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Effects of tiletamine-zolazepam and sevoflurane combination on anaesthetic quality and haemato-biochemical parameters of domestic chicken

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Abstract
The present study was aimed to investigate the effects of tiletamine-zolazepam and sevoflurane combination on quality of anaesthesia, haematological and biochemical parameters in healthy adult domestic chickens. Eighteen birds were randomly divided into three groups and administered zolazepam-tiletamine at doses of 15 mg/kg, 20 mg/kg, and 25 mg/kg, respectively, followed by sevoflurane at dose rate of 1.5% for maintenance. There was non-significant variation of haematological parameters, such as total erythrocytic count, total leucocytic count, haemoglobin, erythrocytic sedimentation rate, packed cell volume, monocyte, lymphocyte, and granulocyte counts was recorded. Biochemical parameters, including blood urea nitrogen, creatinine, alanine transaminase, and aspartate aminotransferase, also remained within normal ranges, except for a significant increase in blood glucose levels. The combination provided adequate analgesia, muscle relaxation, and sedation, with smooth induction and recovery. Induction time was shortest but recovery time was longest in the group administered with 25 mg/kg zolazepam-tiletamine, while it was longest in the group administered with 15 mg/kg zolazepam-tiletamine followed by shortest recovery time. The study concludes that zolazepam-tiletamine and sevoflurane combination is a suitable as anaesthetic protocol for domestic chickens, with potential applications in surgical and diagnostic procedures as well as for transportation of birds.

Keywords: Chicken, tiletamine-zolazepam, sevoflurane, biochemical and haematology.

1. Introduction
Anaesthesia plays a vital role in avian medical treatment, but providing safe and effective anaesthesia for birds remains a significant challenge in veterinary practice. With the growing importance of avian medicine and research, there is an urgent need for reliable anaesthetic agents. Tiletamine-zolazepam combination has been reported to be an effective and safe anaesthetic medication for various wild animals belonging to other classes such as mammals. It is a non-opioid, non-barbiturate injectable anaesthetic which is a combination of equal concentrations of tiletamine and zolazepam in 1:1 ratio. Tiletamine is an atypical dissociative anaesthetic and belongs to the NMDA receptor antagonist class. Tiletamine is a long-acting dissociative anaesthetic agent which is more potent than ketamine (Dugassa and Fromsa, 2018) [6]. Zolazepam is a substituted benzodiazepine, which has a sedative effect and also works in synergy with tiletamine, being a positive allosteric modulator of GABA receptors. Sevoflurane is a halogenated volatile anaesthetic, which has also been shown to be frequently used for birds, in addition to being commonly used for mammals which has low blood/gas and tissue/blood distribution coefficients and a very high bioavailability, providing a shorter induction and recovery period. The present study investigated the efficacy of tiletamine-zolazepam and sevoflurane as anaesthetic combination to evaluate the quality of anaesthesia and haemato-biochemical changes in domestic chickens.

2. Materials and Methods
The study was carried out on 18 healthy adult domestic chickens (*Gallus domesticus*) of similar body weight and of either sex and were randomly divided into 3 groups comprising of 6 birds in each. Following pre-anaesthetic evaluation all the birds fasting was done for 12 hours prior to the experiment; however, water was provided uninterruptedly. Zolazepam-tiletamine was used for induction of anaesthesia. Maintenance of anaesthesia was done using sevoflurane mixed in oxygen. Zolazepam tiletamine was administered intramuscularly at the

dose rate of 15 mg/kg, 20 mg/kg and 25 mg/kg in group I, group II and group III. Sevoflurane was used at a constant rate of 1.5% in all the groups as maintenance of anaesthesia. Blood samples were collected at 0 minutes (before administration of zolazepam-tiletamine), 30 minutes and 60 minutes and 24 hours of anaesthetic procedure. Haematological parameters *viz* Haemoglobin (g/dl), Total Erythrocytic Count (TEC,10^6 /mm^3), Total Leucocytic Count (TLC, 10^3 /mm^3), Packed Cell Volume (PCV, %), Granulocyte (%), Lymphocyte (%), Monocyte (%), Erythrocyte sedimentation rate (ESR, mm/hr) were estimated using automated cell counter (Comptier, Haematology Analyser, Melet Schoesing Laboratories, Made in France, Serial No.6KE368). Biochemical parameter *viz* Alanine Transaminase (ALT, IU/L), Aspartate Aminotransferase (AST, IU/L), Glucose (mg/dl), Blood Urea Nitrogen (BUN, mg/dl), Creatinine (mg/dl) were estimated using spectrophotometer. All the anaesthetic parameters and quality of anaesthesia was recorded as per standard protocol in respect of induction, sedation, analgesia, muscle relaxation and recovery behaviour were recorded at 0 minutes (after endotracheal intubation), 5 minutes after intubation and every 10 minutes during maintenance of anaesthesia for a time period of 60 minutes. The statistical analysis of the data was carried out according to the standard statistical procedure described by Snedecor and Cochran (1994) [10] using S.P.S.S. version 20.

