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Original Article

The Effects of Neostigmine Administered at Different Doses on Hematological and Biochemical Parameters in Sheep

Duygu Durna Çorum[1]✉, Şerafettin Kartal[2], Fatma Akın[3], Hatice Rumeysa Ceyhan[4], Devran Coşkun[5], Orhan Çorum[6], Kamil Uney[7]

[1,6]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Hatay Mustafa Kemal, Hatay, TÜRKİYE
[2]Department of Veterinary Medicine, Samandag Vocational School, University of Hatay Mustafa Kemal, Hatay, TÜRKİYE
[3,5]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Siirt, Siirt, TÜRKİYE
[4]Department of Veterinary Medicine, Health Services Vocational School, University of Osmaniye Korkut Ata, Osmaniye, TÜRKİYE
[7]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Selcuk, Konya, TÜRKİYE

[1]https://orcid.org/0000-0003-1567-991X, [2]http://orcid.org/0000-0003-0935-2590, [3]http://orcid.org/0009-0002-8875-7894, [4]http://orcid.org/0000-0001-9239-5537, [5]https://orcid.org/0000-0003-1151-1861, [6]https://orcid.org/0000-0003-3168-2510, [7]https://orcid.org/0000-0002-8674-4873

✉ddurnacorum@gmail.com

ABSTRACT

This study aimed to assess the impact of varying dosages of neostigmine on hematological and biochemical parameters in sheep. The study was conducted on a total of 12 female Akkaraman sheep aged 2-3 years and weighing 40-50 kg. The sheep were randomly divided into two equal groups. Neostigmine was administered subcutaneously to the first group at 0.025 mg/kg and to the second group at 0.05 mg/kg. Blood samples were collected at 0, 0.5, 1, 3, 6, and 12 hours. Hematological and biochemical analyses were performed on the collected samples. No differences were observed in hematological parameters in the within-group and between-group evaluations. Compared to the 0.025 mg/kg dose group, albumin, triglyceride, and cholesterol levels increased at 0.5 hours in the 0.05 mg/kg dose group, while albumin, lactate dehydrogenase, triglyceride, and urea levels increased at 1 hour. Within-group evaluation showed no difference in the 0.05 mg/kg dose group ($p>0.05$), while in the 0.025 mg/kg dose group, a decrease in cholesterol at 0.5 hours and cholesterol, albumin, and creatine kinase levels at 1 hour was observed ($p<0.05$). In conclusion, while neostigmine administration to sheep at doses of 0.025 mg/kg and 0.05 mg/kg did not cause any differences in hematological parameters, it was determined to cause changes in albumin, triglyceride, cholesterol, and creatine kinase levels. Furthermore, it was observed that these changes in biochemical parameters did not correlate with increasing dose. These results may contribute to the safe use of neostigmine in sheep. However, molecular and histopathological investigation of the safety of neostigmine in sheep is needed.

Key words: Neostigmine, biochemistry, hematology, sheep.

Koyunlarda Farklı Dozlarda Uygulanan Neostigminin Hematolojik ve Biyokimyasal Parametreler Üzerindeki Etkileri

ÖZ

Bu çalışmanın amacı koyunlarda neositigminin farklı dozlarda uygulamasının hematolojik ve biyokimyasal parametrelere etkisini belirlemektir. Araştırma 2-3 yaş ve 40-50 kg canlı ağırlığa sahip toplam 12 adet dişi Akkaraman ırkı koyun üzerinde gerçekleştirildi. Koyunlar rastgele 2 eşit gruba ayrıldı. Birinci gruba 0.025 mg/kg ve ikinci gruba 0.05 mg/kg neostigmin derialtı yolla uygulandı. Kan örnekleri 0, 0.5, 1, 3, 6, ve 12 saatlerde toplandı. Elde edilen örneklerden hematolojik ve biyokimyasal analizler gerçekleştirildi. Grup içi ve gruplar arası değerlendirmede hematolojik parametrelerde herhangi bir farklılık görülmedi. 0.025 mg/kg doz grubu ile karşılaştırıldığında, 0.05 mg/kg doz grubunda albümin, trigliserit ve kolesterol seviyeleri 0.5 saatte yükselirken,

