilmiştir. Suyun sıcaklığı (°C) ve çözünmüş oksijen (O2 mg/L) miktarı OxyGuard® marka oksijen metre ile pH değeri ise 320/ Set/1 WTH marka mikroprocessor pH metre kullanılarak örnek alma esnasında ölçülmüştür. Ca, Mg, Na, K için flame fotometre, nitrat (NO3 -N) için kadmiyum indirgeme, nitrit azotu (NO2-N) için sülfanilamid, amonyum Azotu (NH4-N) için fenat, ortofosfat fosforu için askorbik asit, alkalinite için titrimetrik yöntemler uygulanmıştır (APHA, 1999).

Su analiz sonuçları "Yerüstü Su Kalitesi Yönetmeliği"ne (YSKY) (Anonim, 2023a) ve alabalık yetiştiriciliği (Anonim, 2023b) açısından incelenmiştir.

BULGULAR

Yapılan örnekleme sonucunda seçilen kaynak sularında elde edilen su parametreleri sırasıyla sıcaklık 11.7 ve 11.3, pH 7.12 ve 7.11, çözünmüş oksijen 11.10 mg/L ve 10.63 mg/L olarak

saptanmıştır. Bu parametreler dışında alkalinite 90.39 ve 92.21, nitrat azotu 1.79 mg/L ve 1.65 mg/L, ortofosfat fosforu 0.035 mg/L ve 0.033 mg/L, kalsiyum (Ca) 25.86 mg/L ve 26.08 mg/L, magnezyum (Mg) 15.63 mg/L ve 16.12 mg/L, sodyum (Na) 3.05 mg/L ve 2.94 mg/L, potasyum (K) 1.77 mg/L ve 0.85 mg/L olarak ölçülmüştür. Örnekleme sonucunda analiz edilen su kalitesi parametreleri Tablo 2.'de gösterilmektedir.

Tablo 2: Seçilen kaynak sularında elde edilen su kalitesi parametreleri

Su Parametreleri	Standart Değer
pH	6.5-8.0
Çözünmüş Oksijen (mg/L)	9.2-11.5
Sıcaklık (°C)	9-17
Karbondioksit (CO_2) (mg/L)	<10
Nitrat (mg/L)	<10
Nitrit (mg/L)	<0.2
Fosfat (mg/L)	<0.3
Amonyum (mg/L)	<1

TARTIŞMA

Örnekleme sonucu analiz edilen su kalitesi parametrelerine (Tablo 1) bakıldığında tüm parametrelerin Gökkuşağı alabalığı kültürü için izin verilen sınırlar içinde olduğu saptanmıştır. Örnek alınan istasyonlardan elde edilen su sıcaklığı aralığı sırasıyla 11.7°C ve 11.3°C olarak belirlenmiştir. Gökkuşağı alabalığının 10-18°C su sıcaklığı (Yamazaki, 1991), 6.5-8.5 pH düzeyi ve 8 mg/L'in üzerinde çözünmüş oksijen gereksinimi vardır (Huet, 1975). McGregor ve Nieuwolt (1998), rakımdaki her 100 m artışta hava sıcaklığında 0.65°C düşüş bildirmiştir. Hava sıcaklığı, su sıcaklığı ile ilişkilidir ve su sıcaklığından yüksektir (APHA, 2005). Su sıcaklığı, hava sıcaklığına ve yüksekliğe bağlıdır.

Örnekleme sonucunda her bir istasyon için sırasıyla aşağıdaki özellikler belirlenmiştir; ortalama su sıcaklığı 11.7°C ve 11.3°C, pH 7,12 ve 7,11, çözünmüş oksijen 11,10 mg/L ve 10,63 mg/L. pH, amonyum azotu, nitrat azotu, ortofosfat fosforu YSKY " Tablo 1. Kıtaiçi Yerüstü Su Kaynaklarının Genel Kimyasal ve Fizikokimyasal Parametreler açısından Sınıflarına Göre Kalite Kriterleri"ne (Anonim, 2023a) göre incelendiğinde I. Sınıf (çok iyi) içinde yer almaktadır.

Gökkuşağı alabalığı, 5°C ve üzerindeki su sıcaklığında yaşamını sürdürebilir, ancak büyüme ve gelişme için belirli bir sıcaklığı tercih eder. Suda çözünmüş oksijen seviyesi açısından yüksek düzeyde gereksinim duyar ve optimal konsantrasyon 9 mg/L'den az olmamalıdır. Alabalıklar, saf oksijen ile su doygunluğunu 50 mg/L'ye kadar tolere edebilir. Alabalık için sudaki öldürücü oksijen konsantrasyonu 2,5 mg/L'dir. Yüksek sıcaklıklarda suda çözünmüş oksijen içeriği 9 mg/L'den az olmamalıdır (Munro vd., 1987).

Gökkuşağı alabalığının nehir kıyısındaki en uygun yaşam alanı, berrak, soğuk su; yivli alanlarda alüvyonsuz kayalık alt tabaka; yavaş, derin su alanları ile yaklaşık 1:1 havuz-yiv oranı; iyi bitkilendirilmiş akarsu kıyıları; nispeten sürekli su akışı, sıcaklık rejimleri ile karakterize edilir (Raleigh ve Duff, 1980).

Organik balık yetiştiriciliğin temel ve ilk şartlarından birisi uygun yer seçimidir. Yer seçimindeki en önemli etken ise kaliteli bir su ve su içindeki bazı fiziksel ve kimyasal elementlerin balığın istediği şartlara uygun özelliklerde kirletici etkenlerden uzak olmasını gerektirmektedir (Bengtsson vd., 2005). Hatay ilinde yapılan bir çalışmada, 11 adet alabalık işletmesinde gerçekleştirilen anket sonuçlarına göre, 2 adet işletmenin organik alabalık yetiştiriciliği için uygun olduğu belirlenmiştir (Hasbek, 2011).

Balıklar da dahil olmak üzere çoğu canlı, tek bir değişkene karşı oldukça geniş bir toleransa sahiptir. Örneğin, *Oncorhynchus mykiss* sıcaklık geçmişine (Rodgers ve Griffiths, 1983) ve sıcaklık değişim hızına (Elliott ve Elliott, 1995) bağlı olarak, yaklaşık 0°C ile 29.8°C arasındaki sularda hayatta kalabilmektedir. Ancak, hayatta kalmak için veya başka herhangi bir değişken için bu sıcaklık aralığında *O. mykiss*, büyüme,

üreme ve/veya diğer fizyolojik özelliklerin optimize edildiği tercih edilen bir aralığa sahiptir (Peterson ve Meador, 1994).

Organik yetiştiricilik ilkeleri her ülke standardına bağlı olmakla birlikte değişiklik göstermektedir. En önemli kriterlerden birisi ise yetiştirilecek tür, yaşam döngülerini rahat geçirebileceği yeterli büyüklükte bir yaşam alanına sahip olmasıdır. Bu yaşam alanı da, yeterli miktarda oksijene ve su kalitesine sahip sularda bulunmaktadır (Arslan ve Akhan, 2018). Bu bağlamda her iki istasyondan alınan su örneklerinin analizi sonucu elde edilen parametreler (Tablo 1) göz önüne alındığında, su kalite değerlerinin alabalık yetiştiriciliği için gerekli kriterleri sağladığı ve bölge sularının organik balık yetiştiriciliği için yeterli kriterleri karşıladığı görülmektedir.

Organik su ürünleri yetiştiriciliği, organik tarımın bir parçası olarak kabul edilmekte ve çeşitli önemli katkılar sunmaktadır. Bu sistemler, kırsal kalkınmayı teşvik etmekte, su ürünleri pazarını büyütmekte, toplum sağlığına olumlu etkilerde bulunmakta ve ekolojik çevreyi korumaktadır. Bu nedenle organik su ürünleri yetiştiriciliği, dünya genelinde ve ülkemizde sürdürülebilir su ürünleri yetiştiriciliği için son derece önemli bir konudur (Özlüer Hunt, 2022). Gümüşhane, Artvin ve Rize ekseninde yapılan bir çalışmada, su ürünleri üretimi yapan üreticilerin organik su ürünleri yetiştiriciliği konusunda yetersiz bilgiye sahip oldukları ortaya çıkmıştır. Tesis sahiplerinin %77'si ise organik tarım kavramını daha önce hiç duymamıştır. Organik su ürünleri yetiştiriciliği konusundaki bilgi eksikliğine rağmen, tesis sahiplerinin %82'si, tesislerini organik su ürünlerinin yetiştirilmesine uygun hale getirebileceklerini ifade etmişlerdir (Çavdar vd., 2006).

Azalan balıkçılık hasadı, yabani balık gıda güvenliği sorunları, çevresel kaygılar, artan balık tüketimi ve organik gıdaların artan pazar payı, dikkatleri "organik su ürünleri yetiştiriciliğine" odaklanmasına neden olmuştur. Tüketici talebi, önümüzdeki on yılda balık, kabuklu deniz ürünleri ve diğer su türlerin organik üretimini ana akım haline getirebilecektir. Adana ilinde organik su ürünleri tüketimi üzerine yapılan bir çalışmada, tüketicilerin organik sertifikalı levreklerle ilgili görüşleri incelenmiştir. Araştırmaya katılan tüketicilerin %91,5'i organik balık satın almaya istekli olduklarını ifade etmişlerdir (Budak vd., 2006). Organik su ürünleri yetiştiriciliği, çeşitli akademik disiplinlerden araştırmacıların yanı sıra çevre savunucuları ve girişimci yenilikçilerin de ilgisini çekmiştir. Az sayıda "sertifikalı" ve sertifikasız organik balık ve mikroalg ürünleri, gelişmiş ülkelerde perakende pazarına girmiştir. Düzenleyici spesifikasyonların hala ele alınması gerekse de bu yeni organik pazar alanı gelecekte önemli bir büyüme potansiyeline sahiptir.

SONUÇ

Araştırma sonucunda elde edilen veriler göz önüne alındığında kaynak sularının fizikokimyasal özellikleri açısından yüksek su kalite standardına sahip olduğu görülmektedir. Bu veriler ışığında her iki istasyona giren suların organik balık yetiştiriciliğine uygun olduğu saptanmıştır. Organik balık yetiştiriciliği ile ilgili çalışmaların yetersiz olduğu düşünülmekte ve bu konuyla ilgili araştırmaların gerçekleştirilmesi ve organik su ürünleri standardının belirlenmesi önem arz etmektedir.

AÇIKLAMALAR

Çalışmamız 24-26 Ekim 2018 tarihlerin arasında 3rd International Mediterranean Science and Engineering Congress (IMSEC 2018)'de poster sunumu yapılmıştır.

Finansal destek: Bu çalışma Çukurova Üniversitesi BAP Birimi tarafından FMYO2012BAP1 proje numarası ile desteklenmiştir.

Etik beyan: Bu çalışmada deney hayvanları kullanılmaması nedeniyle Yerel Etik Kurul Onayı alınmamıştır.

Çıkar çatışması: Yazarlar arasında çıkar çatışması bulunmamaktadır.

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Characterization of physical and chemical properties of honey from Northeastern Anatolia of Türkiye

Research Article

ABSTRACT

The aim of this study was to investigate the physical and chemical properties of natural highland honey produced in the Northeastern Anatolia region of Türkiye. In 2020, 24 honey samples collected and sold during the honey harvest season (July and August) were purchased from local vendors. Moisture, color, acidity, pH, conductivity, diastase and invertase activity, C13, C13 protein-honey, C4 analysis and sugar components were analyzed in honey samples. The honey samples were found to contain 17.4% moisture. The mean invertase level were found to be 156.216 U/kg. The freshness and enzymatic activity of the honey were shown by the average diastase number of 12.8 DS. The mineral content and overall purity of the honey was indicated by an electrical conductivity value of 0.17 mS/cm. The average acidity value 15.9 meq/kg, fructose/glucose ratio 1.26% and the average color value 33 mm Pfund determined. The average sugar contents in the honey samples were as follows: fructose 37.4%, glucose 29.5%, sucrose 1.4%, turanose 1.8%, maltose 0.6%, isomaltose 0.2%, erlose 0.3%, trehalose 0% hybriditose 0% maltotriose 0% fructose+glucose. 66.9%, fructose/glucose 1.26, glucose/water 2.0. The mean difference between honey protein and honey delta C13 data is -0.58 and C4 sugar ratio is 0. In conclusion, valuable findings were obtained on the physical and chemical properties of honey from the northeastern Anatolia region of Türkiye. Further research can build on these findings to explore the unique properties and potential benefits of honey from this region and contribute to its value and utilization in various industries.

Keywords: Diastase, honey, moisture, invertase, sugar

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Article info
Submission: 16-11-2023
Accepted: 19-01-2024
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

INTRODUCTION

Honey is a natural sweetener with a unique flavor and scent that is made from the nectar of different flowering plants. Its quality and usefulness for various culinary and therapeutic purposes are significantly influenced by its physical and chemical qualities (Thrasyvoulou et al., 2018). For both consumers and producers, it is essential to comprehend these features. Color, viscosity, and texture of honey are indicators of its quality and can affect how desirable it is for culinary usage (Dominguez and Centurión, 2015). As an illustration, lighter-colored honey is frequently chosen for its mild flavor whereas darker honey has a more strong flavor. Viscosity, or thickness, has an impact on the ease of usage and spreadability in cooking and baking.

However, honey's chemical characteristics, such as its moisture content, pH level, and sugar content, can affect both its stability and possible health advantages (da Silva et al., 2016). While honey's pH level impacts its acidity and preservation, its moisture content is crucial for preventing fermentation and rotting (El Sohaimy et al., 2015). Additionally, the distinct chemical makeup of carbohydrates like fructose, glucose, and sucrose influences the flavor of honey.

How to cite this article
Kılıç Altun, S., Aydemir, ME., (2024). Characterization of physical and chemical properties of honey from Northeastern Anatolia of Türkiye. *Journal of Advances in VetBio Science and Techniques, 9*(1), 26-34. https://doi.org/10.31797/vetbio.1374657

On the other hand, the chemical properties of honey, including its moisture content, pH level, and sugar composition, can influence its stability and potential health benefits (da Silva et al., 2016). The moisture content of honey is critical for preventing fermentation and spoilage, while pH level affects its acidity and preservation. Additionally, the unique composition of sugars, such as fructose, glucose, and sucrose, contributes to honey's taste, texture, and nutritional profile (Aparna and Rajalakshmi, 1999).

Understanding these physical and chemical properties allows consumers and producers to evaluate the quality, authenticity, and potential applications of honey. It helps ensure that honey meets the necessary standards for culinary uses, such as baking, sweetening beverages, or drizzling over foods. Characterization of the physical and chemical properties of honey plays a crucial role in ensuring its quality, authenticity, nutritional value and safety for consumers (Solayman et al., 2016). It is also very important to optimize processing methods and packaging to preserve the composition of honey. Understanding the physical properties of honey, such as moisture content and viscosity, is important for determining its storage requirements and shelf life (Eshete and Eshete, 2019**)**. On the other hand, physical and chemical analyses can help identify the geographical and botanical origin of honey. Analyzing chemical properties can reveal if honey has been adulterated with sugars, syrups, or other substances. Furthermore, studying the properties of honey is essential for exploring its potential medicinal applications, as honey has been used in traditional medicine for its antimicrobial and antioxidant properties (Hamadou et al., 2022).

The types and ratios of sugars in honey are referred to as the honey's sugar composition. Fructose, glucose, and sucrose are the three major sugars found in honey. Since glucose and fructose are monosaccharides, they are simple sugars that cannot be divided into smaller components. They make up the majority of the sugar in honey, making up between 85 and 95 percent of its total sugar content (Al-Farsi et al., 2018). Since fructose is a little bit sweeter than glucose, it is what gives honey its distinctive sweetness. The sugar that gives the body energy and is readily taken into the bloodstream is called glucose. Contrarily, sucrose is a disaccharide made up of one glucose and one fructose molecule. It typically accounts for 1% to 5% of the honey's total sugar content (da Silva et al., 2016). Because bees contain enzymes called invertase that break down sucrose into its separate sugar components during the honey-making process, sucrose is less common in honey than fructose and glucose. The flower source of the nectar, climatic circumstances, and bee foraging habits are only a few of the variables that might affect the relative quantities of these sugars in honey (Machado et al., 2018). Because of this, dependent on their sugar makeup, various types of honey can have marginally different tastes, textures, and crystallization tendencies. The sugar content of honey influences its physical characteristics and overall quality in addition to flavoring it. The proportion of fructose to glucose determines the viscosity and crystallization potential of honey. While a higher glucose level encourages quicker crystallization, a higher fructose content makes honey more viscous (Nurul Zaizuliana et al., 2017). Both consumers and producers should be aware of the sugar content in honey. Customers can choose from a variety of honey types to suit their tastes, and producers can examine the sugar composition to make sure the honey satisfies strict quality requirements and maintains its stability throughout storage.

Türkiye has a long history of beekeeping and honey production, and different locations are renowned for their distinctive honey tastes and

properties. Northeastern Türkiye is a location renowned for its varied flora, which helps to produce many kinds of honey. Beekeeping is a significant agricultural industry in Türkiye, which ranks among the top nations in the world for honey production, according to the Turkish Statistical Institute (TSE, 2020). Based on elements including weather, the accessibility of floral sources, and beekeeping techniques, the precise honey production numbers may change from year to year. In this study, it was aimed to examine the physical and chemical properties of natural highland honey produced in northeastern Anatolia of Türkiye region.

MATERIALS AND METHODS

Collection of honey samples

Following the 2020 honey harvest (July and August), 24 honey samples were purchased from beekeepers who produce and sell honey in Türkiye's Northeastern Anatolia region. The honey samples were collected from Posof (41°30'34.55" N-42° 43' 40.08" E)(n=3), Göle (40° 47' 59.99" N, 42° 36' 59.99" E) (n=5), Hanak (41°14'37.14" N 42°51'6.80" E) (n=2), Damal (41° 20' 26.60"N, 42° 50' 28.28"E)(n=4), Ardahan Province (41° 06' 31.36" N, 42° 42' 7.99" E) (n=6), Olur (40° 49' 59.99" N, 42° 07' 60.00" E)(n=1), and Oltu (40° 32' 26.39" N, 41° 59' 26.39" E) (n=3) regions located in northeastern Anatolia, Türkiye. 400 g of strained honey samples were collected from each producer and stored at room temperature in a dark place until analysis.

Moisture content analysis

The moisture content honey is determined a digital refractometer (Hanna, German), that was regularly calibrated using distilled water or another approved reference material and thermostated at 20°C (Bogdanov and Martin, 2002).

Color analysis

Color analyzes of honey samples were made according to the method based on photometrically reading the color in terms of Pfund Scale (Smetanska et al., 2021).

Sugar analysis

Sugar analysis of the honey samples was carried out using HPLC with a Refractive Index detector. A carbohydrate analysis column (3.9 300 mm) with 10 m-diameter particle size was used for the separation. Throughout the analysis, the column was held at a constant 25°C. Acetonitrile and water made up 80% of the mobile phase. Samples were injected at a flow rate of 2 mL/min in quantities of 25 µL. Peaks in the sample were found by comparing retention times obtained by standards. To confirm the accuracy of the chromatographic peaks, standards were also inserted into the honey samples. Peak quantification was done using average peak areas and duplicate injections. The glucose and maltose standards (Sigma-Aldrich) were used to calculate the sugar content of honey (Bogdanov and Martin, 2002).

pH and acidity analysis

The pH and acidity values of the honey samples were determined using the TSE 3036-2002 technique. A 10% (w/v) solution of honey produced in milli-Q water was tested using a pH meter (Hanna instruments, Italy) (TSE, 2002).