3. Results and Discussion

3.1 Haematological parameters

There was no significant difference ($p>0.05$) in the values of haemoglobin, total erythrocyte count, total leukocyte count, packed cell volume, granulocyte, monocyte, lymphocyte and erythrocyte sedimentation rate between different time interval. A non-significant ($p>0.05$) difference in haemoglobin, total leucocyte count and total erythrocyte count value with time was also reported by Debbarma *et al.* (2023) [3] with the use of tiletamine-zolazepam to produce surgical anaesthesia in domestic chicken. A nonsignificant ($p>0.05$) difference in packed cell volume was also reported by Javdani *et al.* (2014) [7] with the use of ketamine in broiler chicken. Deori *et al.* (2017) [4] reported a nonsignificant ($p>0.05$) difference in the values of granulocyte, monocyte, lymphocyte with time interval with the use of isoflurane in chicken. The values of haematological parameters were under normal physiological limit during the entire period of observation, which indicates the safety of tiletamine-zolazepam and sevoflurane combination as general anaesthesia in domestic chicken.

Table 1: Changes in MEAN ± SE of different haematological parameters:

Parameters	Group	0 minute	30 minutes	60 minutes	24 hours
Haemoglobin (g/dl)	I	15.51±1.61	12.89±1.07	13.81±1.00	13.47±0.95
	II	13.84±1.04	13.34±1.84	13.02±0.74	13.21±1.29
	III	14.87±0.49	14.12±0.99	13.69±0.35	15.02±0.54
Total Erythrocyte Count (10^6 /mm^3)	I	3.28 ± 0.13	3.4 ± 0.19	3.59 ± 0.17	3.39 ± 0.14
	II	3.19 ± 0.17	3.43 ± 0.15	3.63 ± 0.18	3.35 ± 0.14
	III	2.97 ± 0.23	3.14 ± 0.25	3.37 ± 0.26	3.17 ± 0.22
Total Leucocyte Count (10^3 /mm^3)	I	26.73±3.25	20.58±4.82	26.40±3.86	25.58±4.82
	II	21.11±5.19	24.99±2.82	20.94±4.64	17.79±2.78
	III	16.07±1.23	14.41±2.25	14.19±1.85	15.71±0.67
Packed Cell Volume (%)	I	38.03±2.46	39.92±2.39	36.83±1.38	38.31±2.53
	II	36.42±1.72	37.71±3.17	42.07±2.12	40.77±0.98
	III	42.81±2.39	42.14±1.08	45.57±1.94	44.92±1.17
Granulocyte (%)	I	66.57±1.31	66.06±0.72	65.01±1.36	64.45±0.95
	II	64.23±1.15	63.63±0.75	66.57±0.92	64.40±1.08
	III	67.86±1.55	66.47±0.83	66.5±0.52	66.07±0.74
Monocyte (%)	I	4.62±0.28	4.47±0.19	4.42±0.37	4.17±0.31
	II	6.06±0.26	6.38±0.24	6.03±0.24	5.58±0.23
	III	4.23±0.30	4.13±0.16	4.6±0.20	4.63±0.31
Lymphocyte (%)	I	28.8±1.31	29.47±0.82	30.57±0.98	31.38±1.12
	II	29.73±0.68	30.00±0.87	27.4±0.66	30.05±0.40
	III	27.9±1.49	29.47±1.03	28.90±0.62	29.33±1.03
Erythrocyte Sedimentation Rate (mm/hr)	I	3.14±0.33	3.98±0.50	3.58±0.64	3.72±0.69
	II	3.23±0.44	3.53±0.55	3.18±0.50	3.22±0.55
	III	3.01±0.21	3.35±0.18	2.87±0.24	2.99±0.27

3.2 Biochemical parameters

There was no significant difference ($p>0.05$) in the values of blood urea nitrogen, creatinine and aspartate aminotransferase between different time interval; however a non-significant decrease in alanine transaminase level was recorded from 0 minute to 60 minutes. The glucose level increased significantly ($p<0.05$) from 0 minute to 30 minutes, where the value increased from 193.35 ± 5.69 to 203.62±5.10 in group I; from 191.83 ±3.06 to 200.68 ±4.35 in group II and from 189.85±4.80 to 200.48±3.16 in group III. From 30 minutes to 60 minutes the blood glucose level increased non-significantly ($p>0.05$) in all the groups. The increase in glucose level was in agreement with the findings of Chan *et al.* (2013) [2] in crested serpent eagle and Al-Sobayail and Omer in ostriches. Increase in glucose level might be due to anaesthetic stress which inhibits the release of insulin from pancreas which causes an increase in glucose level Zardooz *et al.*, (2010) [11]. A non-significant difference in accordance to