albümin, laktat dehidrogenaz, trigliserit ve üre seviyeleri 1 saatte arttı. Grup içi değerlendireme 0.05 mg/kg doz grubunda herhangi bir farklılık görülmezken ($p>0.05$) 0.025 mg/kg doz grubunda, 0.5 saatte kolesterol ve 1 saatte kolesterol, albümin ve kreatin kinaz seviyelerinde azalma gözlendi ($p<0.05$). Sonuç olarak koyunlara neostigminin 0.025 mg/kg ve 0.05 mg/kg dozlarda uygulaması hematalojik parametrelerde herhangi bir farklılığa neden olmaz iken albümin, trigliserit, kolesterol ve kreatin kinaz sevilerinde değişikliğe neden olduğu belirlendi. Ayrıca biyokimyasal parametrelerdeki bu değişikliğin doz artışı ile paralel olmadığı görüldü. Bu sonuçlar koyunlarda neostigminin güvenle kullanımına katkı sağlayabilir. Ancak koyunlarda neostigminin istenmeyen etkilerinin moleküler ve histopatolojik olarak incelenmesine ihtiyaç vardır.

Anahtar kelimeler: Neostigmin, biyokimya, hematoloji, koyun.

INTRODUCTION

Sheep are a valuable farm animal globally, producing meat, milk, leather, and wool. Sheep meat production has increased by 41.6% over the past 30 years to 9.96 million tons, accounting for 4.8% of total meat production (Coskun et al., 2023). Akkaraman sheep constitute 45% of the sheep breeds in our country. Akkaraman sheep have a high ability to adapt to different climatic conditions, resistance to diseases, and high productivity even under inadequate nutrition conditions (Ozmen et al., 2020).

Neostigmine is a parasympathomimetic drug that is a carbamate derivative belonging to the quaternary ammonium group (CVMP, 1996). Acetylcholine is a neurotransmitter that serves a diverse array of functions in the brain and other organ systems of the body (Sam and Bordoni, 2025). Acetylcholinesterase is an enzyme that catalyzes the hydrolysis of acetylcholine into choline and acetic acid (Neely et al., 2025). Neostigmine reversibly inhibits the acetylcholinesterase enzyme, leading to acetylcholine accumulation at the neuromuscular junction and causing stimulation of nicotinic and muscarinic receptors and the emergence of cholinergic effects (Habib and Gan, 2006; Neely et al., 2025). It is U.S. Food and Drug Administration-approved to counteract the effects of non-depolarizing neuromuscular blocking drugs and is used post-operatively to help muscles contract and facilitate breathing (Neely et al., 2025). Neostigmine exhibits a duration of action of 20 to 30 minutes, with a rapid beginning of effect, reaching its peak at 10 minutes (Ji et al., 2021). In addition, the cholinergic anti-inflammatory pathway is one of the important endogenous immunomodulatory mechanisms in the body, and central cholinergic transmission is activated by electrical stimulation of the vagus nerve or by cholinergic agonists. Neostigmine boosts acetylcholine and activates α7nAChR, resulting in an anti-inflammatory response (Si et al., 2023).

Neostigmine is used in animals to treat gastrointestinal stasis, myasthenia gravis, and curare poisoning (CVMP, 1996). It is also used to accelerate recovery from nondepolarizing neuromuscular blockade, and in conditions such as urinary retention, neurotoxicity due to snakebites, and other cholinergic muscle junction dysfunctions (Nieto et al., 2013; Reddy et al., 2023; Si et al., 2023; Žnidaršič et al., 2023). It is also used in ruminants for non-torsion rumen atony, abomasum atony, and cecal dilation (Reddy et al., 2019; Reddy et al., 2023; Žnidaršič et al., 2023). Neostigmine is recommended for subcutaneous administration in mammals at doses of 0.025-0.05 mg/kg (CVMP, 1996).

The drugs employed in treatment may induce negative consequences alongside the desired effects, and hematological and biochemical indicators can be utilized to assess these adverse effects (Dik et al., 2014). In cattle, neostigmin did not cause any differences in hematological and biochemical parameters after intramuscular and subcutaneous administration (Ismail et al., 2017). Although neostigmine has been reported to have no adverse effects on hemodynamic and respiratory parameters in sheep, no information was available regarding its effects on hematological and biochemical parameters (Hood et al., 1995). Therefore, to safely use neostigmine in sheep, its effects on hematological and biochemical parameters need to be determined. This study hypothesized that increasing the dose of neostigmine could affect hematological and biochemical parameters. This study aimed to assess the impact of subcutaneous neostigmine treatment at doses of 0.025 mg/kg and 0.05 mg/kg on hematological and biochemical markers in sheep.