Electrical conductivity analysis

Using a conductivity meter (Meterlab-CDM230, Türkiye), electrical conductivity levels were evaluated in accordance (Bogdanov and Martin, 2002).

Diastase activity analysis

Handling a UV-Spectrophotometer (Shimadzu UV-1800, Japan) allowed diastase analyses of honey samples were determined in accordance with TSE 3036:2002's recommended procedure. The absorbance levels of the samples were determined using a UV-Spectrophotometer (PerkinElmer Lambda 25) in the 600 nm range in order to identify diastase activity (TSE, 2002).

Invertase activity analysis

According to DIN 10754:2002, the invertase activity of honey samples was measured based on the spectrophotometric detection of p-nitrophenol (DIN, 2002).

C13, C13 protein-honey, and C4 analysis

The official methods of AOAC 998.12 were used to determine the C13, C13 protein-honey, and C4 analyses of honey samples (AOAC, 2008).

Statistical Analysis

All of the calculations were performed in triplicate, and the results were provided as mean ± standard deviation. Mean and standard deviation analyses of honey samples were performed with Microsoft Excel.

RESULTS

The quality of honey is determined by its physical and chemical properties. Determining the properties of honey is very important to know the quality and naturalness of honey. Some of these features are moisture, color, acidity, pH, conductivity, diastase activity, reducing and non-reducing sugar content and invertase activity. Table 1 presented the physical and chemical properties of honey samples.

Table 1. Physical and chemical properties of honey samples

Content	Unit	Mean±Std	Range
Moisture	%	17.4±1.8	14.1 - 18.8
Colour	mm pfund	33±4.2	24.5 - 72.8
pH	meq/kg	3.6±0.4	3.2 - 4.1
Acidity	pH	15.9±1.6	14.2 - 17.8
Invertase activity	U/kg	156.2±16	81.4 - 311.6
Diastase number	DS	12.8±0.4	11.9 - 14.2
Conductivity	mS/cm	0.17±0.2	0.12 - 0.23
C13 Honey	%	-24.91±0.13	-25.5 - -23.6
C13 Protein/honey	%	-0.58±0.24	-0.74 - -0.49
C4	%	0	0
Fructose	%	37.4±0.9	28.8 – 42.4
Glucose	%	29.5±0.6	16.4 - 45.7
Saccarose	%	1.4±0.2	0.7 – 2.3
Turanose	%	1.8±0.3	0.9 – 4.1
Maltose	%	0.6±0.4	0.3 – 1.2
Isomaltose	%	0.2±0.4	0.1 – 0.7
Erlose	%	0.3±0.1	0.2 – 0.5
Trehalose	%	0	0
Melesitose	%	0	0
Maltotriose	%	0	0
Fructose + Glucose	%	66.9±4.8	57.3 – 88.7
Fructose / Glucose	/	1.26±0.08	0.99 – 1.87
Glucose/Water	/	2.0±0.01	1.5 – 2.8

DISCUSSION

In this study, the mean moisture content of honey samples was determined to be 17.4% (Table 1). Honey's moisture content can change depending on where it was made, the climate, how mature it was, and the season (Altun and Aydemir, 2021). The moisture values measured in the honey samples in this study were below the 20% limit determined by the Turkish Food

Codex Honey Communiqué and were determined to be in compliance with the standard (TSE, 2012). According to a study on honey from Algeria, moisture levels in several honey samples ranged from 14% to 18% (Rebiai and Lanez, 2014). Bengü and Kutlu (2018) reported a range of moisture content (14.81-15.91%) in honey produced in Bingöl region of Türkiye. Arıcı and Gökçe (2023) reported the average moisture content of 22 honey samples collected from the center and districts of Bingöl province of Türkiye as 15.43±0.06%. The moisture content of Turkish honey determined in this study (14.1%-18.8%) indicates similar moisture content in other studies. It's important to remember that honey's permissible moisture range often falls below 20%. This limit was acknowledged by international honey quality norms by the European Union Council Directive (Council, 2001). If the moisture content of the honey is higher than this, it could ferment or become spoiled. Consequently, the mean moisture content (17.4%) implies an appropriate level within the desired rage for honey quality.

The color value of honey is an important parameter that reflects its visual appearance and can provide insights into its floral source and processing. The color values ranged from 27 to 198 mm Pfund according to a study on honey samples from various floras of Algeria with a mean level of 81 mm Pfund (Rebiai and Lanez, 2014). Depending on the floral source and processing techniques, honey's color may change. Due to its aesthetic appeal and link to softer flavor profiles, lighter-colored honey is frequently favored, while darker-colored honey is generally perceived to have stronger flavors. Direct comparisons are difficult since different countries and areas may use different color grading systems and standards (Dominguez and Centurión, 2015). The average color value of 33 mm Pfund in the honey samples examined for this study, however, points to a moderate color intensity that may be impacted by the distinctive floral sources and beekeeping procedures used in the northeastern Anatolia of Türkiye.

One of the enzymes secreted from the honeybees' cephalic and thoracic glands, invertase has the highest activity when it comes to honey maturation (Al-Sherif et al., 2017). It was found that all samples fit very well with this measurement scale with an average of 156.216 U/kg when the invertase values obtained within the scope of this study are compared with the ratio of IU 73.45 recommended by the International Honey Commission (IHC) in terms of proof and freshness of honey not being heat-treated (Table 1). The amount of invertase and the overall quality of honey are directly correlated. According to our results, honey samples had an average invertase concentration of 156.2 U/kg. Invertase levels in multifloral honey samples ranged from 29.40 – 166.50 U/kg according to a study on honey from different regions of Bulgaria (Manolova et al., 2018). The invertase concentration in this study within this range, indicating a similar enzymatic activity. An essential enzyme called invertase breaks down sucrose into its glucose and fructose molecules, adding to the sweetness and digestibility of honey. Stronger enzymatic activity and a stronger ability to break down sucrose are indicated by higher invertase levels. It is significant to remember that invertase levels might change based on things like the honey's floral source, the surrounding environment, and beekeeping techniques. Therefore, the precise invertase level of 156.216 U/kg in this study offers important insights into the enzymatic activity and product quality.

An enzyme called diastase is naturally present in honey, and its concentration varies depending on the flora's place of origin and the quantity of heat applied (Çiftçi and Parlat, 2018). The Turkish Food Codex Honey Communiqué states that blossom honey must have 8 or more diastases (TFC, 2012). Diastase numbers ranged from 3.99 DS to 49.42 DS,

according to a study on Andalusian honey samples from Spain (Serrano et al., 2007). Honey samples from Northeastern Anatolia of Türkiye had a mean diastase number of 12.8 DS, which is within this range and suggests a similar level of enzymatic activity. Diastase is an enzyme that converts starches, such maltose in honey, into less complex sugars. The honey's diastase activity level is indicated by the honey's diastase number, which might reveal information about the honey's quality. Diastase activity can vary based on the floral source, weather, and beekeeping procedures; it is crucial to keep in mind. Because of this, honeys' from Northeastern Anatolia of Türkiye average diastase number, which is 12.8 DS, tells us a lot about the honey's enzymatic activity and quality. Similar to the findings of our study, Belli (2019) reported that the number of diastases varied between 3.38-13.18 in honey collected in Muğla province of Türkiye. Kara et al., (2022) reported the mean diastase number of 24 honey samples collected from the center and districts of Tokat province of Türkiye as 0.0-10.9 DS. Bengü and Kutlu (2018) reported diastase number between 16.17-20.61 in honey produced in Bingöl region of Türkiye, which is higher than our findings.

One factor used to differentiate between floral and secretory honeys is electrical conductivity. The electrical conductivity of honey is a measure of its mineral content and can provide insights into its quality and purity. In the honey samples from Northeastern Anatolia of Türkiye which examined in this study, the electrical conductivity is an average level of 0.17 mS/cm, that complies with the Turkish Food Codex Honey Communiqué (TFC, 2012). Electrical conductivity measurements on honey samples from various regions of Vojvodina ranged from 0.08 to 1.99 mS/cm (Živkov-Baloš et al., 2019). Belli (2019) reported that a range of electrical conductivity (0.63-1.67 mS/cm) in honey samples collected in Muğla province of Türkiye. Arıcı and Gökçe (2023) reported the average electrical conductivity of 22 honey samples collected from the center and districts of Bingöl province of Türkiye as 0.228±0.001 (mS/cm). Kara et al., (2022) reported the mean electrical conductivity of 24 honey samples collected from the center and districts of Tokat province of Türkiye as 0.33-0.86 mS/cm. Honey samples from Northeastern Anatolia of Türkiye had a mean electrical conductivity of 0.17 mS/cm, which is within this range and suggests a similar mineral concentration. According to the nectar source and other environmental circumstances, honey with higher electrical conductivity levels may include more minerals. High electrical conductivity, though, can also signal the existence of additional sugars or other impurities.

Honey's acidity value is a crucial indicator of both its pH level and freshness. Depending on the plant source and the region of production, honey's acidity may vary. Honey's total acidity must not exceed more than 50 meq/kg, according to Turkish Food Codex Regulation (TFC, 2012). According to our study, the Turkish Food Codex Honey Communique exceeded the acidity value of honey from the Northeastern Anatolia of Türkiye (14.2-17.8 meq/kg). According to our previous study on samples of honey from Erzurum highland of Türkiye, the acidity ranged from 20.0 meq/kg to 20.8 meq/kg (Altun and Aydemir, 2021). Belli (2019) reported that the acidity value 8.95-27.9 meq/kg in honeysamples collected in Muğla province of Türkiye. Arıcı and Gökçe (2023) reported the mean free acidity values of 22 honey samples collected from the center and districts of Bingöl province of Türkiye as 14.584±0.427 meq kg-1. Kara et al., (2022) reported the mean free acidity values of 24 honey samples collected from the center and

districts of Tokat province of Türkiye as 26.0±0.12-48.0±0.16 meq/kg. Honey from Northeastern Anatolia of Türkiye, with an average acidity value of 15.9 meq/kg, is lower than this range, indicating a comparable acidity level. Honey's acidity is mostly caused by the presence of organic acids like gluconic acid. Higher acidity values could be a sign of increasing honey fermentation or deterioration. It is important to note that permitted amounts of acidity in honey might change based on local laws and standards. However, lower acidity values are typically favored as they signify fresher and better honey. Therefore, honey samples of this study had an average acidity value of 15.9 meq/kg indicates a comparatively low acidity level, indicating good quality and freshness.

The fructose/glucose ratio in honey is a characteristic that reveals both the honey's tendency for crystallization and its place of origin. It also reflects the composition and quality of honey. The Turkish Food Codex Honey Communiqué states that honey should have a fructose/glucose ratio of 0.9 to 1.4 (TFC, 2012). The average fructose/glucose ratio of honey samples analyzed in this study was 1.26. Fructose/glucose ratio in polyfloral honey samples of Romania is 1.29 according to a study (Scripca and Amariei, 2018). The honey samples from this study have an average fructose/glucose ratio of 1.26, which falls within this range, indicating a similar composition. The fructose/glucose ratios in a different study on honey samples from Egyptian and Yemeni honey samples ranged from 0.42 to 2.35 (El Sohaimy et al., 2015). Kara et al., (2022) reported the range of fructose/glucose ratio of 24 honey samples collected from the center and districts of Tokat province of Türkiye as 0.98-2.62. Once more, our study's average fructose/glucose ratio of 1.26% falls within the range that has been previously reported, indicating a comparable composition. The proportion of fructose to glucose in honey is a crucial sign of its floral origin, level of maturity, and level of processing. more fructose/glucose ratios often indicate more fructose content, which contributes to honey's sweetness and stability. They are also correlated with higher honey quality. It's important to remember that fructose/glucose ratios might change based on the type of flower, the area, and the weather. However, the honey samples examined in your study had an average fructose/glucose ratio of 1.26%, which points to a balanced and unique composition.

CONCLUSION

In conclusion, this study on the physical and chemical properties of honey from northeastern Anatolia of Türkiye provides valuable insights into its quality and characteristics. The findings demonstrate that the honey from Northeastern Anatolia of Türkiye possesses certain specific properties that contribute to its overall quality and potential culinary and medicinal applications.

The honey's 17.4% moisture content was found to be an acceptable level of moisture for honey preservation. The analysis of 156.216 U/kg of invertase suggests the existence of enzymes that contribute to the honey's natural sweetness and digestibility. With a diastase number of 12.8 DS, the honey was found to have enzymes that represent its freshness and enzymatic activity. The honey's mineral content and overall purity can be deduced from its electrical conductivity value of 0.17 mS/cm. Finally, the acidity level of the honey is shown by its average value of 15.9 meq/kg; lower values denote a fresher and higher-quality product. The honey's sweetness and stability are attributed to its balanced composition, which is indicated by the fructose/glucose ratio of 1.26%. The honey samples under study had a moderate level of color intensity, as indicated by the color value of 33 mm Pfund.

These results highlight the quality, authenticity, and potential advantages of honey

from Northeastern Anatolia of Türkiye for a variety of culinary and therapeutic uses while also advancing our understanding of the physical and chemical characteristics of this type of honey.

While the study provides valuable insights into the physical and chemical properties of natural highland honey in the northeastern Anatolia region of Türkiye, there are several limitations that should be considered: a) The relatively small sample size may not fully represent the variability that could exist in honey produced in the region, b) The findings may not be generalizable to honey produced in other cities of this region with different environmental conditions and floral sources, c)The study provides only a snapshot of honey properties in 2020 but a longitudinal analysis over multiple years could reveal variations in honey composition. Considering these limits, it is possible to promote the value and application of the honey of this region by conducting additional studies and analyzes on its special qualities and potential areas of use.

ACKNOWLEDGMENT

Financial support: There is no funding source.

Conflict of interest: The authors declare that there is no conflict of interest for this study.

Ethical statement: We declare as the authors of the study that the approval of the Ethics committee is not required within the scope of the presented study.

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PAEP gene restriction fragment length polymorphism and its effects on milk composition in cross-bred Hamdani sheep

ABSTRACT

Beta-lactoglobulin (βLG) stands as the primary whey protein in ruminant milk, synthesized by mammary gland cells during lactation and encoded by the progestagen-associated endometrial protein (*PAEP)* gene. This study aimed to assess the impact of *PAEP* gene exon II polymorphism on milk composition traits in cross-bred Hamdani sheep. Sheep were examined for clinical diseases and mastitis. Milk and blood samples were only collected from healthy ewes. The composition and physical properties of milk were analyzed using milk autoanalyzer. The *PAEP* gene exon II region's 452 bp PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis using the *RsaI* restriction enzyme. Two genotypes, AA and AB, were identified for the *PAEP* gene exon II region, with A and B allele frequencies of 0.7 and 0.3, respectively. Statistical analysis, conducted with Minitab® (Version: 19.2020.2.0), revealed that the AA genotype is associated with a higher milk fat percentage ($p<0.05$). However, no significant genotype effect was observed for other milk composition traits in cross-bred Hamdani sheep. These results suggest that *PAEP* genotypes could serve as valuable indicators for enhancing milk composition in cross-bred Hamdani sheep through breeding programs.

Keywords: Beta-lactoglobulin, cross-bred Hamdani sheep, milk fat, *PAEP*, PCR-RFLP

INTRODUCTION

Sheep milk serves as a significant source of income for rural breeders worldwide. In comparison to cow milk, sheep milk boasts superior nutritional value, with higher fat and protein content crucial for milk products (Wendorff and Haenlein, 2017). Several factors, including lactation stage, management, diseases, and genetic factors, can influence milk production and composition in sheep (Koca et al., 2023; Komprej et al., 2012). The primary components of sheep milk include fat, protein, lactose, and minerals. Numerous studies indicate a strong correlation between milk composition and gene polymorphism in sheep, revealing various genetic variants on milk-related genes (Kusza et al., 2018; Özmen and Kul, 2016; Selvaggi et al., 2014; Yousefi et al., 2013).

Beta-lactoglobulin (βLG), the primary whey protein in ruminant milk, is produced by mammary gland secretory cells during lactation and is encoded by the progestagen-associated endometrial protein (*PAEP)* gene located on chromosome 3, consisting of seven protein-coding exons (Feligini et al., 1998). Polymorphic *PAEP* variants' effects on milk composition have been reported in different sheep breeds, including the

How to cite this article
Turgut, AO., Gülendağ, E., Koca, D., Üner, S., (2024). PAEP gene restriction fragment length polymorphism and its effects on milk composition in cross-bred Hamdani sheep. *Journal of Advances in VetBio Science and Techniques, 9*(1), 35-41. https://doi.org/10.31797/vetbio.1402523

Research Article

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Article info
Submission: 09-12-2023
Accepted: 14-02-2024
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

A, B, and C variants (Kusza et al., 2018; Rustempašić et al., 2018). The genetic variations A and B show a disparity in amino acid position 20, with variant A featuring a histidine instead of threonine found in variant B (Kolde et al., 1983; Anton et al., 1999). Additionally, variant C, a subtype of variant A, involves a sole amino acid substitution, changing arginine to glutamic acid at position 148 (Erhardt, 1989; Anton et al., 1999). While A and B variants are prevalent across all breeds, the C variant is a rare allele specifically identified in the Merino breed (Kusza et al., 2018). Notably, the A variant of *PAEP* is linked to fat and protein, whereas the B variant shows an association with increased milk yield (Selvaggi et al., 2014).

The sheep industry plays a crucial role in Türkiye, contributing significantly to animal products. According to recent data, sheep milk production reached 1.067 million tonnes in 2022, positioning Türkiye as a leading country in sheep milk production (TUIK, 2022). However, limited use of artificial insemination and milking record systems due to traditional breeding practices restricts genetic improvements in sheep milk traits. These limitations underscore the importance of DNA markers influencing milk traits in sheep (Staiger et al., 2010). Cross-bred Hamdani sheep, primarily bred in the Siirt region and its surroundings, exhibit higher fat content compared to other sheep breeds in Türkiye (Turgut et al., 2023). This study aims to identify *PAEP* gene exon II polymorphisms and their associations with milk composition traits in cross-bred Hamdani sheep raised in the Southeastern Anatolia Region of Türkiye.

MATERIALS AND METHODS

Animals and sample collection

In the study, a total of 96 cross-bred Hamdani ewes were used. Animals were fed on pasture and 250 g of barley and 400 g of hay were added to their rations. Blood samples were taken from the *vena jugularis* in 9 mL K_3EDTA-containing tubes (BD Vacutainer®, Becton Dickinson, Türkiye). Blood samples were mixed slightly and stored at -20°C until further examination. Milk samples were collected in sterile 50 mL falcon tubes and transferred to the lab on the ice blocks immediately. Milk samples were collected before the morning and evening milking routine. Sheep were examined for clinical diseases, clinical and subclinical mastitis. And samples were only collected from healthy ewes at 2-3 ages during the spring season. Body condition scores (BCS) of ewes were between 2.75-3. BCS was evaluated according to Russel et al. (1969). All animals were 25-40 days of lactation that represents early lactation stage.