the study conducted in the values of blood urea nitrogen and aspartate aminotransferase was reported by Chan *et al.* (2013) [2] in crested serpent eagle subjected to sevoflurane and isoflurane anaesthesia. A non-significant change in creatinine level was reported by Debbarma *et al.* (2023) [3] under tiletamine and zolazepam anaesthesia in chicken. Chan *et al.* (2013) [2] reported significant decrease in the level of ALT crested serpent eagle. Normal values of BUN, ALT, AST, creatinine during the period of study indicates minimal effect of tiletamine-zolazepam and sevoflurane on the renal system and hepatic system of birds.

Table 2: Changes in MEAN ± SE of different biochemical parameters:

Parameters	Group	0 minute	30 minutes	60 minutes	24 hours
Blood Urea Nitrogen (mg/dl)	I	4.80±0.14	5.35±0.24	5.69±0.27	5.1±0.28
	II	5.22±0.30	5.08±0.18	4.91±0.13	5.01±0.31
	III	4.89±0.27	5.35±0.19	5.18±0.26	5.07±0.28
Creatinine (mg/dl)	I	0.27±0.01	0.26±0.01	0.25±0.01	0.28±0.02
	II	0.26±0.03	0.25±0.02	0.26±0.02	0.25±0.02
	III	0.28±0.02	0.28±0.02	0.30±0.02	0.27±0.02
Glucose (mg/dl)	I	193.35±5.69	203.62±5.10	212.10±3.79	205.55±5.60
	II	191.83±3.06	200.68±4.35	207.22±5.13	210.25±4.23
	III	189.85±4.80	200.48±3.16	206.32±4.21	208.38±3.84
Aspartate Aminotransferase (IU/L)	I	220.67±9.36	234.98±10.48	214.85±10.17	209.22±7.09
	II	221.87±19.71	235.92±19.49	208.27±7.45	201.15±4.56
	III	207.85±11.01	224.70±10.32	215.48±11.31	213.70±7.43
Alanine Transaminase (IU/L)	I	6.00±0.80	5.75±0.78	5.17±0.74	5.50±0.73
	II	6.88±0.44	6.32±0.44	5.83±0.45	6.20±0.39
	III	7.12±0.24	6.32±0.20	5.90±0.15	6.50±0.12

3.3. Quality of anaesthesia

The assessment of quality of sedation score revealed good to excellent in group I, while in group II & III all the birds showed excellent quality of sedation. Author's finding was in accordance with the study of Debbarma *et al.*, (2024) [3] in chicken. The average induction time (min) following injection was recorded as 6.97±0.74 (min), 4.65±0.31 (min) and 2.30±0.24 (min) in group I, II and III respectively. There was a significant difference in respect of average induction time was observed among the groups; the fastest of which was seen in group III, followed by group II and I. The trial showed higher dose rate led to shorter induction time. Similar relation of dose and induction time was also reported by Alves Nicolau *et al.* (1999) [1] with tiletamine and zolazepam in quail. The all the birds of group I showed moderate induction behaviour, followed by partially moderate to smooth in group II and completely smooth in all the birds in group III. Lin *et al*. (1997) [8] also experienced smooth induction behaviour ratite birds with tiletamine-zolazepam induction. The quality of analgesia was graded as good in all the birds of group I, partially good to excellent in group II and excellent in group III. The degree of muscle relaxation also observed in similar trend *viz*, excellent in group III, followed by group II and I. Similar trend of quality of analgesia also reported by Debbarma *et al.* (2024) [3] in chicken exposed to tiletamine-zolazepam combination. Muscle relaxant effect of zolazepam-tiletamine combination can be due to induced inhibition of internuncial neurons at the spinal level (Salve *et al*., 2022) [9]. The Mean ± S.E. in respect of recovery time following tiletamine-zolazepm and sevoflurane administration in group I, II & III was found to be 3.58±1.04 hours, 4.75±0.28 hours and 6.58±0.64 hours respectively. The values were significantly differs among the groups. The recovery time is dose-dependent, with higher doses of dissociative anaesthetics resulting in longer recovery times (Salve *et al.*, 2022) [9]. All the birds irrespective of groups exhibited smooth recovery following anaesthetic protocol.

4. Conclusion

In conclusion it was observed that the combination of tiletamine-zolazepam and sevoflurane showed no deleterious impact on the haematological and biochemical parameters except for transient increase in glucose level during the study period. The overall quality anaesthesia is may be graded as good to excellent without complication in recovery. Thus the anaesthetic protocol used in the study was considered safe for use in chicken.

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