MATERIALS AND METHODS

Animals

The research involved twelve female Akkaraman sheep, aged 2 to 3 years and weighing between 40 and 50 kg. The sheep were sourced from a local farm, and the experimental study was conducted at that farm. The sheep were found to be healthy after taking their medical history and conducting a clinical examination. The animals were separated into individual compartments a week prior to the study and kept there throughout the study period. Numbered collars and ear tags were used for animal classification. The animals were fed

commercial feed appropriate for their age and weight, and water and dry alfalfa were provided ad libitum. Ethical approval (2025/15) for animal experimental investigations was granted by the Local Ethics Committee for Animal Experiments at Selcuk University Faculty of Veterinary Medicine (Konya/Türkiye).

Experimental Procedure

A total of twelve sheep were randomly divided into two equal dose groups. Neostigmine (0.5 mg/mL injectable solution, Adeka, Istanbul/Türkiye) was administered subcutaneously to the sheep in the first group (n=6) at a dose of 0.025 mg/kg and to the sheep in the second group (n=6) at a dose of 0.05 mg/kg. Prior to the study, a catheter was placed in the right jugular vein of the sheep for blood collection. Blood samples (2 mL) were taken before drug administration (0 hours, control) and at 0.5, 1, 3, 6, and 12 hours following drug administration. For hemogram and biochemical analyses, 2 ml of blood were collected into tubes containing EDTA and gel, respectively. Hematological measurements were performed immediately after blood collection. Blood samples were centrifuged (4000 x g for 10 min) to obtain serum samples. Serum samples were stored at -80°C until analysis time. Sheep were clinically observed throughout the study to monitor the clinical effects of different drug doses.

Laboratory Analysis

Hematological and biochemical parameters were analyzed at the times indicated above (0.5, 1, 3, 6, and 12 hours). A hemocell counter (Shenzhen Mindray Bio-Medical Electronics, BC-2800 Auto Hematology Analyzer, China) was used to measure red blood cell, hemoglobin, white blood cell, hematocrit, and platelet parameters. An autoanalyzer (Abbott Architect c8000, Abbott Core Laboratory, USA) device was used to measure biochemical parameters such as urea, creatinine, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholesterol, triglycerides, albumin, total protein, creatine kinase (CK).

Statistical Analysis

Complete blood count and biochemical data were presented as mean ± standard deviation. Statistical analysis was conducted utilizing SPSS software (version 22.0; IBM), and a $P<0.05$ value was considered statistically significant. An independent t-test was used to determine statistical differences between neostigmine dose groups. ANOVA (posthoc Tukey) and posthoc Tukey tests were used to determine statistical differences over time between dose groups.

RESULTS AND DISCUSSION

Results

No differences were observed in the behavior, appetite, feces, or movement of sheep after subcutaneous administration of neostigmine at doses of 0.025 and 0.05 mg/kg. Hematological parameters after administration of neostigmine at doses of 0.025 and 0.05 mg/kg in sheep are presented in Table 1. No differences were found in hematological parameters in intra-group and inter-group evaluations ($p>0.05$).

Table 1. Hematological parameters after subcutaneous administration of neostigmine at doses of 0.025 and 0.5 mg/kg in sheep (n = 6, mean ± SD).

Parameter	Group (mg/kg)	0 h	0.5 h	1 h	3 h	6 h	12 h
WBC (10^9/L)	0.025	12.17±5.78	14.28±6.86	13.12±6.62	11.67±3.85	14.40±6.67	11.77±4.52
	0.05	13.25±4.34	13.83±4.78	12.78±4.3	13.95±4.57	14.10±4.18	14.86±4.41
RBC (10^{12}/L)	0.025	9.48±1.70	10.21±1.65	8.90±1.79	9.36±1.78	9.41±1.43	8.82±1.40
	0.05	9.26±0.58	9.53±0.59	8.97±0.69	9.08±0.84	9.20±0.57	9.14±0.56
HGB (g/dL)	0.025	9.57±1.64	10.30±1.77	8.88±1.89	9.35±1.96	9.32±1.53	8.27±1.19
	0.05	9.02±0.90	9.27±1.07	8.67±1.06	8.83±1.37	9.0±0.99	8.95±1.0
HCT (%)	0.025	30.97±5.52	33.42±4.83	28.92±5.41	30.47±5.29	30.90±4.00	28.32±4.26
	0.05	29.67±3.77	30.03±4.27	28.87±3.95	28.28±4.72	27.90±4.52	28.73±3.78
PLT (10^9/L)	0.025	477.33±170.38	544.50±170.16	435.17±135.29	465.67±102.27	437.50±136.03	480.67±145.10
	0.05	449.17±185.61	443.33±114.71	516.67±121.60	444.00±101.20	369.33±149.71	488.17±177.94