Milk composition analysis

The milk samples underwent analysis using an ultrasonic milk analyzer, specifically the Lactoscan® SA Milk Analyzer (Milkotronic Ltd, Nova Zagora, Bulgaria). The device was calibrated for sheep milk. For each analysis, a total of 15 mL of milk sample was employed, and various parameters were recorded, including milk fat (%), solids-not-fat (SNF) (%), milk protein (%), lactose (%), pH, salt (%), and density (kg/m^3). To ensure the accuracy of the results, the outputs of the milk autoanalyzer were subjected to verification using methods outlined by the Association of Official Analytical Chemists (AOAC). The AOAC Official Method 925.23 was employed for determining total solids in milk. Protein percentage was ascertained using the AOAC official method, known as Kjeldahl's method (Barbano et al., 1990). The fat percentage was assessed using the Gerber method (Kleyn et al., 2001). This dual verification approach, involving both the autoanalyzer and AOAC methods, ensured the reliability and accuracy of the milk composition data. Then, the data obtained from the milk autoanalyzer were utilized for subsequent statistical analyses.

DNA extraction and DNA quality control

Genomic DNA was extracted from blood using a genomic DNA isolation kit (Hibrigen, Hydra Biotechnology, Türkiye) according to the

manufacturer's instruction. The purification and quantity of genomic DNA were evaluated according to optical density at 260/280 nm on a spectrophotometer (Allsheng, Hangzhou, China). DNA integrity was evaluated on %0.8 agarose gel electrophoresis.

Polymerase chain reaction (PCR)

PCR reactions were carried out in 25 µL of total volume; 50-100 ng genomic DNA, 12.5 mL PCR 2X Taq Master Mix (Hibrigen, Hydra Biotechnology, Türkiye), 5 pmol of each primer, and water up to 25 µL. The primer pairs are presented in Table 1.

Table 1. Primers and PCR product size

Gene	Region	Primers (5'→3')	Product size (bp)	References
PAEP	Exon II	F: TTGGGTTCAGTGTGAGTCTGG R: AAAAGCCCTGGGTGGGCAGC	452	Eignatev, (1998)

PCR conditions were as follows; initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 62.2°C for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 7 min on Kyratec SC300G thermal cycler (Kyratec, Queensland, Australia). Following the reaction, 452 bp PCR products of the *PAEP* gene were visualized in %2 agarose gel stained with SYBR Safe (Hibrigen, Hydra Biotechnology, Türkiye) under UV light (Figure 1).

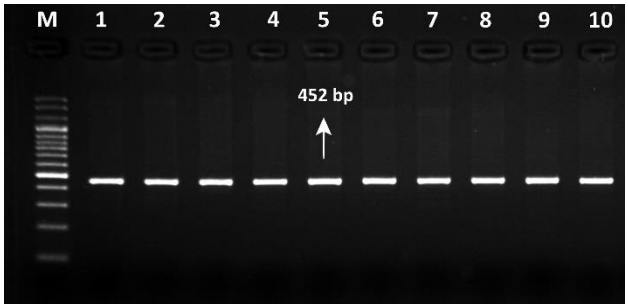

Figure 1. Results of PCR reactions. Line 1-10: 452 bp PCR products of the PAEP gene. M: 100 bp marker.

Genotyping

PAEP genotypes were identified using the restriction fragment length polymorphism (RFLP) method. RFLP reaction was carried out for 15 min at 37 °C in 50 µL of total volume; 1 µg PCR product, 10 units *RsaI* restriction enzyme (NEB, UK), 5 µL 10X rCutSmart buffer (NEB, UK), and water up to 50 µL. Then fragments were analyzed in 4% agarose gel stained with SYBR Safe (Hibrigen, Hydra Biotechnology, Türkiye) under UV light and *PAEP* genotypes were identified.

Statistical Analysis

Statistical analysis was carried out using Minitab® (Version: 19.2020.2.0, Minitab Inc., State College, PA, USA). Allel and genotype frequencies were calculated using Falconer and Mackay (1996) model. The chi-square test was used to evaluate Hardy-Weinberg Equilibrium (HWE) of alleles. Anderson-Darling normality test was applied. Due to normal distribution of the data, independent sample t-test was performed to detect the effects of *PAEP* genotypes on milk composition. The statistical significance level was defined as 0.05.

RESULTS

Allel and genotypes

Following RFLP, allele discrimination was carried out according to fragment size. In cross-bred Hamdani sheep only AA (175, 170, 66, and 41 bp) and AB (236, 175, 170, 66, and 41 bp) genotypes were identified (Figure 2). Allele and genotype frequencies are present in Table 2. Genotype frequencies for AA and AB were 0.403 and 0.597 respectively. Regarding genotypes, A allele frequency (0.7) was higher than B allel frequency (0.3). Allel distribution of *PAEP* was not in HWE ($p<0.05$).

Table 2. Allel and genotype frequencies of *PAEP* gene

		Allele Frequency		Genotypes (Observed)					
PAEP	n	A allele	B allele	AA	AB	BB	Ho	He	p-value
	96	0.7	0.3	38	58	-	0.597	0.421	**0.005**

Ho: Observed heterozygosity, He: Expected heterozygosity

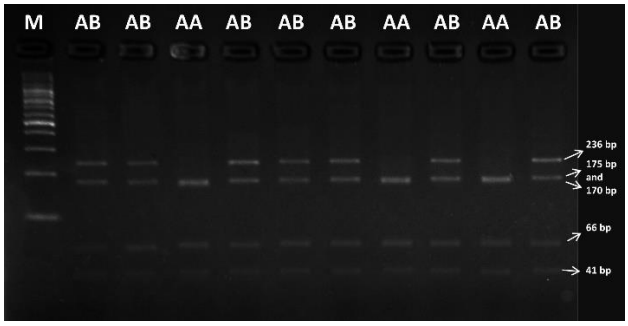

Figure 2. RsaI restriction fragments of 452 bp PCR products of PAEP gene. AA and AB genotypes. M: 100 bp DNA marker

The effects of PAEP genotypes on milk composition

The effects of AA and AB genotypes on milk composition traits are summarized in Table 3. Milk fat content (%) was higher in the AA genotype than in the AB genotype ($p<0.05$). However, there were no significant differences in protein (%), lactose (%), SNF (%), salt (%), pH, and density between AA and AB genotypes.

Table 3. The effects of *PAEP* gene AA and AB genotypes on milk composition (Mean±SE).

	***PAEP* Genotype**			
	AA	**AB**	**BB**	**p-value**
Fat (%)	7.81 ± 0.27	7.07 ± 0.17	-	**0.023**
Protein (%)	4.18 ± 0.06	4.14 ± 0.04	-	>0.05
Lactose (%)	3.94 ± 0.06	3.90 ± 0.04	-	>0.05
SNF (%)	8.78 ± 0.14	8.72 ± 0.10	-	>0.05
pH	6.86 ± 0.03	6.93 ± 0.07	-	>0.05
Density (kg/m^3)	1.0281± 0.0006	1.0283 ± 0.0004	-	>0.05
Salt (%)	0.62 ± 0.12	0.62 ± 0.01	-	>0.05

SE: Standard error, SNF: Solids-non-fat

DISCUSSION

In this study, AA and AB genotypes were identified in the *PAEP* gene exon II region of cross-bred Hamdani sheep. The A allele frequency (0.7) of the *PAEP* gene was greater than the B allele frequency (0.3). Similarly, Özmen and Kul (2016) detected only AA and AB genotypes in Sakız sheep. Moreover, A allele frequency of the *PAEP* gene were higher than B allele frequency in Sakız, Akkaraman, and Awassi sheep, consistent with the data presented in our study (Özmen and Kul, 2016). Comparable results were observed in various sheep breeds, including Egyptian (Othman et al., 2015), Latvian Darkhead (Stambekov et al., 1997), Polish (Kawecka and Radko, 2011), Racka (Barayni et al., 2010), Karagouniko (Triantaphyllopoulus et al., 2017), and Sora (Đokić et al., 2019) and Hamdani (Bayraktar and Shoshin, 2021) sheep breeds. However, Yousefi et al. (2013) reported a contrasting observation, noting that the B allele frequency of the *PAEP* gene exon II was greater than the A allele frequency in Zel sheep. Additionally, studies by Mohammadi et al. (2006), Mele et al. (2007), Dario et al. (2008), Michalcova and Krupova (2009), Corral et al. (2010), and Barayni et al. (2010) reported higher B allele frequencies than A allele frequencies in different sheep breeds. Conversely, Kusza et al. (2018) highlighted variations in A and B allele distribution of the *PAEP* gene in diverse sheep breeds reared in Eastern Europe.

Statistical analysis uncovered a significant association between the AA genotype of the *PAEP* gene and a higher fat percentage in cross-

bred Hamdani sheep. In line with our findings, Özmen and Kul (2016) reported higher milk fat percentages in AA genotypes compared to AB genotypes. In addition, Bayraktar and Shoshin, (2021) reported that milk fat percentage was higher in AA and AB genotypes compared to BB genotype while milk lactose percentage was higher in BB genotype. Fadhil and Dakheel (2022) also observed higher milk fat percentages in the AA genotype compared to the BB genotype. Conversely, Yousefi et al. (2013) noted that ewes with AB genotypes exhibited higher milk fat and lactose percentages in indigenous Zel sheep compared to ewes with the AA genotype. Giambra et al. (2014) similarly found that AB genotypes were associated with fat and lactose percentages in East Frisian dairy sheep.

No significant relationships were observed between *PAEP* genotypes and other milk composition traits in cross-bred Hamdani sheep, consistent with findings in other studies (Kawecka and Radko, 2011; Michalcova and Krupova, 2009; Sumantri et al., 2008). However, Triantaphyllopoulus et al. (2015) reported that AB and BB genotypes were linked to milk lactose and somatic cell score in Karagouniko sheep. Similarly, Fadhil and Dakheel (2022) noted higher milk lactose percentages in AB and BB genotypes compared to the AA genotype in Awassi sheep.

CONCLUSION

In summary, this study identified only AA and AB genotypes in the *PAEP* gene exon II region in cross-bred Hamdani sheep, with a higher A allele frequency. The AA genotype exhibited an association with milk fat percentage, while no such association was observed with other milk composition traits. Consequently, *PAEP* genotypes hold promise for enhancing milk composition in cross-bred Hamdani sheep through breeding programs.

ACKNOWLEDGMENT

The authors thank Assoc. Prof. Dr. Burçak ASLAN ÇELIK and Assoc. Prof. Dr. Behçet INAL for technical support in this study.

Financial support: This study was financially supported by Scientific Research Projects Council of Siirt University (Project No: 2022-SIUVET-041).

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical statement or informed consent: The study was approved by Siirt University Animal Experiments Local Ethics Committee (Approval no: 2023-01-10).

Author contributions: AOT conceived the idea, arranged necessary funding, carried out experimental work and prepared original draft of the manuscript. EG carried out software and preparation of original draft. DK also conceived the idea, helped in experimental work, preparation of original draft. SÜ carried out experimental work. All authors reviewed and approved final version of the manuscript.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Histopathological and biochemical effects of 18β-glycyrrhetinic acid application on lipopolysaccharide-induced kidney toxicity in rats

ABSTRACT

Lipopolysaccharide (LPS) is an endotoxin found in the wall of gram-negative bacteria and causes acute inflammation when it enters the tissues. 18β-glycyrrhetinic acid (18β-GA) is a substance found in licorice root and is responsible for this plant's antiallergic, antioxidant, and anti-inflammatory activity. This study aimed to examine the possible effects of 18β-glycyrrhetinic acid on the damage caused by LPS in kidney tissue. The study was divided into six equal groups containing 48 Sprague Dawley adult male rats (n = 8). The groups were created as follows; the Control group; the group that received 1cc physiological saline throughout the experiment was the DMSO group; DMSO, an intraperitoneal carrier substance, was given. LPS group; A single dose of 7.5 mg/kg intraperitoneal (i.p) LPS was administered. 18β-GA50+LPS group; 18β-glycyrrhetinic acid was given by gavage at 50 mg/kg daily for 10 days, followed by a single dose of 7.5 mg/kg i.p. LPS was administered. 18β-GA100+LPS group; 18β-glycyrrhetinic acid was administered by gavage at 100 mg/kg daily for 10 days, followed by a single dose of 7.5 mg/kg i.p. LPS was administered. 18β-GA100 group; 18β-glycyrrhetinic was given by gavage at 100 mg/kg daily for 10 days. 24 hours after LPS application to all groups, the kidney tissues of the rats were removed under anesthesia and placed in 10% formaldehyde. Histopathological and oxidative stress parameters analyses were performed in kidney tissue. These findings raised the possibility that 18β-GA could be an adjuvant therapy that protects kidney tissue from LPS-induced oxidative and tissue damage effects and reduces its side effects.

Keywords: Histopathology, kidney, lipopolysaccharide, oxidative stress, rat

INTRODUCTION

Sepsis physiopathology is a whole of complex mechanisms that begins with an excessive cellular immunological response against the infection focus that initiates the sepsis process and then damages the host at the level of organs and systems (Uchino et al., 2005). Mediators and cytokines that play a role in intercellular signaling play an important role in the sepsis formation process (Neveu et al., 1996; Silvester et al., 2001). Bacterial products called pathogen-associated molecular structures (PAMPs) can be detected and recognized by the body's natural immunity (Lopes et al., 2009; Oppert et al., 2008). Lipopolysaccharides (LPS) located in the cell walls of gram-negative bacteria are one of the most important PAMPs and play a very important role in triggering the septic process (Cunningham et al., 2002; Knotcke et al., 2001). The event that initiates septic shock is the passage of LPS or toxic cell wall components into the organism's circulatory system as a result of the lysis of bacteria (Morelli et al., 2013). LPS stimulates signaling pathways that lead to the synthesis and

How to cite this article
Erbaş, E., Gelen, V., Yakut, S., Albayrak, K., (2024). Histopathological and biochemical effects of 18β-glycyrrhetinic acid application on lipopolysaccharide-induced kidney toxicity in rats. *Journal of Advances in VetBio Science and Techniques, 9*(1), 42-49. https://doi.org/10.31797/vetbio.1419538

Research Article

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Article info

Submission: 14-01-2024
Accepted: 26-03-2024
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

release of cytokines and other mediators. Thus, TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8) are released from monocytes. IL-1 and IL-6 activate T cells and ensure the secretion of interferon gamma (IFN-γ), interleukin-2 (IL-2) and interleukin-4 (IL-4) (Hagiwara et al., 2009). Mediators such as TNF-α and IL-1 are released within 30 minutes after the appearance of LPS and cause the release of secondary cytokines, lipid mediators, and reactive oxygen metabolites, as well as initiating the release and synthesis of arachidonic acid metabolites, which are extremely important in sepsis (Mori et al., 2011). Since the events occurring in sepsis can affect the entire organism, this situation may extend to multiple organ failure. The most common organ failures in sepsis are lung, kidney, liver, and heart failure (Ogura et al., 2014). Various recent studies have shown that the use of agents with antioxidant and anti-inflammatory effects prevents organ damage in kidney damage occurring in the LPS-induced sepsis model (Gomez et al., 2014). Various studies have reported that Glycyrrhiza glabra L. (licorice root) has antioxidant and anti-inflammatory effects (Eisenbrand, 2006; Kang et al., 2014). The reason why this plant exhibits the mentioned properties is due to the many biological compounds found in its structure (Hasan et al., 2015; Mahmoud and Al Dera**,** 2015; Wu et al., 2015). Its main component is glycyrrhizin, which makes up approximately 10% of the dry weight of licorice root. Glycyrrhizin is a glycyrrhetinic acid glycoside containing two glucuronic acid residues. After oral administration, glycyrrhizin is rapidly and almost completely metabolized to glycyrrhetinic acid by intestinal bacteria (Ishii et al., 2000; Ma et al., 2016). Glycyrrhetinic acid, specifically 18β-Glycyrrhetinic acid, is the main active metabolite of glycyrrhizin and is responsible for most pharmacological properties. Studies have demonstrated the pharmacological and health-promoting effects of 18β-Glycyrrhetinic acid, including antioxidant, anti-inflammation, anticancer, and metabolic regulation (Itoh et al., 1999; Kalaiarasi and Pugalendi, 2009; Young, 1995; Zeller et al., 1984). In line with all this information, present study aims to introduce the possible protective effects of 18β-GA in the LPS-induced acute kidney toxicity model in rats, which has not yet been reported in the literature, and to contribute to filling the gap in this field.

MATERIALS AND METHODS

In the present research, we were studied the renal toxicity model induced by LPS (*O55*:*B5*, Sigma-Aldrich) (7.5 mg/kg, i.p., single dose) in rats, and 18β-GA (Cayman Chemical Company-11845) (50 mg/kg) and 18β-GA (100 mg/kg, dose i.g., 10 days) was applied. Experimental animals were obtained from Atatürk University Medical Experimental Research and Application Center. Rats were fed ad-libitum until the time of study and kept in a ventilated environment with a 12-hour light-dark cycle and a room temperature of approximately 25°C. To provide sufficient kidney tissue samples in each group, 8 rats were used, and 6 groups were formed. A total of 48 12-week-old adult Sprague Dawley male rats weighing 220-250 g were used. The experimental groups were formed as presented in Table 1 and the experimental procedure was applied as written.

All animals were subjected to standard care and feeding conditions. At the end of the experimental applications, after the live weight of the rats was weighed, kidney tissues were taken following intracardiac blood collection and cervical dislocation under sevoflurane anesthesia. After weighing these tissues, a portion of the kidney tissue of 8 rats from each group was immediately placed in 10% formaldehyde after washing with physiological saline for histopathological examinations. The remaining part of the kidney tissue of the rats was washed with physiological saline and then immediately placed in liquid nitrogen and frozen until biochemical analysis.

Table 1: Experimental groups and experimental procedure.

Number of groups	Number of animals	Application
Group 1 (n=8)	Control	i.p saline 10 days
Group 2 (n=8)	DMSO	0.1 ml i.p DMSO injection
Group 3 (n=8)	LPS	7.5mg/kg i.p LPS single dose
Group 4 (n=8)	18β-GA50+LPS	18β-GA at 50 mg/kg i.g dose for 10 days and 7.5mg/kg i.p LPS as a single dose for 10 days
Group 5 (n=8)	18β-GA100+LPS	18β-GA at 100 mg/kg i.g dose for 10 days and 7.5mg/kg i.p LPS as a single dose for 10 days
Group 6 (n=8)	18β-GA100	18β-GA at 100 mg/kg i.g dose for 10 days

Biochemical analyzes

At the end of the experiment, 50 mg of kidney tissue obtained from rats was weighted and homogenized with tissue homogenate buffer at 30 hz for 3 minutes in tissue liser (Qiagen TissueLyser II). It was then centrifuged at 12000 rpm at 4°C for 15 minutes. The supernatant obtained was taken and GSH analysis was performed according to Sedlak et al., 1968. For MDA analysis, 50 mg of kidney tissue obtained from rats at the end of the experiment was weighted and homogenized with tissue homogenate buffer at 30 hz for 3 minutes in tissue liser (Qiagen TissueLyser II). It was then centrifuged at 4000 rpm at 4°C for 15 minutes. The supernatants obtained were analyzed according to the method of Ohkawa et al., (1979).

Histopathological analysis

Rat kidney tissues obtained at the end of the experiment were placed in 10% neutral formaldehyde solution and fixed for 72 hours. Then, they were passed through graded alcohol and xylol series and embedded in paraffin blocks and 5µ thick sections were taken with a microtome device (Leica RM2125 RTS) for histopathologic evaluations. For histopathologic examination, tissue damage was evaluated by staining the sections with Mallory's Triple Staining method modified by Crossman. Each section was scored from 0 to 4 to evaluate histopathologic damage in the kidney tissue. 0 indicates no tissue damage, 1 indicates mild damage, 2 indicates moderate damage, 3 indicates severe damage and 4 indicates very severe damage (Niu et al., 2019). A trinocular microscope (Zeiss AXIO Scope.A1, German) with computer and camera attachment was used for microscopic examination.

RESULTS

Biochemical results

When the MDA level was compared between the groups, we observed that the kidney tissue MDA level of the LPS-treated groups increased significantly compared to the control and other groups. On the other hand, we determined that the application of 18β-GA prevented this LPS-induced increase. When the GSH level was compared between the groups, the kidney tissue GSH level of the LPS-treated groups increased significantly and decreased compared to the control and other groups. On the other hand, we determined that 18β-GA application prevented this LPS-induced decrease. Biochemical results of all groups are presented in Figure 1.

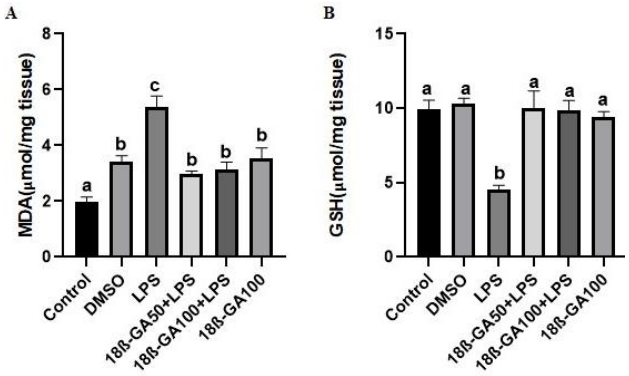

Figure 1. The effects of LPS and 18β-GA administration on MDA (A), and GSH (B) levels in the experimental groups (There are statistically significant differences between the values expressed with different symbols between the control group.

Histopathological results

In the current study, kidney tissue was observed to be normal in the Control, DMSO, and 18β-GA100 groups, and glomerular and tubules in the cortex and medulla tubules were observed to be healthy. In the LPS group, it is observed that

the normal structure of the kidney is completely disrupted and the glomerul and tubules lose their normal structure. It is observed that the Bowman space in the glomerul is widened and the glomerular tangle shrinks and exhibits a degenerative appearance. Widespread hemorrhagic areas are noted in the cortex and medulla.

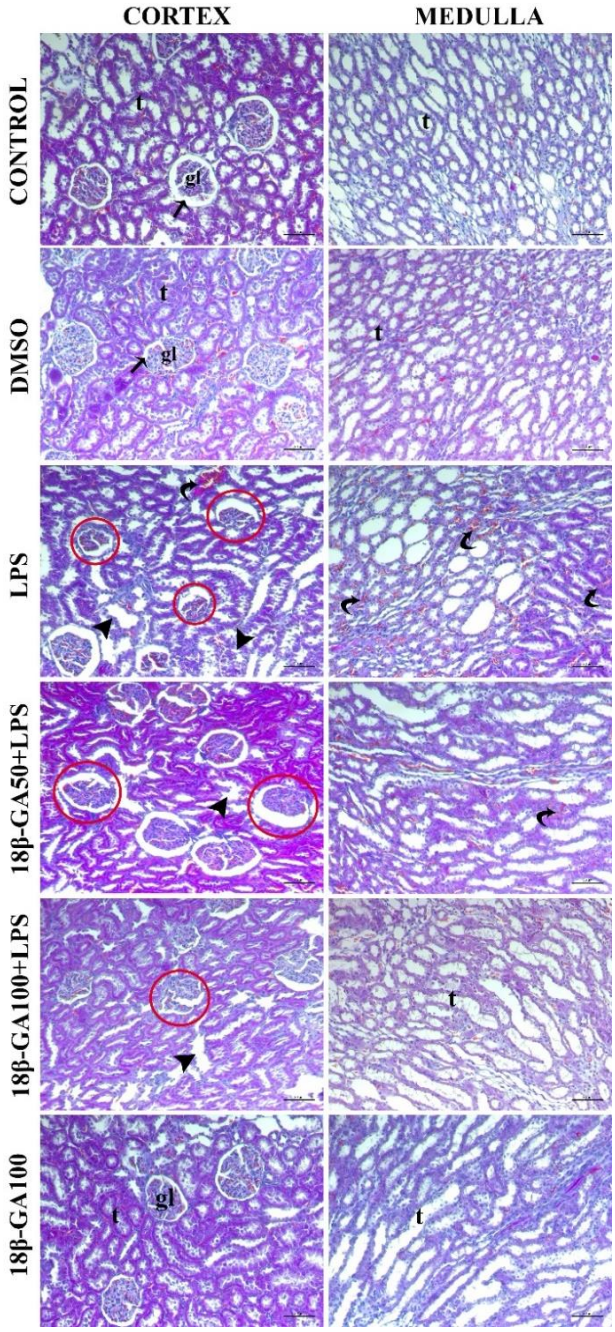

Figure 2. Kidney tissues stained with Mallory's Triple Stain Modified by Crossman. gl: Renal glomerulus, t: Renal tubules, Arrow: Bowman's space, Red circle: Degenerative glomerulus, Arrowhead: Degenerative tubule, Curved arrow: Hemorrhagic area.

While the recovery is better, especially in the 18β-GA100+LPS group, a near-normal appearance is observed in the glomerular and tubules in the 18β-GA50+LPS group. It is noteworthy that the Bowman space is normal and the degeneration in the tubules is reduced. Hemorrhagic areas have decreased considerably. Histopathological evaluation results of all groups are presented in Figure 2 and 3.

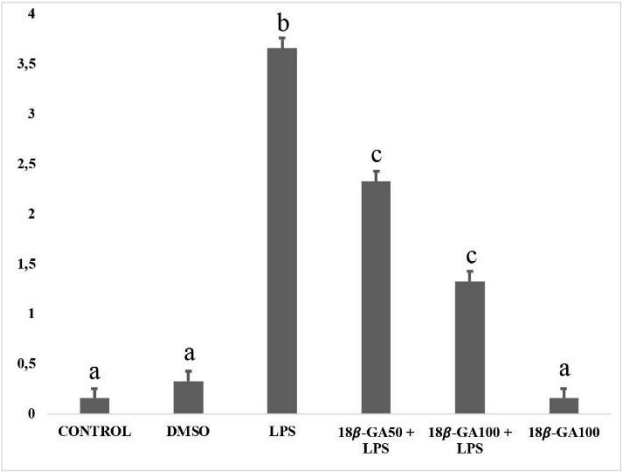

Figure 3. Assessment of renal histopathology.

DISCUSSION

LPS is the structure found in the cell wall of gram-negative bacteria and is responsible for the inflammation and apoptosis caused by these bacteria in the tissue (Hayashi et al., 2001; Tsao et al 2004). LPS administration causes toxicity in the lung, brain, kidney, and testicular tissues as well as the liver (Boveris and Cadenas, 1997; Kadkhodaee and Osami, 2004; Tiwan et al., 2005). If toxicity develops in organs, disruptions in the physiological functions of the organ, loss of function, and organ failure occur (Gündoğdu et al., 2023; Iguchi et al., 1992; Kobayashi et al., 2015). The effects of 18β-GA, a flavonoid compound with antioxidant and anti-inflammatory effects, in experimental organ toxicity models have been reported in many studies (Kao et al., 2010). In the present study, the possible effects of 18β-GA on LPS-induced oxidative stress and tissue damage were investigated.

Imbalances in the typical cellular redox state cause perturbations in biological components such as lipids, proteins, and DNA (Gelen et al., 2023). The extent of ROS production determines the extent of cell membrane damage and leads to the occurrence of lipid peroxidation through oxidative modification of polyunsaturated fatty acids within the composition of the membrane (Alwazeer, 2023; Kara et al., 2016). In the present study, MDA, one of the lipid peroxidation indicators, increased, and 18β-GA application significantly reduced the MDA level. Oxidative stress can be defined as the disproportion between oxidant and antioxidant defense systems. MDA is a suitable lipid peroxidation biomarker (Kara et al., 2023). The elevation observed after LPS kidney injury was significantly attenuated by oral dosage of 18β-GA. Oxidative stress can be described as the disruption of the balance between the mechanisms that produce oxidants and the mechanisms that provide antioxidant protection. The production of reactive oxygen species (ROS) effectively counteracts both enzymatic (such as SOD, GSH-Px, and CAT) and non-enzymatic (such as GSH) antioxidant defenses (Gelen et al., 2021; Gelen et al., 2023). LPS significantly decreased GSH levels while increasing MDA levels in kidney tissue. In a study, it was determined that 18β-GA had an antioxidant role in nephrotoxicity in rats (Abd El-Twab et al., 2016). In the present study, we determined that LPS induces oxidative stress in kidney tissue and 18β-GA application prevents these changes.

In some previous studies, it was observed that LPS application caused congestion, interstitial edema, degeneration of cells, necrosis and calcification in rat kidney tissue (Ban et al., 2022). In the present study, LPS application caused the integrity of the kidney tissue to completely deteriorate and the glomerulus to lose its normal structure. In previous studies, it was determined that LPS administration caused damage to kidney tissue (Raghavan and Weisz, 2015). The data obtained in these studies are compatible with the data obtained in the present study. On the other hand, it was determined that 18β-GA application significantly prevented these changes. Various studies have shown that 18β-GA application prevents kidney tissue damage caused by some toxic agents. These data are compatible with the data we obtained.

CONCLUSION

In conclusion, the findings obtained in this study show that LPS administration triggers ROS production and causes kidney tissue damage. These findings raised the possibility that 18β-GA could be an adjuvant therapy that protects kidney tissue from LPS-induced oxidative and tissue damage effects and reduces its side effects.

ACKNOWLEDGMENT

Financial support: This research was not funded financially by any institution or company.

Conflict of interest: The authors declared that there is no conflict of interest.

Ethical statement or informed consent: The study was approved by the Local Ethics Committee on Animal Experiments of Atatürk University (Decision number: 220, Date: 25.12.2023).

Author contributions: Research and technique and writing innovative validation software; EE, VG, SY, and KA. Writing: EE, VG, SY, and KA; preparing the initial draft Conceptualization: Every author has reviewed and approved the published version of the study.

Availability of data and materials: The article or its supplemental materials include data.

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Bal üretiminin Coğrafi Bilgi Sistemleri (CBS) ile incelenmesi: Ordu ili örneği

Examining honey production with Geographic Information Systems (GIS): The example of Ordu province

ÖZET

Arıcılık toprağa bağlı olmadan elde edilen ürünleriyle ekonomik, tozlaşmadaki katkısı ile de ekolojik bir yetiştiricilik örneğidir. Bu çalışmanın amacı, Türkiye'nin arıcılık faaliyetleri ve bal üretiminde yıllardır ilk sırada yer alan Ordu ilinin 2013-2022 yılları arasındaki bal verileri olarak üretim verileri esas alınarak, Coğrafi Bilgi Sistemleri (CBS) ile incelenmesidir. Ordu ilinde kayıtlı yetiştiricilerin bal üretim verileri değerlendirilerek, bal üretiminin son on yıllık mekânsal projeksiyonunun değerlendirilmesi amacıyla kartografik haritalar üretilmiştir. CBS'ye dayalı bir veri tabanı oluşturulduktan sonra elde edilen çıktıların mekânsal analizi ve haritalanması amacıyla ilçelere göre şekil dosyası (.shp uzantılı) verileri kullanılmıştır. Bu veriler WGS 84 EPSG:4326 koordinat referans sisteminde tanımlandıktan sonra bal üretiminin ve bal veriminin çalışma dönemi boyunca mekânsal dağılımı, açık kaynak QGIS sürüm 3.18.3 yazılımı kullanılarak analiz edilmiştir. En fazla bal üretiminin ilgili zaman aralığında Altınordu ilçesindeki kayıtlı arıcılara ait olduğu belirlenmiştir. Kayıtlı işletme sayısının da yine en çok olduğu ilçe Altınordu'dur. Bu ilçenin komşu ilçelerinden olan Gülyalı ilçesindeki arıcıların bal üretim verileri ve işletme sayılarının Altınordu ilçesinin aksine daha düşük olduğu belirlenmiştir. Altınordu ilçesinden sonra sırasıyla Perşembe, Ulubey, Gürgentepe ve Gölköy ilçelerinin bal üretiminde önemli bir yere sahip olduğu görülmektedir. Bu ilçelerin Gülyalı ilçesi gibi komşu olmalarına rağmen üretimlerinin yüksek olması ilçeler arası iş birliği farklılığından kaynaklanmış olabilir. Sonuç olarak, bal üretimi bakımından önemli bir şehir olan Ordu ili ve ilçelerinde bal üretim faaliyetlerinin ilçelere bağlı değişiklik gösterdiği belirlenmiştir. Dünya'nın yakın gelecekte daha fazla maruz kalacağı öngörülen iklim değişikliği sorunu da arıcılık faaliyetlerini göçer arıcılığa yöneltmektedir. Ordu ilindeki yetiştiricilerin birçoğu gibi diğer şehirlerdeki kayıtlı arıcıların da küresel ısınma tehdidine karşı göçer arıcılığa daha fazla yöneleceği söylenebilir.

Anahtar kelimeler: Altınordu, bal, mekânsal analiz, Ordu, verim.

ABSTRACT

Beekeeping is an example of economic farming with its products that can be done without being dependent on plant and ecological production because of its contribution to pollination. This study aims to examine Ordu province, which has been at the top of Turkey's beekeeping activities and honey production for years, with Geographic Information Systems (GIS) based on honey production data between 2013 and 2022. By evaluating the honey production data of registered breeders in Ordu province, cartographic maps were produced to evaluate the spatial projection of honey production for the last ten years. After creating a GIS-based database, district-based shape file (.shp extension) data was used for spatial analysis and mapping of the obtained outputs. After these data were defined in the WGS 84 EPSG:4326 coordinate reference system, the spatial distribution of honey production and yield throughout the study period was analyzed using the open-source QGIS version 3.18.3 software. It was determined that the highest honey production belonged to registered beekeepers in Altınordu district during the relevant time period. Altınordu is also the district with the highest number of registered enterprises. It was determined that the honey production data and the number of enterprises of the beekeepers in Gülyalı district, which is one of the neighbouring districts of this district, are lower than Altınordu district. After Altınordu district, Perşembe, Ulubey, Gürgentepe and Gölköy districts have an important place in honey production. Although these districts are neighbours like Gülyalı district, their high production may have resulted from the difference in cooperation between the districts. As a result, it was determined that honey production activities in Ordu province and districts, which is an important city in terms of honey production, vary depending on the districts. The problem of climate change, which is predicted that the world will be exposed to more in the near future, also directs beekeeping activities towards nomadic beekeeping. It can be said that like most of the breeders in Ordu province, registered beekeepers in other cities will be more orientated towards nomadic beekeeping against the threat of global warming.

Keywords: Altınordu, honey, spatial analysis, Ordu, yield

How to cite this article

Arslan, E., Bayir, T., İnal, Ş., (2024). Examining honey production with Geographic Information Systems (GIS): The example of Ordu province. *Journal of Advances in VetBio Science and Techniques, 9*(1), 50-58. https://doi.org/10.31797/vetbio.1403373

Research Article

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Article info

Submission: 11-12-2023

Accepted: 29-03-2024

Online First: 25-04-2024

Publication: 30-04-2024

e-ISSN: 2548-1150

doi prefix: 10.31797/vetbio

http://dergipark.org.tr/vetbio

GİRİŞ

Arıcılık toprağa bağlı olmayan bir üretim faaliyeti olduğundan daha az sermaye ile yapılabilmektedir. Bu üretim tipi, orman ve sınır köylerinde tarım ve hayvancılık yapmak için yeterli araziye sahip olmayan kişilere bir iş fırsatı oluşturabilmektedir. Gelişmiş ve gelişmekte olan ülkelerin hem gıda sorununa ilişkin çözüm arayışı hem de küresel çapta yaşanan ekonomik krizlerin son yıllarda arıcılık faaliyetlerinin önemini gün geçtikçe daha da artırdığı görülmektedir (Andaregie ve Astatkie, 2021; Aşkan, 2023; Schouten, 2021).

Arı ürünlerinin hayvan sağlığında da kullanılmasına ilişkin literatür bilgiler oldukça sınırlıdır. Bu ürünlerin sindirim enzimlerini aktive ederek, mikrobiyal dengeyi koruyarak ve vitamin sentezini teşvik ederek kümes hayvanlarının büyümesini ve immun sistemini güçlendirici etkisinin olabileceği öngörülmektedir. Arıcılıktan elde edilen ürünlerin (bal, propolis vd.) etlik piliçlerde büyüme ve gelişmelerini artırıcı etki yapabileceği bildirilmektedir (Abd El-Aziz vd., 2023; Ali ve Mohanny, 2014; Eser ve Erat, 2022).

Arı ailesinin en yüksek bal verimi *Apis mellifera* (bal arısı) 'dan sağlanmaktadır (Breed, 2010). Arıcılık hem sabit hem de göçer olarak yapılabilmektedir. Türkiye, stratejik konumu, bitki vejetasyonun zenginliği ve coğrafi şartları, iklim ve bitki örtüsü açısından arıcılığa elverişli bir ülkedir. Türkiye'de 2022 yılı verilerine göre; 95.386 kişi arıcılık faaliyeti yürütmekte, toplamda 8.984.676 adet kovandan 118.297 ton bal üretilmekte olup bal üretim ortalaması 13,2 kg'dır (Anonim, 2023). Ordu ilinin ortalama bal verimi Türkiye'de ilk sırada olup bölgedeki bal üreticileri genel olarak göçer arıcılık yapmaktadır. Bu bölgede yaşayan insanlar değerlendirildiğinde, özellikle yüksek rakımlı ilçelerinde yaşayan insanların genelinin, arıcılık faaliyetlerini asıl geçim kaynağı olarak gerçekleştirdiği görülmektedir (Sıralı, 2017). Bal tüketenler ise taleplerini karşılamada öncelikleri tanıdıkları yetiştiriciden satın almanın daha güvenli olduğunu düşünmektedir (Dağdemir ve Akdemir, 2021). Bu durumun yerel üreticilerin sürdürülebilir bir üretim yapmalarına katkı sağlayabileceği öngörülmektedir (Dağdemir ve Akdemir, 2021).