WBC; white blood cell, RBC; red blood cell, HGB;hemoglobin, HCT; hematocrit, PLT; platelet, h; hour.

Biochemical parameters after administration of neostigmine at doses of 0.025 and 0.05 mg/kg to sheep are presented in Table 2. Compared to the 0.025 mg/kg dose group, albumin, triglyceride, and cholesterol levels increased at 0.5 hours, and albumin, LDH, triglyceride, and urea levels increased at 1 hour in the 0.05 mg/kg dose group ($p<0.05$). There were no variations in other parameters and time periods ($p>0.05$). At a dose of 0.05 mg/kg, no time-dependent changes were observed in biochemical parameters ($p>0.05$). However, in the 0.025 mg/kg dose group, a decrease in cholesterol was observed at 0.5 hours, and cholesterol, albumin, and creatine kinase levels were observed at 1 hour ($p<0.05$).

Table 2. Biochemical parameters after subcutaneous administration of neostigmine at doses of 0.025 and 0.5 mg/kg in sheep (n = 6, mean ± SD).

Parameter	Group (mg/kg)	0 h	0.5 h	1 h	3 h	6 h	12 h
Albumin (g/dL)	0.025	2.05±0.21^{a}	1.78±0.28^{ab}	1.47±0.61^{b}	2.03±0.27^{a}	2.05±0.15^{a}	2.08±0.12^{a}
	0.05	2.05±0.20	2.20±0.28*	2.10±0.18*	2.02±0.15	2.03±0.18	2.05±0.18
ALP (U/L)	0.025	87.67±33.71	73.50±30.59	60.33±31.76	76.67±36.45	87.67±34.52	89.50±33.32
	0.05	84.50±11.84	82.00±10.60	72.33±9.99	78.17±13.83	82.17±11.81	81.67±11.52
AST (U/L)	0.025	125±47.59	108.50±59.59	95.33±31.24	144.83±57.04	131.0±51.66	120.83±55.41
	0.05	124.5±60.09	136.33±72.42	126±63.47	105.50±34.12	128±55.54	129.67±54.58
ALT (U/L)	0.025	15.67±3.72	12.67±2.42	11.50±4.76	16.17±8.13	14.67±3.50	13.17±5.19
	0.05	15.17±8.75	17.7±10.38	11.33±9.20	10.67±3.01	14.17±7.47	12.17±7.41
LDH (U/L)	0.025	480.62±88.77	412.67±96.17	329.33±147.22	464.33±99.33	475.17±90.11	495.33±39.13
	0.05	511.00±91.54	555.00±124.92	511.67±108.52*	503.67±96.28	514.33±105.59	528.33±100.69
TRIG (mg/dL)	0.025	21.33±12.04	15.83±7.31	9.00±3.29	19.17±7.03	17.83±9.43	17.33±5.68
	0.05	22.00±3.63	26.17±7.99*	17.83±2.64*	21.00±4.00	21.83±5.85	22.83±4.62
CHOL (mg/dL)	0.025	70.17±4.45^{a}	45.67±8.98^{b}	43.83±21.30^{b}	62.17±14.52^{ab}	65.17±9.02^{a}	65.00±9.17^{ab}
	0.05	63.83±8.89	64.83±13.47*	58.00±10.81	59.50±11.61	58.00±11.45	59.00±10.55
CK (U/L)	0.025	111.83±23.44^{ab}	92.67±20.66^{ab}	71.50±31.23^{b}	149.67±95.22^{ab}	81.33±20.49^{a}	88.17±21.99^{a}
	0.05	117.50±32.55	109.83±19.45	104.67±20.14	100.67±11.45	107.50±37.04	101.17±25.00
Creatinine (mg/dL)	0.025	0.71±0.16	0.60±0.22	0.53±0.07	0.64±0.11	0.72±0.18	0.83±0.15
	0.05	0.66±0.13	0.66±0.14	0.66±0.15	0.71±0.18	0.63±0.14	0.65±0.17
Urea (mg/dL)	0.025	14.17±3.06	11.33±3.27	10.33±2.50	13.83±2.04	14.00±3.90	14.67±1.97
	0.05	13.83±2.14	13.67±2.07	13.50±2.07*	14.33±2.66	13.33±1.86	13.00±3.03