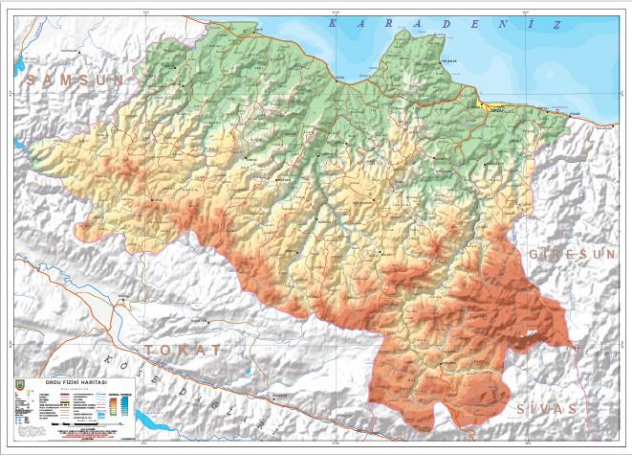
Belirli bir analitik yöntemin seçiminin, istatistik kullanan herhangi bir analizde olduğu gibi, verilerin özelliklerinin ve gözlemlere ilişkin önceki bilgilerin değerlendirilmesiyle belirlenmesi gerekir. Bunlar arasında, Coğrafi Bilgi Sistemleri (GIS) yöntemlerine dayanan mekânsal haritalama, mekânsal analizin başlangıcı için sıklıkla bir temel olarak kullanılmaktadır (Dragicevic, 2004). Bal üretimi bölgeye, iklim yapısına, bitki örtüsüne ve çevre koşullarına göre değişkenlik gösterebilmektedir (Portakal vd., 2023). Bu durumda mekânsal haritalandırmanın önemi ortaya çıkmaktadır. Ülkedeki arıcılık endüstrisini daha iyi organize etmek, kırsal ekonominin büyümesini teşvik etmek ve beslenme gereksinimlerini karşılamak için üstün olanaklar ve hizmetler sağlamak amacıyla, tarım politika yapıcıları ve ilgili hükümetlerin, arıcılık ürünlerinin mekânsal dağılımını değerlendirmelerini fayda sağlayacağı düşünülmektedir. Arıcılık sektörü için en uygun yerlerin belirlenmesi açısından yerel yöneticilerin yeterli mekânsal desen yönetiminin yapılması büyük önem taşımaktadır (Yaman ve Yaman, 2023).

Bu çalışmanın amacı, Türkiye'nin arıcılık faaliyetleri ve bal üretiminde yıllardır ilk sırada yer alan Ordu ilinin 2013-2022 yılları arasındaki kayıtlı arıcılarına ait bal üretim verileri esas alınarak, Coğrafi Bilgi Sistemleri (CBS) ile incelenmesidir.

MATERYAL VE METOT

Çalışma alanı

Doğu Karadeniz'e açılan kapı konumunda bulunan Ordu ili 40'- 41' kuzey paralelleri, 37'-38' doğu meridyenleri arasında yer almaktadır. Batısında Samsun, doğusunda Giresun, güneyinde Sivas ve Tokat illeriyle çevrili olan Ordu ilinin kuzeyinde Karadeniz bulunmaktadır. Ordu ili 751.180 nüfusa ve 5.961 km^2 yüzölçümüne sahip olan bir ildir. Ordu ili merkez ilçesi ile 19 ilçeden oluşmaktadır (Çürüksulu, 2023; Yılmaz, 2022). Bu çalışma, örnek analiz birimi olarak Ordu ili ile ilçelerini merkeze almaktadır. Türkiye'de ilçeler illere göre daha küçük idari ve coğrafi birimlerdir. Ordu ili 751.180 nüfusa ve 5.961 km^2 yüzölçümüne sahiptir. Bu çalışmada, 2013-2022 yılları arasında ilçe düzeyindeki bal üretim verileri Türkiye İstatistik Kurumu'ndan (TÜİK) alınmıştır. Ordu ili fiziki haritası (HGM, 2023) Şekil 1'de verilmiştir.



Şekil 1. Ordu ili fiziki haritası (HGM, 2023).

Mekânsal haritalama

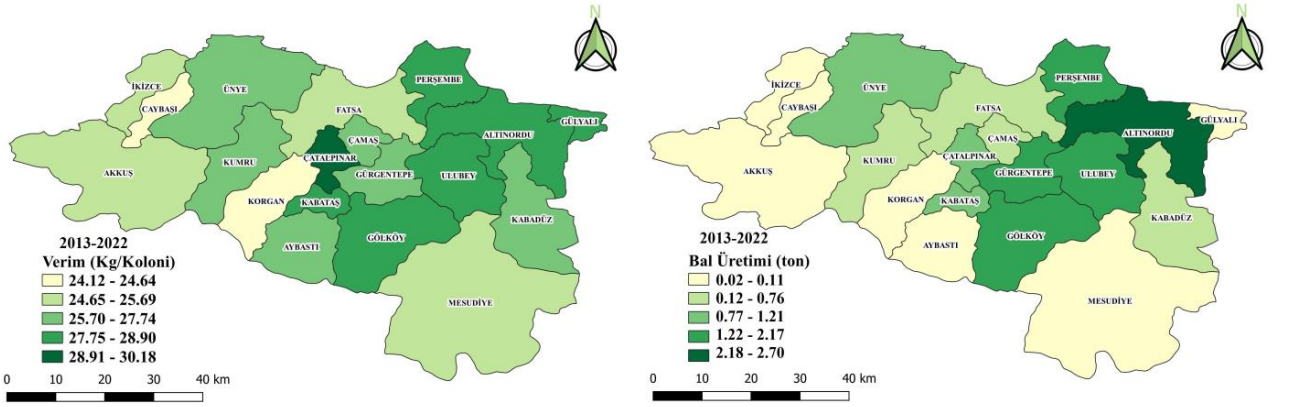
Ordu ilinde bal üretim verileri değerlendirilerek bal üretiminin son on yıllık mekânsal projeksiyonunun değerlendirilmesi amacıyla haritalar üretilmiştir. Bu amaçla Coğrafi Bilgi Sistemlerine (CBS) dayalı bir veri tabanı oluşturulmuştur. Çıktıların mekânsal analizi ve haritalanması amacıyla CBS yazılımında kullanılmak üzere ilçe düzeyinde şekil dosyası (.shp uzantılı) verileri kullanılmıştır. Kullanılan veriler WGS 84 EPSG:4326 koordinat referans sisteminde tanımlanmıştır. Bal üretiminin ve bal veriminin çalışma dönemi boyunca mekânsal dağılımı, açık kaynak QGIS sürüm 3.18.3 yazılımı kullanılarak analiz edilmiştir. Bal verimini hesaplamak için tanımlayıcı yöntemler kullanılmıştır. İlçelere göre Excel formatında oluşturulan bal üretimi (ton) ve kovan verileri kullanılarak bal verimleri hesaplanmıştır. İlçelere göre bal verimi, ilçelere göre üretilen bal miktarlarının (ton) ilçelere göre kovan sayılarına bölünmesiyle hesaplanmıştır.

$$Bal\ verimi = \frac{Bal\ üretimi\ (ton)}{Koloni\ sayısı}$$

BULGULAR

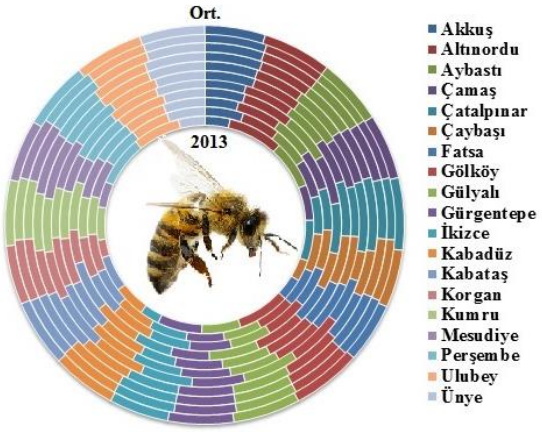
İlçelere göre ayrı ayrı hesaplanan bal verimlerinin on yıllık ortalaması hesaplanıp, genel bir değerlendirme yapılarak mekânsal verim haritası oluşturulmuştur. İlçelere göre ayrı ayrı elde edilen bal üretim (ton) verilerinin dönüşümü yapılarak *(bal üretimi (ton) / 1000)* mekânsal üretim haritası oluşturulmuştur.

Ordu ilinde 2013-2022 yıllarındaki ortalama bal verim (kg/koloni) ve ortalama bal üretim (ton) miktarlarının ilçelere göre haritaları Şekil 2'de verilmiştir. On yıllık değerlendirmede en yüksek üretim ortalamasının Altınordu ilçesinde olduğu belirlenmiştir. Bal verimi değerlendirildiğinde ise verimin en yüksek olduğu ilçe Çatalpınar ilçesi olarak belirlenmiştir. Ordu ilinde 2013-2022 yıllarındaki ilçelere göre bal verimi (kg/koloni) miktarları Şekil 3'te verilmiştir. Bal veriminin yıllara ve ilçelere göre bir ısı haritası ve daire grafiği oluşturularak bal veriminin zaman bazlı daha detaylı değerlendirilmesi yapılmıştır. 2018 yılından sonra 2019 ve 2020 yılında Çatalpınar, Gülyalı, Kabataş, Mesudiye ve Ünye ilçeleri hariç tüm ilçelerde bal veriminde düşüş görülmeye başlanmıştır. 2021 yılında ise tüm ilçelerde %10 oranında bir düşüş tespit edilmiştir. Ancak 2022 yılında tekrar tüm ilçelerde bal veriminin tüm ilçelerde yaklaşık %14 oranında arttığı belirlenmiştir.



Şekil 2. Ordu ilinde 2013-2022 yıllarındaki ortalama bal verim (kg/koloni) ve ortalama bal üretim (ton) miktarlarının ilçelere göre haritaları.

İlçeler	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Ort.
Akkuş	14.00	28.54	23.41	23.69	24.40	30.00	29.07	30.21	18.80	31.34	25.35
Altınordu	31.04	28.51	31.50	30.58	30.16	29.35	27.13	28.31	18.83	31.34	28.68
Aybastı	26.76	28.54	28.64	28.87	26.21	30.00	29.51	28.68	18.82	31.34	27.74
Çamaş	20.00	28.54	28.58	29.42	27.32	30.00	29.85	28.24	18.84	31.34	27.21
Çatalpınar	23.02	28.54	29.54	30.00	32.89	30.00	38.81	38.79	18.84	31.34	30.18
Çaybaşı	12.00	28.54	28.44	22.00	29.41	30.00	16.33	24.27	18.84	31.35	24.12
Fatsa	12.50	28.54	28.34	22.67	27.66	30.00	25.00	25.71	18.76	31.34	25.05
Gölköy	29.54	28.54	29.87	30.39	30.37	30.00	29.89	30.20	18.84	31.34	28.90
Gülyalı	22.00	28.54	28.54	30.00	28.97	30.00	30.71	30.77	18.84	31.34	27.97
Gürgentepe	25.00	28.54	29.54	23.00	28.60	30.00	29.33	29.30	18.84	31.34	27.35
İkizce	11.06	28.41	28.87	22.80	20.98	29.91	28.57	27.73	18.84	31.34	24.85
Kabadüz	17.88	28.54	30.00	28.00	28.12	30.00	29.47	28.10	18.84	31.34	27.03
Kabataş	20.00	28.54	29.54	30.00	30.00	30.00	31.25	32.00	18.84	31.34	28.15
Korgan	10.00	28.54	28.64	20.16	27.96	30.00	26.96	23.97	18.84	31.34	24.64
Kumru	18.65	28.54	28.55	28.88	28.75	30.00	29.88	30.34	18.84	31.34	27.38
Mesudiye	14.51	28.54	29.54	15.00	22.74	30.00	33.64	32.73	18.84	31.34	25.69
Perşembe	28.00	28.54	29.54	29.50	30.50	30.00	29.02	29.01	18.84	31.34	28.43
Ulubey	26.78	28.54	29.74	29.99	30.60	29.99	29.99	29.88	18.83	31.33	28.57
Ünye	15.00	28.54	29.54	25.00	29.87	30.00	32.00	32.09	18.84	31.34	27.22



Şekil 3. Ordu ilinde 2013-2022 yıllarındaki ilçelere göre bal verimi (kg/koloni) miktarları

Son on yılda Ordu ilinde bal üretimi, balmumu üretimi, kovan sayısı (adet) ve işletme sayısının Türkiye geneline oranla bir karşılaştırılması yapılmıştır (Tablo 1).

Türkiye'de 2022 yılında elde edilen toplam bal üretimi içerisinde Ordu ilinin %16.14'lük bir oranla önemli bir yere sahip olduğu görülmektedir. Ordu ilinde aynı yıl için bal mumu üretimi 278 kg, kovan sayısı 609.427 adet ve işletme sayısı ise 3.079 adettir (Tablo 1).

CBS'ye dayalı bir veri tabanı oluşturularak ilçelere göre ayrı ayrı elde edilen bal üretim (ton) verilerinin dönüşümü yapılarak (bal üretimi (ton) / 1000) ve işletme sayıları kullanılarak her yıl için (2013-2022) ayrı ayrı kartografik mekânsal üretim haritaları oluşturulmuştur (Şekil 4). Böylece son on yılın değerlendirilmesi daha detaylı belirlenmeye çalışılmıştır. Tüm yıllarda üretimin en fazla olduğu ilçenin Altınordu olduğu belirlenmiştir. En fazla kayıtlı işletme sayısının da Altınordu ilçesinde olduğu görülmektedir. Altınordu ilçesinin komşu ilçelerinden Gülyalı ve Kabadüz ilçelerinde özellikle Gülyalı ilçesinde üretimin ve işletme sayılarının düşük olduğu belirlenmiştir. 2013 yılında 75 işletmeye sahip olan Gülyalı ilçesinde zamanla işletme sayısının neredeyse %50 oranında azaldığı belirlenmiştir.

Tablo 1. Ordu ili ve Türkiye genelindeki 2013-2022 yıllarında bal üretimi, balmumu üretimi, kovan sayısı ve işletme sayıları

Yıl	Bal Üretimi (ton) (n, %)		Balmumu Üretimi (kg) (n,%)		Kovan Sayısı (adet) (n,%)		İşletme Sayısı (n,%)	
	Ordu	Türkiye (Diğer)	Ordu	Türkiye (Diğer)	Ordu	Türkiye (Diğer)	Ordu	Türkiye (Diğer)
2013	12.865 (13.59)	81.829 (86.41)	200 (4.72)	4.041 (95.28)	519.836 (7.83)	6.121.512 (92.17)	3.881 (4.86)	76.053 (95.14)
2014	15.039 (14.53)	88.486 (85.47)	80 (1.97)	3.973 (98.03)	527.078 (7.44)	6.555.654 (92.56)	2.549 (3.14)	78.559 (96.86)
2015	16.601 (15.35)	91.527 (84.65)	92 (1.93)	4.664 (98.07)	556.593 (7.18)	7.191.694 (92.82)	2.674 (3.20)	80.801 (96.80)
2016	16.278 (15.40)	89.449 (84.60)	89 (2.00)	4.351 (98.00)	577.858 (7.31)	7.322.506 (92.69)	2.783 (3.31)	81.264 (96.69)
2017	16.799 (14.68)	97.672 (85.32)	115 (2.56)	4.373 (97.44)	562.299 (7.04)	7.428.773 (92.96)	2.716 (3.26)	80.494 (96.74)
2018	16.994 (15.75)	90.926 (84.25)	120 (3.01)	3.867 (96.99)	568.547 (7.01)	7.539.877 (92.99)	2.625 (3.21)	79.205 (96.79)
2019	17.057 (15.60)	92.273 (84.40)	120 (3.02)	3.851 (96.98)	573.358 (7.05)	7.555.002 (92.95)	2.636 (3.27)	78.039 (96.73)
2020	17.213 (16.54)	86.864 (83.46)	163 (4.33)	3.602 (95.67)	573.375 (7.01)	7.605.710 (92.99)	2.667 (3.22)	80.195 (96.78)
2021	11.377 (11.81)	84.967 (88.19)	172 (4.57)	3.594 (95.43)	604.213 (6.92)	8.129.181 (93.08)	3.014 (3.37)	86.347 (96.63)
2022	19.098 (16.14)	99.199 (83.86)	278 (6.67)	3.887 (93.33)	609.427 (6.78)	8.375.249 (93.22)	3.079 (3.23)	92.307 (96.77)

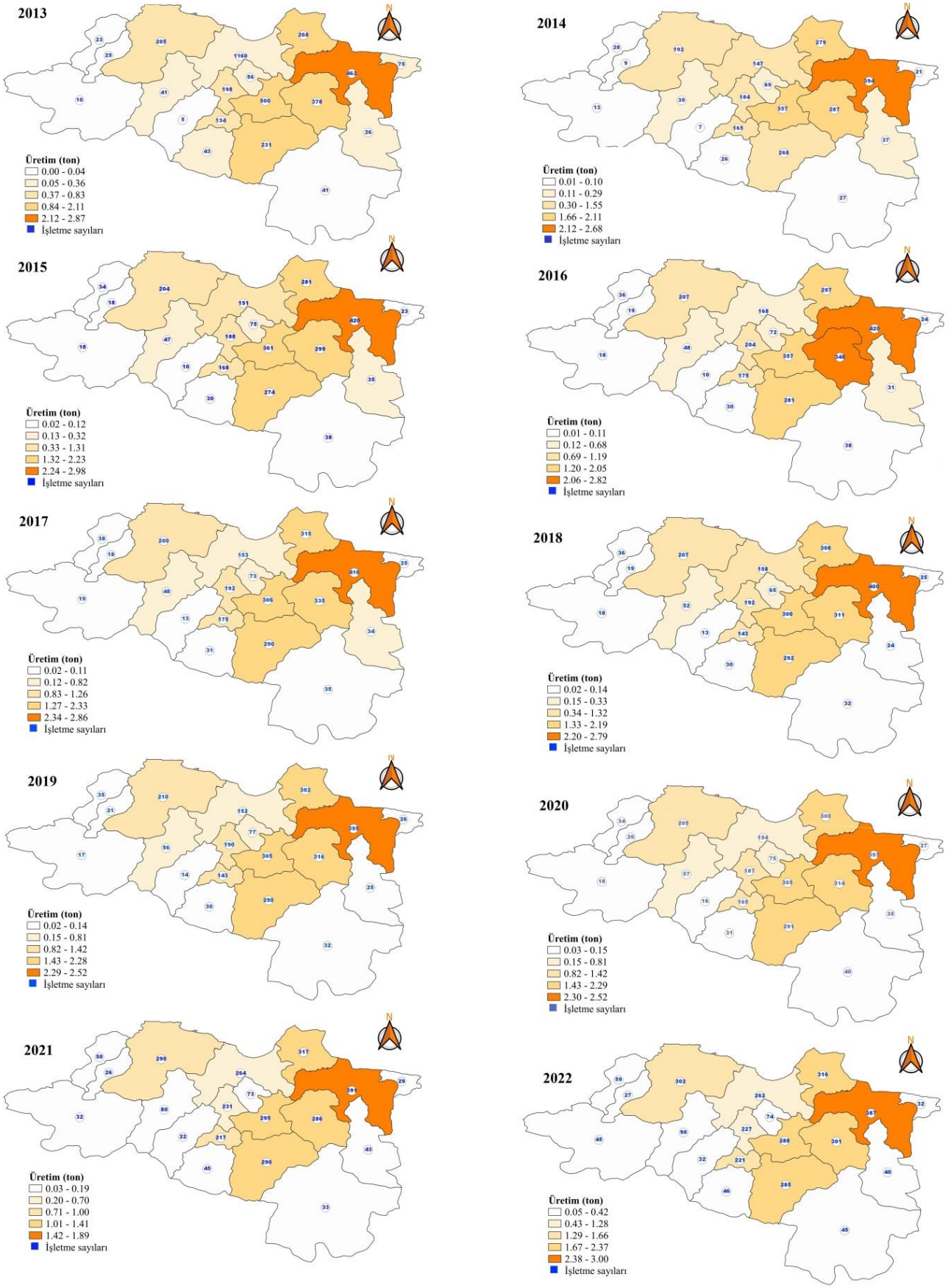
TARTIŞMA

Arılar tozlaşmada görev alarak bitkisel üretimde verimliliğin artırılmasında etkin rol oynamaktadır (Penberthy vd., 2023; Hung vd., 2018; Rollin vd., 2013). Bu çalışmada Türkiye'nin bal üretiminde ilk sırada yer alan Ordu ilindeki arıcılık faaliyetleri Mekânsal Haritalama metodu ile incelenmiştir.