^{a,b}; Shows within-group statistical difference on the same line.
*; Shows between group statistical difference on the same column.
ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine aminotransferase, LDH; lactate dehydrogenase, TRIG; triglyceride, CHOL; cholesterol, CK; creatine kinase, h; hour.

Discussion

Neostigmine is a parasympathomimetic drug that reversibly inhibits the acetylcholinesterase enzyme, leading to acetylcholine accumulation at the neuromuscular junction (Habib and Gan, 2006; Si et al., 2023). Neostigmine causes contractions in smooth muscles, leading to increased peristaltic movements in the intestines (10–30 minutes after parenteral administration), increased body secretions, increased ciliary activity and bronchial secretions in the respiratory system, and bladder emptying (Anonymous, 2026). Although neostigmine is used in the treatment of rumen and intestinal atony in sheep, no studies have been found regarding its effects on hematological and biochemical parameters. This study is the first to determine the effects of different doses of neostigmine on hematological and biochemical parameters.

Neostigmine has been reported to be used subcutaneously in all mammalian species at doses of 0.025-0.05 mg/kg (CVMP, 1996). Studies have shown that it has been used at doses of 0.02 mg/kg in cattle (Reddy et al., 2019; Reddy et al. 2023), 0.02-0.04 mg/kg in horses (Nieto et al., 2013), and 0.02-0.07 mg/kg in dogs (Martin-Flores et al., 2017). The commercial preparation of neostigmine is recommended for use in sheep at a dose of 0.022 mg/kg via subcutaneous or intramuscular injection (Anonymous, 2026). Thus, this study preferred subcutaneous injection of neostigmine at doses of 0.025 mg/kg and 0.05 mg/kg.

The adverse effects of neostigmine are due to excessive cholinergic stimulation (CVMP, 1996). Neostigmine causes cholinergic side effects in many tissues and organs. The main side effects of neostigmine include bradyarrhythmias such as complete heart block and asystole, hypertension, bronchoconstriction in the bronchi, increased saliva and other body secretions, miosis, nausea, vomiting, colic, and increased peristalsis in

the gastrointestinal system. The most significant side effect of neostigmine is cholinergic crisis, which can lead to loss of function in many muscle groups, including respiratory muscles, and may result in death (Neely et al., 2025; Anonymous, 2026).

Neostigmine has been reported to have no adverse effects on hemodynamic and respiratory parameters when administered intrathecally at doses of 0.2 and 2 mg in sheep (Hood et al., 1995); increased rumen and bowel motility and decreased systolic blood pressure when administered intramuscularly and subcutaneously at a dose of 0.02 mg/kg in cattle (Ismail et al., 2017); not caused spinal tissue damage after intrathecal administration in rats and dogs (Yaksh et al., 1995); and may cause neurotoxicity with repeated administration in rabbits (Demirel et al., 2006).

In this study, subcutaneous administration of neostigmine at 0.025 mg/kg and 0.05 mg/kg to sheep did not cause any differences in hemogram parameters (red blood cell, hemoglobin, white blood cell, hematocrit, and platelet, Table 1, $p>0.05$). The subcutaneous and intramuscular administration of neostigmine at a dose of 0.02 mg/kg in cattle did not result in any variations in hemoglobin, , red blood cell, or white blood cell levels (Ismail et al., 2017). Complete blood count parameters primarily provide information about bone marrow function. They also provide information about fluid and electrolyte balance, liver (site of coagulation factor synthesis), and kidney (site where erythropoietin is activated) functions in the patient (Corum et al., 2015). The absence of any change in hemogram parameters at doses of 0.025-0.05 mg/kg indicates that neostigmine has no adverse effects on bone marrow and electrolyte balance in sheep.