Ordu ilindeki kayıtlı arıcılar için arıcılık faaliyetleri Doğu Karadeniz bölgesindeki Giresun, Trabzon, Rize, Artvin, Gümüşhane, Bayburt illerinden farklı olarak ana geçim kaynağını oluşturmaktadır. Ordu ilinde arıcılığın ana geçim kaynağı olmasının nedenleri arasında; işletmelerin kapasitesi, bölge yetiştiricilerinin sabit arıcılık yerine göçer arıcılığı tercih etmesi ve buna bağlı farklı bitkilerden daha yüksek bal üretilmesi gösterilmektedir (Kuvancı vd., 2017; Sıralı, 2017). Altınordu ilçesinde bal üretiminin daha fazla olması (Şekil.2), kayıtlı işletme sayısının daha yüksek olmasından ya da göçer arıcılık faaliyetini gerçekleştiren arıcıların daha fazla olmasından kaynaklanmış olabilir.

Ordu ilindeki arıcılar yaz aylarında genellikle doğu illerini tercih etmektedir (Koday ve Karadağ, 2020). Bal üreticileri Muş, Erzincan, Erzurum, Yozgat, Sivas, Ağrı, Kars, Hakkâri, Çankırı illerinde göçer arıcılık yaptıkları bir kısmının ise iki farklı ilde de göçer arıcılık yaptıkları bildirilmiştir (Kuvancı vd., 2017).

Arıcılık faaliyetleri, tarımda tozlaşmadaki görevi ve insan sağlığı için bal, balmumu, arı sütü, polen ve arı zehri gibi arıcılık ürünlerinin üretilmesini sağlamaktadır. Arıcılık faaliyetleri özellikle gelişmekte olan ülke ekonomilerinde kırsal kalkınmaya da katkıda bulunan bir üretim koludur (Damián ve Lankreijer, 2016; Lee vd., 2008; Sarı vd., 2020; Wright vd., 2018). Arıcılığın tedavi, apiterapi, turizm, gastronomi ve ekolojik sağlığı destek dâhil olmak üzere farklı faydaları da bulunmaktadır (Akpınar ve Bozkurt, 2021; Bozkurt, 2019). Ordu ilinin Türkiye'deki bal üretimindeki payının %11.81-16.54 arasında değişen bir paya sahip olduğu belirlenmiştir. 2021 yılında arıcılık faaliyetlerinin 2020 ve 2022 yılları ile kıyaslandığında daha az olması pandemiden kaynaklanmış olabilir (Tablo 1, Şekil 3).



Şekil 4. Ordu ilinde 2013-2022 yıllarında bal üretim (ton) miktarlarının yıl bazında haritalanması.

Arıcılar özellikle COVID-19 döneminde, sadece bağışıklık sistemini güçlendirici arı ürünlerini tedarik etmede değil, aynı zamanda doğal antiviral ajanlar olarak da işlev görebilen ürünleri sağlama noktasında oldukça etkili olmuşlardır. Bu bağlamda insan ve hayvan sağlığının sürdürülmesine katkı sağladıkları ifade edilmektedir (Attia vd., 2019; El-Sabrout vd., 2023). Bu nedenle, 2021 yılında küresel çapta yaşanan pandemi nedeniyle azalmış olabileceği varsayılan bal üretimi, pandemi döneminde tüketicilerin talep ettiği bal, polen ve propolis ürünlerinden dolayı (Özbakır vd., 2021) 2022 yılı itibariyle tekrar artış eğilimi göstermiş olabilir.

Bu çalışmada bal verimleri değerlendirildiğinde 2013 yılına kıyasla bal verimlerinin ciddi düzeyde arttığı belirlenmiştir. Sezgin ve Kara (2011)'in Ağrı, Kars, Ardahan ve Iğdır illerinde arıcılar ile yaptıkları bir çalışmada, bölgede yetiştirilen en yaygın arı ırkının Kafkas arısı ve bal verimlerinin 15 kg altında olduğu bildirilmiştir. Şekil 3'te görüldüğü üzere Ordu ilindeki yetiştiricilerin Sezgin ve Kara (2011)'in bildirdiği bal verimlerinden daha yüksek değerlere ulaştıkları tespit edilmiştir. Bu farklılık bölgenin coğrafik yapısından, bitki örtüsünden ya da arıcıların sertifika sahibi olup olmaması ya da arıcılığı ana geçim kaynağı olarak belirlemelerinden kaynaklanmış olabilir (Sezgin ve Kara, 2011). Bu çalışmada Ordu ilinin Çatalpınar ilçesinde son on yıl boyunca diğer ilçelere göre bal veriminin daha yüksek olduğu belirlenmiştir. Bal veriminin ilçelere göre değişiklik göstermesinin nedenleri arasında; arıcıların deneyiminin, bölge ikliminin diğer ilçelere göre daha ılıman olmasının, hastalık ve zararlıların, kraliçe arıların düşük verimliliğinin, iklim değişikliği ve arılar için sınırlı, azalan veya düşük kaliteli çiçek kaynakları gibi faktörlerin etkili olduğu düşünülmektedir (Guler, 2010; Schouten, 2021).

Ordu ilinde 2013-2022 yılları arasında bal üretim verilerinin mekânsal projeksiyonu değerlendirildiğinde, Altınordu ilçesinden sonra Perşembe, Ulubey, Gürgentepe ve Gölköy ilçelerinin bal üretiminde önemli bir yere sahip olduğu görülmektedir. Bunun sebeplerinden biri en yüksek üretime sahip Altınordu ilçesinin kendisine komşu olan Perşembe, Ulubey ve Gürgentepe ilçelerinde işletme sayılarının yüksek olmasının yanı sıra ilçeler arasında iş birliklerinin sağlanmış olmasından da kaynaklanabilir (Bayramoğlu vd., 2016). İlçelerdeki kayıtlı arıcılar arasında sağlıklı iletişimin ve bilgi aktarımının sağlanmasıyla Altınordu'da yüksek olan arıcılık faaliyetleri komşu ilçeleri de olumlu yönde etkilemiş olabilir. Ana geçim kaynağı olarak görülen arıcılıktan sağlanan kazanç ile kırsal kesimdeki ailelerin refah düzeyinin iyileşmesi komşu ilçelerde arıcılık ile uğraşan yetiştiricilere pozitif anlamda katkı sağlayabilir. Ancak bu durum çevre illerle sağlanan iş birlikleriyle etkili ve ulaşılabilir bir hale getirilebilir. Sonuç olarak, diğer ilçeler ile arıcılık faaliyetleri yüksek ilçeler arasında sağlanacak iş birlikleri ile komşu ilçelerde üretimin nasıl arttırılacağının planlanması konusunda gelecekteki çalışmalar açısından önemli kazanımlar elde edilebilecektir.

SONUÇ

Ordu ili ve ilçelerindeki kayıtlı arıcıların göçer arıcılığı ve bu mesleği ana geçim kaynağı olarak benimsemeleri, bal üretiminde bu bölgenin gelecekte de önemli bir potansiyele sahip olacağının bir göstergesidir.

Dünya'nın yakın gelecekte daha fazla maruz kalacağı öngörülen iklim değişikliği arıcılık faaliyetlerini de olumsuz etkileyebilir. Buna bağlı olarak yakın gelecekte Ordu ilindeki kayıtlı arıcılar gibi ülke genelinde diğer arıcıların da göçer arıcılık faaliyetlerini sabit arıcılığa göre daha fazla tercih etmesi beklenebilir. Bal bir ülkenin hem döviz kaynağı hem de insanlar için kıymetli bir gıdadır. Arıcılık faaliyetlerinin detaylı ele alındığı yeni mekânsal araştırmalara ihtiyaç vardır. Araştırıcıların bal verimlerini etkileyen faktörlere daha fazla odaklanılması ve

bununla ilgili yeni çalışmaların yapılması önerilebilir.

AÇIKLAMALAR

Finansal destek: Bu çalışmada herhangi bir mali kaynak kullanılmamıştır.

Etik beyan: Bu çalışma için etik belgesi gerekmemektedir.

Çıkar çatışması: Yazarların araştırma ile ilgili olarak bir çıkar çatışması yoktur.

Veri sağlama durumu: Çalışmada bulunan bilgi ve veriler akademik etik kurallarına uygun bir şekilde verilmiştir. Araştırma verileri Türkiye İstatistik Kurumu (TÜİK) veri tabanından alınmıştır. Türkiye İstatistik Kurumu'nun (TÜİK) resmi internet sitesinden derlenen veriler kamu kullanımına ait verilerdir.

Yazar katkıları: E.A. ve Ş.İ. çalışmayı tasarladı, verileri topladı ve düzenledi. T.B. mekânsal haritalandırmaları gerçekleştirdi. E.A makaleyi yazdı. Tüm yazarlar makalenin nihai halini okudu ve onayladı.

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The antidepressant efficacy of flurbiprofen in mice: Behavioral assessment

Research Article

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Article info
Submission: 31-10-2023
Accepted: 16-03-2024
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

ABSTRACT

Flurbiprofen is a nonsteroidal anti-inflammatory medication (NSAID). The psychological effect of nonsteroidal anti-inflammatory drugs (NSAIDs) is a source of contention based on clinical and experimental evidence. As a result, the goal of our study was evaluated the antidepressant effects of various flurbiprofen doses in male mice. We evaluated the effect of oral administration of flurbiprofen at 10 , 20 , and 40 mg/kg in the tail suspension and forced swimming tests after 1 h of treatment. Fluoxetine (10 mg/kg, i.p.) was used as a positive control. Flurbiprofen at 40 mg/kg showed a significant antidepressant effect, which was revealed by a significant decrease in immobility time compared with the control group, with the group administered flurbiprofen 10 mg/kg, and with the group given flurbiprofen 20 mg/kg in the tail suspension test. Flurbiprofen at 40 mg/kg showed an antidepressant effect, which was revealed by a significant decrease in immobility time compared with the control group and with the group given flurbiprofen 10 mg/kg. Flurbiprofen at 20 mg/kg had a minimal antidepressant effect in the swimming forced test, which was reflected by a non-significant decrease in immobility time compared with the control group. In conclusion, our results showed that relatively high therapeutic doses of flurbiprofen might have an antidepressant effect in mice model and we recommended for conducting other in vivo studies to clarify the variation in dose response.

Keywords: Antidepressant; flurbiprofen; forced swimming test; tail suspension test

INTRODUCTION

Depression is a serious and prevalent mental illness that can impair daily functioning and quality of life. According to the World Health Organization, depression will soon overtake heart disease as the second most common cause of the disease burden. Only 60–70% of depressed patients experience remission with antidepressant therapy, despite the fact that effective treatments like serotonin selective reuptake inhibitors (SSRIs) have improved the safety and tolerability of antidepressant medications (Rush et al., 2006). Recently, neuroimmune disorders that cause depression have received considerable attention. Numerous preclinical and clinical studies have examined the possible antidepressant effects of various anti-inflammatory medications. However, the outcomes of these trials vary widely (Bay-Richter and Wegener, 2022). Evidence suggests that the pathogenesis of depression involves inflammatory processes (the cytokine hypothesis of depression). First, proinflammatory cytokine administration causes depressive symptoms (Schiepers et al., 2005; Young et al., 2014). Second, proinflammatory cytokines can induce animal behaviors that are strikingly similar to those observed in depressed individuals (Dantzer et al., 1999; Miller and O'Callaghan, 2005). Third, proinflammatory

How to cite this article
Alberifki, NM., Naser, AS., (2024). The antidepressant efficacy of flurbiprofen in mice: Behavioral assessment. *Journal of Advances in VetBio Science and Techniques, 9*(1), 59-64. https://doi.org/10.31797/vetbio.1383152

cytokines increase the HPA axis' hyperactivity, which is frequently seen in depressive disorders(Yang et al., 2014; Zhou et al., 2022). Fourth, some cytokines impair serotonergic neurotransmission, which also happens to be a factor in depression (Dunn et al., 1999; Höglund et al., 2019; Wilson et al., 2002). Low dosages of flurbiprofen may reduce anxiety in male mice; however, the anti-anxiety activity does not manifest statistically significant with chronic or repeated treatment of flurbiprofen.(Albrefkani and Naser, 2023) . A recent study demonstrates that flurbiprofen exhibits significant dose-dependent anticonvulsant activity in mice (Naser and Alberifkani, 2023) . Another study found that flurbiprofen has a wide range of efficacy and is an effective painkiller for peripheral and abdominal pain , furthermore the study indicated the synergistic effect of flurbiprofen and alpha-lipoic acid as analgesic ,the synergistic effect of flurbiprofen and alpha-lipoic acid may have clinical benefits, including the reduction of flurbiprofen dose when used jointly (Alberifki and Naser, 2023). In the current study, we used tail suspension and forced swim tests to clarify how flurbiprofen affected depressive-like behavior in murine models.

MATERIALS AND METHODS

Experimental animals

All experiments were conducted on eight- to ten-week-old male Swiss albino mice (*Mus musculus*) in plastic cages measuring 32 × 18 × 24 cm, which were housed in groups of 4-5 in a cage with free access to food and water. Mice were purchased from the Laboratory Animal House of the University of Mosul College of Veterinary Medicine. They were kept in a climate-controlled room with a constant temperature of 23±2 °C and a 12-hour light/dark cycle.

Ethical approval

The University of Mosul College of Veterinary Medicine's animal care and use committees approved all animal use and procedures in accordance with the National Institutes of Health's standards (Date: 15/3/2022, Number: UM.VET.2022.056).

Drugs

Flurbiprofen (fortine 100mg film-coated tablet, Bilim Pharmaceuticals Industry/ Turkey) was orally administered after dilution in distilled water. Fluoxetine (APO fluoxetine 20mg capsule Bristol Laboratories Limited / UK) was diluted in distilled water and administered intraperitoneally (i.p.) at a volume of administration of 2 mL/kg body weight. An oral dose of flurbiprofen was administered 60 minutes before the test.

Study design

Twenty-five mice were allocated into five groups, as below:

1st group was given distilled water orally (negative control).

2nd group was administered fluoxetine at 10 mg/kg intraperitoneally (positive control).

3rd group was administered flurbiprofen at 10 mg/kg orally.

4th group was administered flurbiprofen at 20 mg/kg orally.

5th group was administered flurbiprofen at 40 mg/kg orally.

An hour after the treatment of the five groups, the following two experiments were performed:

Tail suspension test

The tail suspension test, which has been previously described by (Umemura et al., 2017; Ueno et al.,2022; Onouchi et al., 2014), was used to examine depression-like behavior. White acrylic walls (20 × 40 × 60 cm) with one open side that allowed for video recording of the animals made up the test apparatus. Using adhesive tape placed approximately 1 cm from the tip of the mouse's tail, each mouse was suspended by the tail 60 cm above the chamber floor. A video camera records the subsequent behavior for 5 minutes. The following parameters were later determined by analyzing the behavior: total time spent immobile. The total time that each mouse was immobile was measured in seconds, and the percentage of total time per

minute was calculated. The term "immobile time" was used in this test to describe the time when the animals were motionless for less than one second.

Forced swim test

The forced swim test was also used to look for signs of depression. The device was a cylinder (20 cm in height by 10 cm in diameter). Cylinder was positioned in the middle of the device, which was made up of a square area enclosed by white acrylic walls measuring 20 × 40 × 60 cm, one of which was open to record the behavior. Based on previous studies (Abbas et al., 2015; Matsuda et al. 2016), the cylinder was filled with water (23 °C) to a depth of 7.5 cm. Five minutes of video were recorded while the mice were inside the cylinder. The total duration of immobility was one of the parameters determined by analyzing mouse behavior. Each mouse's immobility time was measured in seconds and expressed as a percentage of the total time. The term "immobile period" was used in this test to describe the time when the animals were motionless for less than one second.

$$\% \text{ Immobility Time} = \frac{\text{Total time (300sec)} - \text{Immobility time}}{\text{Total time (300sec)}} \times 100$$

Statistical analysis

Data were analyzed statistically and expressed as mean ±standard error of the mean (SEM). All data were subjected to one-way ANOVA, followed by a post-hoc LSD test for multiple comparisons. Probability *p≤ 0.05 was statistically significant, and this was done using the Statistical Package for Social Sciences program version 16.

RESULTS

Flurbiprofen at 40 mg/kg showed a significant antidepressant effect, which was revealed by a significant decrease in immobility time (48.60±11.05) compared with the control group (155.20±22.00), with the group administered flurbiprofen 10 mg/kg (193.40±13.73), and with the group given flurbiprofen 20 mg/kg (189.40±12.58) in the tail suspension test (Table 1).

Table 1. The antidepressant effect of acute administration of flurbiprofen by tail suspension test

Groups	Immobility time (sec) in tail suspension test	Percentage of immobility time
Control	155.20±22.00	52%
Fluoxetine10mg/kg	65.80±12.11*	22%
Flu10mg/kg	193.40±13.73a	64%
Flu20mg/kg	189.40±12.58a	63%
Flu40mg/kg	48.60±11.05*bc	16%

Values are referred to mean ± SE of five male mice/group. * Referred to significantly dissimilar from the control values, p ≤ 0.05. a Referred to significantly dissimilar from the values of the fluoxetine 10 mg/kg group, p ≤ 0.05. b Referred to significantly dissimilar from the values of the Flu 10mg group ,p ≤ 0.05. c Referred to significantly dissimilar from the values of the Flu 20 mg group ,p ≤ 0.05.

Table 2. The antidepressant effect of acute administration of flurbiprofen by forced swim test

Groups	Immobility time (sec) in forced swim test	Percentage of immobility time
Control	144.20±12.85	48%
Fluoxetine10mg/kg	73.20±4.14*	25%
Flu10mg/kg	94.20±3.76*	31%
Flu20mg/kg	118.40±17.56 a	39%
Flu40mg/kg	67.80±8.23*b	23%

Values are referred to mean ± SE of five male mice/group. * Referred to significantly dissimilar from the control values, p ≤ 0.05. a Referred to significantly dissimilar from the values of the fluoxetine 10 mg/kg group, p ≤ 0.05. b Referred to significantly dissimilar from the values of the Flu 10mg group ,p ≤ 0.05.

Flurbiprofen at 40 mg/kg showed an antidepressant effect, which was revealed by a significant decrease in immobility time (67.80±8.23) compared with the control group

(144.20±12.85) and with the group given flurbiprofen 10 mg/kg (94.20±3.76). Flurbiprofen at 20 mg/kg had a minimal antidepressant effect in the swimming forced test, which was reflected by a non-significant decrease in immobility time (118.40±17.56) compared with the control group (144.20±12.85) (Table 2).