Following subcutaneous injection of neostigmine to sheep at doses of 0.025 mg/kg and 0.05 mg/kg, no variations were observed in albumin, ALP, AST, ALT, and creatinine levels in both intra-group and inter-group assessments. In the 0.05 mg/kg dose group, albumin, triglyceride, and cholesterol levels elevated after 0.5 hours, while albumin, LDH, triglyceride, and urea levels rose at 1 hour, in comparison to the 0.025 mg/kg dose group ($p<0.05$). In the 0.025 mg/kg dose group, a decrease in cholesterol was observed at 0.5 hours, and cholesterol, albumin, and creatine kinase levels were observed at 1 hour ($p<0.05$). However, it has been reported that subcutaneous and intramuscular administration of neostigmine at a dose of 0.02 mg/kg in cattle did not cause any differences in creatinine, AST, ALP, ALT, total protein, albumin, blood urea nitrogen, calcium, potassium, magnesium, sodium, and chloride values (Ismail et al., 2017). In this study, the differences in biochemical parameters returned to their normal values at 3 hours, indicating that the increase in these biochemical parameters was temporary.

CONCLUSION

Neostigmine did not cause any differences in hemogram parameters in sheep at doses of 0.025 mg/kg and 0.05 mg/kg. Significant dose- and time-dependent changes in some biochemical parameters were observed immediately (0.5-1 h) after neocytigmine administration, but these changes returned to normal at 3 hours. Subcutaneous injection of neostigmine at doses of 0.025 and 0.05 mg/kg in sheep did not cause significant adverse effects in hemogram parameters, but it was determined to lead to temporary changes in some biochemical parameters. These findings support the use of neostigmine in sheep; nonetheless, molecular and histological evaluations of its safety after both single and multiple doses are needed.

Declaration of interests

The authors declared that there is no conflict of interest.

Author Contributions

Duygu DURNA ÇORUM **:**Conceptualization; data curation; formal analysis; investigation; methodology; project administration; writing— original draft; writing—review and editing.
Şerafettin KARTAL **:**Data curation; formal analysis; investigation; methodology; writing— original draft; writing—review and editing.
Fatma AKIN: Data curation; formal analysis; investigation; methodology; writing— original draft; writing—review and editing.
Hatice Rumeysa CEYHAN**:** Data curation; formal analysis; investigation; methodology; writing— original draft; writing—review and editing.
Devran COŞKUN**:** Data curation; formal analysis; investigation; methodology; writing— original draft; writing—review and editing.

***Orhan ÇORUM*:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; writing— original draft; writing—review and editing.
***Kamil UNEY* :**Conceptualization; data curation; formal analysis; investigation; methodology; project administration; writing— original draft; writing—review and editing.

ORCID

Duygu DURNA ÇORUM https://orcid.org/0000-0003-1567-991X
Şerafettin KARTAL http://orcid.org/0000-0003-0935-2590
Fatma AKIN http://orcid.org/0009-0002-8875-7894
Hatice Rumeysa CEYHAN http://orcid.org/0000-0001-9239-5537
Devran COŞKUN https://orcid.org/0000-0003-1151-1861
Orhan ÇORUM https://orcid.org/0000-0003-3168-2510
Kamil UNEY https://orcid.org/0000-0002-8674-4873

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REFERENCES

Anonymous, (2026) https://medicines.health.europa.eu/veterinary/en/600000017531 (Accessed date: 13.02.2026)

Corum, O., Er, A., Dik, B., Eser, H., Bahcivan, E., & Yazar, E. (2015). Determination of the safety of tulathromycin in sheep. *Eurasian Journal of Veterinary Sciences*, *31*, 152-157.

Coskun, D., Corum, O., Durna Corum, D., & Uney, K. (2023). Comparative pharmacokinetics of intravenous enrofloxacin in one-six-and twelve-month-old sheep. *Current Drug Metabolism*, *24*, 780-785. https://doi.org/10.2174/0113892002278220231208072351.

CVMP.(1996).https://www.ema.europa.eu/en/documents/mrl-report/neostigmine-summary-report committee-veterinary-medicinal-products_en.pdf (Accessed date: 13.02.2026)

Demirel, E., Ugur, H. C., Dolgun, H., Kahilogullari, G., Sargon, M. F., Egemen, N., & Kecik, Y. (2006). The neurotoxic effects of intrathecal midazolam and neostigmine in rabbits. *Anaesthesia and Intensive Care*, *34*, 218-223. https://doi.org/10.1177/0310057X0603400204.