DISCUSSION

In this study, we assessed the antidepressant effects of single doses of flurbiprofen (a non-selective reversible COX inhibitor) in a mouse model. Our results revealed a different perception about the doses of flurbiprofen, with the low dose failing to demonstrate an antidepressant effect, while the high dose produced a notable antidepressant effect in the two depression models. When comparing the results of our study with other studies on the same topic, our results were consistent with a study conducted on mice injected with colon-26 adenocarcinoma cells and administered ibuprofen at 10 mg/kg through drinking water. Ibuprofen showed an antidepressant effect in the forced swimming test (Norden et al,. 2015). Other researchers have reported that ibuprofen enhanced the performance of mice in the passive avoidance trial while also reducing anxiety and antidepressant behaviors. However, ibuprofen did not improve spatial memory in the Morris water maze experiment or recognition ability in the novel object recognition test (Salmani et al., 2021). Another study was conducted to determine the effect of repeated administration for seven days of ketoprofen in mice, where ketoprofen showed an antidepressant effect represented by increasing the time of immobility in the forced swimming test, and this study suggested that the reason for this activity is the effect on the serotonin pathway, either receptors or metabolism (Răducanu et al., 2012). Other researchers mention that the co-administration of flurbiprofen and fluoxetine in mice did not has a synergistic effect against depression (Alboni et al., 2018). Anti-inflammatory drugs exert their therapeutic effects in part by regulating cytokine formation. The observation that depressed people have higher plasma levels of certain cytokines than healthy controls lends support to the "cytokine hypothesis" of depression (Warner-Schmidt et al., 2011). In rodent models, p11, a member of the S100 protein family, is a critical molecule of depressive-like states and antidepressant reactions, p11, also known as S100A10, is a tiny acidic protein that reacts with unique serotonin receptors to start regulating trafficking and control cell-surface localization. (Svenningsson et al., 2006; Warner-Schmidt et al., 2010, 2009). This activity alters the firing rate of the cells, resulting in significant behavioral outcomes. In classic behavioral paradigms such as the tail suspension and forced swim tests, p11 knockout (KO) mice exhibit a depressive-like phenotype, whereas p11 overexpression mice exhibit antidepressant-like responses (Warner-Schmidt et al., 2010). According to a study conducted in mice, tumor necrosis factor-alpha (TNFα) and interferon act on p11 to mediate their antidepressant activity. IFN interacts directly with IFN binding sites on the p11 promoter to increase p11 levels (Warner-Schmidt et al., 2011). Our study has some limitations such as the number of animals that have been used, the regime of dosing of animals, and finally, the use of only two tests for assaying antidepressant effects.

CONCLUSION

In conclusion, we conclude from our study that a single dose of flurbiprofen 40 mg/kg decreased immobility time in both the tail suspension test and the forced swim test, so we demonstrate that this dose can generate an antidepressant effect in mice model.

ACKNOWLEDGMENT

We appreciate the support from the College of Veterinary Medicine at the University of Mosul in helping us prepare the workplace and the materials needed to complete this work.

Financial support: The authors did not take any support payments from any institution in conducting the study.

Conflict of interest: The authors declare that we have no competing interest.

Ethical statement or informed consent: The University of Mosul College of Veterinary Medicine's animal care and use committees approved all animal use and procedures in accordance with the National Institutes of Health's standards (Date: 15/3/2022, Number: UM.VET.2022.056).

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The effects of letrozole on liver function and some biochemical parameters in rats

ABSTRACT

Letrozole (LTZ), is an aromatase inhibitor, that has been widely used in a variety of diseases such as polycystic ovary syndrome, endometriosis, and breast cancer. LTZ is received via the oral route and metabolized in the liver. Therefore, LTZ may have toxic effects like other drugs metabolized in the liver. Based on this, our study aimed to investigate the effect of LTZ on liver function and biochemical parameters. For this purpose, 16 Wistar albino female rats were divided into two groups (n=8): Control and LTZ respectively. The rats in the letrozole group were administered with 2 mL/kg LTZ by oral gavage once a day for 21 days. The Control group received the vehicle once a day for 21 days. Blood samples were collected on the 22nd day of the experiment. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), albumin (ALB), alkaline phosphatase (ALP), direct bilirubin and total bilirubin were measured. Biochemical analysis indicated that ALT, AST, LDH, ALP, and total bilirubin levels were significantly higher in the LTZ administrated group compared to the Control ($p>0.05$). ALB levels decreased in the LTZ group ($p>0.05$). In conclusion, it was determined that LTZ has toxic and detrimental effects on the liver. We suggested that long-term LTZ-administered patients should be under control against liver damage and may have liver-supporting adjuvant therapies for robust liver functions.

Keywords: Albumin, hepatotoxicity, letrozole, liver function tests

INTRODUCTION

Aromatase belongs to the cytochrome P450 system and is expressed by various tissues such as ovaries, adipose tissue, muscle, liver, and breast (Sun et al., 2007). Androgen precursors such as testosterone are involved in the converting reactions of enone rings to phenols for estrogen synthesis. The aromatase enzyme catalyzes above mentioned indispensable steps. Therefore, in pathologies including breast cancer, aromatase inhibitors (AI) are preferred for the inhibition of estrogen production and estrogen receptors. Recently, various steroidal and nonsteroidal AI treatments have been reported in most of the studies. Those in the first, second, and third-generation drugs among the several AI drugs that limit both genomic and non-genomic effects of estrogen, have been approved by the US Food and Drug Administration (FDA). Drugs in the third group are generally used as standard treatment for postmenopausal breast cancer (Kharb et al., 2020; Ratre et al., 2020). Additionally, they are effective as adjuvant therapy and generally well tolerated. Recently, these drugs have been commonly preferred in pathologies such as polycystic ovary syndrome and endometriosis in premenopausal non-fertile women (Barnhart et al., 2003; Bulun and Simpson, 1994; Makav et al., 2023; Mukherjee et al., 2022; Sun et al., 2007).

How to cite this article
Dolanbay, Y., Makav, M., Vural, A., Cumaoğlu, MO., Yuceer, Ö., Başer, L., Tetik Metin, H., (2024). The effects of letrozole on liver function and some biochemical parameters in rats. *Journal of Advances in VetBio Science and Techniques*, *9*(1), 65-72. https://doi.org/10.31797/vetbio.1451147

Research Article

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Article info
Submission: 11-03-2024
Accepted: 30-04-2024
Online First: 30-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

Letrozole, as the marketing name Fermara, is a third-generation non-steroidal aromatase inhibitor. The chemical formula of Fermara is (4,4′-[(1H-1,2,4-triazol-1-yl) methylene] bis-benzonitrile) (Mukherjee et al., 2022). LTZ, a triazole derivative, inhibits the conversion of testosterone to estradiol and androstenedione to estrone. Thus, LTZ has been reported to inhibit aromatase activity by 99% and endogenous estrogen synthesis by 97-99% (Wit et al., 2012). LTZ is one of the most potent AIs and is widely used. LTZ has been found to have various positive effects on breast cancer, anovulatory infertility, and spermatogenesis. It is approved for the treatment of hormone receptor-positive, metastatic breast cancer in postmenopausal women. It has a high potential for use in both prevention of the androgenic steroids converting to estrogen and to prevent or reduce the side effects of androgenic steroids (Aydin et al., 2011; Mukherjee et al., 2022; Sun et al., 2007).

In addition to the positive effects of LTZ, many studies have claimed that it may have side effects (Čustović et al., 2019; Perez et al., 2006; Sharma et al., 2014;). The prolonged administration of LTZ has different side effects. The short-term use of LTZ has lower serious side effects. The most common side effects are headache (7%), nausea (6%), fatigue (5%), hot flushes (5%), peripheral edema (6%), rash (2.7%), drowsiness (3.2%) and vomiting (3.8%). Long-term use has been shown to cause more serious side effects. Generally, in breast cancer patients, bone pain, hot flushes, back pain, nausea, and dyspnea (Barnhart et al., 2003; Bulun and Simpson, 1994; Makav et al., 2023; Mukherjee et al., 2022; Sun et al., 2007). Many studies have indicated that LTZ administration leads to toxicity in several tissues and organs. The liver is one of the toxicity targets of LTZ. LTZ use has been shown to increase liver enzymes and cause hepatoxicity. In addition, it has been determined that LTZ may have detrimental effects on the endothelial layer of the central vein of the liver. LTZ has been reported to imbalance the serum lipid profile by an unknown mechanism (Aydin et al., 2011; Gharia et al., 2017; Mukherjee et al., 2022; Moy et al., 2014). The most crucial liver enzymes are considered to be aspartate aminotransferase (AST) and alkaline phosphatase (ALT) (Deveci et al., 2021; Karapehlivan et al., 2023; Kuru et al., 2022)

Based on the mentioned information, our study aimed to investigate the effects of LTZ on liver function and biochemical parameters in rats.

MATERIALS AND METHODS

Animals and ethical procedures

The ethical approval (KAÜ-HADYEK/2024-023) was obtained from Kafkas University Experimental Research Application and Research Center. Considering the principle of reduction from the 4R rule, serum samples of the Control and LTZ-treated groups were obtained from the previous study approved by Kafkas University Experimental Research Application and Research Center with the number 2021/156 for the present study. All procedures were in line with the TR Law 6343/2; 6.7.26 Veterinary Deontology and Helsinki World Medicine Organisation Declaration.

For his study, Wistar Albino female rats were purchased from Kafkas University Experimental Research Application and Research Center. All stages of the study were performed at the same center and under the same conditions. Rats were housed at 22±2 ˚C temperature and 12 h/12 h light/dark cycle. During the experiment, rats were fed with standard food pellets and water ad libitum. Before the experiment procedure rats were fasted for 12 h, allowed for only water.

Groups

In this study, 16 Wistar Albino female rats (200-250 grams and 4 months old) were used. The rats were divided into two groups randomly as given below.

- Control (n=8): Group given vehicle at a dose of 2 ml/kg for 21 days

- Letrozole (n=8): Group given LTZ at a dose of 2 ml/kg for 21 days

LTZ Administration

LTZ (Femara®, Novartis, Istanbul, Turkey) was dissolved in a 1% carboxymethylcellulose (CMC) solution as previously described. The solution was administered to the rats in the Letrozole group by oral gavage at a dose of 2 mL/kg once a day for 21 days (Kafali et al., 2004). The Control group received 1% CMC solution as a vehicle.

Tissue harvesting

The euthanasia procedure was performed on the 22nd day of the experiment, under general anesthesia (ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg) intramuscular) by cervical dislocation. Serum samples were obtained and stored at -80ºC for biochemical analysis.

Biochemical analyses

ALT, AST, GGT, LDH, ALB, ALP, direct bilirubin, and total bilirubin were analyzed spectrophotometrically with a Beckman-Coulter AU5800 autoanalyzer (Beckman Coulter®, U.S.).

Statistical analysis

GraphPad 8.1 (San Diego, CA, USA) was used for statistical analyses. The difference between the two groups of biochemical parameters was analyzed by independent samples t-test. The significance was accepted as $p<0.05$.

RESULTS

In this study, ALT, AST, GGT, LDH, ALB, ALP, direct bilirubin, and total bilirubin levels were measured. There was a statistically significant increase in serum ALT levels of the Letrozole group compared to the Control ($p<0.05$). Similarly, AST values showed a statistically significant increase in the Letrozole group compared to the Control ($p<0.05$). There was a statistically significant difference in LDH levels between the Control and Letrozole groups ($p<0.05$). On the other hand, there was no significant difference in GGT levels between the Control and Letrozole groups ($p<0.05$; Figure 1).

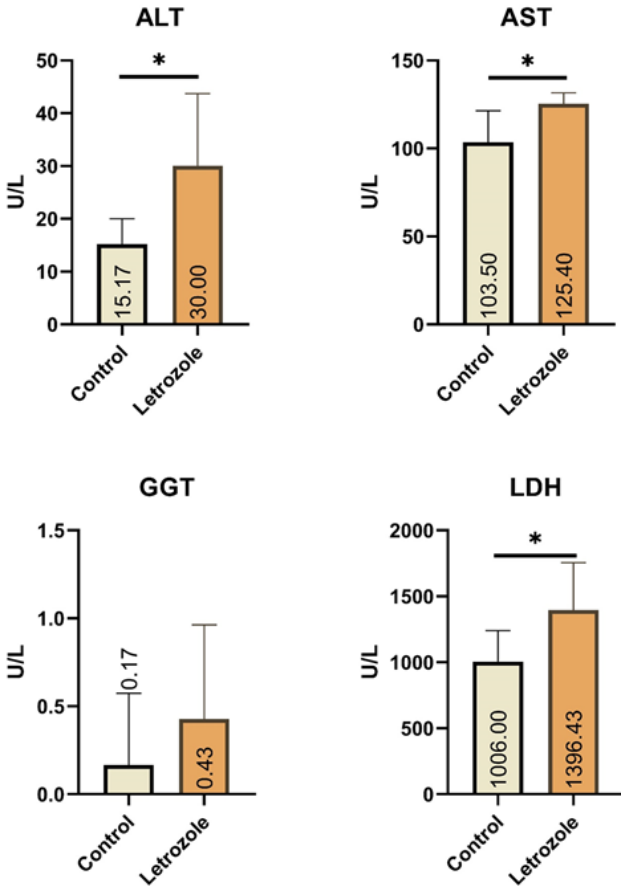

Figure 1. Comparison of serum ALT, AST, GGT, and LDH levels of control and Letrozole groups (*: $p<0.05$). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase.

A statistically significant decrease was observed in serum ALB levels in the Letrozole group compared to the Control ($p<0.01$). When serum ALP levels were analyzed, there was a statistical increase in the Letrozole group compared to the Control ($p<0.05$). Similarly, the total bilirubin level was increased in the Letrozole group compared to the Control ($p<0.01$). However, there was no statistically

significant difference between the Control and Letrozole groups in direct bilirubin levels ($p<0.05$; Figure 2).

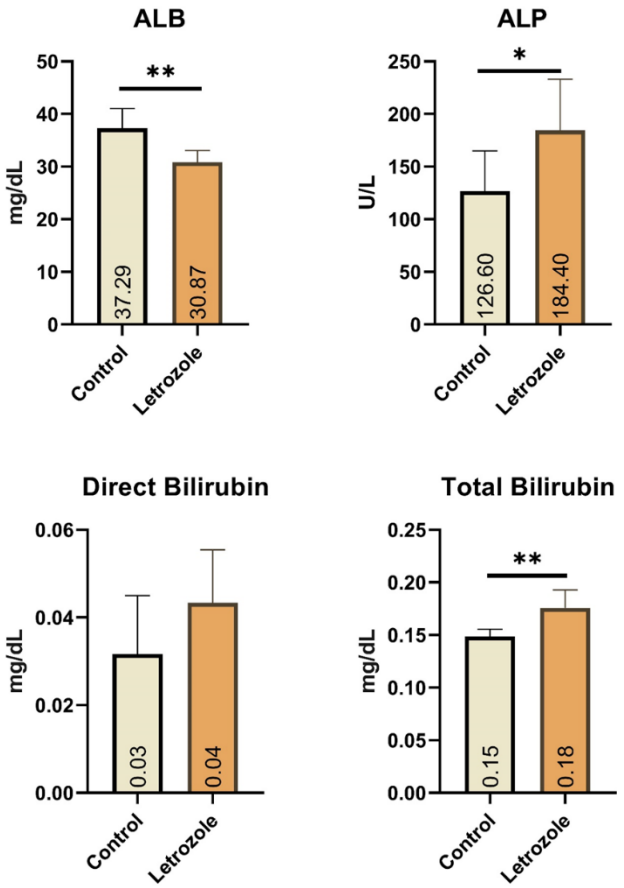

Figure 2. Graphical representation of ALB, ALP, direct, and total bilirubin values of control and Letrozol groups (*: $p<0.05$; **: $p<0.01$). ALB: Albumin, ALP: Alkaline phosphatase.

DISCUSSION

LTZ is a third-generation aromatase inhibitor, generally preferred for its reversibility, selectivity, and efficacy. It is widely used in many conditions including polycystic ovary syndrome, intrauterine insemination, endometriosis, and breast cancer (Jin et al., 2012; Requena et al., 2008; Taniguchi et al., 2011). LTZ is used via the oral route and metabolized in the liver after absorption from the gastrointestinal tract (Mukherjee et al., 2022; Taniguchi et al., 2011). Depending on the metabolism of most drugs in the liver may cause liver toxicity (Akşit et al., 2015; Kutlubay et al., 2009). LTZ is one of the above-mentioned drugs and has the potential to lead to liver toxicity (Aydin et al., 2011). Therefore, in our study, the effects of experimental LTZ-induced liver toxicity in rats were investigated through serum ALT, AST, GGT, LDH, ALB, ALP, direct bilirubin, and total bilirubin levels measurement. Interestingly, there was a significant increase in ALT, AST, LDH, ALB, ALP, and total bilirubin levels in the Letrozole administrated group.

Aminotransferases are enzymes involved in the interconverting of amino and keto acids in carbohydrates and nitrogen. Aminotransferases may be beneficial for the diagnosis of liver diseases such as hepatitis and are a sensitive indicator of liver cell damage. The increase in serum aminotransferase level may be due to the passage of the enzyme in the cell into the serum as a result of hepatocellular necrosis, or it may be due to increased membrane permeability in a level of cell damage that does not end with necrosis. Aminotransferase elevation can generally be assessed at three different levels: severely elevated (usually more than 15 times the normal value), moderately elevated (5-15 times the normal value), and mild elevated (less than 5 times the normal value). ALT and AST are enzymes from the aminotransferase group. ALT which is secreted from hepatocytes is a cytosolic enzyme and relatively more specific to the liver. AST is both cytosolic and mitochondrial sourced. It is also found in striated muscles, the brain, the pancreas, and blood cells as well as the liver (Ersoy, 2012; Green and Flamm, 2002). In our study, we evaluated the effects of LTZ on serum ALT and AST levels. Both enzyme levels were increased. This is a clear indication that LTZ causes liver damage. However, we found that ALT levels increased approximately 2-fold at the end of the 21 days of the experiment. The increase in ALT levels, which is more specific to the liver, caused by LTZ suggested that long-term use of LTZ may cause more serious damage.

ALP is an enzyme synthesized in bone and liver. ALP, which plays an important role in the hydrolysis of phosphate groups of nucleic acids, proteins, and other substrates, is found in the

canalicular membranes of hepatocytes and on the luminal surface of biliary epithelial cells. Serum ALP activity is primarily used as an indicator of hepatic diseases (Fernandez and Kidney, 2007; Wang et al., 2021). Dramatic ALP increases are commonly seen following obstructive biliary disorders, tumor infiltration, and metastasis to the liver (Limdi and Hyde, 2003). GGT is a key enzyme in response to the transpeptidation of functional gamma-glutamyl groups. All mammalian tissues contain GGT but the liver has the greater levels of GGT. GGT is a key marker for most liver pathologies but does not directly specify liver damage. However, increased levels of GGT together with other liver enzymes indicate that the source of damage is the liver (Brennan et al., 2022; Limdi and Hyde, 2003). The fact that serum ALT, AST, ALP, and GGT levels were increased together concluded that LTZ causes liver damage. In addition, GGT and ALP enzymes synthesized in bile duct epithelial cells suggest that LTZ causes severe liver and biliary tract damage.

LDH, an oxidoreductase enzyme, can be synthesized in different tissues. In particular, LDH is the main activator of the pyruvate-to-lactate converting enzymatic reaction. LDH is typically released from necrotic cells (Chaudhary and Chauhan, 2015; Faloppi et al., 2016). In our study, it was determined that the amount of LDH increased with liver-specific enzymes in the LTZ toxicity group. This result indicated that LTZ may create necrotic regions during liver toxicity.

ALB is the most abundant protein in plasma with a concentration of 30-50 g/L, corresponding to 50% of all plasma proteins. ALB is synthesized predominantly in the liver, with a synthesis rate of 150 mg/kg/day at about 10-15 grams per day and in response to the synthesis of 10% of hepatic proteins. ALB enters the bloodstream and helps transport vitamins, enzymes, and other important substances. The final concentration of plasma ALB is balanced through albumin synthesis, intravascular and interstitial influx, catabolism, and loss via renal or intestinal routes (Carvalho and Machado, 2018; Yılmaz et al., 2020). During liver damage, albumin production decreases. In the clinic, ALB is the most frequently used marker to measure the functionality of the liver (Eren et al., 2007; Yılmaz et al., 2020). In our study, serum ALB levels were significantly lower in the LTZ-induced toxicity group. This suggests that ALB levels were decreased secondary to LTZ-induced hepatotoxicity.

Bilirubin is an orange-yellow bile pigment sourced from the catabolism of various heme-containing proteins, particularly hemoglobin. Heme turns into biliverdin, which is converted to unconjugated or indirect bilirubin (UCB). UCB is an insoluble structure and is bound to albumin and circulate. In the liver, glucuronic acid is added to UCB to enhance water-solubility and direct bilirubin. As a result of this conjugation, it is excreted in bile or urine (Guerra Ruiz et al., 2021). Liver lesions cause a decrease in the number of hepatocytes and conclude the uridine diphosphate glucuronic transferase (UDPGT) enzyme, which is involved in conjugation, is not to be produced in sufficient amounts. Therefore, the amount of UCB increases (Işık et al., 2020; Kıncı et al., 2021). In our study, direct and total bilirubin levels were evaluated, and it was revealed that total bilirubin levels increased significantly in the LTZ group. According to our findings, serum total bilirubin levels were increased, and direct bilirubin was not increased indicating that it caused an increase in the amount of unconjugated bilirubin. In conclusion, we suggest that LTZ leads a widespread damage in the liver tissue through deficiency in the UDPGT enzyme.

Taken together, all of our findings indicate that even short-term LTZ administration causes an increase in both liver-specific and other tissue

damage markers. Our findings suggested that damage to the bile ducts, hepatocytes, and necrotic areas in the liver may be present. Aydın et al. (2013) reported that LTZ affected AST, LDH, ALP, and bilirubin values in rats. In addition, hematoxylin and eosin staining of liver tissue revealed congestion, thrombosis, and detached endothelial layer of the central vein (Aydin et al., 2011). These outcomes are in line with the results of our study and indicate that LTZ may result in severe liver damage in long-term use. Gharia et al. (2017) reported that an LTZ-administrated patient was admitted to a hospital with severe liver failure. Li et al. (2023) evaluated the efficacy of AI use, including LTZ, on liver function in cancer patients. AI users have been shown to have worsening liver function after 6 months compared to baseline (Yuechong et al., 2023). In addition, researchers who have studied the pharmacokinetics of LTZ believe that liver failure may significantly increase the half-life of LTZ. Therefore, it is also stated that LTZ-administered patients should be warned about liver damage (Bhatnagar, 2007).

CONCLUSION

In conclusion, LTZ affects the liver at therapeutic doses and may cause severe liver damage at toxic doses. Therefore, we believe that in patients with indications for long-term use of LTZ, an AI, physicians should check the liver function of patients before drug use and these tests should be repeated at regular intervals and adjuvant treatments should be initiated to support the liver when necessary.

ACKNOWLEDGMENT

Financial support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest: Authors declare no conflict of interest.

Ethical statement or informed consent: The ethical approval was obtained from Kafkas University Experimental Research Application and Research Center (KAÜ-HADYEK/2024-023).

Author Contributions: Study design, experimental process, sample collection, and performed the analysis: TA, MM, AV, MOC, ÖY, and LB; Analysis of Data and writing: HTM; Revisions and Proof Reading: All authors.

Availability of data and materials: All data and materials of the study are available in contact with the corresponsible author.

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A case of Brucellosis with neurological and dermatological findings in a thoroughbred Arabian horse

Case Report

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Article info
Submission: 06-01-2024
Accepted: 22-03-2024
Online First: 25-04-2024
Publication: 30-04-2024

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
http://dergipark.org.tr/vetbio

ABSTRACT

Brucellosis, a zoonotic infection, exhibits varying prevalence across different geographical regions, with a notable incidence in Mediterranean countries, contributing to considerable economic losses. This study focuses on an 11-year-old purebred male Arabian horse, presenting symptoms including weight loss, weakness, environmental indifference, joint swelling, and diverse dermatological manifestations despite sufficient feed intake. A pivotal clinical observation from anamnesis and examination is the animal's inclination to lean its head against walls or fixed objects. In conjunction with hematological and biochemical analyses, the Brucella Rose Bengal plate test (RBPT) and serum agglutination test (SAT) were conducted, revealing seropositivity for brucellosis in the patient. Consequently, it is imperative for professionals in the equine breeding sector, particularly clinical veterinarians, to adhere to biosafety protocols concerning brucellosis cases exhibiting atypical clinical symptoms in horses. Proper diagnostic methods should be employed to assess suspicious cases, underscoring the significance of safeguarding both public and animal health.

Keywords: *Brucella*, horse, dermatologic, neurologic

INTRODUCTION

Brucellosis constitutes a significant zoonotic infection affecting both humans and diverse animal species, prevalent in various regions worldwide, particularly in Mediterranean countries, despite successful eradication efforts in certain nations (Corbel, 1997; Pappas et al., 2006). Brucella species, characterized by a Gram-negative, non-spore-forming, non-encapsulated, immotile, aerobic, and facultative anaerobic small rod morphology, play a crucial role in the etiology of the disease (Morgan et al., 1990; Quinn et al., 2011; Lotfi et al., 2022).

The clinical manifestations of brucellosis in animals encompass symptoms such as mastitis, diminished milk yield, calving difficulties, infertility, and arthritis, leading to substantial economic losses (Aydın, 2006). Additionally, brucellosis poses a notable threat to public health, being transmissible to humans both directly and through the consumption of contaminated foods. Consequently, there exists a correlation between the prevalence of brucellosis in humans and animals that share the same geographical region and maintain contact with each other (Aşkar et al., 2013). The horse, since its domestication, has played a pivotal role in contributing significantly to military, social, and economic aspects of human society. In view of the close relationship between horses and humans in daily life, cases of brucellosis in these animals are deemed a noteworthy risk that merits consideration for human health (Ribeiro et al., 2008; Silva et al., 2022).

How to cite this article
Uzlu, E., Kaya, F., Ünal, BK., Özkurşun, F., İlhan, Z., (2024). A case of Brucellosis with neurological and dermatological findings in a thoroughbred Arabian horse. *Journal of Advances in VetBio Science and Techniques, 9*(1), 73-78. https://doi.org/10.31797/vetbio.1415732

Brucella abortus, *B. suis*, and *B. canis* predominantly account for brucellosis cases in horses. The infection can be transmitted through various routes, both direct and indirect, involving contaminated materials, encompassing digestion, inhalation, and mating (Alexander et al., 1981; Godfroid, 2002). Brucellosis in horses typically exhibits a latent progression, with animals that appear clinically normal demonstrating a positive antibody titer. Nonetheless, certain horses may manifest nonspecific symptoms such as weakness, depression, muscle stiffness, intermittent fever, and movement disorders. Notably, occurrences of bursitis, carpal bursitis, tenosynovitis, osteomyelitis, osteoarthritis, and, infrequently, reproductive disorders may also be observed (Cohn et al., 1992; Ocholi et al., 2004; Cvetnic et al., 2005; Amini et al., 2024). In humans, neurological and dermatological symptoms in brucellosis infection have been reported in limited cases. However, cases presenting with neurological and dermatological lesions in horses are exceedingly rare. We aim to present this case report to veterinary clinicians for consideration.

CASE HISTORY AND FINDING

The subject of this case study was an 11-year-old purebred male Arabian horse, obtained from a local equestrian and traditional horse archery facility in the Soma district of Manisa, and brought to the Internal Medicine Clinic at Balıkesir University, Faculty of Veterinary Medicine. The horse's medical history included reported symptoms of weakness, malaise, and a tendency to lean its head against the wall (Figure 1) despite maintaining normal appetite.

Upon clinical examination, several notable findings were observed, including hair loss with itching in the tail region, wounds in the left fossa paralumbalis region (Figure 2) and left hind foot tarsal joint region, a mixed hair structure, and polyarthritis in both the forelimbs and hindlimbs, which exhibited partial spontaneous drainage. Furthermore, superficial lymph nodes were within normal limits, the conjunctival mucosa showed slight dirtiness, hyperemia, and a slightly yellowish tint. Physiological parameters were measured, revealing a pulsation rate of 40/min, respiration rate of 8/min, and a body temperature of 37.4°C.

Figure 1. For a long time, tendency to lean its head against the wall.

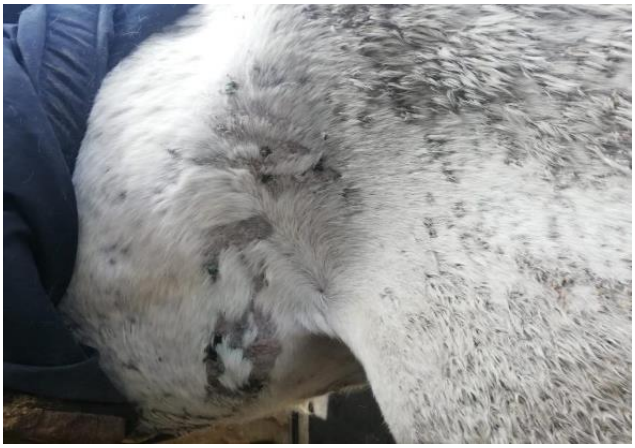

Figure 2. Extensive dermatologic lesions.

Native parasitologic examination identified the presence of *Strongylus* spp. eggs. For comprehensive hematologic, biochemical, and serologic analyses, blood samples were obtained from the vena jugularis, utilizing 8 mL K3 EDTA and 8 mL dry biochemistry tubes. The results of the complete blood count and biochemical analyses are detailed in Table 1 and Table 2, respectively.

Table 1. Hemogram analysis results.

Parameters	Result (Reference ranges)
RBC (10^6/L)	7.97 (6.8-12.9)
HGB (g/dL)	12.3 (11-19)
HCT (%)	36.44 (32-53)
MCV (fl)	46 (36-50)
MCH (pg)	15.5 (12.3-19.7)
MCHC (g/dL)	33.9 (31-39)
WBC (10^3/L)	15.25 (5.4-14.3)
LYM (10^3/L)	2.07 (1.5-6.0)
MON (10^3/L)	0.58 (0-1.5)
NEU (10^3/L)	12.58 (2.3-9.5)
EOS (10^3/L)	0.03 (0.1-1)
BAS (10^3/L)	0.00 (0-0.5)
LY (%)	13.5 (17-68)
MO (%)	3.8 (0-14)
NE (%)	82.5 (22-80)
EO (%)	0.2 (1-8)
BA (%)	0.0 (0-3)
PLT (10^9/L)	162 (90-350)
PCT (%)	0.13
MPV (fl)	8.2

Abbrevetion: RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell, LYM: Lymphocyte, MON: Monocyte, NEU: Neutrophil, EOS: Eosinophile, BAS: Basophile, PLT: Platelet, PCT: Plateletcrit, MPV: Mean platelet volume (Turgut, 2000).

Table 2. Biochemical analysis results.

Parameters	Result (Reference ranges)
GLU (mg/dL)	35.7 (62-134)
ALP (U/L)	88 (143-395)
TP (g/dL)	6.1 (5.6-7.6)
GGT (U/L)	19 (6.0-32)
AST (U/L)	141 (160-412)
ALT (U/L)	2.2 (34-113)
BUN (mg/dL)	10.18 (10-24)
CREA (mg/dL)	0.10 (0.4-2.2)
BUN/ CREA	101.81
ALB (g/dL)	1.8 (2.6-4.1)
GLOB (mg/dL)	4.30 (2.6-4.0)
ALB/GLOB	0.42

Abbrevetion: GLU: Glucose, ALP: Alkaline phosphatase, TP: Total protein, GGT: Gamma-glutamyl transferase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CREA: Creatinine, ALB: Albumin, GLOB: Globulin (Turgut, 2000).

The serum sample was analyzed by Rose Bengal plate test (RBPT) and serum agglutination tests (SAT). Test antigens were obtained from Istanbul Pendik Veterinary Control Institute. Both tests were performed according to the conventional method. The results were compared with positive and negative control sera and evaluated by naked eye. RBPT was performed on a clean slide using 30 µL of serum and antigen. A positive result was observed in approximately 2 min (Figure 3). SAT was performed in 8 tubes and the titer was determined as 1/160 (Morgan et al., 1990; İlhan et al., 1999).

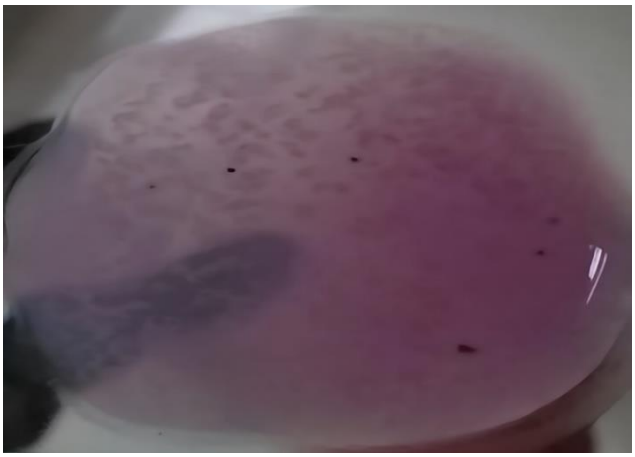

Figure 3. Rose Bengal plate test (RBPT) +++ in horse serum.

DISCUSSION

Brucellosis, recognized as a zoonotic infection, exhibits global prevalence, particularly in Mediterranean countries, and is associated with substantial economic repercussions (Acosta-González et al., 2006; Acosta-González et al., 2009). Research on animal brucellosis has predominantly focused on cattle, sheep, and goats, with investigations into etiological, pathogenic, epidemiological, diagnostic, and preventive aspects (Khan and Zahoor, 2018; Rossetti et al., 2022). Contrastingly, studies on horses primarily emphasize epidemiological data, a trend attributed to the comparatively lesser impact of brucellosis on equine reproductive health, resulting in a reduced

economic burden (Ribeiro et al., 2008; Sanchez-Villalobos et al., 2010; Tahamtan et al., 2010).

A study conducted in Pakistan aimed to assess the seroprevalence of brucellosis and analyze hematological and biochemical parameters in positive animals. Among the 50 horses that tested positive by RBPT and SAT, the mean WBC value was 8.25±2.79 (10^9/L), total protein 8.57±0.78 g/dL, ALT 28.18±5.71 U/L, AST 236.82±22.80 U/L, and ALP 92.36±13.84 U/L. In this particular study, a horse was identified as positive for brucellosis based on RBPT (++++) and SAT (1/320), with corresponding values of WBC 15.25 (10^9/L), total protein 6.1 g/dL, ALT 2.2 U/L, AST 141 U/L, and ALP 88 U/L. A comparative analysis of findings between the two studies reveals the consistent use of the same serological diagnostic methods (RBPT and SAT), while hematological and biochemical parameters exhibit partial similarities. Discrepancies in these parameters may be attributed to the fact that only one animal was tested in the present study, or could potentially be linked to variations in breed, diet, and age among the animals tested in both studies.

Brucellosis typically does not manifest with typical clinical signs in horses. However, affected animals may exhibit serofibrinous inflammatory reactions and, occasionally, purulent localized lesions in tendon sheaths, ligaments, bursae, synovia, joints, and shoulders (Dorneles et al., 2023). The distinctive clinical finding in the current study was the neurological symptom observed when the animal leaned its head against the wall. Notably, literature on brucellosis in horses does not commonly report neurological findings. Conversely, central nervous system (CNS) involvement is rarely observed in human brucellosis cases, raising concerns regarding prognosis (Tuncel et al., 2008; Maji et al., 2020).

The prevalence of human neurobrucellosis is reported to range between 1.7% and 10% worldwide and between 2.7% and 17.8% in Turkey (Maji et al., 2020; Bodur et al., 2003; Heper et al., 2004). In the present study, the sole neurological symptom observed was the animal's head position against the wall. On the other hand, *Equine Herpesvirus Type 1* (EHV-1) in horses commonly induces respiratory symptoms, abortions, and various neurological manifestations, varying from ataxia to paralysis of the forelimbs and hindlimbs (particularly hindlimbs), fecal and/or urinary incontinence, tail paralysis, and blindness (Jackson and Kendrick, 1971; van Maanen et al., 2001; Borchers et al., 2006; Gryspeerdt et al., 2011). As a result of the clinical observation and gain of anamnesis on the horse farm, no findings or disease history related to EHV-1 were determined. This observation underscores the importance of considering such manifestations among the clinical symptoms of brucellosis in horses, particularly for clinician veterinarians.

When the literature on the subject was examined, no study was found with dermatologic findings caused by brucellosis in horses. However, in human studies, cutaneous skin lesions have been reported in 5%-10% of *Brucella spp*. positive patients. (Berger et al., 1981, Ariza et al., 1989). In a study analyzing a total of 436 human brucellosis cases, 27 (6.1%) of the patients reported diffuse erythematous and papulonodular lesions. (Ariza et al., 1989).

The dermatological findings observed in this study could be attributed to alternative causes or potentially serve as symptoms associated with brucellosis, a condition rarely observed in horses. Conducting further studies on the subject would contribute to a more comprehensive interpretation of the results.

CONCLUSION

Brucellosis remains a threat to both public and animal health across numerous regions globally, with the exception of some countries. While the infection induces significant clinical signs in farm animals like cattle, sheep, and goats, it does not typically manifest typical clinical signs in

horses. Consequently, individuals involved in horse breeding, particularly clinician veterinarians, play a crucial role in safeguarding public and animal health by exercising caution. It is imperative for them to evaluate cases with an appropriate diagnostic method, especially when faced with suspicious circumstances.

ACKNOWLEDGMENT

Financial support: None.

Conflict of interest: The authors declared that there is no conflict of interest.

Ethical statement or informed consent: In accordance with the "Clinical applications for diagnostic and therapeutic purposes" mentioned in Article 1 of the "Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees" published in the Official Gazette No. 28914 in 2014, all examination materials evaluated in this study were obtained from the samples taken for diagnostic purposes during routine health examination from horse brought to Balıkesir Veterinary Faculty Animal Hospital, Internal Diseases Polyclinic by animal owner, again with the permission of animal owner.

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