Dik, B., Er, A., & Corum, O. (2014). Effect of alcoholic extract of Tarantula cubensis (Theranekron®) on serum thiobarbituric acid-reactive species concentrations in sheep. *Eurasian Journal of Veterinary Sciences*, *30*, 68-71.

Habib, A.S., & Gan, T.J. (2006). Use of neostigmine in the management of acute postoperative pain and labour pain: a review. *CNS Drugs*, *20*, 821-839. https://doi.org/10.2165/00023210-200620100-00004.

Hood, D.D., Eisenach, J.C., Tong, C., Tommasi, E., & Yaksh, T.L. (1995). Cardiorespiratory and spinal cord blood flow effects of intrathecal neostigmine methylsulfate, clonidine, and their combination in sheep. *Anesthesiology*, *82*, 428-435. https://doi.org/10.1097/00000542-199502000-00013.

Ismail, Z.B., Al-Zghoul, A., & Al-Zoubi, I. (2017). Clinical, behavioral, hematological and serum biochemical effects of neostigmine in normal dairy cows following subcutaneous and intramuscular administration of single dose. *Research Opinions in Animal & Veterinary Sciences*, *7,* 49-53.

Ji, W., Zhang, X., Liu, J., Sun, G., Wang, X., Bo, L., & Deng, X. (2021). Efficacy and safety of neostigmine for neuromuscular blockade reversal in patients under general anesthesia: a systematic review and meta-analysis. *Annals of Translational Medicine*, *9,* 1691. https://doi.org/10.21037/atm-21-5667.

Martin-Flores, M., Lorenzutti, A.M., Litterio, N.J., Rossetti, V.L., Zarazaga, M.P., Bonetto, C.C., & Aguirre, G.E. (2017). Speed of reversal of vecuronium neuromuscular block with different doses of neostigmine in anesthetized dogs. *Veterinary Anaesthesia and Analgesia*, *44*, 28-34. https://doi.org/10.1111/vaa.12395.

Neely, G.A., Sabir, S., & Kohli, A. (2025). Neostigmine. [Updated 2025 Jan 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Nieto, J.E., Morales, B., Yamout, S.Z., Stanley, S.D., Harmon, F.A., & Snyder, J.R. (2013). In vivo and in vitro effects of neostigmine on gastrointestinal tract motility of horses. *American Journal of Veterinary Research*, *74*, 579-588. https://doi.org/10.2460/ajvr.74.4.579.

Ozmen, O., Kul, S., & Gok, T. (2020). Determination of genetic diversity of the Akkaraman sheep breed from Turkey. *Small Ruminant Research*, *182*, 37-45. https://doi.org/10.1016/j.smallrumres.2019.10.009.

Reddy, B.S., Sivajothi, S., & Swetha, K. (2023). Ultrasonographic assessment of effect of neostigmine on abomasal atony in young calves. *Buffalo Bulletin*, *42*, 391-396. https://doi.org/10.56825/bufbu.2023.4233968.

Reddy, B.S., Vijayakumar, G., Balasubramaniam, G.A., Sivaraman, S., & Kathirvel, S. (2019). Efficacy of neostigmine and azithromycin in buffaloes with functional ileus. *Buffalo Bulletin*, *38*, 649-652.

Sam C, Bordoni B. Physiology, Acetylcholine. [Updated 2023 Apr 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025

Si, S., Zhao, X., Su, F., Lu, H., Zhang, D., Sun, L., Wang, F., & Xu, L. (2023). New advances in clinical application of neostigmine: no longer focusing solely on increasing skeletal muscle strength. *Frontiers in Pharmacology*, *14*, 1227496. https://doi.org/10.3389/fphar.2023.1227496.

Yaksh, T.L., Grafe, M.R., Malkmus, S., Rathbun, M.L., & Eisenach, J.C. (1995). Studies on the safety of chronically administered intrathecal neostigmine methylsulfate in rats and dogs. *Anesthesiology*, *82*, 412-427. https://doi.org/10.1097/00000542-199502000-00012.

Žnidaršič, N., Štrbenc, M., Grgurevič, N., & Snoj, T. (2023). Potential revival of cholinesterase inhibitors as drugs in veterinary medicine. *Frontiers in Veterinary Science*, *10*, 1125618. https://doi.org/10.3389/fvets.2023.1125618